

# The Alzheimer Protocols: A Nanorobotic Cure for Alzheimer's Disease and Related Neurodegenerative Conditions

© 2016 Robert A. Freitas Jr. All Rights Reserved.

**Abstract.** Alzheimer's disease (AD), along with many other neurodegenerative disorders including Parkinson's disease, is presently incurable despite many decades of research and many billions of dollars invested in the effort. Future developments of conventional technologies now on the long-term R&D horizon – including pharmaceuticals, nanoparticles, gene therapies, stem cells, and anti-aging drugs – will likely require huge investments, many decades of further development, and (if history is a guide) may fail to provide a complete cure because Alzheimer's is a highly complex, highly multifactorial disorder with dense causal networks that will probably resist any attempt to find a single “magic bullet” drug or simple curative treatment. But the advent of the nanofactory – a proposed new technology for atomically precise manufacturing – will make possible a revolutionary new paradigm in human health care: medical nanorobotics. Medical nanorobotics will provide a single powerful general-purpose therapeutic platform that can simultaneously address many different kinds of biological malfunctions, using platform variants specifically and efficiently targeted to each of the multifactorial pathologies comprising Alzheimer's disease. The proposed nanorobotic treatment for AD, called the Alzheimer Protocols, can be conceptually organized as a series of three specific protocols aimed at three distinct clinical objectives: (1) genetic derisking, (2) tissue rejuvenation, and (3) neural reconstruction. The outcome of these simultaneous nanomedical interventions, likely performed on an existing or preclinical Alzheimer's patient over a period of perhaps one week using a few trillion nanorobots in an appropriate clinical setting, will be a halt to the ongoing damage and a reversal of existing damage caused by AD, resulting in a complete cure for Alzheimer's disease and related neurodegenerative conditions.

**Cite as:** Robert A. Freitas Jr., “The Alzheimer Protocols: A Nanorobotic Cure for Alzheimer's Disease and Related Neurodegenerative Conditions,” IMM Report No. 48, June 2016; <http://www.imm.org/Reports/rep048.pdf>.

## Table of Contents

<b>Chapter 1. Introduction .....</b>	<b>5</b>
<b>Chapter 2. Background on Alzheimer’s Disease.....</b>	<b>8</b>
<b>2.1 Prevalence and Social Cost of the Disease .....</b>	<b>10</b>
<b>2.2 Prevention of Alzheimer’s Disease .....</b>	<b>13</b>
2.2.1 Preventative Medications.....	14
2.2.2 Preventative Lifestyle .....	16
2.2.3 Preventative Diet .....	17
<b>2.3 Four Progressive Stages of Alzheimer’s Disease .....</b>	<b>23</b>
<b>2.4 Molecular Pathologies of Alzheimer’s Disease .....</b>	<b>30</b>
2.4.1 Amyloid Pathologies .....	31
2.4.2 Tau Protein Pathologies.....	39
2.4.3 Other Pathologies and Biochemical Events in Alzheimer’s Disease.....	45
2.4.4 Aging as a Risk Factor.....	58
2.4.5 Genetics and Epigenetics .....	61
2.4.6 Does Mercury Contribute to Alzheimer’s Disease? .....	66
2.4.7 Lipofuscin Hypothesis of Alzheimer’s Disease.....	72
2.4.8 A Synoptic View of the Etiology of Alzheimer’s Disease .....	79
<b>Chapter 3. Conventional Treatments for Alzheimer’s Disease .....</b>	<b>89</b>
<b>3.1 Current Pharmaceutical Treatments for Alzheimer’s Disease.....</b>	<b>90</b>
<b>3.2 Future Conventional Treatments for Alzheimer’s Disease .....</b>	<b>95</b>
3.2.1 Reduce Amyloid- $\beta$ Production .....	97
3.2.2 Prevent Amyloid- $\beta$ Aggregation.....	101
3.2.3 Facilitate Amyloid- $\beta$ Clearance .....	104
3.2.4 Reduce Pathological Tau Production.....	113
3.2.5 Prevent Pathological Tau Aggregation .....	115
3.2.6 Facilitate Pathological Tau Clearance .....	117
3.2.7 Control Inflammation .....	119
3.2.8 Modulate Complement Cascade .....	123
3.2.9 Neuroprotective Agents .....	125
3.2.10 Antibiotic and Antiviral Medications .....	133
3.2.11 Nanoparticle Therapies .....	135
3.2.12 Gene Therapies .....	140
3.2.13 Telomere Extension Therapy.....	144
3.2.14 Stem Cell Transplantation .....	146
3.2.15 Anti-Aging Drugs .....	153

<b>Chapter 4. NEW APPROACH: Medical Nanorobotics .....</b>	<b>155</b>
<b>4.1 Atomically Precise Manufacturing using Nanofactories .....</b>	<b>156</b>
4.1.1 Scanning Probe Microscopy .....	157
4.1.2 Mechanosynthesis .....	162
4.1.3 Nanofactories: Manufacturing Medical Nanorobots .....	167
<b>4.2 Medical Nanorobots: Background .....</b>	<b>172</b>
4.2.1 Spirocyte-Class Nanorobots.....	176
4.2.2 Microbivore-Class Nanorobots.....	179
4.2.3 Chromalloycyte-Class Nanorobots .....	182
4.2.4 Medical Nanorobot Biocompatibility .....	186
4.2.4.1 Immune System Reactions .....	188
4.2.4.2 Inflammation .....	190
4.2.4.3 Phagocytosis.....	192
4.2.5 Advantages of Medical Nanorobots .....	194
4.2.6 Nanorobotic Cell Mills .....	200
<b>4.3 Medical Nanorobots: Ingress to, and Egress from, the Brain.....</b>	<b>206</b>
4.3.1 Blood-Brain Barrier (BBB) Penetration .....	207
4.3.2 Blood-CSF Barrier (BCB) Penetration.....	221
4.3.3 CSF-Brain Interface Penetration.....	226
4.3.4 Nanorobotic Transit Through Extracellular Spaces.....	230
4.3.5 Nanocatheter Transport into the Brain.....	232
<b>Chapter 5. Nanorobotic Treatment: The Alzheimer Protocols .....</b>	<b>239</b>
<b>5.1 First Alzheimer Protocol: Genetic Derisking .....</b>	<b>240</b>
5.1.1 Replace Familial Alzheimer’s Gene Mutations.....	242
5.1.2 Replace Apolipoprotein ApoE4 Gene .....	244
5.1.3 Replace Other High-Risk Genes.....	247
5.1.4 Eliminate Pathological Genetic Mosaicism.....	251
<b>5.2 Second Alzheimer Protocol: Tissue Rejuvenation .....</b>	<b>252</b>
5.2.1 Remove Extracellular Aggregates .....	255
5.2.1.1 Amyloid Plaques .....	256
5.2.1.2 Glutamate .....	259
5.2.2 Remove Intracellular Aggregates .....	260
5.2.2.1 Tau Protein and Neurofibrillary Tangles.....	261
5.2.2.2 Lipofuscin Granules .....	265
5.2.2.3 $\alpha$ -Synuclein and Lewy Bodies.....	269
5.2.3 Correct Cancer, Nuclear Mutations and Epimutations .....	272
5.2.4 Replace Mutant Mitochondria .....	275
5.2.5 Repair or Eliminate Toxic, Dysfunctional, and Senescent Cells .....	278
5.2.6 Restore Essential Lost or Atrophied Cells .....	282
5.2.7 Remove Extracellular Crosslinks.....	286
<b>5.3 Third Alzheimer Protocol: Neural Reconstruction.....</b>	<b>290</b>
5.3.1 Brain Mapping and Neural Repair Plan.....	293
5.3.2 Reconstruction of Neural Tissues .....	318
5.3.2.1 Replacement Cell Manufacture .....	319
5.3.2.2 Intracranial Debridement.....	320
5.3.2.3 Cell Insertion and Emplacement .....	327
5.3.2.4 Neural Incorporation .....	333

5.3.3 Neural Network Retraining.....	347
5.3.3.1 Environmental Enrichment.....	349
5.3.3.2 Brain Memory: Data Recovery .....	354
5.3.3.3 Brain Memory: Algorithm Recovery .....	358
<b>Chapter 6. Treating Parkinson’s and Related Neurodegenerative Conditions .....</b>	<b>361</b>
<b>6.1 Parkinson’s Disease.....</b>	<b>362</b>
6.1.1 Genetic Causes of Parkinson’s Disease .....	363
6.1.2 Some Details of the Neuropathology.....	365
6.1.3 Current Treatments for Parkinson’s Disease .....	368
6.1.4 Nanorobotic Treatment for Parkinson’s Disease .....	376
<b>6.2 Other Neurodegenerative Conditions .....</b>	<b>378</b>
6.2.1 Trinucleotide Repeat Disorders (including Huntington’s Disease) .....	380
6.2.2 Amyotrophic Lateral Sclerosis .....	385
6.2.3 Vascular Dementia.....	388
6.2.4 Cerebral Amyloidosis .....	390
6.2.5 Neurodegenerative Tauopathies .....	393
6.2.6 Neuronal Ceroid Lipofuscinoses .....	399
6.2.7 Neural Autoimmune Disorders.....	401
6.2.8 Multiple System Atrophy.....	403
<b>Chapter 7. Conclusions.....</b>	<b>405</b>
<b>Appendix A. Normal Physiological Roles of Amyloid Beta (A<math>\beta</math>).....</b>	<b>407</b>
<b>Appendix B. Alzheimer’s Drugs in Development as of 2013 .....</b>	<b>422</b>
<b>Appendix C. Molecular Sorting Rotors .....</b>	<b>428</b>

## Chapter 1. Introduction

The purpose of this book is to present the technical opportunities for the development of a new method for preventing the initial onset, and for completely arresting and curing the progression of an existing case, of Alzheimer's disease, Parkinson's disease, and related neurodegenerative conditions in human patients.

Alzheimer's disease (AD) is the most common form of dementia in the elderly and accounts for the majority of all cases. By 2030 it is estimated that more than 65 million people will be living with dementia, with projections almost doubling every 20 years.<sup>1</sup> AD is a disorder characterized by the loss of neurons, mainly in the hippocampus and cerebral cortex.<sup>2</sup> The symptoms of the disease include erosion of memory and of other cognitive functions such as language and reasoning, as well as neuropsychiatric manifestations such as delusions and apathy.<sup>3</sup> In addition to its role in neurodegeneration, Alzheimer's is the fourth most common cause of death in industrialized nations, preceded only by cardiovascular disease, cancer and stroke.<sup>4</sup> The increasing burden of AD has spurred intensive efforts by the scientific community to develop therapeutic agents to prevent the progression of the disease.



Unfortunately, the history of the multi-decade search for Alzheimer's disease treatments is a record of soaring hope followed by crushing failure. Over and over again, laboratory "breakthroughs" based on results from mouse or other animal models, or from early clinical trials, are breathlessly announced in the media, only to have all hope dashed a year or two afterwards when later-phase human trials show no cognitive improvements in AD patients. This has led some dispirited researchers to proclaim that future money should be spent on caring for Alzheimer's sufferers, rather than on fruitless and expensive searches for pharmaceutical-based cures for what appears to be a complex and intractable disease.

---

<sup>1</sup> Prince M, Albanese E, Guerchet M, Prina M. World Alzheimer's Report 2014: Dementia and Risk Reduction. [www.alz.co.uk/research/world-report](http://www.alz.co.uk/research/world-report). Prince M, *et al.* World Alzheimer Report 2009: Alzheimer's Disease International. [www.alz.co.uk/research/files/WorldAlzheimerReport.pdf](http://www.alz.co.uk/research/files/WorldAlzheimerReport.pdf).

<sup>2</sup> Nussbaum RL, Ellis CE. Alzheimer's disease and Parkinson's disease. *N Engl J Med.* 2003 Apr 3;348(14):1356-64; <http://www.nejm.org/doi/full/10.1056/NEJM2003ra020003>.

<sup>3</sup> Prince M, *et al.* World Alzheimer Report 2009: Alzheimer's Disease International. [www.alz.co.uk/research/files/WorldAlzheimerReport.pdf](http://www.alz.co.uk/research/files/WorldAlzheimerReport.pdf). Carter MD, Simms GA, Weaver DF. The development of new therapeutics for Alzheimer's disease. *Clin Pharmacol Ther.* 2010 Oct;88(4):475-86; <http://www.ncbi.nlm.nih.gov/pubmed/20811351>. Prince M, *et al.* World Alzheimer Report 2009: Alzheimer's Disease International. [www.alz.co.uk/research/files/WorldAlzheimerReport.pdf](http://www.alz.co.uk/research/files/WorldAlzheimerReport.pdf).

<sup>4</sup> Nussbaum RL, Ellis CE. Alzheimer's disease and Parkinson's disease. *N Engl J Med.* 2003 Apr 3;348(14):1356-64; <http://www.nejm.org/doi/full/10.1056/NEJM2003ra020003>.

As a case in point, Peter Whitehouse, a physician at Case Western Reserve University in Cleveland, laments: “Care needs to be dominant over cure, but it’s not. What people with Alzheimer’s disease and their families really need is help with the day-to-day issues of care.” Whitehouse thinks a lot of the money now put into research could be better spent in other ways. “Research money might be better spent in promoting physical exercise and overall well-being – factors known to keep people healthier as they age. Those things are always likely to be more effective than any biological therapy we could produce.”<sup>5</sup>

Such defeatist thinking reminds physician and anti-aging researcher Michael Fossel of an earlier episode in medical history:<sup>6</sup>

“Consider an apt parallel in how we once thought of other diseases, such as polio in the late 1940’s. A careful review of the medical literature of those days, much of it still on paper rather than electronic, is revealing.”

“Before the Salk vaccine, there was an almost universally pessimistic (and often unspoken) assumption that polio must be accepted. The question wasn’t one of cure, but of care. The most we could do was to improve iron lungs, leg braces, and nursing care. The medical literature addressed ways to improve pulmonary care for those in iron lungs and medical economists fretted over the likely future costs of long-term nursing care for polio victims. Few people, among them Jonas Salk, believed that the disease might ever be prevented or cured. Most people were wrong: polio is now rare.”

“More than half a century later, there is an almost universally pessimistic (and often unspoken) assumption that dementia is inevitable. The question isn’t one of cure, but of care. The most we can do is perhaps slow the inevitable decline, marginally improve memory, and provide better nursing care. The medical literature is full of ways to address beta amyloid deposition and medical economists fret over the likely future costs of nursing home care for Alzheimer’s patients. Few people truly believe that Alzheimer’s can be prevented or cured. [But again] most people are wrong: Alzheimer’s may be prevented and cured.”

We strongly agree with Fossel’s conclusion. This book will explain our unique approach to achieving this highly desirable objective.

We start with a brief background on the nature of Alzheimer’s disease (Chapter 2) and a summary of the methods and limitations of current and anticipated conventional future treatments (Chapter 3). Please note that in June 2016, PubMed listed 53,602 papers with “Alzheimer” in the title and 107,529 papers with “Alzheimer” in the title or abstract. Obviously this is a vast literature that no one person can completely comprehend. As a result, the summaries given in the present document may omit mention of issues, factors, theories, drugs, or therapeutic approaches that are known to the reader but not to us. Our discussion of the current state of knowledge in this field is therefore best regarded as representative and not comprehensive.

---

<sup>5</sup> Joseph Shapiro, “Emphasis of Alzheimer’s Cure Debated,” NPR Books, 6 Nov 2006; <http://www.npr.org/templates/story/story.php?storyId=6430300>.

<sup>6</sup> <http://www.michaelfossel.com/blog/?p=64>.

In Chapter 4, we introduce the concept of “atomically precise manufacturing” of products built to atomic precision using nanofactories. The key product in the present context is medical nanorobots. This Chapter features discussions of several representative nanorobot systems, biocompatibility of nanorobots, methods by which nanorobots can enter and exit the human brain, and methods for manufacturing living biological cells in a modified nanofactory.

Chapter 5 presents the three Alzheimer Protocols – a new approach for arresting the progression of Alzheimer’s disease and repairing most of the neurological damage caused by the disease even in its more advanced stages. The tasks of the Alzheimer Protocols include genetic de-risking, tissue rejuvenation, and neural reconstruction.

Chapter 6 describes how the same protocols can provide cures for Parkinson’s disease and a wide range of related neurodegenerative conditions.

Chapter 7 presents our final conclusions. The key conclusion: Medical nanorobotics will almost certainly provide a complete cure for Alzheimer’s disease and related neurodegenerative conditions. All that remains is to execute the technical plan for implementation.

The author acknowledges helpful comments by Tad Hogg and James Ryley on an earlier draft of this manuscript.

## Chapter 2. Background on Alzheimer's Disease

Alzheimer's disease (AD), also known as Alzheimer disease or just Alzheimer's, accounts for 60% to 80% of cases of dementia, with a lifetime AD risk of 17% for women and 9% for men. It is the 6th leading cause of death worldwide.<sup>7</sup> AD was initially described by, and later named after, German psychiatrist and pathologist Alois Alzheimer, who published the first case description in 1907.<sup>8</sup>



Alzheimer's disease is usually preceded by a condition called Mild Cognitive Impairment (MCI), characterized by bothersome memory loss and cognitive difficulty that does not yet constitute dementia. Full-blown Alzheimer's, on the other hand, is characterized by short- and long-term memory loss, confusion, aggression and irritability, mood swings, language breakdown, and the general withdrawal of an Alzheimer's patient from social interactions as the disease progresses.



AD is a chronic neurodegenerative disease that usually starts slowly and gets worse over time. The most common early symptom is difficulty in remembering recent events (short term memory loss). As the disease advances, symptoms can include: problems with language, disorientation (including easily getting lost), mood swings, loss of motivation, not managing self care, and behavioral issues. As a person's condition declines, he or she often withdraws from family and society. Gradually, bodily functions are lost, ultimately leading to death.<sup>9</sup> Although the speed of progression can vary, the average life expectancy following diagnosis is three to nine years.<sup>10</sup>

<sup>7</sup> Burns A, Iliffe S. Alzheimer's disease. *BMJ*. 2009 Feb 5;338:b158; <http://www.ncbi.nlm.nih.gov/pubmed/19196745>. "Dementia Fact Sheet N°362". who.int. Apr 2012; <http://www.who.int/mediacentre/factsheets/fs362/en/>. 2011 Alzheimer's Disease Facts and Figures; [http://www.alz.org/downloads/Facts\\_Figures\\_2011.pdf](http://www.alz.org/downloads/Facts_Figures_2011.pdf).

<sup>8</sup> Alzheimer A. Über eine eigenartige Erkrankung der Hirnrinde [About a peculiar disease of the cerebral cortex]. *Allgemeine Zeitschrift für Psychiatrie und Psychisch-Gerichtlich Medizin*. 1907;64(1-2):146-148, in German. Translated at: Alzheimer A. About a Peculiar Disease of the Cerebral Cortex. *Alzheimer Disease and Associated Disorders*. 1987;1(1):3-8; <http://www.ncbi.nlm.nih.gov/pubmed/3331112>.

<sup>9</sup> "About Alzheimer's Disease: Symptoms," National Institute on Aging, 2016; <http://www.nia.nih.gov/alzheimers/topics/symptoms>.

<sup>10</sup> Querfurth, HW, LaFerla, FM. Alzheimer's disease. *New Eng J Med*. 2010 Jan 28;362(4):329-44; <http://www.razipath.com/fa/upload/collections/29961916/Alzheimer%20disease.pdf>. Todd S, Barr S, Roberts M, Passmore AP. Survival in dementia and predictors of mortality: a review. *Int J Geriatr Psychiatry*. 2013 Nov;28(11):1109-24; <http://www.ncbi.nlm.nih.gov/pubmed/23526458>.

The cause of Alzheimer's disease is poorly understood. Many believe the majority of the risk to be genetic with many genes usually involved.<sup>11</sup> Others ascribe the disease to risk factors such as a history of head injuries, depression, and hypertension. The disease process is associated with pathological microscopic "plaques" and "tangles" in brain tissue. A probable diagnosis is based on the history of the illness and cognitive testing, combined with medical imaging and blood tests to rule out other possible causes.<sup>12</sup> Initial symptoms are often mistaken for normal aging, and until fairly recently a direct physical examination of brain tissue was typically needed for a definite diagnosis.

In this Chapter, we discuss the prevalence and social cost of Alzheimer's disease (Section 2.1) and list a great many possible preventative measures that have been tried or considered (Section 2.2). A brief recital of the four clinical stages of AD (Section 2.3) is followed by a lengthy technical description of the primary molecular pathologies of the disease (Section 2.4).

---

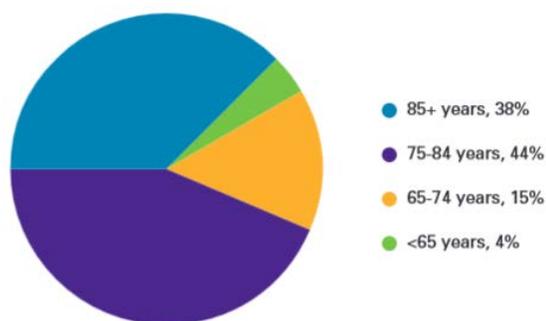
<sup>11</sup> Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E. Alzheimer's disease. *Lancet*. 2011 Mar 19;377(9770):1019-31; <http://www.ncbi.nlm.nih.gov/pubmed/21371747>.

<sup>12</sup> "Dementia diagnosis and assessment," National Institute for Health and Care Excellence, 2015; <http://pathways.nice.org.uk/pathways/dementia/dementia-diagnosis-and-assessment.pdf>.

## 2.1 Prevalence and Social Cost of the Disease

In 2015, there were between 28 and 33 million people worldwide with AD,<sup>13</sup> with 5 million in the U.S. alone. The disease most often manifests in people over 65 years of age, although 4% to 5% of cases are early-onset Alzheimer's which begin before this.<sup>14</sup> The age distribution of people with Alzheimer's disease in the United States is shown in the pie chart below.<sup>15</sup> One in nine people age 65 and older (11%) has Alzheimer's disease. About 1/3 of people age 85 and older (32%) have Alzheimer's disease. Of those with Alzheimer's disease, the vast majority (82%) are age 75 or older. In 2010, dementia resulted in about 486,000 deaths worldwide.<sup>16</sup> (Interestingly, if you've had cancer you are 50% less likely to get Alzheimer's disease; and if you have Alzheimer's disease, you're 60-70% less likely to get cancer.<sup>17</sup>)

Dementia, and specifically Alzheimer's disease, may be among the most costly diseases for society in developed countries such as Europe and the United States,<sup>18</sup> while their cost in developing countries such as Argentina<sup>19</sup> or



<sup>13</sup> Querfurth, HW, LaFerla, FM. Alzheimer's disease. *New Eng J Med*. 2010 Jan 28;362(4):329-44; <http://www.razipath.com/fa/upload/collections/29961916/Alzheimer%20disease.pdf>. "About Alzheimer's Disease: Symptoms," National Institute on Aging; <http://www.nia.nih.gov/alzheimers/topics/symptoms>. Dementia Fact Sheet No. 362, World Health Organization, Mar 2015; <http://www.who.int/mediacentre/factsheets/fs362/en/>.

<sup>14</sup> Mendez MF. Early-onset Alzheimer's disease: nonamnestic subtypes and type 2 AD. *Arch Med Res*. 2012 Nov;43(8):677-85; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3532551/>.

<sup>15</sup> "2014 Alzheimer's Disease Facts and Figures," Alzheimer's Association, 2014; [https://www.alz.org/downloads/Facts\\_Figures\\_2014.pdf](https://www.alz.org/downloads/Facts_Figures_2014.pdf).

<sup>16</sup> Lozano R, *et al*. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012 Dec 15;380(9859):2095-128; <http://www.ncbi.nlm.nih.gov/pubmed/23245604>.

<sup>17</sup> Catalá-López F, Crespo-Facorro B, Vieta E, Valderas JM, Valencia A, Tabarés-Seisdedos R. Alzheimer's disease and cancer: current epidemiological evidence for a mutual protection. *Neuroepidemiology*. 2014;42(2):121-2; <http://www.karger.com/Article/FullText/355899>.

<sup>18</sup> Bonin-Guillaume S, Zekry D, Giacobini E, Gold G, Michel JP. Impact économique de la démence (English: The Economical Impact of Dementia). *Presse Médicale*. 2005;34(1):35-41, in French; <http://www.ncbi.nlm.nih.gov/pubmed/15685097>. Meek PD, McKeithan K, Schumock GT. Economic Considerations in Alzheimer's Disease. *Pharmacotherapy*. 1998;18(2 Pt 2):68-73; discussion 79-82; <http://www.ncbi.nlm.nih.gov/pubmed/9543467>.

<sup>19</sup> Allegri RF, Butman J, Arizaga RL, Machnicki G, Serrano C, Taragano FE, Sarasola D, Lon L. Economic Impact of Dementia in Developing Countries: An Evaluation of Costs of Alzheimer-type Dementia in

South Korea<sup>20</sup> is also high and rising. These costs will probably increase with the aging of society, becoming an important social problem.<sup>21</sup> AD-associated costs include direct medical costs such as nursing home care, direct nonmedical costs such as in-home day care, and indirect costs such as lost productivity of both patient and caregiver. Numbers vary between studies but dementia costs worldwide had reached \$160 billion a decade ago,<sup>22</sup> while costs of Alzheimer's disease to Medicare in the United States alone may now exceed \$150 billion each year.

The greatest cost for society is long-term AD care by health care professionals and particularly institutionalization, which contributes 2/3 of the total costs for society. The cost of living at home is also very high, especially when informal costs for the family, such as caregiving time and caregiver's lost earnings, are taken into account.<sup>23</sup> Dementia caregivers are subject to high rates of physical and mental disorders.<sup>24</sup> Factors associated with greater psychosocial problems of the primary caregivers include having an affected person at home, the carer being a spouse, demanding behaviors of the cared person such as depression, behavioral disturbances, hallucinations, sleep problems or walking disruptions, and social isolation.<sup>25</sup> Regarding economic problems, family caregivers often give up time from work to spend 47 hours per week

Argentina. *Int Psychogeriatr*. 2007 Aug;19(4):705-18;  
<http://www.cemic.edu.ar/descargas/2007%20Costos%20%28B%29.pdf>.

<sup>20</sup> Suh GH, Knapp M, Kang CJ. The Economic Costs of Dementia in Korea, 2002. *Int J Geriatr Psychiatry*. 2006 Aug;21(8):722-8; <http://www.ncbi.nlm.nih.gov/pubmed/16858741>.

<sup>21</sup> There is at least one report that the overall incidence of dementia might not be increasing in the U.K., with later-born cohorts experiencing lower incidence than older cohorts. See: Matthews FE, Arthur A, Barnes LE, Bond J, Jagger C, Robinson L, Brayne C; Medical Research Council Cognitive Function and Ageing Collaboration. A two-decade comparison of prevalence of dementia in individuals aged 65 years and older from three geographical areas of England: results of the Cognitive Function and Ageing Study I and II. *Lancet*. 2013 Oct 26;382(9902):1405-12; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3906607/>.  
 Matthews FE, Stephan BC, Robinson L, Jagger C, Barnes LE, Arthur A, Brayne C; Cognitive Function and Ageing Studies (CFAS) Collaboration. A two decade dementia incidence comparison from the Cognitive Function and Ageing Studies I and II. *Nat Commun*. 2016 Apr 19;7:11398;  
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4838896/>.

<sup>22</sup> Wimo A, Jonsson L, Winblad B. An Estimate of the Worldwide Prevalence and Direct Costs of Dementia in 2003. *Dement Geriatr Cogn Disord*. 2006;21(3):175-81;  
<http://www.karger.com/Article/Pdf/90733>.

<sup>23</sup> Moore MJ, Zhu CW, Clipp EC. Informal costs of dementia care: estimates from the National Longitudinal Caregiver Study. *J Gerontol B Psychol Sci Soc Sci*. 2001 Jul;56(4):S219-28;  
<https://psychogerontology.oxfordjournals.org/content/56/4/S219.full>.

<sup>24</sup> Ritchie K, Lovestone S. The dementias. *Lancet*. 2002 Nov 30;360(9347):1759-66;  
<http://www.ncbi.nlm.nih.gov/pubmed/12480441>.

<sup>25</sup> Brodaty H, Hadzi-Pavlovic D. Psychosocial effects on carers of living with persons with dementia. *Aust N Z J Psychiatry*. 1990 Sep;24(3):351-61; <http://www.ncbi.nlm.nih.gov/pubmed/2241719>.  
 Donaldson C, Tarrrier N, Burns A. Determinants of carer stress in Alzheimer's disease. *Int J Geriatr Psychiatry*. 1998 Apr;13(4):248-56; <http://www.ncbi.nlm.nih.gov/pubmed/9646153>.

on average with the person suffering from AD, while the costs of caring for them are high. Direct and indirect costs of caring for an Alzheimer's patient average between \$18,000 and \$77,500 per year in the United States, depending on the study.<sup>26</sup> The average lifetime cost of care for a person with Alzheimer's disease is \$250,000, including the cost of drugs, nursing homes or assisted living facilities, uncompensated care, paid caregivers, etc.<sup>27</sup>

Costs increase with dementia severity and the presence of behavioral disturbances<sup>28</sup> and are related to the increased caregiving time required for the provision of physical care. Any treatment that slows cognitive decline, delays institutionalization, or reduces caregivers' hours will have economic benefits.

---

<sup>26</sup> Moore MJ, Zhu CW, Clipp EC. Informal costs of dementia care: estimates from the National Longitudinal Caregiver Study. *J Gerontol B Psychol Sci Soc Sci.* 2001 Jul;56(4):S219-28; <https://psychogerontology.oxfordjournals.org/content/56/4/S219.full>. "The MetLife study of Alzheimer's disease: The caregiving experience," MetLife Mature Market Institute. Aug 2006; <http://www.metlife.com/assets/cao/mmi/publications/studies/mmi-alzheimers-disease-caregiving-experience-study.pdf>.

<sup>27</sup> [http://www.alz.org/documents/grva/did\\_you\\_know\\_2012\\_new\\_format\(1\).pdf](http://www.alz.org/documents/grva/did_you_know_2012_new_format(1).pdf)

<sup>28</sup> Jönsson L, Eriksson Jönhagen M, Kilander L, Soininen H, Hallikainen M, Waldemar G, Nygaard H, Andreasen N, Winblad B, Wimo A. Determinants of Costs of Care for Patients with Alzheimer's Disease. *Int J Geriatr Psychiatry.* 2006 May;21(5):449-59; <http://www.ncbi.nlm.nih.gov/pubmed/16676288>.

## 2.2 Prevention of Alzheimer's Disease

At present, there is no definitive evidence that any particular measure is effective in preventing AD.<sup>29</sup> Mental exercise and avoiding obesity may decrease the risk of AD,<sup>30</sup> and physical exercise programs are beneficial with respect to activities of daily living and may potentially improve outcomes.<sup>31</sup> However, global studies of measures to prevent or delay the onset of AD have produced inconsistent results, and a 2010 panel of experts reported that there were no medications or supplements with clear evidence to support their use as a preventative measure for AD.<sup>32</sup> Epidemiological studies have proposed relationships between certain modifiable factors, such as diet, cardiovascular risk, pharmaceutical products, or intellectual activities among others, along with a given population's likelihood of developing AD. Only further research, including clinical trials, will reveal whether any of these factors can truly help to prevent AD.<sup>33</sup>

In this Section, we review what is currently known about the AD preventative potential of medications (Section 2.1.1), lifestyle (Section 2.1.2), and diet (Section 2.1.3).

---

<sup>29</sup> Luchsinger JA, Mayeux R. Dietary Factors and Alzheimer's Disease. *Lancet Neurology*. 2004;3(10):579-87; <http://www.ncbi.nlm.nih.gov/pubmed/15380154>. Kawas CH. Medications and Diet: Protective Factors for AD?. *Alzheimer Disease and Associated Disorders*. 2006;20(3 Suppl 2):S89-96; <http://www.ncbi.nlm.nih.gov/pubmed/16917203>. Luchsinger JA, Noble JM, Scarmeas N. Diet and Alzheimer's Disease. *Curr Neurol Neurosci Rep*. 2007 Sep;7(5):366-72; <http://www.ncbi.nlm.nih.gov/pubmed/17764625>. "Independent Panel Finds Insufficient Evidence to Support Preventive Measures for Alzheimer's Disease," Press Release, National Institutes of Health, 28 Apr 2010; <http://www.nih.gov/news/health/apr2010/od-28.htm>. Daviglus ML, *et al.* "NIH State-of-the-Science Conference: Preventing Alzheimer's Disease and Cognitive Decline," 26-28 Apr 2010; <http://consensus.nih.gov/2010/alzstatement.htm>.

<sup>30</sup> Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E. Alzheimer's disease. *Lancet*. 2011 Mar 19;377(9770):1019-31; <http://www.ncbi.nlm.nih.gov/pubmed/21371747>.

<sup>31</sup> Forbes D, Thiessen EJ, Blake CM, Forbes SC, Forbes S. Exercise programs for people with dementia. *Cochrane Database Syst Rev*. 2013 Dec 4;12:CD006489; <http://www.ncbi.nlm.nih.gov/pubmed/24302466>.

<sup>32</sup> "More research needed on ways to prevent Alzheimer's, panel finds," National Institute on Aging, 15 Jun 2010; <http://www.nia.nih.gov/alzheimers/announcements/2010/06/more-research-needed-ways-prevent-alzheimers-panel-finds>.

<sup>33</sup> Szekely CA, Breitner JC, Zandi PP. Prevention of Alzheimer's Disease. *Int Rev Psychiatry*. 2007 Dec;19(6):693-706; <http://www.ncbi.nlm.nih.gov/pubmed/18092245>.

## 2.2.1 Preventative Medications

Cardiovascular risk factors, such as hypercholesterolemia, hypertension, diabetes, and smoking, are associated with a higher risk of onset and course of AD.<sup>34</sup> However, statins, which are cholesterol lowering drugs, have not been effective in preventing or improving the course of Alzheimer's disease.<sup>35</sup>

Long-term usage of non-steroidal anti-inflammatory drugs (NSAIDs) has sometimes been associated with a reduced likelihood of developing AD,<sup>36</sup> and some evidence has supported the notion that NSAIDs might reduce inflammation related to amyloid plaques.<sup>37</sup> Even if not useful as a treatment, NSAIDs could have preventative effects.<sup>38</sup> However, a recently-completed 12-year NSAID prevention trial found no overall risk reduction for AD.<sup>39</sup>

Hormone replacement therapy, although previously used for the management and prevention of cardiovascular disease, osteoporosis and dementia in older women, may increase the risk of dementia and is no longer recommended for preventing deterioration of cognitive function in postmenopausal women.<sup>40</sup>

---

<sup>34</sup> Rosendorff C, Beeri MS, Silverman JM. Cardiovascular risk factors for Alzheimer's disease. *Am J Geriatr Cardiol.* 2007 May-Jun;16(3):143-9; <http://www.ncbi.nlm.nih.gov/pubmed/17483665>. Patterson C, Feightner JW, Garcia A, Hsiung GY, MacKnight C, Sadovnick AD. Diagnosis and treatment of dementia: 1. Risk assessment and primary prevention of Alzheimer disease. *CMAJ.* 2008 Feb 26;178(5):548-56; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2244657/>.

<sup>35</sup> Kuller LH. Statins and Dementia. *Curr Atheroscler Rep.* 2007 Aug;9(2):154-61; <http://www.ncbi.nlm.nih.gov/pubmed/17877925>. Reiss AB, Wirkowski E. Role of HMG-CoA reductase inhibitors in neurological disorders: progress to date. *Drugs.* 2007;67(15):2111-20; <http://www.ncbi.nlm.nih.gov/pubmed/17927279>.

<sup>36</sup> Szekely CA, Town T, Zandi PP. NSAIDs for the chemoprevention of Alzheimer's disease. *Subcell Biochem.* 2007;42:229-48; <http://www.ncbi.nlm.nih.gov/pubmed/17612054>.

<sup>37</sup> Szekely CA, Town T, Zandi PP. NSAIDs for the chemoprevention of Alzheimer's disease. *Subcell Biochem.* 2007;42:229-48; <http://www.ncbi.nlm.nih.gov/pubmed/17612054>.

<sup>38</sup> Hoozemans JJ, Veerhuis R, Rozemuller JM, Eikelenboom P. Soothing the inflamed brain: effect of non-steroidal anti-inflammatory drugs on Alzheimer's disease pathology. *CNS Neurol Disord Drug Targets.* 2011 Feb;10(1):57-67; <http://www.ncbi.nlm.nih.gov/pubmed/21143138>.

<sup>39</sup> Breitner J, *et al.* Results of a follow-up study to the randomized Alzheimer's Disease Anti-inflammatory Prevention Trial (ADAPT). *Alzheimers Dement.* 2013 Nov;9(6):714-23; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3823756/>.

<sup>40</sup> Marjoribanks J, Farquhar C, Roberts H, Lethaby A. Long term hormone therapy for perimenopausal and postmenopausal women. *Cochrane Database Syst Rev.* 2012 Jul 11;7:CD004143; <http://www.ncbi.nlm.nih.gov/pubmed/22786488>.

Treatment of behavioral problems or psychosis due to dementia with antipsychotic drugs is common but not usually recommended because there is often little benefit coupled with an increased risk of early death.<sup>41</sup>

An FDA panel voted unanimously to recommend approval of florbetapir, an imaging agent that can help to detect Alzheimer's brain plaques, but the agent will require additional clinical research before it can be made available commercially.<sup>42</sup> With knowledge that a potential AD patient has the tell-tale plaques, a 2015 review suggested that "mindfulness-based interventions" might prevent or delay the onset of mild cognitive impairment and Alzheimer's disease.<sup>43</sup>

---

<sup>41</sup> "Information for Healthcare Professionals: Conventional Antipsychotics," U.S. Food and Drug Administration, 16 Jun 2008; <http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm124830.htm>. "Low-dose antipsychotics in people with dementia," National Institute for Health and Clinical Excellence, 15 Jan 2015; <https://www.nice.org.uk/advice/ktt7/resources/non-guidance-lowdose-antipsychotics-in-people-with-dementia-pdf>.

<sup>42</sup> Clark CM, Schneider JA, Bedell BJ, Beach TG, Bilker WB, Mintun MA, Pontecorvo MJ, Hefti F, Carpenter AP, Flitter ML, Krautkramer MJ, Kung HF, Coleman RE, Doraiswamy PM, Fleisher AS, Sabbagh MN, Sadowsky CH, Reiman EP, Zehntner SP, Skovronsky DM; AV45-A07 Study Group. Use of florbetapir-PET for imaging beta-amyloid pathology. *JAMA*. 2011 Jan 19;305(3):275-83; <http://jama.jamanetwork.com/article.aspx?articleid=645188>. "FDA recommends approval of new compound in Alzheimer's detection," *USA Today*, 21 Jan 2011; [http://usatoday30.usatoday.com/yourlife/health/medical/alzheimers/2011-01-22-alzheimersfda21\\_ST\\_N.htm](http://usatoday30.usatoday.com/yourlife/health/medical/alzheimers/2011-01-22-alzheimersfda21_ST_N.htm).

<sup>43</sup> Larouche E, Hudon C, Goulet S. Potential benefits of mindfulness-based interventions in mild cognitive impairment and Alzheimer's disease: an interdisciplinary perspective. *Behav Brain Res*. 2015 Jan 1;276:199-212; [https://www.researchgate.net/profile/Eddy\\_Larouche/publication/262764588\\_Potential\\_benefits\\_of\\_mindfulness-based\\_interventions\\_in\\_mild\\_cognitive\\_impairment\\_and\\_Alzheimer's\\_disease\\_An\\_interdisciplinary\\_perspective/links/545669190cf2cf516480304c.pdf](https://www.researchgate.net/profile/Eddy_Larouche/publication/262764588_Potential_benefits_of_mindfulness-based_interventions_in_mild_cognitive_impairment_and_Alzheimer's_disease_An_interdisciplinary_perspective/links/545669190cf2cf516480304c.pdf).

## 2.2.2 Preventative Lifestyle

People who engage in intellectual activities such as reading, playing board games, completing crossword puzzles, playing musical instruments, or regular social interaction show a reduced risk for Alzheimer's disease.<sup>44</sup> This is compatible with the cognitive reserve theory, which states that some life experiences result in more efficient neural functioning providing the individual a cognitive reserve that delays the onset of dementia manifestations.

Physical activity is associated with a reduced risk of AD. Education delays the onset of AD syndrome but is not related to earlier death after diagnosis.<sup>45</sup> Learning a second language even later in life might also delay getting Alzheimer's disease.<sup>46</sup>



Other unusual lifestyle choices have been suggested. For example, wearing an experimental helmet (image, right) for 10 minutes every day that “bathes the brain with infra-red light and stimulates the growth of brain cells” was claimed by one U.K. medical research company, Virulite, to reverse the symptoms of dementia (such as memory loss and anxiety) after only 4 weeks of use.<sup>47</sup>

Perhaps the oddest approach that has some basis in fact is “young blood” transfusion. Years of animal experiments have shown that an infusion of young blood in older mice can improve cognition, physical endurance, and the health of several organs, and even makes the animals look younger. In 2016 will come the first results from a trial that has given “young blood,” donated by volunteers aged 30 or younger, to people with mild to moderate Alzheimer's in the hope that it will improve their symptoms.<sup>48</sup> What might make young blood so revitalizing is unclear, but transfusions of young blood that reverse heart decline in old mice is an effect that can be mimicked by giving mice just GDF11 – a growth factor in the blood that decreases with age.

---

<sup>44</sup> Stern Y. Cognitive reserve and Alzheimer disease. *Alzheimer Dis Assoc Disord.* 2006 Jul-Sep;20(3 Suppl 2):S69-74; <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.327.9576&rep=rep1&type=pdf>.

<sup>45</sup> Paradise M, Cooper C, Livingston G. Systematic review of the effect of education on survival in Alzheimer's disease. *Int Psychogeriatr.* 2009 Feb;21(1):25-32; <http://www.ncbi.nlm.nih.gov/pubmed/19026089>.

<sup>46</sup> Lauran Neergaard, “Speaking 2 Languages May Delay Getting Alzheimer's,” *The Denver Post*, Associated Press, 19 Feb 2011; [http://www.denverpost.com/ci\\_17427474](http://www.denverpost.com/ci_17427474).

<sup>47</sup> David Derbyshire, “The helmet that could turn back the symptoms of Alzheimer's,” *DailyMail.com*, 25 Jan 2008; <http://www.dailymail.co.uk/health/article-510172/The-helmet-turn-symptoms-Alzheimers.html>.

<sup>48</sup> Helen Thomson, “First results from human young blood rejuvenation trial expected,” *New Scientist*, 15 Dec 2015; <https://www.newscientist.com/article/mg22830522-000-first-results-from-human-young-blood-rejuvenation-trial-expected/>.

### 2.2.3 Preventative Diet

Those who eat a diet high in saturated fats and simple carbohydrates have a higher risk of AD.<sup>49</sup> But people who eat a healthy Japanese or Mediterranean diet have a lower risk of AD.<sup>50</sup> Specifically, a Mediterranean diet may improve outcomes in those with the disease.<sup>51</sup> The Mediterranean diet's beneficial cardiovascular effect has been proposed as the mechanism of action.<sup>52</sup> A high-fat ketogenic diet might be also helpful for AD.<sup>53</sup>

Conclusions on dietary components have been difficult to ascertain as results have differed between population-based studies and randomized controlled trials.<sup>54</sup> There is limited evidence that light-to-moderate use of alcohol, particularly red wine, is associated with lower risk of AD.<sup>55</sup>

---

<sup>49</sup> Kanoski SE, Davidson TL. Western diet consumption and cognitive impairment: links to hippocampal dysfunction and obesity. *Physiol Behav.* 2011 Apr 18;103(1):59-68; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3056912/>.

<sup>50</sup> Hu N, Yu JT, Tan L, Wang Y-L, Sun L, Tan L. Nutrition and the risk of Alzheimer's disease. *Biomed Res Int.* 2013;2013:524820; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3705810/>.

<sup>51</sup> Solfrizzi V, Panza F, Frisardi V, Seripa D, Logroscino G, Imbimbo BP, Pilotto A. Diet and Alzheimer's disease risk factors or prevention: the current evidence. *Expert Rev Neurother.* 2011 May;11(5):677-708; [https://www.researchgate.net/profile/Francesco\\_Panza/publication/51095382\\_Diet\\_and\\_Alzheimer's\\_disease\\_risk\\_factors\\_or\\_prevention/The\\_current\\_evidence/links/02e7e530c60d26aece000000.pdf](https://www.researchgate.net/profile/Francesco_Panza/publication/51095382_Diet_and_Alzheimer's_disease_risk_factors_or_prevention/The_current_evidence/links/02e7e530c60d26aece000000.pdf).

<sup>52</sup> Solfrizzi V, Capurso C, D'Introno A, Colacicco AM, Santamato A, Ranieri M, Fiore P, Capurso A, Panza F. Lifestyle-related factors in predementia and dementia syndromes. *Expert Rev Neurother.* 2008 Jan;8(1):133-58; <http://www.ncbi.nlm.nih.gov/pubmed/18088206>.

<sup>53</sup> Gasior M, Rogawski MA, Hartman AL. Neuroprotective and disease-modifying effects of the ketogenic diet. *Behav Pharmacol.* 2006 Sep;17(5-6):431-9; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2367001/>.

<sup>54</sup> Hu N, Yu JT, Tan L, Wang Y-L, Sun L, Tan L. Nutrition and the risk of Alzheimer's disease. *Biomed Res Int.* 2013;2013:524820; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3705810/>.

<sup>55</sup> Panza F, Capurso C, D'Introno A, Colacicco AM, Frisardi V, Lorusso M, Santamato A, Seripa D, Pilotto A, Scafato E, Vendemiale G, Capurso A, Solfrizzi V. Alcohol drinking, cognitive functions in older age, predementia, and dementia syndromes. *J Alzheimers Dis.* 2009;17(1):7-31; [https://www.researchgate.net/profile/Francesco\\_Panza/publication/26264835\\_Alcohol\\_Drinking\\_Cognitive\\_Functions\\_in\\_Older\\_Age\\_Predementia\\_and\\_Dementia\\_Syndromes/links/059e7a33215d9b61fc200d40.pdf](https://www.researchgate.net/profile/Francesco_Panza/publication/26264835_Alcohol_Drinking_Cognitive_Functions_in_Older_Age_Predementia_and_Dementia_Syndromes/links/059e7a33215d9b61fc200d40.pdf).

There is tentative evidence that caffeine and coffee may be protective.<sup>56</sup> A number of foods high in flavonoids such as cocoa, red wine, and tea may decrease the risk of AD.<sup>57</sup>

Reviews on the use of vitamins and minerals have not found enough consistent evidence to justify adding these to the diet. This applies to vitamin A,<sup>58</sup> vitamin C,<sup>59</sup> vitamin E,<sup>60</sup> selenium,<sup>61</sup> zinc,<sup>62</sup> and folic acid with or without vitamin B12.<sup>63</sup> Vitamin E is also associated with health risks.<sup>64</sup>

---

<sup>56</sup> Santos C, Costa J, Santos J, Vaz-Carneiro A, Lunet N. Caffeine intake and dementia: systematic review and meta-analysis. *J Alzheimers Dis.* 2010;20 Suppl 1:S187-204; <http://content.iospress.com/download/journal-of-alzheimers-disease/jad091387?id=journal-of-alzheimers-disease%2Fjad091387>.

<sup>57</sup> Stoclet JC, Schini-Kerth V. Dietary flavonoids and human health. *Ann Pharm Fr.* 2011 Mar;69(2):78-90, in French; <http://www.ncbi.nlm.nih.gov/pubmed/21440100>. Nehlig A. The neuroprotective effects of cocoa flavanol and its influence on cognitive performance. *Br J Clin Pharmacol.* 2013 Mar;75(3):716-27; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3575938/>.

<sup>58</sup> Lerner AJ, Gustaw-Rothenberg K, Smyth S, Casadesus G. Retinoids for treatment of Alzheimer's disease. *Biofactors.* 2012 Mar-Apr;38(2):84-9; <http://www.ncbi.nlm.nih.gov/pubmed/22419567>. Ono K, Yamada M. Vitamin A and Alzheimer's disease. *Geriatr Gerontol Int.* 2012 Apr;12(2):180-8; <http://www.ncbi.nlm.nih.gov/pubmed/22221326>.

<sup>59</sup> Boothby LA, Doering PL. Vitamin C and Vitamin E for Alzheimer's disease. *Ann Pharmacother.* 2005 Dec;39(12):2073-80; [https://www.researchgate.net/profile/Paul\\_Doering/publication/7539821\\_Vitamin\\_C\\_and\\_Vitamin\\_E\\_for\\_Alzheimer's\\_Disease/links/53f4d7030cf2fceacc6eaab6.pdf](https://www.researchgate.net/profile/Paul_Doering/publication/7539821_Vitamin_C_and_Vitamin_E_for_Alzheimer's_Disease/links/53f4d7030cf2fceacc6eaab6.pdf). Heo JH, Lee KM. The possible role of antioxidant vitamin C in Alzheimer's disease treatment and prevention. *Am J Alzheimers Dis Other Demen.* 2013 Mar;28(2):120-5; <http://www.ncbi.nlm.nih.gov/pubmed/23307795>.

<sup>60</sup> Boothby LA, Doering PL. Vitamin C and Vitamin E for Alzheimer's disease. *Ann Pharmacother.* 2005 Dec;39(12):2073-80; [https://www.researchgate.net/profile/Paul\\_Doering/publication/7539821\\_Vitamin\\_C\\_and\\_Vitamin\\_E\\_for\\_Alzheimer's\\_Disease/links/53f4d7030cf2fceacc6eaab6.pdf](https://www.researchgate.net/profile/Paul_Doering/publication/7539821_Vitamin_C_and_Vitamin_E_for_Alzheimer's_Disease/links/53f4d7030cf2fceacc6eaab6.pdf). Isaac MG, Quinn R, Tabet N. Vitamin E for Alzheimer's disease and mild cognitive impairment. *Cochrane Database Syst Rev.* 2008 Jul 16;(3):CD002854; <http://www.ncbi.nlm.nih.gov/pubmed/18646084>. Dysken MW, *et al.* Effect of vitamin E and memantine on functional decline in Alzheimer disease: the TEAM-AD VA cooperative randomized trial. *JAMA.* 2014 Jan 1;311(1):33-44; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4109898/>.

<sup>61</sup> Loeff M, Schrauzer GN, Walach H. Selenium and Alzheimer's disease: a systematic review. *J Alzheimers Dis.* 2011;26(1):81-104; <http://www.ncbi.nlm.nih.gov/pubmed/21593562>.

<sup>62</sup> Loeff M, von Stillfried N, Walach H. Zinc diet and Alzheimer's disease: a systematic review. *Nutr Neurosci.* 2012 Sep;15(5):2-12; [https://www.researchgate.net/profile/Harald\\_Walach/publication/224956695\\_Zinc\\_diet\\_and\\_Alzheimer's\\_disease\\_A\\_systematic\\_review/links/0fcfd51066c11139e8000000.pdf](https://www.researchgate.net/profile/Harald_Walach/publication/224956695_Zinc_diet_and_Alzheimer's_disease_A_systematic_review/links/0fcfd51066c11139e8000000.pdf).

<sup>63</sup> Malouf R, Grimley Evans J. Folic acid with or without vitamin B<sub>12</sub> for the prevention and treatment of healthy elderly and demented people. *Cochrane Database Syst Rev.* 2008 Oct 8;(4):CD004514; <http://www.ncbi.nlm.nih.gov/pubmed/18843658>.

Trials examining folic acid (B9)<sup>65</sup> and other B vitamins failed to show any significant association with cognitive decline, although at least some favorable results for cognitive function have been reported for vitamin B2, vitamin B6, and vitamin B12.<sup>66</sup> In patients already afflicted with Alzheimer's, the addition of docosahexaenoic acid, an omega-3 fatty acid, to the diet has not been found to slow decline.<sup>67</sup> Long-term use of the NSAID ibuprofen<sup>68</sup> may slightly reduce the probability of getting AD during the course of treatment, but aspirin has no useful benefit.<sup>69</sup>

Curcumin (aka. turmeric)<sup>70</sup> has not shown definitive benefit in people<sup>71</sup> even though there is tentative evidence in animals.<sup>72</sup> There is inconsistent and unconvincing evidence that ginkgo

<sup>64</sup> Boothby LA, Doering PL. Vitamin C and vitamin E for Alzheimer's disease. *Ann Pharmacother.* 2005 Dec;39(12):2073-80; [https://www.researchgate.net/profile/Paul\\_Doering/publication/7539821\\_Vitamin\\_C\\_and\\_Vitamin\\_E\\_for\\_Alzheimer's\\_Disease/links/53f4d7030cf2fceacc6eaab6.pdf](https://www.researchgate.net/profile/Paul_Doering/publication/7539821_Vitamin_C_and_Vitamin_E_for_Alzheimer's_Disease/links/53f4d7030cf2fceacc6eaab6.pdf).

<sup>65</sup> Wald DS, Kasturiratne A, Simmonds M. Effect of folic acid, with or without other B vitamins, on cognitive decline: meta-analysis of randomized trials. *Am J Med.* 2010 Jun;123(6):522-527; <http://www.ncbi.nlm.nih.gov/pubmed/20569758>.

<sup>66</sup> Kim H, Kim G, Jang W, Kim SY, Chang N. Association between intake of B vitamins and cognitive function in elderly Koreans with cognitive impairment. *Nutr J.* 2014 Dec 17;13(1):118; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4290102/>.

<sup>67</sup> Cunnane SC, Chouinard-Watkins R, Castellano CA, Barberger-Gateau P. Docosahexaenoic acid homeostasis, brain aging and Alzheimer's disease: Can we reconcile the evidence? *Prostaglandins Leukot Essent Fatty Acids.* 2013 Jan;88(1):61-70; [https://www.researchgate.net/profile/Christian-Alexandre-Castellano/publication/224938889\\_Docosahexaenoic\\_acid\\_homeostasis\\_brain\\_aging\\_and\\_Alzheimer's\\_disease\\_Can\\_we\\_reconcile\\_the\\_evidence/links/0c960527d6fe03a512000000.pdf](https://www.researchgate.net/profile/Christian-Alexandre-Castellano/publication/224938889_Docosahexaenoic_acid_homeostasis_brain_aging_and_Alzheimer's_disease_Can_we_reconcile_the_evidence/links/0c960527d6fe03a512000000.pdf).

<sup>68</sup> Vlad SC, Miller DR, Kowall NW, Felson DT. Protective effects of NSAIDs on the development of Alzheimer disease. *Neurology.* 2008 May 6;70(19):1672-7; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2758242/>.

<sup>69</sup> Bentham P, Gray R, Sellwood E, Hills R, Crome P, Raftery J. Aspirin in Alzheimer's disease (AD2000): a randomised open-label trial. *Lancet Neurol.* 2008 Jan;7(1):41-9; <http://www.ncbi.nlm.nih.gov/pubmed/18068522>. Richard E, Kuiper R, Dijkgraaf MG, Van Gool WA; Evaluation of Vascular care in Alzheimer's disease. Vascular care in patients with Alzheimer's disease with cerebrovascular lesions-a randomized clinical trial. *J Am Geriatr Soc.* 2009 May;57(5):797-805; <http://www.ncbi.nlm.nih.gov/pubmed/19484836>. Thoonsen H, Richard E, Bentham P, Gray R, van Geloven N, De Haan RJ, Van Gool WA, Nederkoorn PJ. Aspirin in Alzheimer's disease: increased risk of intracerebral hemorrhage: cause for concern? *Stroke.* 2010 Nov;41(11):2690-2; <http://stroke.ahajournals.org/content/41/11/2690.long>.

<sup>70</sup> <http://www.best-alzheimers-products.com/can-turmeric-cure-alzheimers.html>

<sup>71</sup> Apetz N, Munch G, Govindaraghavan S, Gyengesi E. Natural compounds and plant extracts as therapeutics against chronic inflammation in Alzheimer's disease – a translational perspective. *CNS Neurol Disord Drug Targets.* 2014;13(7):1175-91; [https://www.researchgate.net/profile/Erika\\_Gyengesi/publication/265734665\\_Natural\\_Compounds\\_and\\_Plant\\_Extracts\\_as\\_Therapeutics\\_Against\\_Chronic\\_Inflammation\\_in\\_Alzheimer's\\_Disease\\_-\\_A\\_Translational\\_Perspective/links/542258620cf290c9e3a79365.pdf](https://www.researchgate.net/profile/Erika_Gyengesi/publication/265734665_Natural_Compounds_and_Plant_Extracts_as_Therapeutics_Against_Chronic_Inflammation_in_Alzheimer's_Disease_-_A_Translational_Perspective/links/542258620cf290c9e3a79365.pdf).

biloba might have some positive effect on cognitive impairment and dementia.<sup>73</sup> As of 2014 there was no conclusive evidence that cannabinoids are effective in improving the symptoms of AD or dementia,<sup>74</sup> though some research in its early stages looks promising.<sup>75</sup>

A growing number of herbal remedies,<sup>76</sup> dietary supplements, nutraceuticals<sup>77</sup> and “medical foods” are being promoted as memory enhancers or treatments to delay or prevent Alzheimer’s disease and related dementias, including: acetyl-L-carnitine (aka. carnitine, ALC, ALCAR),<sup>78</sup>

<sup>72</sup> Hamaguchi T, Ono K, Yamada M. REVIEW: Curcumin and Alzheimer’s disease. *CNS Neurosci Ther.* 2010 Oct;16(5):285-97; <http://www.ncbi.nlm.nih.gov/pubmed/20406252>.

<sup>73</sup> Birks J, Grimley Evans J. Ginkgo biloba for cognitive impairment and dementia. *Cochrane Database Syst Rev.* 2009 Jan 21;(1):CD003120; <http://www.ncbi.nlm.nih.gov/pubmed/19160216>.

<sup>74</sup> Krishnan S, Cairns R, Howard R. *Cochrane Database Syst Rev.* 2009 Apr 15;(2):CD007204; <https://www.theroc.us/images/Cannabinoids%20for%20the%20treatment%20of%20dementia.pdf>. Van den Elsen GA, Ahmed AI, Lammers M, Kramers C, Verkes RJ, van der Marck MA, Rikkert MG. Efficacy and safety of medical cannabinoids in older subjects: a systematic review. *Ageing Res Rev.* 2014 Mar;14:56-64; [https://www.researchgate.net/profile/Geke\\_Van\\_Den\\_Elsen/publication/260130280\\_Efficacy\\_and\\_safety\\_of\\_medical\\_cannabinoids\\_in\\_older\\_subjects\\_A\\_systematic\\_review/links/5538c3b10cf226723ab63b6b.pdf](https://www.researchgate.net/profile/Geke_Van_Den_Elsen/publication/260130280_Efficacy_and_safety_of_medical_cannabinoids_in_older_subjects_A_systematic_review/links/5538c3b10cf226723ab63b6b.pdf).

<sup>75</sup> Bilkei-Gorzo A. The endocannabinoid system in normal and pathological brain ageing. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* 2012;367(1607):3326–41; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3481530/>. Karl T, Cheng D, Garner B, Arnold JC. The therapeutic potential of the endocannabinoid system for Alzheimer’s disease. *Expert Opin Ther Targets.* 2012 Apr;16(4):407-20; <http://www.ncbi.nlm.nih.gov/pubmed/22448595>. Velayudhan L, Van Diepen E, Marudkar M, Hands O, Suribhatla S, Prettyman R, Murray J, Baillon S, Bhattacharyya S. Therapeutic potential of cannabinoids in neurodegenerative disorders: a selective review. *Curr Pharm Des.* 2014;20(13):2218-30; <http://www.ncbi.nlm.nih.gov/pubmed/23829360>.

<sup>76</sup> <http://grazoph.com/>; <http://www.freep.com/story/news/health/2015/03/11/researcher-hopes-plant-compound-works-alzheimers/70179346/>.

<sup>77</sup> Mecocci P, Tinarelli C, Schulz RJ, Polidori MC. Nutraceuticals in cognitive impairment and Alzheimer’s disease. *Front Pharmacol.* 2014 Jun 23;5:147; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4066843/>.

<sup>78</sup> Thal LJ, Carta A, Clarke WR, Ferris SH, Friedland RP, Petersen RC, Pettegrew JW, Pfeiffer E, Raskind MA, Sano M, Tuszynski MH, Woolson RF. A 1-year multicenter placebo-controlled study of acetyl-L-carnitine in patients with Alzheimer’s disease. *Neurology.* 1996 Sep;47(3):705-11; <http://www.ncbi.nlm.nih.gov/pubmed/8797468>. Bianchetti A, Rozzini R, Trabucchi M. Effects of acetyl-L-carnitine in Alzheimer’s disease patients unresponsive to acetylcholinesterase inhibitors. *Curr Med Res Opin.* 2003;19(4):350-3; <http://www.ncbi.nlm.nih.gov/pubmed/12841930>. Remington R, Chan A, Paskavitz J, Shea TB. Efficacy of a vitamin/nutraceutical formulation for moderate-stage to later-stage Alzheimer’s disease: a placebo-controlled pilot study. *Am J Alzheimers Dis Other Demen.* 2009 Feb-Mar;24(1):27-33; <http://dev.thinkperceptiv.com/wp-content/uploads/AJADOD-2009-Placebo-Study.pdf>. GavriloVA SI, Kalyn IaB, Kolykhalov IV, Roshchina IF, Selezneva ND. Acetyl-L-carnitine (carnitine) in the treatment of early stages of Alzheimer’s disease and vascular dementia. *Zh Nevrol Psikhiatr Im S S Korsakova.* 2011;111(9):16-22, in Russian; <http://www.ncbi.nlm.nih.gov/pubmed/22027664>.

ashwagandha (withanamides),<sup>79</sup> bacopa, caprylic acid, cinnamon extract,<sup>80</sup> cocoa (chocolate),<sup>81</sup> coconut oil,<sup>82</sup> coenzyme Q10, coral calcium, daffodil extract (galanthamine),<sup>83</sup> fullerene C<sub>60</sub>,<sup>84</sup> ginger root, green tea, kangkong (Ipomoea aquatic),<sup>85</sup> lemon balm (Melissa officinalis),<sup>86</sup> magnesium,<sup>87</sup> nicotinamide (aka. niacinamide, Vitamin B3),<sup>88</sup> olive oil,<sup>89</sup> phosphatidylserine, red

---

<sup>79</sup> <http://msutoday.msu.edu/news/2015/promising-alzheimers-treatment-moves-toward-clinical-trials/>.

<sup>80</sup> Rao PV, Gan SH. Cinnamon: a multifaceted medicinal plant. *Evid Based Complement Alternat Med*. 2014;2014:642942; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4003790/>. Malik J, Munjal K, Deshmukh R. Attenuating effect of standardized lyophilized Cinnamomum zeylanicum bark extract against streptozotocin-induced experimental dementia of Alzheimer's type. *J Basic Clin Physiol Pharmacol*. 2014 Oct 10; <http://www.ncbi.nlm.nih.gov/pubmed/25301673>.

<sup>81</sup> Wang J, Varghese M, Ono K, Yamada M, Levine S, Tzavaras N, Gong B, Hurst WJ, Blitzer RD, Pasinetti GM. Cocoa extracts reduce oligomerization of amyloid- $\beta$ : implications for cognitive improvement in Alzheimer's disease. *J Alzheimers Dis*. 2014;41(2):643-50; [https://www.researchgate.net/profile/Giulio\\_Pasinetti/publication/263356475\\_Cocoa\\_Extracts\\_Reduce\\_Oligomerization\\_of\\_Amyloid-beta\\_Implications\\_for\\_Cognitive\\_Improvement\\_in\\_Alzheimer's\\_Disease/links/53cffe9a0cf2fd75bc5b183e.pdf](https://www.researchgate.net/profile/Giulio_Pasinetti/publication/263356475_Cocoa_Extracts_Reduce_Oligomerization_of_Amyloid-beta_Implications_for_Cognitive_Improvement_in_Alzheimer's_Disease/links/53cffe9a0cf2fd75bc5b183e.pdf). Nehlig A. The neuroprotective effects of cocoa flavanol and its influence on cognitive performance. *Br J Clin Pharmacol*. 2013 Mar;75(3):716-27; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3575938/>.

<sup>82</sup> <http://www.amazon.com/Alzheimers-Disease-What-There-Cure/dp/1591202930>, <http://www.agingcare.com/Articles/coconut-oil-and-alzheimers-157103.htm>.

<sup>83</sup> "Treating Alzheimer's Disease with daffodils," Joint Nature Conservation Committee, 28 Jun 2013; <http://jncc.defra.gov.uk/page-5721>.

<sup>84</sup> "Fullerene C60," Alzheimer's Drug Discovery Foundation, 10 Apr 2014; <http://www.alzdiscovery.org/cognitive-vitality/report/fullerene-c60>. "Drinking Ukrainian Buckyballs to Treat Alzheimer's Disease," *Wired*, 11 Feb 2013; <http://www.wired.com/2013/02/drinking-ukrainian-buckyballs-to-treat-alzheimers-disease/>. Vince Giuliano, "Buckyballs, health and longevity – state of knowledge," 12 Nov 2012; <http://www.anti-agingfirewalls.com/2012/11/12/1424/>.

<sup>85</sup> <http://www.scidev.net/asia-pacific/disease/news/common-plant-could-hold-key-to-alzheimer-s-treatment.html>.

<sup>86</sup> Akhondzadeh S, Noroozian M, Mohammadi M, Ohadinia S, Jamshidi AH, Khani M. Melissa officinalis extract in the treatment of patients with mild to moderate Alzheimer's disease: a double blind, randomised, placebo controlled trial. *J Neurol Neurosurg Psychiatry*. 2003 Jul;74(7):863-6; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1738567/>.

<sup>87</sup> Martin Alessio, "Novel Magnesium Compound Reverses Neurodegeneration," *Life Extension Magazine*, Feb 2012; <http://www.lifeextension.com/Magazine/2012/2/Novel-Magnesium-Compound-Reverses-Neurodegeneration/Page-01>.

<sup>88</sup> Rennie G, Chen AC, Dhillon H, Vardy J, Damian DL. Nicotinamide and neurocognitive function. *Nutr Neurosci*. 2015 Jul;18(5):193-200; <http://www.ncbi.nlm.nih.gov/pubmed/24559077>.

<sup>89</sup> Casamenti F, Grossi C, Rigacci S, Pantano D, Luccarini I, Stefani M. Oleuropein Aglycone: A Possible Drug against Degenerative Conditions. *In Vivo Evidence of its Effectiveness against Alzheimer's Disease*. *J*

beets, red cabbage, sage oil, salmon, and walnuts. According to the Alzheimer's Association,<sup>90</sup> claims about the safety and effectiveness of these products are based largely on testimonials, tradition, and a rather small body of actual scientific research: "Although some of these remedies may be valid candidates for treatments, there are legitimate concerns about using these drugs [or foods] as an alternative or in addition to physician-prescribed therapy."

---

Alzheimers Dis. 2015;45(3):679-88;  
[https://flore.unifi.it/retrieve/handle/2158/997207/31316/JAD2015\\_Casamenti%20et.%20al.pdf](https://flore.unifi.it/retrieve/handle/2158/997207/31316/JAD2015_Casamenti%20et.%20al.pdf).

<sup>90</sup> "Alternative Treatments," Alzheimer's Association, 2016;  
[http://www.alz.org/alzheimers\\_disease\\_alternative\\_treatments.asp](http://www.alz.org/alzheimers_disease_alternative_treatments.asp).

## 2.3 Four Progressive Stages of Alzheimer’s Disease

Following diagnosis, Alzheimer’s patients typically lose ~10% of their cognitive abilities per year. The course of the disease is sometimes divided into four stages<sup>91</sup> with a progressive pattern of cognitive and functional impairment, as follows:

**Stage 1: Pre-dementia.** The first symptoms are often mistakenly attributed to aging or stress.<sup>92</sup> Detailed neuropsychological testing can reveal mild cognitive difficulties up to eight years before a person fulfills the clinical criteria for a diagnosis of AD.<sup>93</sup> These early symptoms can affect the most complex daily living activities.<sup>94</sup> The most noticeable deficit is short term memory loss, which shows up as difficulty in remembering recently learned facts and an inability to acquire new information.<sup>95</sup>

Subtle problems with the executive functions of attentiveness, planning, flexibility, and abstract thinking, or impairments in semantic memory (memory of meanings and concept relationships) can also be symptomatic of the early stages of AD.<sup>96</sup> Apathy can be observed at this stage, and

---

<sup>91</sup> “Evaluating Prescription Drugs Used to Treat: Alzheimer’s Disease,” Consumer Reports Health, May 2012; <http://www.consumerreports.org/health/resources/pdf/best-buy-drugs/AlzheimersFINAL.pdf>.

<sup>92</sup> Waldemar G, Dubois B, Emre M, Georges J, McKeith IG, Rossor M, Scheltens P, Tariska P, Winblad B; EFNS. Recommendations for the diagnosis and management of Alzheimer’s disease and other disorders associated with dementia: EFNS guideline. *Eur J Neurol*. 2007 Jan;14(1):e1-26; <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.549.3628&rep=rep1&type=pdf>.

<sup>93</sup> Bäckman L, Jones S, Berger AK, Laukka EJ, Small BJ. Multiple cognitive deficits during the transition to Alzheimer’s disease. *J Intern Med*. 2004 Sep;256(3):195-204; <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2796.2004.01386.x/epdf>.

<sup>94</sup> Nygård L. Instrumental activities of daily living: a stepping-stone towards Alzheimer’s disease diagnosis in subjects with mild cognitive impairment? *Acta Neurol Scand Suppl*. 2003;179:42-6; <http://www.ncbi.nlm.nih.gov/pubmed/12603250>.

<sup>95</sup> Arnáiz E, Almkvist O. Neuropsychological features of mild cognitive impairment and preclinical Alzheimer’s disease. *Acta Neurol Scand*. 2003;179:34-41; <http://www.ncbi.nlm.nih.gov/pubmed/12603249>. Bäckman L, Jones S, Berger AK, Laukka EJ, Small BJ. Multiple cognitive deficits during the transition to Alzheimer’s disease. *J Intern Med*. 2004 Sep;256(3):195-204; <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2796.2004.01386.x/epdf>.

<sup>96</sup> Arnáiz E, Almkvist O. Neuropsychological features of mild cognitive impairment and preclinical Alzheimer’s disease. *Acta Neurol Scand*. 2003;179:34-41; <http://www.ncbi.nlm.nih.gov/pubmed/12603249>. Bäckman L, Jones S, Berger AK, Laukka EJ, Small BJ. Multiple cognitive deficits during the transition to Alzheimer’s disease. *J Intern Med*. 2004 Sep;256(3):195-204; <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2796.2004.01386.x/epdf>.

remains the most persistent neuropsychiatric symptom throughout the course of the disease.<sup>97</sup> Depressive symptoms, irritability and reduced awareness of subtle memory difficulties are also common.<sup>98</sup> The preclinical stage of the disease has also been termed mild cognitive impairment (MCI).<sup>99</sup> This is often found to be a transitional stage between normal aging and dementia. MCI can present with a variety of symptoms, and when memory loss is the predominant symptom, it is termed “amnesic MCI” and is frequently seen as a prodromal stage of Alzheimer’s disease.<sup>100</sup>

**Stage 2: Early AD.** In people with AD, the increasing impairment of learning and memory eventually leads to a definitive diagnosis. In a small percentage of cases, difficulties with language, executive functions, perception (agnosia), or execution of movements (apraxia) are more prominent than memory problems.<sup>101</sup> AD does not affect all memory capacities equally. Older memories of the person’s life (episodic memory), facts learned (semantic memory), and implicit memory (the memory of the body on how to do things, such as using a fork to eat) are affected to a lesser degree than new facts or memories.<sup>102</sup>

Language problems are mainly characterized by a shrinking vocabulary and decreased word fluency, leading to a general impoverishment of oral and written language. In this stage, the person with Alzheimer’s is usually capable of communicating basic ideas adequately.<sup>103</sup> While

---

<sup>97</sup> Landes AM, Sperry SD, Strauss ME, Geldmacher DS. Apathy in Alzheimer’s disease. *J Am Geriatr Soc.* 2001 Dec;49(12):1700-7; <http://www.ncbi.nlm.nih.gov/pubmed/11844006>.

<sup>98</sup> Murray ED, Buttner N, Price BH. Depression and psychosis in neurological practice. In Bradley WG, Daroff RB, Fenichel GM, Jankovic J. *Bradley’s Neurology in Clinical Practice*, 6th ed., Elsevier/Saunders, Philadelphia PA, 2012.

<sup>99</sup> Arnáiz E, Almkvist O. Neuropsychological Features of Mild Cognitive Impairment and Preclinical Alzheimer’s Disease. *Acta Neurol Scand Suppl.* 2003;179:34-41; <http://www.ncbi.nlm.nih.gov/pubmed/12603249>.

<sup>100</sup> Grundman M, *et al.* Mild cognitive impairment can be distinguished from Alzheimer disease and normal aging for clinical trials. *Arch Neurol.* 2004 Jan;61(1):59-66; <http://archneur.jamanetwork.com/article.aspx?articleid=785241>.

<sup>101</sup> Förstl H, Kurz A. Clinical features of Alzheimer’s disease. *Eur Arch Psychiatry Clin Neurosci.* 1999;249(6):288-90; <http://www.ncbi.nlm.nih.gov/pubmed/10653284>.

<sup>102</sup> Carlesimo GA, Oscar-Berman M. Memory deficits in Alzheimer’s patients: a comprehensive review. *Neuropsychol Rev.* 1992 Jun;3(2):119-69; <http://www.ncbi.nlm.nih.gov/pubmed/1300219>. Jelacic M, Bonebakker AE, Bonke B. Implicit memory performance of patients with Alzheimer’s disease: a brief review. *Int Psychogeriatr.* 1995 Fall;7(3):385-92; <http://www.ncbi.nlm.nih.gov/pubmed/8821346>.

<sup>103</sup> Frank EM. Effect of Alzheimer’s disease on communication function. *J S C Med Assoc.* 1994 Sep;90(9):417-23; <http://www.ncbi.nlm.nih.gov/pubmed/7967534>. Förstl H, Kurz A. Clinical features of Alzheimer’s disease. *Eur Arch Psychiatry Clin Neurosci.* 1999;249(6):288-90; <http://www.ncbi.nlm.nih.gov/pubmed/10653284>. Taler V, Phillips NA. Language performance in Alzheimer’s disease and mild cognitive impairment: a comparative review. *J Clin Exp Neuropsychol.* 2008 Jul;30(5):501-56; <http://www-psychology.concordia.ca/fac/phillips/files/TalerPhillipsJCEN2008.pdf>.

performing fine motor tasks such as writing, drawing or dressing, certain movement coordination and planning difficulties (apraxia) may be present, but they are commonly unnoticed. As the disease progresses, people with AD can often continue to perform many tasks independently, but may need assistance or supervision with the most cognitively demanding activities.

**Stage 3: Moderate AD.** Progressive deterioration eventually hinders independence, with subjects being unable to perform most common activities of daily living.<sup>104</sup> Speech difficulties become evident due to an inability to recall vocabulary, which leads to frequent incorrect word substitutions (paraphasias). Reading and writing skills are also progressively lost. Complex motor sequences become less coordinated as time passes and the AD progresses, so the risk of falling increases. During this phase, memory problems worsen and the person may fail to recognize close relatives. Long-term memory, which was previously intact, becomes impaired.

Behavioral and neuropsychiatric changes become more prevalent. Common manifestations are wandering, irritability and labile affect (i.e., emotions easily aroused or freely expressed), leading to crying, outbursts of unpremeditated aggression, or resistance to caregiving. Sundowning (i.e., “good” mornings, “bad” evenings) can also appear.<sup>105</sup> Approximately 30% of people with AD develop illusionary misidentifications and other delusional symptoms.<sup>106</sup> Subjects lose insight on their disease process and limitations (anosognosia), and urinary incontinence can develop. These symptoms create stress for relatives and carers, which can be reduced by moving the person from home care to a long-term care facility.<sup>107</sup>

**Stage 4: Advanced AD.** During the final stages,<sup>108</sup> the patient is completely dependent upon caregivers. Language is reduced to simple phrases or even single words, eventually leading to complete loss of speech. Despite the loss of verbal language abilities, people can often

---

<sup>104</sup> Frank EM. Effect of Alzheimer’s disease on communication function. *J S C Med Assoc.* 1994 Sep;90(9):417-23; <http://www.ncbi.nlm.nih.gov/pubmed/7967534>. Förstl H, Kurz A. Clinical features of Alzheimer’s disease. *Eur Arch Psychiatry Clin Neurosci.* 1999;249(6):288-90; <http://www.ncbi.nlm.nih.gov/pubmed/10653284>.

<sup>105</sup> Volicer L, Harper DG, Manning BC, Goldstein R, Satlin A. Sundowning and circadian rhythms in Alzheimer’s disease. *Am J Psychiatry.* 2001 May;158(5):704-11; <http://www-syst.bu.edu/alzresearch/research/publications/documents/VolicerSundowningandAD2001.pdf>.

<sup>106</sup> Förstl H, Kurz A. Clinical features of Alzheimer’s disease. *Eur Arch Psychiatry Clin Neurosci.* 1999;249(6):288-90; <http://www.ncbi.nlm.nih.gov/pubmed/10653284>.

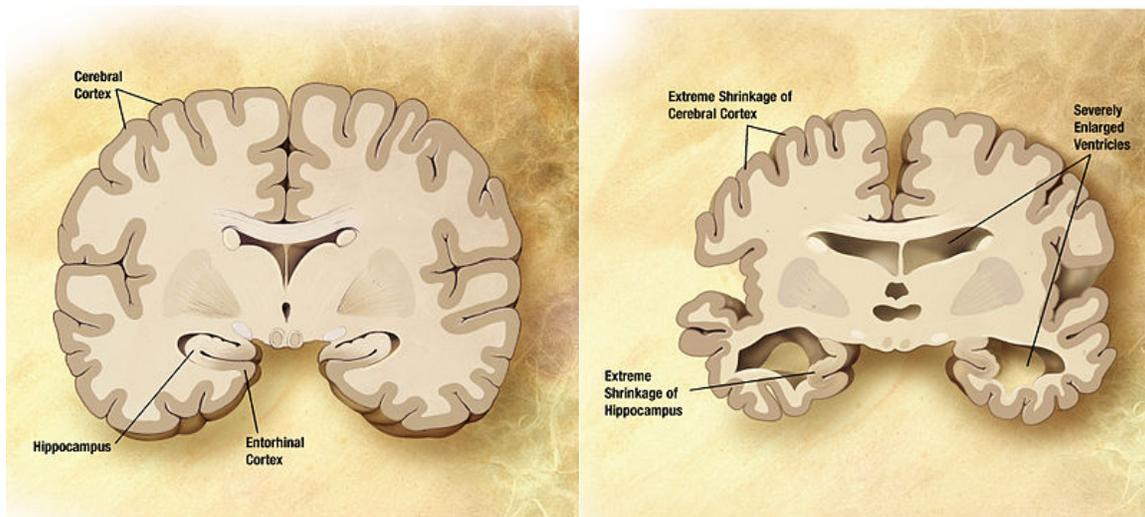
<sup>107</sup> Gold DP, Reis MF, Markiewicz D, Andres D. When home caregiving ends: a longitudinal study of outcomes for caregivers of relatives with dementia. *J Am Geriatr Soc.* 1995 Jan;43(1):10-6; <http://www.ncbi.nlm.nih.gov/pubmed/7806732>.

<sup>108</sup> Frank EM. Effect of Alzheimer’s disease on communication function. *J S C Med Assoc.* 1994 Sep;90(9):417-23; <http://www.ncbi.nlm.nih.gov/pubmed/7967534>. Förstl H, Kurz A. Clinical features of Alzheimer’s disease. *Eur Arch Psychiatry Clin Neurosci.* 1999;249(6):288-90; <http://www.ncbi.nlm.nih.gov/pubmed/10653284>.

understand and return emotional signals. Although aggressiveness can still be present, extreme apathy and exhaustion are much more common symptoms. People with Alzheimer's disease will eventually be unable to perform even the simplest tasks independently. Muscle mass and mobility deteriorate to the point where patients are bedridden and unable to feed themselves. The immediate cause of death is usually an external factor (e.g., infection from pressure ulcers, pneumonia).

The brain of a late-stage AD patient undergoes significant anatomical changes as illustrated in **Figure 1**. AD causes a large loss in brain weight and volume, and affects some brain regions and neuronal populations more than others.<sup>109</sup> Although AD clearly causes loss of neurons in specific brain regions (e.g., of pyramidal cells in lamina II of the entorhinal cortex and in the CA1 region of the hippocampus), much of the overall loss of brain volume appears to be due to the shrinkage and loss of neuronal processes.

*Figure 1. A normal aged brain (left) and the brain of a person with Alzheimer's disease (right).<sup>110</sup>*

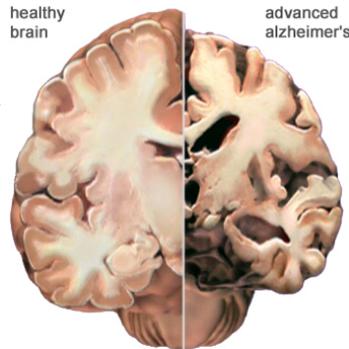


Anatomically, Alzheimer's disease is characterized by a loss of neurons and synapses in the cerebral cortex and certain subcortical regions. This loss results in gross atrophy of the affected regions, including degeneration in the temporal lobe and parietal lobe, and parts of the frontal

<sup>109</sup> Gomez-Isla T, Price JL, McKeel DW, Jr, Morris JC, Growdon JH, Hyman BT. Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *J Neurosci*. 1996;16:4491-4500; <http://www.jneurosci.org/content/16/14/4491.long>.

<sup>110</sup> [http://commons.wikimedia.org/wiki/File:Alzheimer%27s\\_disease\\_brain\\_comparison.jpg](http://commons.wikimedia.org/wiki/File:Alzheimer%27s_disease_brain_comparison.jpg).

cortex and cingulate gyrus.<sup>111</sup> Degeneration is also present in brainstem nuclei like the locus coeruleus.<sup>112</sup> Studies using MRI and PET have documented reductions in the size of specific brain regions in people with AD as they progressed from mild cognitive impairment to Alzheimer's disease, and in comparison with similar images from healthy older adults.<sup>113</sup>



The early stages of Alzheimer's disease have traditionally been difficult to diagnose. A definitive diagnosis is usually made once cognitive impairment compromises daily living activities, although the person may still be living independently. The symptoms will progress from mild cognitive problems, such as memory loss, through increasing stages of cognitive and non-cognitive disturbances thus eliminating any possibility of independent living, especially in the late stages of the disease.<sup>114</sup> Somewhat more recently, initial experiments using serum samples from 90 human subjects identified a small panel of ten autoantibody (IgG) biomarkers that could distinguish people with Alzheimer's disease from healthy people with 90% accuracy, and could also differentiate Alzheimer's from Parkinson's patients with 86% accuracy.<sup>115</sup> Several studies now underway are also looking at new technology that will enable ophthalmologists to detect beta amyloid plaque via the retina,<sup>116</sup> with FDA approval expected in 2016.<sup>117</sup>

<sup>111</sup> Wenk GL. Neuropathologic changes in Alzheimer's disease: potential targets for treatment. *J Clin Psychiatry*. 2006;67 Suppl 3:3-7; <http://faculty.psy.ohio-state.edu/wenk/documents/JClinPsychiatry2003.pdf>.

<sup>112</sup> Braak H, Del Tredici K. Where, when, and in what form does sporadic Alzheimer's disease begin? *Curr Opin Neurol*. 2012 Dec;25(6):708-14; [https://www.researchgate.net/profile/Kelly\\_Del\\_Tredici/publication/233537724\\_Where\\_when\\_and\\_in\\_what\\_form\\_does\\_sporadic\\_Alzheimer's\\_disease\\_begin/links/5475d0040cf245eb43710cab.pdf](https://www.researchgate.net/profile/Kelly_Del_Tredici/publication/233537724_Where_when_and_in_what_form_does_sporadic_Alzheimer's_disease_begin/links/5475d0040cf245eb43710cab.pdf).

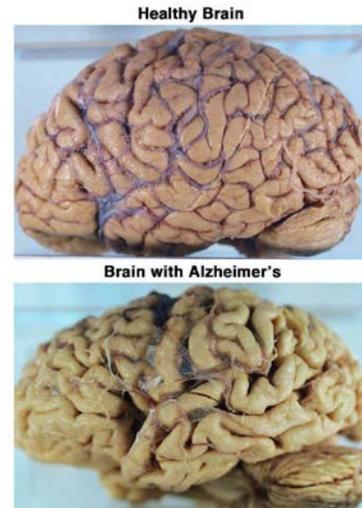
<sup>113</sup> Desikan RS, Cabral HJ, Hess CP, Dillon WP, Glastonbury CM, Weiner MW, Schmansky NJ, Greve DN, Salat DH, Buckner RL, Fischl B. Automated MRI measures identify individuals with mild cognitive impairment and Alzheimer's disease. *Brain*. Aug 2009;132(Pt 8):2048-57; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2714061/>. Moan R. MRI software accurately IDs preclinical Alzheimer's disease. *Diagnostic Imaging*. 20 Jul 2009; <http://www.diagnosticimaging.com/news/display/article/113619/1428344>.

<sup>114</sup> Förstl H, Kurz A. Clinical features of Alzheimer's disease. *Eur Arch Psychiatry Clin Neurosci*. 1999;249(6):288-90; <http://www.ncbi.nlm.nih.gov/pubmed/10653284>.

<sup>115</sup> Nagele E, Han M, Demarshall C, Belinka B, Nagele R. Diagnosis of Alzheimer's disease based on disease-specific autoantibody profiles in human sera. *PLoS One*. 2011;6(8):e23112; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3149629/>.

<sup>116</sup> Heaton GR, Davis BM, Turner LA, Cordeiro MF. Ocular biomarkers of Alzheimer's disease. *Cent Nerv Syst Agents Med Chem*. 2015;15(2):117-25; <http://www.ncbi.nlm.nih.gov/pubmed/25788142>. Bambo MP, Garcia-Martin E, Gutierrez-Ruiz F, Pinilla J, Perez-Olivan S, Larrosa JM, Polo V, Pablo L. Analysis of optic disk color changes in Alzheimer's disease: A potential new biomarker. *Clin Neurol Neurosurg*. 2015 Mar 9;132:68-73; <http://www.ncbi.nlm.nih.gov/pubmed/25797847>.

Life expectancy of the population with AD is reduced,<sup>118</sup> with a mean life expectancy following diagnosis of 5.7 years in one study.<sup>119</sup> Fewer than 3% of people with AD live more than fourteen years.<sup>120</sup> Disease features that are significantly associated with reduced survival are: an increased severity of cognitive impairment, decreased functional level, history of falls, and disturbances in the neurological examination. Other coincident diseases such as heart problems, diabetes or history of alcohol abuse are also related to AD, with shortened survival.<sup>121</sup> While the earlier the age at onset, the higher the total survival years, life expectancy is reduced in comparison to the healthy younger population.<sup>122</sup> Men also have a less favorable survival prognosis than women.<sup>123</sup>



<sup>117</sup> “Noninvasive retinal imaging device detects Alzheimer’s 20 years in advance,” 22 Jul 2014; <http://www.kurzweilai.net/noninvasive-retinal-imaging-device-detects-alzheimers-20-years-in-advance>.

<sup>118</sup> Bowen JD, Malter AD, Sheppard L, Kukull WA, McCormick WC, Teri L, Larson EB. Predictors of mortality in patients diagnosed with probable Alzheimer’s disease. *Neurology*. 1996 Aug;47(2):433-9; <http://www.ncbi.nlm.nih.gov/pubmed/8757016>. Dodge HH, Shen C, Pandav R, DeKosky ST, Ganguli M. Functional transitions and active life expectancy associated with Alzheimer disease. *Arch Neurol*. 2003 Feb;60(2):253-9; <http://archneur.jamanetwork.com/article.aspx?articleid=783753>.

<sup>119</sup> Mölsä PK, Marttila RJ, Rinne UK. Survival and cause of death in Alzheimer’s disease and multi-infarct dementia. *Acta Neurol Scand*. 1986 Aug;74(2):103-7; <http://www.ncbi.nlm.nih.gov/pubmed/3776457>.

<sup>120</sup> Mölsä PK, Marttila RJ, Rinne UK. Long-term survival and predictors of mortality in Alzheimer’s disease and multi-infarct dementia. *Acta Neurol Scand*. 1995 Mar;91(3):159-64; <http://www.ncbi.nlm.nih.gov/pubmed/7793228>.

<sup>121</sup> Jagger C, Clarke M, Stone A. Predictors of survival with Alzheimer’s disease: a community-based study. *Psychol Med*. 1995 Jan;25(1):171-7; <http://www.ncbi.nlm.nih.gov/pubmed/7792352>. Bowen JD, Malter AD, Sheppard L, Kukull WA, McCormick WC, Teri L, Larson EB. Predictors of mortality in patients diagnosed with probable Alzheimer’s disease. *Neurology*. 1996 Aug;47(2):433-9; <http://www.ncbi.nlm.nih.gov/pubmed/8757016>. Larson EB, Shadlen MF, Wang L, McCormick WC, Bowen JD, Teri L, Kukull WA. Survival after initial diagnosis of Alzheimer disease. *Ann Intern Med*. 2004 Apr 6;140(7):501-9; <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.537.9578&rep=rep1&type=pdf>.

<sup>122</sup> Dodge HH, Shen C, Pandav R, DeKosky ST, Ganguli M. Functional transitions and active life expectancy associated with Alzheimer disease. *Arch Neurol*. 2003 Feb;60(2):253-9; <http://archneur.jamanetwork.com/article.aspx?articleid=783753>.

<sup>123</sup> Mölsä PK, Marttila RJ, Rinne UK. Long-term survival and predictors of mortality in Alzheimer’s disease and multi-infarct dementia. *Acta Neurol Scand*. 1995 Mar;91(3):159-64; <http://www.ncbi.nlm.nih.gov/pubmed/7793228>. Ganguli M, Dodge HH, Shen C, Pandav RS, DeKosky ST. Alzheimer disease and mortality: a 15-year epidemiological study. *Arch Neurol*. 2005 May;62(5):779-84; <http://archneur.jamanetwork.com/article.aspx?articleid=788478>.

When a cure for AD is found, it will be important to begin treatment as early as possible, ideally well before pathological mental symptoms appear. Even those with mild Alzheimer's disease have profound cell loss. One study<sup>124</sup> found that at time of death, AD patients with the mildest clinically detectable dementia had 32% fewer entorhinal cortex neurons than controls (vs. 69% for severe AD brains), 40% fewer layer IV neurons (vs. 70% for severe AD) and 60% fewer layer II neurons (vs. 90% for severe AD).

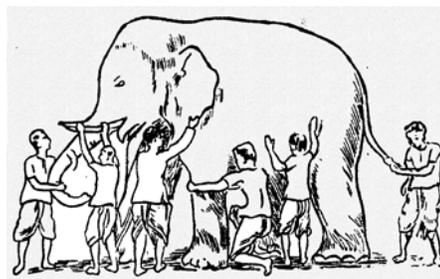
---

<sup>124</sup> Gomez-Isla T, Price JL, McKeel DW, Jr, Morris JC, Growdon JH, Hyman BT. Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *J Neurosci.* 1996;16:4491-4500; <http://www.jneurosci.org/content/16/14/4491.long>.

## 2.4 Molecular Pathologies of Alzheimer's Disease

While scientists know that Alzheimer's disease involves progressive brain cell failure, the complete story of why brain cells fail is not yet fully established. Like many other chronic conditions, experts have long believed that Alzheimer's develops as a complex result of multiple factors rather than from any one overriding cause.

After spending time digging through the literature, a newcomer quickly realizes that discussions of the nature of Alzheimer's disease are reminiscent of the ancient parable of the elephant.<sup>125</sup> According to a Jain version of the story, six blind men were asked to determine what an elephant looked like by feeling different parts of the elephant's body. The blind man who feels a leg says the elephant is like a pillar; the one who feels the tail says the elephant is like a rope; the one who feels the trunk says the elephant is like a tree branch; the one who feels the ear says the elephant is like a hand fan; the one who feels the belly says the elephant is like a wall; and the one who feels the tusk says the elephant is like a solid pipe. Of course the elephant is actually all of these things, and more, but is just too big and complex for a single person to fully grasp.



In similar manner, all of the many theories about what causes AD seem to have some good measure of truth to them, but none seems to encompass the entire story. In the first seven parts of Section 2.4, we describe the causes of Alzheimer's disease as they are commonly presented in the literature, allowing the reader to gradually become familiar with most of the molecular actors that have been implicated in the disease while experiencing firsthand the multifactorial complexity that has long existed in this field. In particular, amyloid (Section 2.4.1) and tau protein (Section 2.4.2) aggregation are the two most prominent molecular pathologies associated with AD. Their plaques and tangles are widely considered as examples of "misfolded proteins" that are associated with Alzheimer's.<sup>126</sup> A variety of additional molecular pathologies and pathways to AD are described in Section 2.4.3. Other AD theories having some measure of general currency include the effects of aging (Section 2.4.4) and genetics and epigenetics (Section 2.4.5). We then provide one example (of many possible) of a well-conceived but controversial theory of AD (Section 2.4.6). The lipofuscin hypothesis (Section 2.4.7) is also included here for completeness. These seven Sections complete our analogy to the parable of the blind men examining the elephant.

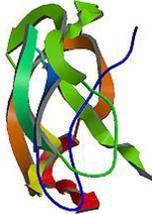
In the final Section 2.4.8, we present an eagle's-eye view of the elephant – what appears to be the most coherent synthesis of the root molecular causes and subsequent etiology of Alzheimer's disease that is currently available. Knowledgeable readers already intimately familiar with the literature on the molecular pathologies of AD may wish to skip directly to Section 2.4.8.

<sup>125</sup> "Elephant and the Blind Men", 2011; <http://www.jainworld.com/education/stories25.asp>.

<sup>126</sup> Herczenik E, Gebbink MF. Molecular and cellular aspects of protein misfolding and disease. *FASEB J*. 2008 Jul;22(7):2115-33; <http://www.fasebj.org/content/22/7/2115.full>. See also: <http://neurophage.com/science/protein-misfolding-diseases/>.

## 2.4.1 Amyloid Pathologies

The original amyloid cascade hypothesis postulated that extracellular amyloid beta ( $A\beta$ ) deposition was the fundamental cause of AD, leading to a pathological cascade of events including tau protein phosphorylation and tangle formation, followed by neuronal death.<sup>127</sup>



Amyloid beta, also written  $A\beta$ , is a short 4 kDa peptide,<sup>128</sup> 39-43 amino acids in length, that is a proteolytic byproduct of the transmembrane protein called amyloid precursor protein (APP; image, left) that penetrates through the neuron's membrane. APP is critical to neuron growth, survival and post-injury repair.<sup>129</sup> APP itself is not neurotoxic, and does not produce  $A\beta$  until it undergoes proteolytic cleavage. APP is produced in considerable quantities in neurons, where it is sorted via the Golgi and then shipped toward the axons.<sup>130</sup>

$A\beta$  monomer is derived from APP through sequential cleavage by  $\beta$ - and  $\gamma$ -secretases.<sup>131</sup>  $A\beta$  monomer is primarily produced in neurons<sup>132</sup> and is released extracellularly following cleavage by the two secretases. The two most prevalent isoforms of  $A\beta$  are 40 and 42 residues in length.<sup>133</sup>  $A\beta_{40}$  ( $\geq 90\%$  under physiological conditions)<sup>134</sup> is produced by cleavage in the trans-Golgi network (TGN) of the neuron and packaged into post-TGN secretory vesicles.  $A\beta_{42}$  ( $\leq 5\%$ , and more fibrillogenic than  $A\beta_{40}$ ) is produced both in the endoplasmic reticulum (where it is sequestered in an insoluble state) and in the TGN (where it is packaged into post-TGN secretory

<sup>127</sup> Hardy J, Allsop D. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends Pharmacol Sci.* 1991 Oct;12(10):383-8; <http://www.ncbi.nlm.nih.gov/pubmed/1763432>.

<sup>128</sup> Glenner GG, Wong CW. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem Biophys Res Commun.* 1984 May 16;120(3):885-90; <http://www.ncbi.nlm.nih.gov/pubmed/6375662>.

<sup>129</sup> Turner PR, O'Connor K, Tate WP, Abraham WC. Roles of amyloid precursor protein and its fragments in regulating neural activity, plasticity and memory. *Prog Neurobiol.* 2003 May;70(1):1-32; <http://www.ncbi.nlm.nih.gov/pubmed/12927332>. Priller C, Bauer T, Mitteregger G, Krebs B, Kretschmar HA, Herms J. Synapse formation and function is modulated by the amyloid precursor protein. *J Neurosci.* 2006 Jul 5;26(27):7212-21; <http://www.jneurosci.org/content/26/27/7212.long>.

<sup>130</sup> Koo EH, Sisodia SS, Archer DR, Martin LJ, Weidemann A, Beyreuther K, Fischer P, Masters CL, Price DL. Precursor of amyloid protein in Alzheimer disease undergoes fast anterograde axonal transport. *Proc Natl Acad Sci U S A.* 1990 Feb;87(4):1561-5; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC53515/>.

<sup>131</sup> Zhang X, Li Y, Xu H, Zhang YW. The  $\gamma$ -secretase complex: from structure to function. *Front Cell Neurosci.* 2014 Dec 11;8:427; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4263104/>.

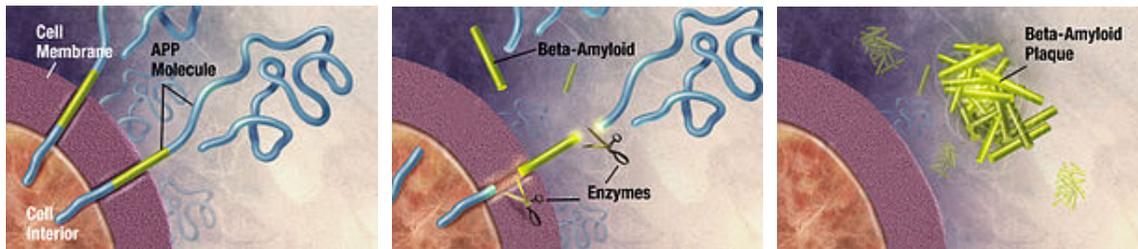
<sup>132</sup> Nathalie P, Jean-Noël O. Processing of amyloid precursor protein and amyloid peptide neurotoxicity. *Curr Alzheimer Res.* 2008 Apr;5(2):92-9; <http://www.ncbi.nlm.nih.gov/pubmed/18393795>.

<sup>133</sup>  $A\beta_{1-40}$ , aka.  $A\beta_{40}$ : DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV  
 $A\beta_{1-42}$ , aka.  $A\beta_{42}$ : DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

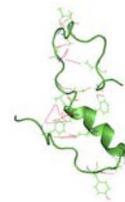
<sup>134</sup> Sun X, Chen WD, Wang YD.  $\beta$ -Amyloid: the key peptide in the pathogenesis of Alzheimer's disease. *Front Pharmacol.* 2015 Sep 30;6:221; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4588032/>.

vesicles).<sup>135</sup> Non-neuronal cells produce significant amounts of both isoforms only at the cell surface, as illustrated in **Figure 2**.<sup>136</sup>

*Figure 2. Enzymes act on the APP (amyloid precursor protein) and cut it into fragments. The amyloid beta fragment is crucial in the ultimate formation of senile plaques in AD.*



The monomer concentration in AD patient cerebrospinal fluid is 6 ng/ml for the more common  $A\beta_{40}$  (image, right), the same as in normal brain, but 1.1 ng/ml (for the denser and more aggregable  $A\beta_{42}$ ) vs. 1.5 ng/ml for normal brain.<sup>137</sup> However, it should be noted that these are *extracellular* concentrations of  $A\beta$  monomer. The toxicity of *intracellular*  $A\beta_{42}$  is at least 100,000 times greater than for extracellular  $A\beta_{42}$ . Directly microinjecting as little as 1-100 pM (0.005-0.5 ng/ml) of intracellular  $A\beta_{42}$  induces 50% (1 pM) to 90% (100 pM) cell death in cultured human neurons; yet these neurons do not undergo cell death even with a 10  $\mu$ M (4500 ng/ml) dose of extracellular  $A\beta_{42}$ .<sup>138</sup> Extracellular  $A\beta_{40}$  is perhaps only about half as neurotoxic as  $A\beta_{42}$ .<sup>139</sup>  $A\beta_{1-42}$  and  $A\beta_{1-43}$  are cleared from tissues more slowly than  $A\beta_{1-39}$  and  $A\beta_{1-40}$ .<sup>140</sup>



<sup>135</sup> Greenfield JP, Tsai J, Gouras GK, Hai B, Thinakaran G, Checler F, Sisodia SS, Greengard P, Xu H. Endoplasmic reticulum and trans-Golgi network generate distinct populations of Alzheimer beta-amyloid peptides. Proc Natl Acad Sci U S A. 1999 Jan 19;96(2):742-7; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC15207/>. Hartmann T, Bieger SC, Brühl B, Tienari PJ, Ida N, Allsop D, Roberts GW, Masters CL, Dotti CG, Unsicker K, Beyreuther K. Distinct sites of intracellular production for Alzheimer's disease A beta40/42 amyloid peptides. Nat Med. 1997 Sep;3(9):1016-20; <https://www.ncbi.nlm.nih.gov/pubmed/9288729>.

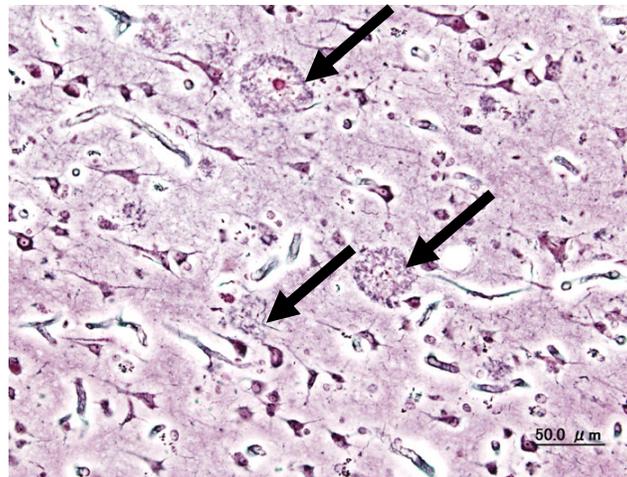
<sup>136</sup> Hartmann T, Bieger SC, Brühl B, Tienari PJ, Ida N, Allsop D, Roberts GW, Masters CL, Dotti CG, Unsicker K, Beyreuther K. Distinct sites of intracellular production for Alzheimer's disease A beta40/42 amyloid peptides. Nat Med. 1997 Sep;3(9):1016-20; <https://www.ncbi.nlm.nih.gov/pubmed/9288729>.

<sup>137</sup> Oe T, Ackermann BL, Inoue K, Berna MJ, Garner CO, Gelfanova V, Dean RA, Siemers ER, Holtzman DM, Farlow MR, Blair IA. Quantitative analysis of amyloid beta peptides in cerebrospinal fluid of Alzheimer's disease patients by immunoaffinity purification and stable isotope dilution liquid chromatography/negative electrospray ionization tandem mass spectrometry. Rapid Commun Mass Spectrom. 2006;20(24):3723-35; <http://www.ncbi.nlm.nih.gov/pubmed/17117458>.

<sup>138</sup> Zhang Y, McLaughlin R, Goodyer C, LeBlanc A. Selective cytotoxicity of intracellular amyloid beta peptide1-42 through p53 and Bax in cultured primary human neurons. J Cell Biol. 2002 Feb 4;156(3):519-29; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2173346/>.

A $\beta$  monomers are soluble with a structure that is largely alpha helical in membranes.<sup>141</sup> However, once free of the constraints of the APP parent protein these monomers refold in the extracellular spaces to form a hairpin. The hairpins, in turn, form medium-sized oligomers, apparently via  $\beta$ -sheet bonds. At sufficiently high concentration the oligomers further undergo a dramatic conformational change to form a beta sheet-rich tertiary structure that aggregates to form amyloid fibrils.<sup>142</sup> These fibrils deposit outside neurons (1) in dense formations known as senile plaques or neuritic plaques (almost exclusively in the brain's gray matter, not the white matter), (2) in less dense aggregates as diffuse plaques, and sometimes (3) in the walls of small blood vessels in the brain in a process called amyloid angiopathy or congophilic angiopathy. Amyloid plaques are dense, mostly insoluble deposits of beta-amyloid peptide and cellular material outside and around neurons (**Figure 3**).

*Figure 3. Histopathologic image (silver impregnation) of early senile A $\beta$  plaques, indicated by arrows, as seen in the cerebral cortex of a person with pre-senile onset of Alzheimer's disease.*



<sup>139</sup> Liao MQ, Tzeng YJ, Chang LY, Huang HB, Lin TH, Chyan CL, Chen YC. The correlation between neurotoxicity, aggregative ability and secondary structure studied by sequence truncated Abeta peptides. FEBS Lett. 2007 Mar 20;581(6):1161-5; <http://www.sciencedirect.com/science/article/pii/S0014579307001822>.

<sup>140</sup> Cameron DJ, Galvin C, Alkam T, Sidhu H, Ellison J, Luna S, Ethell DW. Alzheimer's-related peptide amyloid- $\beta$  plays a conserved role in angiogenesis. PLoS One. 2012;7(7):e39598; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3392248/>.

<sup>141</sup> Tomaselli S, Esposito V, Vangone P, van Nuland NA, Bonvin AM, Guerrini R, Tancredi T, Temussi PA, Picone D. The alpha-to-beta conformational transition of Alzheimer's A $\beta$ (1-42) peptide in aqueous media is reversible: a step by step conformational analysis suggests the location of beta conformation seeding. ChemBiochem. 2006 Feb;7(2):257-67; <http://dspace.library.uu.nl/bitstream/handle/1874/20092/497.pdf?sequence=1>.

<sup>142</sup> Ohnishi S, Takano K. Amyloid fibrils from the viewpoint of protein folding. Cell Mol Life Sci. 2004 Mar;61(5):511-24; <http://www.ncbi.nlm.nih.gov/pubmed/15004691>.

While plaques may displace and distort neuronal processes, experimental vaccines that clear amyloid plaques in trials in dogs<sup>143</sup> and humans<sup>144</sup> have had no significant effect on dementia, and A $\beta$  plaque density is not highly correlated with nerve cell loss or cognitive decline.<sup>145</sup> This has led some researchers to suspect that non-plaque A $\beta$  oligomers (aggregates of many monomers) may be the primary pathogenic form of A $\beta$ , not the amyloid plaques. These toxic A $\beta$  oligomers, also referred to as amyloid-derived diffusible ligands (aka. ADDLs), bind to a surface receptor on neurons and change the structure of the synapse, thereby disrupting neuronal communication.<sup>146</sup> ADDLs can also induce abnormal expression of insulin receptors and interrupt normal insulin signaling, potentially contributing to central insulin resistance that can occur during the progression of AD.<sup>147</sup>

One of the earliest signs of Alzheimer's disease is the loss of synapses,<sup>148</sup> which can be linked to toxicity mediated by A $\beta$  oligomers.<sup>149</sup> In AD brain, one early study found ADDLs accumulate

---

<sup>143</sup> Head E, Pop V, Vasilevko V, Hill M, Saing T, Sarsoza F, Nistor M, Christie LA, Milton S, Glabe C, Barrett E, Cribbs D. A two-year study with fibrillar beta-amyloid (Abeta) immunization in aged canines: effects on cognitive function and brain Abeta. *J Neurosci*. 2008 Apr 2;28(14):3555-66; <http://www.jneurosci.org/content/28/14/3555.long>.

<sup>144</sup> Holmes C, Boche D, Wilkinson D, Yadegarfar G, Hopkins V, Bayer A, Jones RW, Bullock R, Love S, Neal JW, Zotova E, Nicoll JA. Long-term effects of A $\beta$ <sub>42</sub> immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled Phase I trial. *Lancet*. 2008 Jul 19;372(9634):216-23; [http://www.thelancet.com/pdfs/journals/lancet/PIIS0140-6736\(08\)61075-2.pdf](http://www.thelancet.com/pdfs/journals/lancet/PIIS0140-6736(08)61075-2.pdf).

<sup>145</sup> Terry RD. Cell death or synaptic loss in Alzheimer disease. *J Neuropathol Exp Neurol*. 2000 Dec;59(12):1118-9; <http://www.ncbi.nlm.nih.gov/pubmed/11138931>.

<sup>146</sup> Lacor PN, Buniel MC, Furlow PW, Clemente AS, Velasco PT, Wood M, Viola KL, Klein WL. Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. *J Neurosci*. 2007 Jan 24;27(4):796-807; <http://www.jneurosci.org/content/27/4/796.full>.

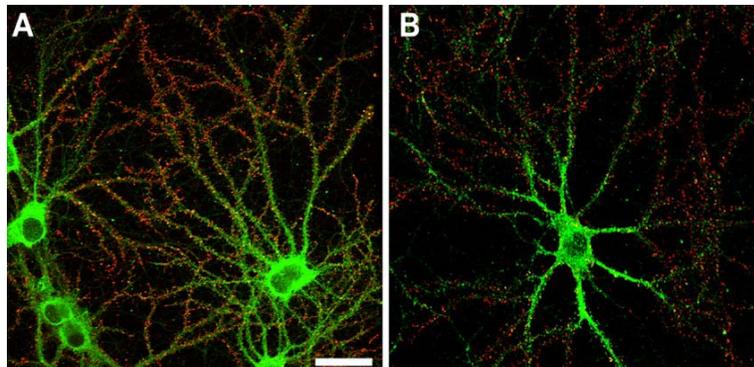
<sup>147</sup> Liu X, Teng Z, Cui C, Wang R, Liu M, Zhang Y. Amyloid beta-derived diffusible ligands (ADDLs) induce abnormal expression of insulin receptors in rat hippocampal neurons. *J Mol Neurosci*. 2014 Jan;52(1):124-30; <http://www.ncbi.nlm.nih.gov/pubmed/24402880>.

<sup>148</sup> Scheff SW, Price DA. Synaptic pathology in Alzheimer's disease: a review of ultrastructural studies. *Neurobiol Aging*. 2003 Dec;24(8):1029-46; <http://www.ncbi.nlm.nih.gov/pubmed/14643375>.

<sup>149</sup> Lacor PN, Buniel MC, Furlow PW, Clemente AS, Velasco PT, Wood M, Viola KL, Klein WL. Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. *J Neurosci*. 2007 Jan 24;27(4):796-807; <http://www.jneurosci.org/content/27/4/796.full>. Pham E, Crews L, Ubhi K, Hansen L, Adame A, Cartier A, Salmon D, Galasko D, Michael S, Savas JN, Yates JR, Glabe C, Masliah E. Progressive accumulation of amyloid-beta oligomers in Alzheimer's disease and in amyloid precursor protein transgenic mice is accompanied by selective alterations in synaptic scaffold proteins. *FEBS J*. 2010 Jul;277(14):3051-67; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2933033/>. Wilcox KC, Lacor PN, Pitt J, Klein WL. A $\beta$  oligomer-induced synapse degeneration in Alzheimer's disease. *Cell. Mol. Neurobiol*. 2011;31:939-948; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3146579/>.

primarily as A $\beta$ -12mers (~54 kDa) and can be found in dot-like clusters distinct from senile plaques (**Figure 4**).<sup>150</sup> Exposure to ADDLs also can induce marked changes in spine shape throughout the dendritic arbors (**Figure 5**).<sup>151</sup>

*Figure 4. ADDLs (red) bind to neurons expressing N-methyl-D-aspartate receptor (NMDA-R) subunits, such as NR1 (A, green) and NR2B (B, green), using cultured hippocampal cells exposed to 500 nM ADDLs for 15 min that were fixed and immunolabeled using an oligomer-raised antibody. Scale bar is 30  $\mu$ m.*<sup>152</sup>

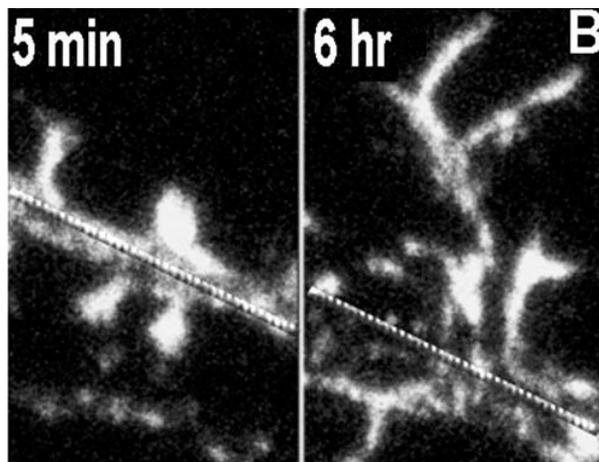


<sup>150</sup> Viola KL, Velasco PT, Klein WL. Why Alzheimer's is a disease of memory: the attack on synapses by A beta oligomers (ADDLs). *J Nutr Health Aging*. 2008 Jan;12(1):51S-7S; <http://www.ncbi.nlm.nih.gov/pubmed/18165846>.

<sup>151</sup> Lacor PN, Buniel MC, Furlow PW, Clemente AS, Velasco PT, Wood M, Viola KL, Klein WL. Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. *J Neurosci*. 2007 Jan 24;27(4):796-807; <http://www.jneurosci.org/content/27/4/796.full>.

<sup>152</sup> Lacor PN, Buniel MC, Furlow PW, Clemente AS, Velasco PT, Wood M, Viola KL, Klein WL. Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. *J Neurosci*. 2007 Jan 24;27(4):796-807; <http://www.jneurosci.org/content/27/4/796.full>.

*Figure 5. ADDL-induced aberrations in dendritic spine morphology and density: images of zoomed dendritic branches harboring “spines” demonstrate dramatic lengthening of dendritic protrusions after 6 hr of ADDL treatment. The line marks the dendritic shaft.<sup>153</sup>*



It is becoming more widely accepted that ADDLs, soluble oligomeric assemblies of the amyloid beta peptide, play a prominent role in triggering the cognitive deficits and neurodegeneration that constitute Alzheimer’s disease. Within the past decade, the longstanding emphasis on fibrillar deposits and neuronal death has given way to a more general paradigm involving ADDL-triggered aberrant synaptic signaling and consequent memory malfunction and neurodegeneration.<sup>154</sup> Today the array of known polymorphic forms of A $\beta$  aggregates, aside from the classic monomers and fibrils, includes oligomers, protofibrils, annular structures, ADDLs, and globulomers.<sup>155</sup>

---

<sup>153</sup> Lacor PN, Buniel MC, Furlow PW, Clemente AS, Velasco PT, Wood M, Viola KL, Klein WL. Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer’s disease. *J Neurosci*. 2007 Jan 24;27(4):796-807; <http://www.jneurosci.org/content/27/4/796.full>.

<sup>154</sup> Krafft GA, Klein WL. ADDLs and the signaling web that leads to Alzheimer’s disease. *Neuropharmacology*. 2010 Sep-Oct;59(4-5):230-42; <http://www.ncbi.nlm.nih.gov/pubmed/20650286>.

<sup>155</sup> Miller Y, Ma B, Nussinov R. Polymorphism in Alzheimer Abeta amyloid organization reflects conformational selection in a rugged energy landscape. *Chem Rev*. 2010 Aug 11;110(8):4820-38; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2920034/>.

A $\beta$  oligomers, but not monomers, inhibit the normal degradation of ubiquitinated proteins and misfolded proteins in intracellular proteasomes (image, right) by impairing proteasome activity both *in vitro* and in AD mice, leading to additional amyloid and tau accumulation.<sup>156</sup> A $\beta$  oligomers have many other pathological effects in the brain. For example, soluble A $\beta$  oligomers contribute importantly to synaptotoxicity in Alzheimer's disease.<sup>157</sup> A $\beta$  oligomers cause mitochondrial structural and functional damage,<sup>158</sup> with ADDLs producing mitochondrial fragmentation, reduced mitochondrial density in neuronal processes, and synaptic change (i.e., loss of dendritic spines and puncta of postsynaptic density protein 95) correlated with abnormal mitochondrial distribution.<sup>159</sup> A $\beta$  oligomers act as apoptotic ligands that can trigger cytochrome c-dependent apoptosis.<sup>160</sup> Intracerebroventricular infusion of AD-associated A $\beta$  oligomers (aka. A $\beta$ Os) in mice triggers peripheral glucose intolerance, and A $\beta$ Os cause internalization and cellular redistribution of insulin receptors, block downstream hippocampal insulin signaling, and cause hippocampal endoplasmic reticulum (ER) stress.<sup>161</sup>



One receptor for A $\beta$  oligomers may be the prion protein, the same protein that has been linked to mad cow disease and the related human condition, Creutzfeldt-Jakob disease (Section 6.2.4), thus potentially linking the underlying mechanism of these neurodegenerative disorders with that of Alzheimer's disease.<sup>162</sup> Another recent study reported the detection of self-replicating A $\beta$ <sub>42</sub> oligomers (12-24 mer), implicating a prion-like mechanism for A $\beta$  oligomer propagation.<sup>163</sup>

<sup>156</sup> Almeida CG, Takahashi RH, Gouras GK. Beta-amyloid accumulation impairs multivesicular body sorting by inhibiting the ubiquitin-proteasome system. *J Neurosci*. 2006 Apr 19;26(16):4277-88; <http://www.jneurosci.org/content/26/16/4277.long>. Tseng BP, Green KN, Chan JL, Blurton-Jones M, LaFerla FM. Abeta inhibits the proteasome and enhances amyloid and tau accumulation. *Neurobiol Aging*. 2008 Nov;29(11):1607-18; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2664168/>.

<sup>157</sup> Brouillette J. The effects of soluble A $\beta$  oligomers on neurodegeneration in Alzheimer's disease. *Curr Pharm Des*. 2014;20(15):2506-19; <http://www.ncbi.nlm.nih.gov/pubmed/23859546>.

<sup>158</sup> Reddy PH. Amyloid beta, mitochondrial structural and functional dynamics in Alzheimer's disease. *Exp Neurol*. 2009 Aug;218(2):286-92; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2710427/>.

<sup>159</sup> Wang X, Su B, Lee HG, Li X, Perry G, Smith MA, Zhu X. Impaired balance of mitochondrial fission and fusion in Alzheimer's disease. *J Neurosci*. 2009 Jul 15;29(28):9090-103; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2735241/>.

<sup>160</sup> Kim J, Yang Y, Song SS, Na JH, Oh KJ, Jeong C, Yu YG, Shin YK. Beta-amyloid oligomers activate apoptotic BAK pore for cytochrome c release. *Biophys J*. 2014 Oct 7;107(7):1601-8; <http://www.ncbi.nlm.nih.gov/pubmed/25296312>.

<sup>161</sup> Clarke JR, Lyra E Silva NM, Figueiredo CP, Frozza RL, Ledo JH, Beckman D, Katashima CK, Razolli D, Carvalho BM, Frazão R, Silveira MA, Ribeiro FC, Bomfim TR, Neves FS, Klein WL, Medeiros R, LaFerla FM, Carvalheira JB, Saad MJ, Munoz DP, Velloso LA, Ferreira ST, De Felice FG. Alzheimer-associated A $\beta$  oligomers impact the central nervous system to induce peripheral metabolic deregulation. *EMBO Mol Med*. 2015 Jan 23;7(2):190-210; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4328648/>.

<sup>162</sup> Laurén J, Gimbel DA, Nygaard HB, Gilbert JW, Strittmatter SM. Cellular Prion Protein Mediates Impairment of Synaptic Plasticity by Amyloid- $\beta$  Oligomers. *Nature*. 2009;457(7233):1128-32; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2748841/>.

A $\beta$  monomers are normally present in the brain at modest concentrations. Natural degradation pathways exist to eliminate normal amounts of it, including about a dozen proteases or enzymes involved in the cleavage of AB peptide.<sup>164</sup>



Most notably, **neprilysin** (image, left) is a zinc-dependent metalloprotease that cleaves peptides at the amino side of hydrophobic residues and degrades the amyloid beta peptide. Neprilysin-deficient knockout mice show both Alzheimer's-like behavioral impairment and amyloid-beta deposition in the brain,<sup>165</sup> providing strong evidence for the protein's association with the Alzheimer's disease process. Indeed, some believe that neprilysin is the rate-limiting molecule in amyloid beta degradation.<sup>166</sup> One hypothesis for the strong dependence of Alzheimer's

incidence on age focuses on the declining production of the neuropeptide somatostatin (an inhibitory hormone) in the brains of elderly people, which depresses the activity of neprilysin and promotes aggregation of unprocessed amyloid beta.<sup>167</sup> Declining neprilysin activity with increasing age may also be explained by oxidative damage, another known causative factor in Alzheimer's disease. Higher levels of inappropriately oxidized neprilysin have been found in Alzheimer's patients compared to cognitively normal elderly people.<sup>168</sup>

<sup>163</sup> Kumar A, Pate KM, Moss MA, Dean DN, Rangachari V. Self-propagative replication of A $\beta$  oligomers suggests potential transmissibility in Alzheimer disease. PLoS One. 2014 Nov 3;9(11):e111492; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4218758/>.

<sup>164</sup> Sun X, Chen WD, Wang YD.  $\beta$ -Amyloid: the key peptide in the pathogenesis of Alzheimer's disease. Front Pharmacol. 2015 Sep 30;6:221; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4588032/>.

<sup>165</sup> Madani R, Poirier R, Wolfer DP, Welzl H, Groscurth P, Lipp HP, Lu B, El Mouedden M, Mercken M, Nitsch RM, Mohajeri MH. Lack of neprilysin suffices to generate murine amyloid-like deposits in the brain and behavioral deficit *in vivo*. J Neurosci Res. 2006 Dec;84(8):1871-8; <http://www.ncbi.nlm.nih.gov/pubmed/16998901>.

<sup>166</sup> Iwata N, Tsubuki S, Takaki Y, Watanabe K, Sekiguchi M, Hosoki E, Kawashima-Morishima M, Lee HJ, Hama E, Sekine-Aizawa Y, Saido TC. Identification of the major Abeta1-42-degrading catabolic pathway in brain parenchyma: suppression leads to biochemical and pathological deposition. Nat Med. 2000 Feb;6(2):143-50; <http://www.ncbi.nlm.nih.gov/pubmed/10655101>.

<sup>167</sup> Hama E, Saido TC. Etiology of sporadic Alzheimer's disease: somatostatin, neprilysin, and amyloid beta peptide. Med Hypotheses. 2005;65(3):498-500; <http://www.ncbi.nlm.nih.gov/pubmed/15921860>.

<sup>168</sup> Wang DS, Iwata N, Hama E, Saido TC, Dickson DW. Oxidized neprilysin in aging and Alzheimer's disease brains. Biochem Biophys Res Commun. 2003 Oct 10;310(1):236-41; <http://www.ncbi.nlm.nih.gov/pubmed/14511676>.

## 2.4.2 Tau Protein Pathologies

For years, Alzheimer's researchers have debated whether amyloid or tau protein pathologies have primacy in causing the disease, and which one should be targeted for therapeutic intervention. Decades of failed attempts to control AD using pharmaceuticals aimed at amyloid have convinced many to re-examine tau as a promising target, prompting some researchers to move further in this direction.<sup>169</sup> For example, a 2015 Mayo Clinic study examined 3,618 brains in its postmortem brain bank, of which 1,375 brains were Alzheimer's from patients who died at different ages and at different stages of dementia.<sup>170</sup> This allowed the time course of the disease to be tracked. The lead researcher explains what they found:

Tau can be compared to railroad ties that stabilize a train track that brain cells use to transport food, messages and other vital cargo throughout neurons. In Alzheimer's, changes in the tau protein cause the tracks to become unstable in neurons of the hippocampus, the center of memory. The abnormal tau builds up in neurons, which eventually leads to the death of these neurons. Evidence suggests that abnormal tau then spreads from cell to cell, disseminating pathological tau in the brain's cortex – the cortex is the outer part of the brain that is involved in higher levels of thinking, planning, behavior and attention – mirroring later behavioral changes in Alzheimer's patients.

Amyloid, on the other hand, starts accumulating in the outer parts of the cortex and then spreads down to the hippocampus and eventually to other areas. Our study shows that the accumulation of amyloid has a strong relationship with a decline in cognition. When you account for the severity of tau pathology, however, the relationship between amyloid and cognition disappears – which indicates tau is the driver of Alzheimer's.

So, what is tau? Every neuron has a cytoskeleton, an internal support structure partly made up of structures called microtubules. These microtubules act like tracks, guiding nutrients and molecules from the body of the cell to the ends of the axon and back. Tau protein is a microtubule-associated protein,<sup>171</sup> expressed in neurons and glia, that stabilizes microtubules in the cell cytoskeleton – especially in axons, where it appears to play a role in establishing neuronal

---

<sup>169</sup> Giacobini E, Gold G. Alzheimer disease therapy – moving from amyloid- $\beta$  to tau. *Nat Rev Neurol*. 2013 Dec;9(12):677-86; <http://www.ncbi.nlm.nih.gov/pubmed/24217510>.

<sup>170</sup> Murray ME, Lowe VJ, Graff-Radford NR, Liesinger AM, Cannon A, Przybelski SA, Rawal B, Parisi JE, Petersen RC, Kantarci K, Ross OA, Duara R, Knopman DS, Jack CR Jr, Dickson DW. Clinicopathologic and 11C-Pittsburgh compound B implications of Thal amyloid phase across the Alzheimer's disease spectrum. *Brain*. 2015 Mar 23; <http://brain.oxfordjournals.org/content/early/2015/03/20/brain.awv050.long>.

<sup>171</sup> Binder LI, Frankfurter A, Rebhun LI. The distribution of tau in the mammalian central nervous system. *J Cell Biol*. 1985;101:1371-8; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2113928/pdf/jc10141371.pdf>.

polarity and axonal identity.<sup>172</sup> Like other microtubule-associated proteins, tau is normally regulated by phosphorylation.

In AD patients, tau protein undergoes chemical changes, becoming hyperphosphorylated by a factor of 3-4 fold. The hyperphosphorylated tau is less soluble and accumulates as paired helical filaments<sup>173</sup> that, in turn, aggregate or “mis-fold” into masses inside nerve cell bodies known as neurofibrillary tangles (NFTs), disintegrating the neuron’s internal transport system (**Figure 6**).<sup>174</sup> Tau aggregates also appear as dystrophic neurites associated with amyloid plaques.

Hyperphosphorylation of tau has an impact on microtubule stability and axonal transport, dendritic positioning and synaptic health, cell signaling at plasma membranes, protection of DNA from cell stressors, and release of tau.<sup>175</sup> Why does hyperphosphorylation occur? A large number of different kinases (enzymes that add phosphate) and phosphatases (enzymes that remove phosphate) have been shown to regulate tau phosphorylation, and an imbalance in tau kinase and phosphatase activity is believed to result in tau hyperphosphorylation in disease. Importantly, many of these enzymes have been implicated in pathways affected by A $\beta$  oligomers in models of AD.<sup>176</sup> According to prevailing hypotheses, the presence of amyloid oligomers<sup>177</sup> or plaques<sup>178</sup> triggers tau protein hyperphosphorylation which in turn causes the protein to

---

<sup>172</sup> Caceres A, Kosik KS. Inhibition of neurite polarity by tau antisense oligonucleotides in primary cerebellar neurons. *Nature*. 1990;343:461-3; <http://www.ncbi.nlm.nih.gov/pubmed/2105469>.

<sup>173</sup> Goedert M, Klug A, Crowther RA. Tau protein, the paired helical filament and Alzheimer’s disease. *J Alzheimers Dis*. 2006;9(3 Suppl):195-207; <http://www.ncbi.nlm.nih.gov/pubmed/16914859>.

<sup>174</sup> Hernández F, Avila J. Tauopathies. *Cell Mol Life Sci*. 2007 Sep;64(17):2219-33; <http://www.ncbi.nlm.nih.gov/pubmed/17604998>. Iqbal K, Liu F, Gong CX, Grundke-Iqbal I. Tau in Alzheimer disease and related tauopathies. *Curr Alzheimer Res*. 2010 Dec;7(8):656-64; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3090074/>.

<sup>175</sup> Noble W, Hanger DP, Miller CC, Lovestone S. The importance of tau phosphorylation for neurodegenerative diseases. *Front Neurol*. 2013 Jul 1;4:83; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3696910/>.

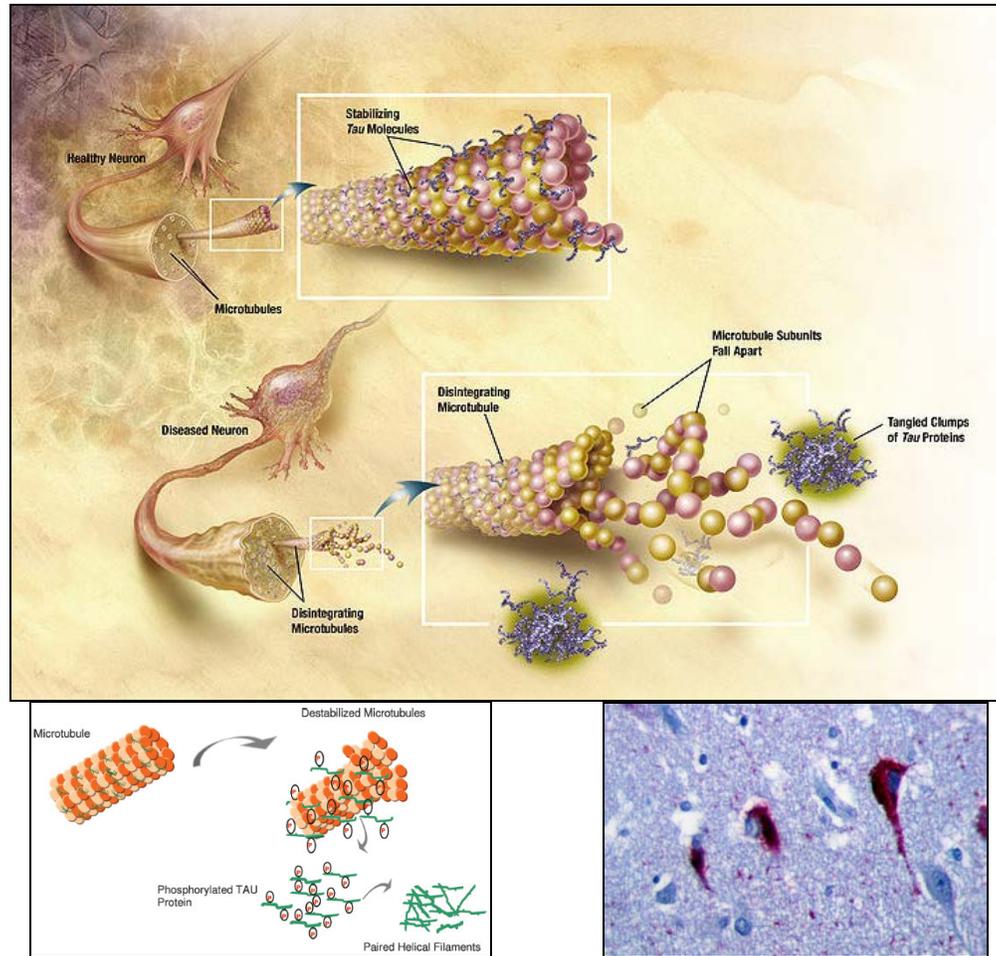
<sup>176</sup> For example: Mairet-Coello G, Courchet J, Pieraut S, Courchet V, Maximov A, Polleux F. The CAMKK2-AMPK kinase pathway mediates the synaptotoxic effects of A $\beta$  oligomers through tau phosphorylation. *Neuron*. 2013 Apr 10;78(1):94-108; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3784324/>.

<sup>177</sup> De Felice FG, Wu D, Lambert MP, Fernandez SJ, Velasco PT, Lacor PN, Bigio EH, Jerecic J, Acton PJ, Shughrue PJ, Chen-Dodson E, Kinney GG, Klein WL. Alzheimer’s disease-type neuronal tau hyperphosphorylation induced by A beta oligomers. *Neurobiol Aging*. 2008 Sep;29(9):1334-47; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3142933/>.

<sup>178</sup> Bierer LM, Hof PR, Purohit DP, Carlin L, Schmeidler J, Davis KL, Perl DP. Neocortical neurofibrillary tangles correlate with dementia severity in Alzheimer’s disease. *Arch Neurol*. 1995 Jan;52(1):81-8; <http://www.ncbi.nlm.nih.gov/pubmed/7826280>. Brion JP. Neurofibrillary tangles and Alzheimer’s disease. *Eur Neurol*. 1998 Oct;40(3):130-40; <http://www.ncbi.nlm.nih.gov/pubmed/9748670>.

accumulate and form neurofibrillary tangles, leading to synaptic dysfunction and contributing considerably to AD symptoms.  $A\beta$  oligomers may even seed tau oligomerization.<sup>179</sup>

Figure 6. In Alzheimer's disease, aggregating tau proteins cause microtubules to disintegrate.



It's not yet entirely clear how the hyperphosphorylation trigger works. One possibility derives from the widespread occurrence of 3-nitrotyrosine, an indicator of peroxynitrite, in neurons of AD patients, suggesting that peroxynitrite is excessively produced in the AD brain. Peroxynitrite is a strong oxidant, and it is formed from NO and superoxide at a high rate.  $A\beta$  deposited as senile plaque in AD brain can stimulate production of NO and superoxide through activating

<sup>179</sup> Lasagna-Reeves CA, Castillo-Carranza DL, Guerrero-Muoz MJ, Jackson GR, Kaye R. Preparation and characterization of neurotoxic tau oligomers. *Biochemistry*. 2010 Nov 30;49(47):10039-41; <http://www.ncbi.nlm.nih.gov/pubmed/21047142>.

microglia and astrocytes.<sup>180</sup> Thus the formation of peroxynitrite from excessive production of NO and superoxide in AD brains may be an upstream effector for both tau hyperphosphorylation and nitration.<sup>181</sup> If so, this effector might be neutralized by the use of peroxynitrite scavengers.<sup>182</sup>

The tau that polymerizes into NFTs, looking like a ball of yarn inside the cell, is apparently inert and neither binds to tubulin nor promotes its assembly into microtubules. As much as 40% of the abnormally hyperphosphorylated tau in AD brain is present in the cytosol and is not polymerized into paired helical filaments (PHFs), aka. neurofibrillary tangles or NFTs.<sup>183</sup> The neuronal concentration of normal ~45 kD 441-residue human tau protein is ~2  $\mu\text{M}$  and it binds to microtubules at a  $K_d$  of ~100 nM, thus practically all normal tau is likely to be microtubule-bound in the cell. An early brain-tissue study found 31 mg tau/gm protein in control brain and 12 mg tau/gm protein in AD brain, and 2.1 mg/100 gm tissue of normal tau and 0.8 mg/100 gm tissue of hyperphosphorylated tau in AD human cerebral cortex tissue.<sup>184</sup>

Besides hyperphosphorylation, tau acetylation also occurs in tau pathologies related to AD and may represent a turning point that accelerates tau toxicity.<sup>185</sup> Acetylation may be an intermediate step in tangle formation from threads and pre-tangle structures, thus playing a mechanistic role in driving tau polymerization into neurofibrillary pathology and tau mediated neurodegeneration.<sup>186</sup> There is some evidence that tau itself possesses acetyltransferase activity and thus is capable of catalyzing self-acetylation, possibly exciting a positive feedback loop if tau levels exceed some critical threshold concentration.<sup>187</sup>

---

<sup>180</sup> Wang Q, Rowan MJ, Anwyl R. Beta-amyloid-mediated inhibition of NMDA receptor-dependent long-term potentiation induction involves activation of microglia and stimulation of inducible nitric oxide synthase and superoxide. *J Neurosci.* 2004 Jul 7;24(27):6049-56; <http://www.jneurosci.org/content/24/27/6049.long>.

<sup>181</sup> Zhang YJ, Xu YF, Liu YH, Yin J, Li HL, Wang Q, Wang JZ. Peroxynitrite induces Alzheimer-like tau modifications and accumulation in rat brain and its underlying mechanisms. *FASEB J.* 2006 Jul;20(9):1431-42; <http://www.fasebj.org/content/20/9/1431.long>.

<sup>182</sup> Klotz LO, Sies H. Defenses against peroxynitrite: selenocompounds and flavonoids. *Toxicol Lett.* 2003 Apr 11;140-141:125-32; <http://www.ncbi.nlm.nih.gov/pubmed/12676458>.

<sup>183</sup> Iqbal K, Liu F, Gong CX, Grundke-Iqbal I. Tau in Alzheimer disease and related tauopathies. *Curr Alzheimer Res.* 2010;7:656-664; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3090074/>.

<sup>184</sup> Kopke E, Tung YC, Shaikh S, Alonso AC, Iqbal K, Grundke-Iqbal I. Microtubule-associated protein tau. Abnormal phosphorylation of a non-paired helical filament pool in Alzheimer disease. *J Biol Chem.* 1993;268:24374-24384; <http://www.jbc.org/content/268/32/24374.long>.

<sup>185</sup> Kovacs GG. Invited review: Neuropathology of tauopathies: principles and practice. *Neuropathol Appl Neurobiol.* 2015 Feb;41(1):3-23; <http://onlinelibrary.wiley.com/doi/10.1111/nan.12208/full>.

<sup>186</sup> Irwin DJ, Cohen TJ, Grossman M, Arnold SE, Xie SX, Lee VM, Trojanowski JQ. Acetylated tau, a novel pathological signature in Alzheimer's disease and other tauopathies. *Brain.* 2012 Mar;135(Pt 3):807-18; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3286338/>.

<sup>187</sup> Tenreiro S, Eckermann K, Outeiro TF. Protein phosphorylation in neurodegeneration: friend or foe? *Front Mol Neurosci.* 2014 May 13;7:42; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4026737/>.

In addition to hyperphosphorylation and acetylation, further posttranslational modifications of tau include N- and C-terminal truncation, glycosylation, oxidative and nitrative injuries, transglutamination, deamidation, and formation of tau oligomers.<sup>188</sup>

As with amyloid oligomers (e.g., ADDLs and A $\beta$ Os; Section 2.4.1), tau oligomers appear to be the toxic form of tau in neurodegenerative disease.<sup>189</sup> Cell death can occur prior to tau tangle formation in AD,<sup>190</sup> suggesting that NFTs are not the pathogenic species responsible for the spread of the disease. Indeed, recently completed *in vitro* and *in vivo* studies suggest that tau oligomers, intermediate in size between the monomeric form and NFTs, are the true toxic species in disease and the best targets for anti-tau therapies. For instance, healthy mice develop memory problems one week after their brains are injected with tau oligomers from AD patients; tissue samples showed toxic tau throughout the animals' brains.<sup>191</sup> Thus NFTs might actually be neuroprotective by avoiding the accumulation of toxic oligomeric tau.<sup>192</sup>

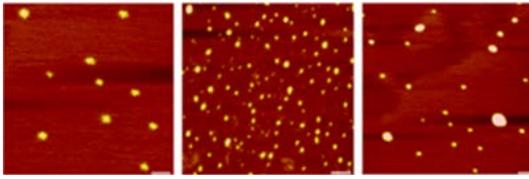
<sup>188</sup> Martin L, Latypova X, Terro F. Post-translational modifications of tau protein: implications for Alzheimer's disease. *Neurochem Int.* 2011 Mar;58(4):458-71; <http://www.ncbi.nlm.nih.gov/pubmed/21215781/>.

<sup>189</sup> Guzmán-Martínez L, Farías GA, Maccioni RB. Tau oligomers as potential targets for Alzheimer's diagnosis and novel drugs. *Front Neurol.* 2013 Oct 28;4:167; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3808896/>. Cárdenas-Aguayo Mdel C, Gómez-Virgilio L, DeRosa S, Meraz-Ríos MA. The role of tau oligomers in the onset of Alzheimer's disease neuropathology. *ACS Chem Neurosci.* 2014 Dec 17;5(12):1178-91; <http://www.ncbi.nlm.nih.gov/pubmed/25268947>.

<sup>190</sup> Gómez-Isla T, Hollister R, West H, Mui S, Growdon JH, Petersen RC, Parisi JE, Hyman BT. Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. *Ann Neurol.* 1997 Jan;41(1):17-24; <http://www.ncbi.nlm.nih.gov/pubmed/9005861>. Morsch R, Simon W, Coleman PD. Neurons may live for decades with neurofibrillary tangles. *J Neuropathol Exp Neurol.* 1999 Feb;58(2):188-97; <http://www.ncbi.nlm.nih.gov/pubmed/10029101>. Haroutunian V, Davies P, Vianna C, Buxbaum JD, Purohit DP. Tau protein abnormalities associated with the progression of Alzheimer disease type dementia. *Neurobiol Aging.* 2007 Jan;28(1):1-7; <http://www.ncbi.nlm.nih.gov/pubmed/16343696>.

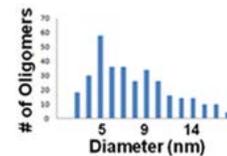
<sup>191</sup> Castillo-Carranza DL, Gerson JE, Sengupta U, Guerrero-Muñoz MJ, Lasagna-Reeves CA, Kaye R. Specific targeting of tau oligomers in Htau mice prevents cognitive impairment and tau toxicity following injection with brain-derived tau oligomeric seeds. *J Alzheimers Dis.* 2014;40 Suppl 1:S97-S111; <http://www.ncbi.nlm.nih.gov/pubmed/24603946>.

<sup>192</sup> Tenreiro S, Eckermann K, Outeiro TF. Protein phosphorylation in neurodegeneration: friend or foe? *Front Mol Neurosci.* 2014 May 13;7:42; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4026737/>.



However, the exact mechanism by which the spread of tau pathology occurs is unknown. Evidence suggests that tau oligomers (AFM scan images, left) may act as templates for the misfolding of native tau, thereby seeding the spread of the toxic forms of the protein.<sup>193</sup>

Researchers have recently reported the ability of tau oligomers (3-16 nm in size; chart, right) to enter and exit cells, propagating from disease-affected regions to unaffected areas.<sup>194</sup> While the exact mechanism by which the spreading of misfolded tau occurs has yet to be elucidated, there are a few different models which have been proposed, including cell membrane stress and pore-formation, endocytosis and exocytosis, and non-traditional secretion of protein not enclosed by a membrane. Coming to an understanding of how toxic tau species seed and spread through the brain would seem to be a necessary precondition for finding effective conventional treatments for neurodegenerative tauopathies.<sup>195</sup>



Several recent findings have challenged the traditional view that tau functions primarily only to stabilize microtubules and that its aggregation in AD causes deficits through a loss-of-function mechanism. Studies in cell culture and genetically modified mouse models suggest that tau may normally facilitate or enhance excitatory neurotransmission by regulating the distribution of synaptic activity-related signaling molecules.<sup>196</sup> However, when it is abnormally modified and assumes pathogenic conformations, tau becomes enriched in dendritic spines where it can interfere with neurotransmission.<sup>197</sup> Other research suggests that tau might be released extracellularly by an exosome-based mechanism in Alzheimer's disease.<sup>198</sup>

<sup>193</sup> Gerson JE, Kaye R. Formation and propagation of tau oligomeric seeds. *Front Neurol.* 2013 Jul 17;4:93; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3713404/>.

<sup>194</sup> Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, Guerrero-Munoz MJ, Kiritoshi T, Neugebauer V, Jackson GR, Kaye R. Alzheimer brain-derived tau oligomers propagate pathology from endogenous tau. *Sci Rep.* 2012;2:700; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3463004/>.

<sup>195</sup> Cárdenas-Aguayo Mdel C, Gómez-Virgilio L, DeRosa S, Meraz-Ríos MA. The role of tau oligomers in the onset of Alzheimer's disease neuropathology. *ACS Chem Neurosci.* 2014 Dec 17;5(12):1178-91; <http://www.ncbi.nlm.nih.gov/pubmed/25268947>.

<sup>196</sup> Morris M, Maeda S, Vossel K, Mucke L. The many faces of tau. *Neuron.* 2011;70:410-426; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3319390/>.

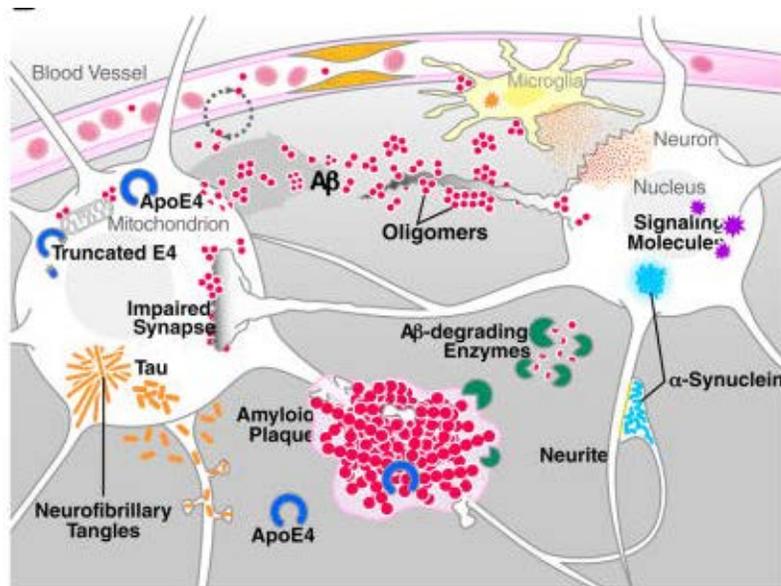
<sup>197</sup> Hoover BR, Reed MN, Su J, Penrod RD, Kotilinek LA, Grant MK, Pitstick R, Carlson GA, Lanier LM, Yuan LL, Ashe KH, Liao D. Tau mislocalization to dendritic spines mediates synaptic dysfunction independently of neurodegeneration. *Neuron.* 2010 Dec 22;68(6):1067-81; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3026458/>.

<sup>198</sup> Saman S, Kim W, Raya M, Visnick Y, Miro S, Saman S, Jackson B, McKee AC, Alvarez VE, Lee NC, Hall GF. Exosome-associated tau is secreted in tauopathy models and is selectively phosphorylated in cerebrospinal fluid in early Alzheimer disease. *J Biol Chem.* 2012 Feb 3;287(6):3842-9; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3281682/>.

### 2.4.3 Other Pathologies and Biochemical Events in Alzheimer's Disease

One recent overview of the classical “big picture” of the cellular and molecular pathologies of AD is summarized in **Figure 7** and is discussed in more detail in the numbered items below.

*Figure 7. Diagram showing two neurons and a capillary blood vessel in the brain, illustrating key cellular and molecular pathologies in Alzheimer's disease (from Huang and Mucke, 2012).<sup>199</sup>*



(1) Amyloid beta ( $A\beta$ ) fragments form in the extracellular space and accumulate there, whether because of (a) increased neuronal production of  $A\beta$ , (b) decreased degradation by  $A\beta$ -degrading enzymes, or (c) reduced clearance across the blood-brain barrier. These monomer fragments of  $A\beta$  can later aggregate into  $A\beta$  dimers, trimers, and larger amyloid oligomers (ADDLs).<sup>200</sup>

(2) Amyloid in the extracellular space triggers the release of neurotoxic mediators from glial cells.<sup>201</sup>

<sup>199</sup> Huang Y, Mucke L. Alzheimer mechanisms and therapeutic strategies. *Cell*. 2012;148(6):1204-22; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3319071/>.

<sup>200</sup> Walsh DM, Klyubin I, Fadeeva JV, Rowan MJ, Selkoe DJ. Amyloid-beta oligomers: their production, toxicity and therapeutic inhibition. *Biochem Soc Trans*. 2001;30:552-557; <http://www.ncbi.nlm.nih.gov/pubmed/12196135>.

<sup>201</sup> Hashimoto Y, Nawa M, Chiba T, Aiso S, Nishimoto I, Matsuoka M. Transforming growth factor beta2 autocrinally mediates neuronal cell death induced by amyloid-beta. *J Neurosci Res*. 2006 May 1;83(6):1039-47;

(3) Amyloid monomers migrate into the intracellular space and oligomerize into ADDLs, impairing synaptic functions and related signaling pathways, and changing other neuronal activities. Amyloid beta protein is neurotoxic to mature neurons in culture at higher concentrations; in differentiated neurons, amyloid beta protein causes dendritic and axonal retraction followed by neuronal death.<sup>202</sup> A $\beta$  also selectively builds up in the mitochondria in the cells of AD-affected brains, where it increases mitochondrial stress by inhibiting enzyme activity, releasing reactive oxygen species, and inhibiting certain enzyme functions and the utilization of glucose by neurons.<sup>203</sup>

(4) Amyloid oligomers in the extracellular space further aggregate into large fibrillar amyloid plaques or “senile plaques”, displacing and distorting neuronal processes. There is ~10 mg of deposited A $\beta$ <sub>1-42</sub>,<sup>204</sup> mostly in the gray matter of the cortex (42% by weight of brain), in a human AD brain of assumed mass 1150 gm.<sup>205</sup> Amyloid fibrils effectively catalyze the formation of neurotoxic A $\beta$  oligomers.

(5) The lipid transport protein ApoE4 impairs A $\beta$  clearance and promotes A $\beta$  deposition. When expressed within stressed neurons, ApoE4 is cleaved, to a much greater extent than ApoE3, into neurotoxic ApoE fragments that disrupt the cytoskeleton and impair mitochondrial functions.

(6) Tau protein, which is normally most abundant in axons, becomes mislocalized to the neuronal soma and dendritic spines and forms inclusions called neurofibrillary tangles that disrupt

[https://www.researchgate.net/profile/Tomohiro\\_Chiba/publication/7537999\\_Transforming\\_growth\\_factor\\_beta2\\_is\\_a\\_neuronal\\_death-inducing\\_ligand\\_for\\_amyloid-beta\\_precursor\\_protein/links/09e4150f53befb58af000000.pdf](https://www.researchgate.net/profile/Tomohiro_Chiba/publication/7537999_Transforming_growth_factor_beta2_is_a_neuronal_death-inducing_ligand_for_amyloid-beta_precursor_protein/links/09e4150f53befb58af000000.pdf).

<sup>202</sup> Yankner BA, Duffy LK, Kirschner DA. Neurotrophic and neurotoxic effects of amyloid beta protein: reversal by tachykinin neuropeptides. *Science*. 1990 Oct 12;250(4978):279-82; [https://www.researchgate.net/profile/Daniel\\_Kirschner/publication/20936592\\_Neurotrophic\\_and\\_neurotoxic\\_effects\\_of\\_amyloid\\_beta\\_protein\\_reversal\\_by\\_tachykinin\\_neuropeptides/links/547c80570cf27ed978602020.pdf](https://www.researchgate.net/profile/Daniel_Kirschner/publication/20936592_Neurotrophic_and_neurotoxic_effects_of_amyloid_beta_protein_reversal_by_tachykinin_neuropeptides/links/547c80570cf27ed978602020.pdf).

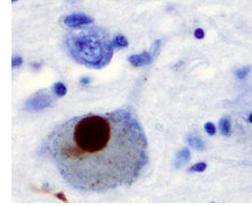
<sup>203</sup> Chen X, Yan SD. Mitochondrial Abeta: a potential cause of metabolic dysfunction in Alzheimer’s disease. *IUBMB Life*. 2006 Dec;58(12):686-94; <http://onlinelibrary.wiley.com/doi/10.1080/15216540601047767/epdf>.

<sup>204</sup> Karran E, Mercken M, De Strooper B. The amyloid cascade hypothesis for Alzheimer’s disease: an appraisal for the development of therapeutics. *Nat Rev Drug Discov*. 2011 Aug 19;10(9):698-712; <http://www.nature.com/nrd/journal/v10/n9/full/nrd3505.html>.

<sup>205</sup> Arnold SE, Hyman BT, Flory J, Damasio AR, Van Hoesen GW. The topographical and neuroanatomical distribution of neurofibrillary tangles and neuritic plaques in the cerebral cortex of patients with Alzheimer’s disease. *Cereb Cortex*. 1991 Jan-Feb;1(1):103-16; <http://www.ncbi.nlm.nih.gov/pubmed/1822725>.

the cytoskeleton. Tau is present in both white (at ~0.19 mg/ml) and gray (at ~0.08 mg/ml) matter in bovine brain tissue.<sup>206</sup>

(7) The 140-residue human presynaptic-terminal  $\alpha$ -synuclein protein self-assembles into pathogenic oligomers and forms larger aggregates (Lewy bodies; image at right, stained brown, in brain cell of the substantia nigra in Parkinson's disease; Section 6.1), disrupting neuron and dendritic function. A large proportion of AD cases have abnormal accumulations of the presynaptic protein  $\alpha$ -synuclein in brain cells.<sup>207</sup>  $A\beta$  enhances the misfolding and accumulation of  $\alpha$ -synuclein *in vitro* and *in vivo*, and studies in doubly transgenic mice suggest that these molecules can synergize to more severely impair neuronal functions.<sup>208</sup> Lewy bodies are not rare in the brains of people with AD,<sup>209</sup> but Lewy body dementia or LBD (Section 6.1.2) is usually distinguished




---

<sup>206</sup> Binder LI, Frankfurter A, Rebhun LI. The distribution of tau in the mammalian central nervous system. *J Cell Biol.* 1985;101:1371-8;

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2113928/pdf/jc10141371.pdf>.

<sup>207</sup> Higashi S, Iseki E, Yamamoto R, Minegishi M, Hino H, Fujisawa K, Togo T, Katsuse O, Uchikado H, Furukawa Y, Kosaka K, Arai H. Concurrence of TDP-43, tau and alpha-synuclein pathology in brains of Alzheimer's disease and dementia with Lewy bodies. *Brain Res.* 2007 Dec 12;1184:284-94;

<http://www.ncbi.nlm.nih.gov/pubmed/17963732>.

<sup>208</sup> Masliah E, Rockenstein E, Veinbergs I, Sagara Y, Mallory M, Hashimoto M, Mucke L.  $\beta$ -Amyloid peptides enhance  $\alpha$ -synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease. *Proc Natl Acad Sci USA.* 2001;98:12245-50;

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC59799/>.

<sup>209</sup> Kotzbauer PT, Trojanowski JQ, Lee VM. Lewy body pathology in Alzheimer's disease. *J Mol Neurosci.* 2001 Oct;17(2):225-32; <http://www.ncbi.nlm.nih.gov/pubmed/11816795>.

from Alzheimer's disease.<sup>210</sup>  $\alpha$ -synuclein oligomers also induce tau aggregation and the formation of  $\beta$ -sheet-rich neurotoxic tau oligomers.<sup>211</sup>

(8) A $\beta$ , tau,  $\alpha$ -synuclein and other proteins associated with neurodegenerative disorders can be released into the extracellular space, where they may spread to other cells and seed abnormal protein aggregation in experimental models. For example, tau, when present in the extracellular space, is toxic to neurons.<sup>212</sup> This may be one important mechanism by which AD spreads throughout the brain. These properties have been compared to those of prions, different forms of which cause Jacob-Creutzfeldt disease, scrapie and mad-cow disease (Section 6.2.4).<sup>213</sup> However, prions differ fundamentally from the other proteins in that they cause diseases that are communicable to other people, while it is generally accepted that AD and most other neurodegenerative disorders are not.<sup>214</sup>

---

<sup>210</sup> Dementia with Lewy bodies (DLB), also known under a variety of other names including Lewy body dementia (LBD), diffuse Lewy body disease, cortical Lewy body disease, and senile dementia of Lewy type, is a type of dementia closely associated with Parkinson's disease that affects 1.3 million individuals in the United States alone. LBD is characterized by the development of abnormal proteinaceous ( $\alpha$ -synuclein) cytoplasmic inclusions, called Lewy bodies, throughout the brain. These inclusions have similar structural features to "classical" Lewy bodies seen subcortically in Parkinson's disease. Additionally, a loss of dopamine-producing neurons (in the substantia nigra) occurs, similar to that seen in Parkinson's disease, and a loss of acetylcholine-producing neurons (in the basal nucleus of Meynert and elsewhere) similar to that seen in Alzheimer's disease. Cerebral atrophy (or shrinkage) also occurs as the cerebral cortex degenerates. Autopsy series reveal that when Lewy body inclusions are found in the cortex, they often co-occur with Alzheimer's disease pathology found primarily in the hippocampus, including senile plaques, and granulovacuolar degeneration (grainy deposits within and a clear zone around hippocampal neurons). Neurofibrillary tangles (abnormally phosphorylated tau protein) are less common in LBD, although they are known to occur. It is presently not clear whether LBD is an Alzheimer's variant or a separate disease entity. Unlike Alzheimer's disease, the LBD brain may appear grossly normal with no visible signs of atrophy. [http://en.wikipedia.org/wiki/Dementia\\_with\\_Lewy\\_bodies](http://en.wikipedia.org/wiki/Dementia_with_Lewy_bodies).

<sup>211</sup> Lasagna-Reeves CA, Castillo-Carranza DL, Guerrero-Muoz MJ, Jackson GR, Kaye R. Preparation and characterization of neurotoxic tau oligomers. *Biochemistry*. 2010 Nov 30;49(47):10039-41; <http://www.ncbi.nlm.nih.gov/pubmed/21047142>.

<sup>212</sup> Gómez-Ramos A, Díaz-Hernández M, Cuadros R, Hernández F, Avila J. Extracellular tau is toxic to neuronal cells. *FEBS Lett*. 2006;580:4842-50; <http://www.ncbi.nlm.nih.gov/pubmed/16914144>.

<sup>213</sup> Braak H, Del Tredici K. Alzheimer's pathogenesis: Is there neuron-to-neuron propagation? *Acta Neuropathol*. 2011 May;121(5):589-95; [https://www.researchgate.net/profile/Kelly\\_Del\\_Tredici/publication/51073537\\_Alzheimer's\\_pathogenesis\\_is\\_there\\_neuron-to-neuron\\_propagation/links/550013320cf260c99e8f2aaf.pdf](https://www.researchgate.net/profile/Kelly_Del_Tredici/publication/51073537_Alzheimer's_pathogenesis_is_there_neuron-to-neuron_propagation/links/550013320cf260c99e8f2aaf.pdf). Jucker M, Walker LC. Pathogenic protein seeding in Alzheimer disease and other neurodegenerative disorders. *Ann Neurol*. 2011 Oct;70(4):532-40; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3203752/>.

<sup>214</sup> One dissenting view: Jaunmuktane Z, Mead S, Ellis M, Wadsworth JD, Nicoll AJ, Kenny J, Launchbury F, Linehan J, Richard-Loendt A, Walker AS, Rudge P, Collinge J, Brandner S. Evidence for human transmission of amyloid- $\beta$  pathology and cerebral amyloid angiopathy. *Nature*. 2015 Sep 10;525(7568):247-50; <http://www.nature.com/nature/journal/v525/n7568/full/nature15369.html>. See also commentary: "Alzheimer's disease may be transmittable," *Washington Post*, 10 Sep 2015; <http://www.heraldnet.com/article/20150910/NEWS02/150919927>.

(9) Observation of tau present in the nuclei of human neuroblastoma cells suggested that tau might have novel functions mediated by interactions with DNA or RNA.<sup>215</sup> Full-length tau was identified in neuronal nuclei, where it co-localizes with the chromosome scaffold, nuclear and nucleolar organization centers.<sup>216</sup> The microtubule-binding domain of tau can bind RNA<sup>217</sup> and both single-stranded<sup>218</sup> and double-stranded<sup>219</sup> DNA. The interaction of tau with DNA results in conformational changes in DNA and protects DNA from heat damage and oxidative stress.<sup>220</sup> Nuclear tau appears to be largely dephosphorylated, suggesting that increased tau phosphorylation in diseased states could interfere with protective functions of non-phosphorylated tau in neuronal nuclei.

(10) Extracellular misfolded A $\beta$ <sub>1-40</sub> and  $\alpha$ -synuclein proteins can form 3D morphologically compatible ion-channel-like structures in reconstituted membranes and elicit single ion-channel currents.<sup>221</sup> These spurious transmembrane ion channels could destabilize cellular ionic homeostasis and hence induce cell pathophysiology and degeneration in amyloid diseases like AD.

---

<sup>215</sup> Loomis PA, Howard TH, Castleberry RP, Binder LI. Identification of nuclear tau isoforms in human neuroblastoma cells. *Proc Natl Acad Sci U S A*. 1990 Nov;87(21):8422-6; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC54968/>.

<sup>216</sup> Qu MH, Li H, Tian R, Nie CL, Liu Y, Han BS, He RQ. Neuronal tau induces DNA conformational changes observed by atomic force microscopy. *Neuroreport*. 2004 Dec 22;15(18):2723-7; <http://www.ncbi.nlm.nih.gov/pubmed/15597042>.

<sup>217</sup> Kampers T, Friedhoff P, Biernat J, Mandelkow EM, Mandelkow E. RNA stimulates aggregation of microtubule-associated protein tau into Alzheimer-like paired helical filaments. *FEBS Lett*. 1996 Dec 16;399(3):344-9; <http://www.ncbi.nlm.nih.gov/pubmed/8985176>.

<sup>218</sup> Krylova SM, Musheev M, Nutiu R, Li Y, Lee G, Krylov SN. Tau protein binds single-stranded DNA sequence specifically – the proof obtained *in vitro* with non-equilibrium capillary electrophoresis of equilibrium mixtures. *FEBS Lett*. 2005 Feb 28;579(6):1371-5; <http://www.ncbi.nlm.nih.gov/pubmed/15733843>.

<sup>219</sup> Li W, Wang XS, Qu MH, Liu Y, He RQ. Human protein tau represses DNA replication *in vitro*. *Biochim Biophys Acta*. 2005 Nov 30;1726(3):280-6; <http://www.ncbi.nlm.nih.gov/pubmed/16226838>.

<sup>220</sup> Sultan A, Nessler F, Violet M, Bégard S, Loyens A, Talahari S, Mansuroglu Z, Marzin D, Sergeant N, Humez S, Colin M, Bonnefoy E, Buée L, Galas MC. Nuclear tau, a key player in neuronal DNA protection. *J Biol Chem*. 2011 Feb 11;286(6):4566-75; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3039398/>.

<sup>221</sup> Quist A, Doudevski I, Lin H, Azimova R, Ng D, Frangione B, Kagan B, Ghiso J, Lal R. Amyloid ion channels: a common structural link for protein-misfolding disease. *Proc Natl Acad Sci U S A*. 2005 Jul 26;102(30):10427-32; <http://www.pnas.org/content/102/30/10427.full>.

(11) Vascular abnormalities (e.g., partial blockages) can impair the supply of nutrients and removal of metabolic byproducts, cause microinfarcts, and promote the activation of microglial cells.<sup>222</sup>

(12) Levels of the neurotransmitter acetylcholine are reduced. (The “cholinergic hypothesis” for AD, on which most currently available drug therapies are based, proposed that AD is caused by reduced synthesis of the neurotransmitter acetylcholine,<sup>223</sup> but this hypothesis has not maintained widespread support largely because medications intended to treat acetylcholine deficiency have not been very effective.) Levels of the neurotransmitters serotonin, norepinephrine, and somatostatin are also often reduced.

(13) There is reduced clearance of A $\beta$  from brain tissues due to a functional decline in protein waste removal via paravascular pathways (aka. the glymphatic system) that facilitate convective exchange of water and soluble contents between cerebrospinal fluid (CSF) and interstitial fluid (ISF) through astroglial water channels lining the paravascular CSF pathways.<sup>224</sup>

(14) The data are unclear as to whether non-neuronal neuroglial brain cells are affected similarly as neurons during AD. One study of the human principal inferior olivary nucleus found oligodendrocytes (-46%) were lost in similar proportions as neurons (-34%), whereas the number of astrocytes decreased only slightly.<sup>225</sup> Another study of neocortical cells found identical -11% drops in glial and neuron counts during AD.<sup>226</sup> Yet another study found an increase in non-neuronal cell numbers in the cerebral cortex and subcortical white matter of demented patients with Alzheimer’s disease when compared with asymptomatic subjects with Alzheimer’s disease and control subjects.<sup>227</sup>

---

<sup>222</sup> McGeer PL, McGeer EG. The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases. *Brain Res Brain Res Rev.* 1995 Sep;21(2):195-218; <http://www.ncbi.nlm.nih.gov/pubmed/8866675>.

<sup>223</sup> Francis PT, Palmer AM, Snape M, Wilcock GK. The cholinergic hypothesis of Alzheimer’s disease: a review of progress. *J Neurol Neurosurg Psychiatry.* 1999 Feb;66(2):137-47; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1736202/>.

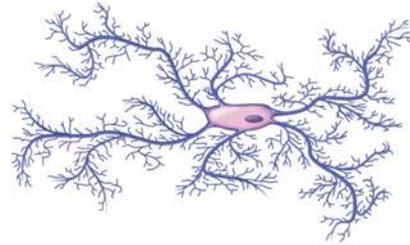
<sup>224</sup> Kress BT, Iliff JJ, Xia M, Wang M, Wei HS, Zeppenfeld D, Xie L, Kang H, Xu Q, Liew JA, Plog BA, Ding F, Deane R, Nedergaard M. Impairment of paravascular clearance pathways in the aging brain. *Ann Neurol.* 2014 Dec;76(6):845-61; <http://www.ncbi.nlm.nih.gov/pubmed/25204284>.

<sup>225</sup> Lasn H, Winblad B, Bogdanovic N. Neuroglia in the inferior olivary nucleus during normal aging and Alzheimer’s disease. *J Cell Mol Med.* 2006 Jan-Mar;10(1):145-56; <http://www.ncbi.nlm.nih.gov/pubmed/16563227>.

<sup>226</sup> Pelvig DP, Pakkenberg H, Regeur L, Oster S, Pakkenberg B. Neocortical glial cell numbers in Alzheimer’s disease. A stereological study. *Dement Geriatr Cogn Disord.* 2003;16(4):212-9; <http://www.ncbi.nlm.nih.gov/pubmed/14512716>.

<sup>227</sup> Andrade-Moraes CH1, Oliveira-Pinto AV, Castro-Fonseca E, da Silva CG, Guimarães DM, Szczupak D, Parente-Bruno DR, Carvalho LR, Polichiso L, Gomes BV, Oliveira LM, Rodriguez RD, Leite RE, Ferretti-Rebustini RE, Jacob-Filho W, Pasqualucci CA, Grinberg LT, Lent R. Cell number changes in Alzheimer’s disease relate to dementia, not to plaques and tangles. *Brain.* 2013 Dec;136(Pt 12):3738-52; <http://www.ncbi.nlm.nih.gov/pubmed/24136825>.

(15) Microglia, representing 10%-15% of all brain cells, are immune cells that act as a frontline defense, looking for suspicious activities and materials. When such is detected, microglia trigger the release of substances that recruit other microglia to the scene which then destroy and get rid of any foreign invaders. Microglia also work as garbage collectors and prevent inflammation by chewing up dead cells, misfolded proteins, and other molecular debris



strewn among living cells including A $\beta$  clusters that aggregate as gummy deposits and break the connections between neurons, causing loss of memory and spatial awareness. One recent study at Stanford University<sup>228</sup> found that microglia keep the sticky plaques under control in young mice, but in older mice, the heightened signaling activity of a single receptor protein that sits on the surface of microglial and nerve cells, called EP2, retards microglia from producing enzymes that can digest the plaques. Previous work has shown that the EP2 molecule has a strong potential to cause inflammation when activated by binding to a substance called prostaglandin E2, or PGE2. Mice that have been genetically engineered not to have EP2 don't develop severe memory loss even when injected with a solution of A $\beta$ , suggesting that their cells are getting rid of the protein naturally. The suspicion is that heightened EP2 activity may be a symptom of cellular aging.

(16) Neuron and synapse loss. The distribution of neuronal cell death and synapse loss is similar to that of NFT (tau tangles).<sup>229</sup> In typical AD, the death of neurons in the nucleus basalis of Meynert leads to a deficit in acetylcholine, a neurotransmitter involved in memory. This cholinergic deficit is the target of most current treatments. In the brainstem, loss of median raphe and locus coeruleus neurons leads to deficits in serotonin and norepinephrine, respectively. Abnormal cerebral serotonergic and adrenergic activity likely contribute to dysphoria and insomnia in AD.<sup>230</sup>

(17) Disruption of BBB. A $\beta$  can also disrupt the blood-brain barrier (Section 4.3.1), increasing vascular permeability and causing BBB leakage.<sup>231</sup>

<sup>228</sup> Johansson JU, Woodling NS, Wang Q, Panchal M, Liang X, Trueba-Saiz A, Brown HD, Mhatre SD, Loui T, Andreasson KI. Prostaglandin signaling suppresses beneficial microglial function in Alzheimer's disease models. *J Clin Invest*. 2015 Jan;125(1):350-64; <http://www.jci.org/articles/view/77487>.

<sup>229</sup> Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E. Alzheimer's disease. *Lancet* 2011; 377:1019-1031; <http://www.ncbi.nlm.nih.gov/pubmed/21371747>.

<sup>230</sup> Meeks TW, Ropacki SA, Jeste DV. The neurobiology of neuropsychiatric syndromes in dementia. *Curr Opin Psychiatry* 2006 Nov; 19(6):581-586; <http://www.ncbi.nlm.nih.gov/pubmed/17012935>.

<sup>231</sup> Biron KE, Dickstein DL, Gopaul R, Jefferies WA. Amyloid triggers extensive cerebral angiogenesis causing blood brain barrier permeability and hypervascularity in Alzheimer's disease. *PLoS One*. 2011;6(8):e23789; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3166122/>. Hartz AM, Bauer B, Soldner EL, Wolf A, Boy S, Backhaus R, Mihaljevic I, Bogdahn U, Klünemann HH, Schuierer G, Schlachetzki F. Amyloid- $\beta$  contributes to blood-brain barrier leakage in transgenic human amyloid precursor protein mice and in humans with cerebral amyloid angiopathy. *Stroke*. 2012 Feb;43(2):514-23; <http://stroke.ahajournals.org/content/43/2/514.long>. Keane J, Walsh DM, O'Malley T, Hudson N, Crosbie

A long list of other inflammatory processes, cytokines, and environmental factors not illustrated in **Figure 7** may also have some role in the pathology of Alzheimer's disease, including, e.g.:

(a) Inflammation is a general marker of tissue damage in any disease, and may be either secondary to tissue damage in AD or a marker of an immunological response.<sup>232</sup> Systemic markers of the innate immune system are risk factors for late-onset AD.<sup>233</sup>

(b) AD is characterized by the accumulation and aggregation of misfolded proteins. Disturbed homeostasis in the endoplasmic reticulum leads to accumulation of misfolded proteins, triggering a stress response called the unfolded protein response (UPR) that protects the cell against the toxic buildup of misfolded proteins.<sup>234</sup>

(c) Alterations in the distribution of different neurotrophic factors and in the expression of their receptors such as the brain-derived neurotrophic factor (BDNF) have been described in AD.<sup>235</sup>

(d) Herpes simplex virus type 1 (HSV-1) has been proposed to play a causative role in people carrying the susceptible versions of the ApoE4 gene.<sup>236</sup> In further work, the same

DE, Loftus T, Sheehan F, McDaid J, Humphries MM, Callanan JJ, Brett FM, Farrell MA, Humphries P, Campbell M. Autoregulated paracellular clearance of amyloid- $\beta$  across the blood-brain barrier. *Sci Adv*. 2015 Sep 4;1(8):e1500472; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4610013/>.

<sup>232</sup> Greig NH, Mattson MP, Perry T, Chan SL, Giordano T, Sambamurti K, Rogers JT, Ovadia H, Lahiri DK. New therapeutic strategies and drug candidates for neurodegenerative diseases: p53 and TNF-alpha inhibitors, and GLP-1 receptor agonists. *Ann N Y Acad Sci*. 2004 Dec;1035:290-315; <http://www.ncbi.nlm.nih.gov/pubmed/15681814>.

<sup>233</sup> Eikelenboom P, van Exel E, Hoozemans JJ, Veerhuis R, Rozemuller AJ, van Gool WA. Neuroinflammation – an early event in both the history and pathogenesis of Alzheimer's disease. *Neurodegener Dis*. 2010;7(1-3):38-41; [https://www.researchgate.net/profile/Willem\\_Van\\_Gool/publication/48322453\\_Neuroinflammation\\_-\\_An\\_Early\\_Event\\_in\\_Both\\_the\\_History\\_and\\_Pathogenesis\\_of\\_Alzheimer's\\_Disease/links/554a223c0cf21ed21358112a.pdf](https://www.researchgate.net/profile/Willem_Van_Gool/publication/48322453_Neuroinflammation_-_An_Early_Event_in_Both_the_History_and_Pathogenesis_of_Alzheimer's_Disease/links/554a223c0cf21ed21358112a.pdf).

<sup>234</sup> Hoozemans JJ, van Haastert ES, Nijholt DA, Rozemuller AJ, Scheper W. Activation of the unfolded protein response is an early event in Alzheimer's and Parkinson's disease. *Neurodegener Dis*. 2012;10(1-4):212-5; <http://www.ncbi.nlm.nih.gov/pubmed/22302012>.

<sup>235</sup> Schindowski K, Belarbi K, Buée L. Neurotrophic factors in Alzheimer's disease: role of axonal transport. *Genes Brain Behav*. 2008 Feb;7 Suppl 1:43-56; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2228393/>. Tapia-Arancibia L, Aliaga E, Silhol M, Arancibia S. New insights into brain BDNF function in normal aging and Alzheimer disease. *Brain Res Rev*. 2008 Nov;59(1):201-20; <http://www.ncbi.nlm.nih.gov/pubmed/18708092>.

<sup>236</sup> Wozniak MA, Itzhaki RF, Shipley SJ, Dobson CB. Herpes simplex virus infection causes cellular beta-amyloid accumulation and secretase upregulation. *Neurosci Lett*. 2007 Dec 18;429(2-3):95-100; <http://www.ncbi.nlm.nih.gov/pubmed/17980964>. Itzhaki RF, Wozniak MA. Herpes simplex virus type 1 in

researchers discovered a striking localization of HSV-1 DNA within amyloid plaques. Specifically, in AD brains, 90% of the plaques contain the viral DNA and 72% of the DNA is plaque-associated, whereas in normal brains, 80% of plaques contain HSV-1 DNA but only 24% of the viral DNA are plaque-associated. This suggests that in normal aged individuals, there is a lesser production and/or greater removal of A $\beta$ , so that less of the viral DNA is seen to be associated with A $\beta$  in the brain.<sup>237</sup>

(e) The cellular homeostasis of ionic copper, iron, and zinc is disrupted in AD, though it remains unclear whether this is produced by or causes the changes in proteins. These ions affect and are affected by tau, APP, and ApoE.<sup>238</sup> Some studies have shown an increased risk of developing AD with environmental factors such as the intake of metals, particularly aluminum.<sup>239</sup> Some have hypothesized that dietary copper may play a causal role.<sup>240</sup> But the quality of some of these studies has been criticized,<sup>241</sup> and other studies have concluded that there is no relationship between these environmental factors and the development of AD.<sup>242</sup> According to the Alzheimer's Association, it is a "myth" that aluminum from pots and pans, aluminum and mercury in flu shots, and silver-mercury amalgam dental fillings (cf. Section 2.4.6) increase the risk of AD.<sup>243</sup>

(f) While a few studies suggest that extremely low frequency electromagnetic fields may increase the risk for Alzheimer's disease,<sup>244</sup> reviewers found that further epidemiological and

Alzheimer's disease: the enemy within. *J Alzheimers Dis.* 2008 May;13(4):393-405;  
<http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.468.3722&rep=rep1&type=pdf>.

<sup>237</sup> Wozniak MA, Mee AP, Itzhaki RF. Herpes simplex virus type 1 DNA is located within Alzheimer's disease amyloid plaques. *J Pathol.* 2009 Jan;217(1):131-8;  
<http://www.ncbi.nlm.nih.gov/pubmed/18973185>.

<sup>238</sup> Xu H, Finkelstein DI, Adlard PA. Interactions of metals and Apolipoprotein E in Alzheimer's disease. *Front Aging Neurosci.* 2014 Jun 12;6:121; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4054654/>.

<sup>239</sup> Shcherbatykh I, Carpenter DO. The role of metals in the etiology of Alzheimer's disease. *J Alzheimers Dis.* 2007 May;11(2):191-205; <http://www.ncbi.nlm.nih.gov/pubmed/17522444>.

<sup>240</sup> Brewer GJ. Copper excess, zinc deficiency, and cognition loss in Alzheimer's disease. *Biofactors.* 2012 Mar-Apr;38(2):107-13; <http://www.ncbi.nlm.nih.gov/pubmed/22438177>.

<sup>241</sup> Santibáñez M, Bolumar F, García AM. Occupational risk factors in Alzheimer's disease: a review assessing the quality of published epidemiological studies. *Occup Environ Med.* 2007 Nov;64(11):723-32; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2078415/>.

<sup>242</sup> Rondeau V. A review of epidemiologic studies on aluminum and silica in relation to Alzheimer's disease and associated disorders. *Rev Environ Health.* 2002 Apr-Jun;17(2):107-21; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4764671/>.

<sup>243</sup> "Alzheimer's Myths," Alzheimer's Association, 2016;  
[http://www.alz.org/alzheimers\\_disease\\_myths\\_about\\_alzheimers.asp](http://www.alz.org/alzheimers_disease_myths_about_alzheimers.asp).

<sup>244</sup> Kheifets L, Bowman JD, Checkoway H, Feychting M, Harrington JM, Kavet R, Marsh G, Mezei G, Renew DC, van Wijngaarden E. Future needs of occupational epidemiology of extremely low frequency

laboratory investigations of this hypothesis are needed,<sup>245</sup> and some contrary evidence has been reported.<sup>246</sup>

(g) Smoking is a significant AD risk factor.<sup>247</sup>

(h) Another hypothesis asserts that the disease may be caused by age-related myelin breakdown in the brain. Iron released during myelin breakdown is hypothesized to cause further damage. Homeostatic myelin repair processes may contribute to the development of proteinaceous deposits such as beta-amyloid and tau.<sup>248</sup>

(i) Drebrin A is one of the main neuronal actin binding proteins,<sup>249</sup> regulating actin's role in cell architecture and functions. AD has been linked to the loss of dendritic spines and/or deformation of these spines in the patient's frontal and temporal cortices, and to a decrease in the expression of drebrin<sup>250</sup> (a protein thought to play a role in long-term potentiation) which could cause neurons to lose plasticity and have trouble forming new connections. This malfunction

electric and magnetic fields: review and recommendations. *Occup Environ Med.* 2009 Feb;66(2):72-80; <http://www.ncbi.nlm.nih.gov/pubmed/18805878>.

<sup>245</sup> Scientific Committee on Emerging and Newly Identified Health Risks-SCENIHR. Health Effects of Exposure to EMF. Jan 2009:4-5; [http://ec.europa.eu/health/ph\\_risk/committees/04\\_scenihr/docs/scenihr\\_o\\_022.pdf](http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_022.pdf).

<sup>246</sup> Arendash GW, Sanchez-Ramos J, Mori T, Mamcarz M, Lin X, Runfeldt M, Wang L, Zhang G, Sava V, Tan J, Cao C. Electromagnetic field treatment protects against and reverses cognitive impairment in Alzheimer's disease mice. *J Alzheimers Dis.* 2010;19(1):191-210; <http://www.ncbi.nlm.nih.gov/pubmed/20061638>. Arendash GW, Mori T, Dorsey M, Gonzalez R, Tajiri N, Borlongan C. Electromagnetic treatment to old Alzheimer's mice reverses  $\beta$ -amyloid deposition, modifies cerebral blood flow, and provides selected cognitive benefit. *PLoS One.* 2012;7(4):e35751; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3338462/>.

<sup>247</sup> Cataldo JK, Prochaska JJ, Glantz SA. Cigarette smoking is a risk factor for Alzheimer's disease: An analysis controlling for tobacco industry affiliation. *J Alzheimers Dis.* 2010;19(2):465-80; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2906761/>.

<sup>248</sup> Bartzokis G, Lu PH, Mintz J. Quantifying age-related myelin breakdown with MRI: novel therapeutic targets for preventing cognitive decline and Alzheimer's disease. *J Alzheimers Dis.* 2004 Dec;6(6 Suppl):S53-9; <http://www.ncbi.nlm.nih.gov/pubmed/15665415>. Bartzokis G, Lu PH, Mintz J. Human brain myelination and amyloid beta deposition in Alzheimer's disease. *Alzheimers Dement.* 2007 Apr;3(2):122-5; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2442864/>. Bartzokis G. Alzheimer's disease as homeostatic responses to age-related myelin breakdown. *Neurobiol Aging.* 2011 Aug;32(8):1341-71; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3128664/>.

<sup>249</sup> Sharma S, Grintsevich EE, Hsueh C, Reisler E, Gimzewski JK. Molecular cooperativity of drebrin1-300 binding and structural remodeling of F-actin. *Biophys J.* 2012 Jul 18;103(2):275-83; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3400778/>.

<sup>250</sup> Ivanov A, Esclapez M, Ferhat L. Role of drebrin A in dendritic spine plasticity and synaptic function: Implications in neurological disorders. *Commun Integr Biol.* 2009 May;2(3):268-70; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2717538/>.

presents itself in the form of helical filaments (distinct from amyloid and tau aggregates) that tangle together in the neuropil.<sup>251</sup>

(j) Oxidative stress and dys-homeostasis of biometal metabolism may be significant in the formation of Alzheimer's disease.<sup>252</sup> If true, low molecular weight antioxidants such as melatonin might provide promising approaches to treatment.<sup>253</sup>

(k) AD individuals exhibit a 70% loss of locus coeruleus cells that provide norepinephrine (more known for its neurotransmitter role) that locally diffuses from "varicosities" as an endogenous anti-inflammatory agent in the microenvironment around the neurons, glial cells, and blood vessels in the neocortex and hippocampus. It has been shown that norepinephrine stimulates mouse microglia to suppress A $\beta$ -induced production of cytokines and their phagocytosis of A $\beta$ . This suggests that degeneration of the locus coeruleus might be responsible for increased A $\beta$  deposition in AD brains.<sup>254</sup>

(l) There is tentative evidence that exposure to air pollution may be a contributing factor in the development of Alzheimer's disease.<sup>255</sup>

(m) Other potential environmental risk factors for late-onset AD include head injury (leading to tauopathy),<sup>256</sup> low educational levels, hyperlipidemia, hypertension, homocysteinemia, diabetes mellitus, and obesity.<sup>257</sup> However, several of these associations

<sup>251</sup> Braak H, Braak E, Grundke-Iqbal I, Iqbal K. Occurrence of neuropil threads in the senile human brain and in Alzheimer's disease: a third location of paired helical filaments outside of neurofibrillary tangles and neuritic plaques. *Neurosci Lett*. 1986 Apr 24;65(3):351-5; <http://www.ncbi.nlm.nih.gov/pubmed/2423928>.

<sup>252</sup> Su B, Wang X, Nunomura A, Moreira PI, Lee HG, Perry G, Smith MA, Zhu X. Oxidative stress signaling in Alzheimer's disease. *Curr Alzheimer Res*. 2008 Dec;5(6):525-32; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2780015/>. Kastenholz B, Garfin DE, Horst J, Nagel KA. Plant metal chaperones: a novel perspective in dementia therapy. *Amyloid*. 2009;16(2):81-3; <http://www.ncbi.nlm.nih.gov/pubmed/20536399>. Pohanka M. Alzheimer's disease and oxidative stress: a review. *Curr Med Chem*. 2014;21(3):356-64; <http://www.ncbi.nlm.nih.gov/pubmed/24059239>.

<sup>253</sup> Pohanka M. Alzheimer's disease and related neurodegenerative disorders: implication and counteracting of melatonin. *J Appl Biomed* 2011;9:185-196; <http://www.sciencedirect.com/science/article/pii/S1214021X14600387>.

<sup>254</sup> Heneka MT, Nadrigny F, Regen T, Martinez-Hernandez A, Dumitrescu-Ozimek L, Terwel D, Jardanhazi-Kurutz D, Walter J, Kirchhoff F, Hanisch UK, Kummer MP. Locus ceruleus controls Alzheimer's disease pathology by modulating microglial functions through norepinephrine. *Proc Natl Acad Sci U S A*. 2010 Mar 30;107(13):6058-63; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2851853/>.

<sup>255</sup> Moulton PV, Yang W. Air pollution, oxidative stress, and Alzheimer's disease. *J Environ Public Health*. 2012;2012:472751; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3317180/>.

<sup>256</sup> Mckee AC, Daneshvar DH. The neuropathology of traumatic brain injury. *Handb Clin Neurol*. 2015;127:45-66; <http://www.ncbi.nlm.nih.gov/pubmed/25702209>.

<sup>257</sup> Rosendorff C, Beerl MS, Silverman JM. Cardiovascular risk factors for Alzheimer's disease. *Am J Geriatr Cardiol*. 2007 May-Jun;16(3):143-9; <http://www.ncbi.nlm.nih.gov/pubmed/17483665>. Van Den

remain controversial.<sup>258</sup> Combinations of ApoE4 with one or more of these environmental risk factors may further increase the risks for late-onset AD and age-related cognitive decline.<sup>259</sup>

(n) The links between type 2 diabetes and AD have become sufficiently strong for AD to be described by some investigators as a form of “neural diabetes”.<sup>260</sup> The idea is that it is not amyloid plaques themselves that cause AD symptoms but rather their precursors – small, soluble clumps of A $\beta$  oligomers. The same enzymes (e.g., insulin-degrading enzyme, or IDE) break down both insulin and A $\beta$  oligomers, and oligomers also prevent insulin from binding to its receptors in the hippocampus. So when there is too much insulin around – as in patients with type 2 diabetes – those enzymes are working flat out to break it down. This preferential treatment of insulin over oligomers could leave the oligomers to form clumps, which then keep insulin from its receptors, causing a vicious spiral of impaired brain insulin signaling coupled with cognitive decline.<sup>261</sup>

(o) Elevated serum lipid ceramide levels, triggered by excess amyloid plaque production in mice, appears to support the theory that Alzheimer’s could be an autoimmune disease characterized by the immune system producing antibodies against ceramide in a patient’s tissue.<sup>262</sup>

Heuvel C, Thornton E, Vink R. Traumatic brain injury and Alzheimer’s disease: A review. *Prog Brain Res.* 2007;161:303-316; <http://www.ncbi.nlm.nih.gov/pubmed/17618986>. Barnes DE, Yaffe K. The projected effect of risk factor reduction on Alzheimer’s disease prevalence. *Lancet Neurol.* 2011 Sep;10(9):819-828; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3647614/>. Sharp ES, Gatz M. Relationship between education and dementia: an updated systematic review. *Alzheimer Dis Assoc Disord.* 2011 Oct-Dec;25(4):289-304; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3193875/>.

<sup>258</sup> Daviglus ML, Bell CC, Berrettini W, Bowen PE, Connolly ES Jr, Cox NJ, Dunbar-Jacob JM, Granieri EC, Hunt G, McGarry K, Patel D, Potosky AL, Sanders-Bush E, Silberberg D, Trevisan M. National Institutes of Health State-of-the-Science Conference statement: Preventing Alzheimer disease and cognitive decline. *Ann Intern Med.* 2010 Aug 3;153(3):176-81; <http://www.ncbi.nlm.nih.gov/pubmed/20547888>.

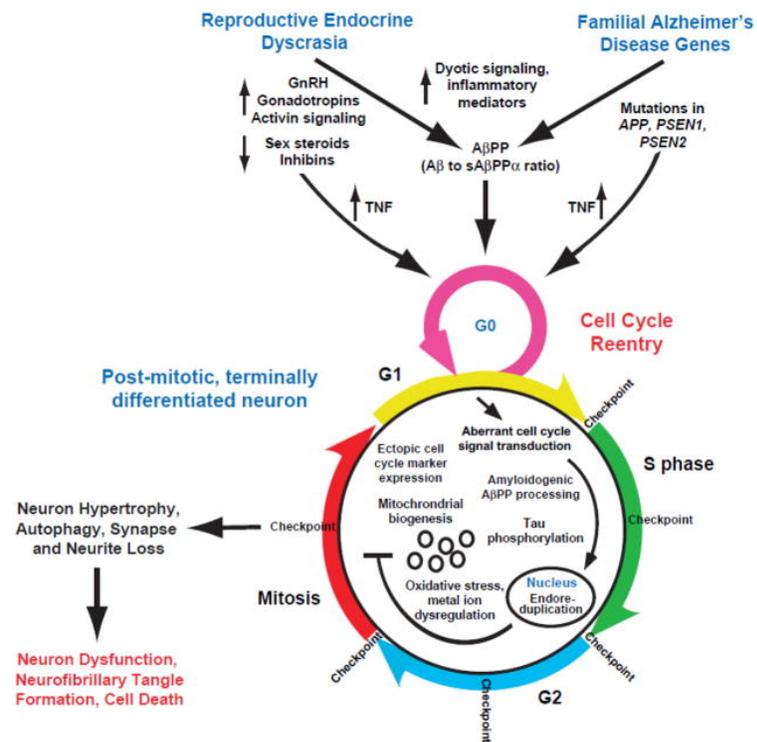
<sup>259</sup> Haan MN, Shemanski L, Jagust WJ, Manolio TA, Kuller L. The role of APOE epsilon4 in modulating effects of other risk factors for cognitive decline in elderly persons. *JAMA.* 1999 Jul 7;282(1):40-6; [http://idealab.ucdavis.edu/pubs/papers/haan\\_shemanski\\_et\\_al\\_JAMA1999.pdf](http://idealab.ucdavis.edu/pubs/papers/haan_shemanski_et_al_JAMA1999.pdf). Caselli RJ, Dueck AC, Locke DE, Sabbagh MN, Ahern GL, Rapcsak SZ, Baxter LC, Yaari R, Woodruff BK, Hoffman-Snyder C, Rademakers R, Findley S, Reiman EM. Cerebrovascular risk factors and preclinical memory decline in healthy APOE  $\epsilon$ 4 homozygotes. *Neurology.* 2011 Mar 22;76(12):1078-84; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3068011/>.

<sup>260</sup> de la Monte SM, Wands JR. Alzheimer’s disease is type 3 diabetes-evidence reviewed. *J Diabetes Sci Technol.* 2008 Nov;2(6):1101-13; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2769828/>.

<sup>261</sup> McNay EC, Recknagel AK. Brain insulin signaling: a key component of cognitive processes and a potential basis for cognitive impairment in type 2 diabetes. *Neurobiol Learn Mem.* 2011 Oct;96(3):432-42; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3190063/>.

<sup>262</sup> Dinkins MB, Dasgupta S, Wang G, Zhu G, He Q, Kong JN, Bieberich E. The 5XFAD Mouse Model of Alzheimer’s Disease Exhibits an Age-Dependent Increase in Anti-Ceramide IgG and Exogenous Administration of Ceramide Further Increases Anti-Ceramide Titers and Amyloid Plaque Burden. *J Alzheimers Dis.* 2015;46(1):55-61; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4593501/>.

(p) The aberrant re-expression of various cell cycle control elements (e.g., localization of various cyclins, cyclin-dependent kinases, and cyclin inhibitors) in neurons may be an attempt to re-enter the cell cycle.<sup>263</sup> Given that primary neurons are terminally differentiated and post-mitotic, any attempted re-entry into the cell division cycle will be dysregulated and deleterious. Inappropriate re-entry into the cell cycle then resembles an abortive oncogeny (tumor formation) involving virtually the entire spectrum of the described pathological events in Alzheimer disease including, ultimately, cell death.<sup>264</sup>



Atwood and Bowen hypothesize<sup>265</sup> that such cell-cycle re-entry could be the underlying cause for most of the pathologies of Alzheimer's disease (see diagram, above): "Endocrine dyscrasia<sup>266</sup> and AD-related genetic mutations intersect at the cell cycle to drive neurodegeneration and cognitive decline in AD [as shown in this] model of aberrant cell cycle reentry initiated by (1) aging-related endocrine dyscrasia leading to late-onset sporadic AD and (2) genetic mutations in APP, PS1, and PS2 leading to early-onset familial AD."

<sup>263</sup> Raina AK, Zhu X, Rottkamp CA, Monteiro M, Takeda A, Smith MA. Cyclin' toward dementia: cell cycle abnormalities and abortive oncogenesis in Alzheimer disease. *J Neurosci Res.* 2000 Jul 15;61(2):128-33; <http://www.ncbi.nlm.nih.gov/pubmed/10878584/>.

<sup>264</sup> Raina AK, Zhu X, Monteiro M, Takeda A, Smith MA. Abortive oncogeny and cell cycle-mediated events in Alzheimer disease. *Prog Cell Cycle Res.* 2000;4:235-42; <http://www.ncbi.nlm.nih.gov/pubmed/10740829/>.

<sup>265</sup> Atwood CS, Bowen RL. A unified hypothesis of early- and late-onset Alzheimer's disease pathogenesis. *J Alzheimers Dis.* 2015;47(1):33-47; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4807856/>.

<sup>266</sup> Dyscrasia, an ancient term, now refers to a general morbid state resulting from the presence of abnormal material in the blood, including excessive numbers of blood cells or a severe imbalance in blood chemistry or in molecular components in the blood.

## 2.4.4 Aging as a Risk Factor

Although Alzheimer's is not traditionally regarded as a normal part of growing older, the greatest risk factor for the disease is increasing age. After age 65, the risk of Alzheimer's doubles every five years. After age 85 the risk reaches nearly 50 percent,<sup>267</sup> and after age 90 the five-year AD risk-doubling continues (at least in women),<sup>268</sup> making aging the most important known non-genetic risk factor for late-onset AD. Biogerontologist Aubrey de Grey believes that AD is a disease that everyone would get eventually, if only they lived long enough.<sup>269</sup>

It is well established that many neurodegenerative disorders are strongly linked to aging. However, it remains uncertain whether this link is specifically caused by aging-related processes or simply reflects the time required for the relevant pathogenic processes to unfold, given that children with progeria (a "premature aging" disease) have no apparent cognitive deterioration and no pathologic evidence of dementia or Alzheimer-type changes.<sup>270</sup> Genetic changes (Section 2.4.5) that accelerate the accumulation of pathogenic proteins in the brain can clearly override the aging "requirement" and cause AD or other neurodegenerative conditions in middle-aged or even young people. Nonetheless, diverse lines of experimental evidence support the notion that AD and other neurodegenerative conditions may be enabled by specific aging-related factors, such as gradual failure of neuroprotective or protein clearance mechanisms (Section 2.4.8) and the emergence of co-morbidities.<sup>271</sup> Inflammation<sup>272</sup> along with oxidative stress and altered

---

<sup>267</sup> "Risk Factors," Alzheimer's Association, 2015; [http://www.alz.org/alzheimers\\_disease\\_causes\\_risk\\_factors.asp](http://www.alz.org/alzheimers_disease_causes_risk_factors.asp).

<sup>268</sup> Corrada MM, Brookmeyer R, Berlau D, Paganini-Hill A, Kawas CH. Prevalence of dementia after age 90: results from the 90+ study. *Neurology*. 2008 Jul 29;71(5):337-43; <http://www.ncbi.nlm.nih.gov/pubmed/18596243/>.

<sup>269</sup> "All of the major diseases of old age are things that we would all get if we didn't die of something else first. They result from the gradual lifelong accumulation of precursors of those diseases. Those precursors continue to accumulate as inevitable side effects of normal metabolism, so inevitably we're going to get them, once we live long enough, as long as we don't die of something else first." See: Big Think interview with Aubrey de Grey; <http://bigthink.com/videos/big-think-interview-with-aubrey-de-grey>. See also: <http://bigthink.com/articles/will-everyone-get-alzheimers-if-they-live-long-enough>.

<sup>270</sup> Ullrich NJ, Gordon LB. Hutchinson-Gilford progeria syndrome. *Handb Clin Neurol*. 2015;132:249-64; <http://www.ncbi.nlm.nih.gov/pubmed/26564085>.

<sup>271</sup> Herrup K. Reimagining Alzheimer's disease – an age-based hypothesis. *J Neurosci*. 2010 Dec 15;30(50):16755-62; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3004746/>. Mawuenyega KG, Sigurdson W, Ovod V, Munsell L, Kasten T, Morris JC, Yarasheski KE, Bateman RJ. Decreased clearance of CNS beta-amyloid in Alzheimer's disease. *Science*. 2010 Dec 24;330(6012):1774; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3073454/>. Villeda SA, Luo J, Mosher KI, Zou B, Britschgi M, Bieri G, Stan TM, Fainberg N, Ding Z, Eggel A, Lucin KM, Czirr E, Park JS, Couillard-Després S, Aigner L, Li G, Peskind ER, Kaye JA, Quinn JF, Galasko DR, Xie XS, Rando TA, Wyss-Coray T. The ageing systemic milieu negatively regulates neurogenesis and cognitive function. *Nature*. 2011 Aug 31;477(7362):90-4; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3170097/>.

cholesterol metabolism leading to hypercholesterolemia<sup>273</sup> might also be key components of unhealthy aging leading to Alzheimer's.

The immune system may be implicated in AD. The hypothesized actor is C1q, a protein component of complement factor C1 known as the initiator of the “classical pathway” of the complement system in the human immune response. A 2013 study of brain tissue in mice of varying ages, as well as postmortem samples of a 2-month-old human infant and an elderly person, found that C1q exponentially increases in the aging brain, producing as much as a 300-fold buildup.<sup>274</sup> This is potentially significant, since most known age-associated increases of protein concentrations in the brain are only three- or four-fold in magnitude. The research also revealed that C1q accumulates around the brain's synapses – contact points that connect the brain's nerve cells to one another – as the brain ages. Rather than being naturally cleared by the brain, the C1q sticks, making these synapses more vulnerable to destruction from the brain's immune cells.

The progressive shortening of telomeres (the protective “end caps” on our chromosomes) with age might also be implicated in the onset of Alzheimer's disease via the declining capacity of microglial cells. Notes Michael Fossel, an expert in telomere-based aging:<sup>275</sup>

Alzheimer's disease begins in our glial cells. These cells together form 90% of our brains, while neurons are only a small minority in the nervous system. One set of these glial cells, the microglia, have the critical job of protecting the neurons and supporting them metabolically. These are the cells that, among dozens of other functions, are responsible for clearing metabolic waste products and recycling the extracellular proteins that surround the neurons. Unfortunately, as we age, the microglial cells not only fail to divide, but gradually lose telomere length. By itself, telomere loss is unimportant, but this loss begins a cascade of crucial changes in our cells. As these telomeres shorten, they trigger a gradual shift in gene expression throughout the entire microglial cell. Proteins that are critical to DNA repair, to making our mitochondria work, to holding free radical damage to a minimum, begin to become scant. Where once, a young microglial cell would recycle proteins quickly and efficiently – including beta amyloid proteins – as the cell ages, the rate of turnover slows to a crawl...One-by-one our neurons are snuffed out, submerged under the rising effects of beta amyloid and tau proteins, and all of this, the plaques,

---

<sup>272</sup> Sastre M, Richardson JC, Gentleman SM, Brooks DJ. Inflammatory risk factors and pathologies associated with Alzheimer's disease. *Curr Alzheimer Res.* 2011 Mar;8(2):132-41; <http://www.ncbi.nlm.nih.gov/pubmed/21345169>.

<sup>273</sup> Gamba P, Testa G, Gargiulo S, Staurengi E, Poli G, Leonarduzzi G. Oxidized cholesterol as the driving force behind the development of Alzheimer's disease. *Front Aging Neurosci.* 2015 Jun 19;7:119; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4473000/>.

<sup>274</sup> Stephan AH, Madison DV, Mateos JM, Fraser DA, Lovelett EA, Coutellier L, Kim L, Tsai HH, Huang EJ, Rowitch DH, Berns DS, Tenner AJ, Shamloo M, Barres BA. A dramatic increase of C1q protein in the CNS during normal aging. *J Neurosci.* 2013 Aug 14;33(33):13460-74; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3742932/>.

<sup>275</sup> Michael Fossel, “Reversing Alzheimer's by Lengthening Telomeres,” *Humanity+ Media*, 8 Oct 2015; <http://hplusmagazine.com/2015/10/08/reversing-alzheimers-by-lengthening-telomeres/>.

the tangles, and the dying neurons characteristic of Alzheimer's can be traced back to the failing microglial cells.

However, the direct evidence for glial and leukocyte telomere length shortening in Alzheimer's<sup>276</sup> and Parkinson's<sup>277</sup> patients remains inconsistent.

Yet another interesting idea involving aging as the possible ultimate cause of late-onset sporadic AD is the mitochondrial cascade hypothesis.<sup>278</sup> In this model, "the inherited, gene-determined make-up of an individual's electron transport chain sets basal rates of reactive oxygen species (ROS) production, which determines the pace at which acquired mitochondrial damage accumulates. Oxidative mitochondrial DNA, RNA, lipid, and protein damage amplifies ROS production and triggers three events: (1) a *reset response* in which cells respond to elevated ROS by generating the beta-sheet protein (beta amyloid), which further perturbs mitochondrial function, (2) a *removal response* in which compromised cells are purged via programmed cell death mechanisms, and (3) a *replace response* in which neuronal progenitors unsuccessfully attempt to re-enter the cell cycle, with resultant aneuploidy, tau phosphorylation, and neurofibrillary tangle formation."

In addition to defining a role for aging in AD pathogenesis, the mitochondrial cascade hypothesis allows and accounts for histopathologic overlap between the late-onset and early-onset forms of the disease. The theory's proponents specify the molecular substrate of the human aging clock (mainly mitochondrial DNA or mtDNA) and the primary influence on the clock speed setting (the rate of accumulation of change as determined by genetically determined ROS production rates), and tie this in with secondary phenomena (both adaptive and maladaptive) such as A $\beta$  accumulation, tangle formation, and others. In this view it is possible that during aging, the accumulation of oxidative stress-induced mtDNA damage and subsequent mitochondrial dysfunction may serve as a trigger for the appearance of the main AD histopathologic markers.<sup>279</sup>

---

<sup>276</sup> Eitan E, Hutchison ER, Mattson MP. Telomere shortening in neurological disorders: an abundance of unanswered questions. *Trends Neurosci.* 2014 May;37(5):256-263; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4008659/>.

<sup>277</sup> Forero DA, González-Giraldo Y, López-Quintero C, Castro-Vega LJ, Barreto GE, Perry G. Telomere length in Parkinson's disease: A meta-analysis. *Exp Gerontol.* 2016 Mar;75:53-5; <http://www.ncbi.nlm.nih.gov/pubmed/26772888>.

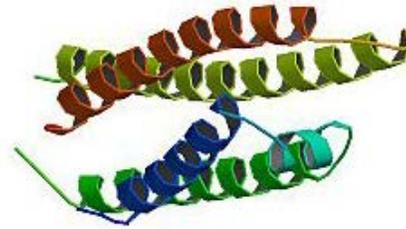
<sup>278</sup> Swerdlow RH, Khan SM. A "mitochondrial cascade hypothesis" for sporadic Alzheimer's disease. *Med Hypotheses.* 2004;63(1):8-20; <http://www.ncbi.nlm.nih.gov/pubmed/15193340>. Swerdlow RH, Khan SM. The Alzheimer's disease mitochondrial cascade hypothesis: an update. *Exp Neurol.* 2009 Aug;218(2):308-15; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2710413/>. Swerdlow RH, Burns JM, Khan SM. The Alzheimer's disease mitochondrial cascade hypothesis: progress and perspectives. *Biochim Biophys Acta.* 2014 Aug;1842(8):1219-31; <http://www.ncbi.nlm.nih.gov/pubmed/24071439>.

<sup>279</sup> Santos RX, Correia SC, Wang X, Perry G, Smith MA, Moreira PI, Zhu X. Alzheimer's disease: diverse aspects of mitochondrial malfunctioning. *Int J Clin Exp Pathol.* 2010 Jun 25;3(6):570-81; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2907118/>.

## 2.4.5 Genetics and Epigenetics

Aside from environmental factors, AD might ultimately be caused by complex interactions among multiple genetic and epigenetic factors. The Alzheimer's Genome Project, sponsored by the Cure Alzheimer's Fund,<sup>280</sup> tentatively identified more than 100 genes that might be associated with Alzheimer's disease.<sup>281</sup>

Most notably, apolipoprotein E (APOE; image, right) is 299 amino acids long and transports lipoproteins, fat-soluble vitamins, and cholesterol in the blood. There are three isoforms of the protein, called ApoE2, ApoE3, and ApoE4, that differ by only 1-2 amino acids, with the gene residing on Chromosome 19. ApoE3 is the



“neutral” variant, with an allele frequency of 79%. The ApoE4 form (frequency 14%), which differs from ApoE3 only by having the amino acid arginine instead of cysteine at position 112, has been genetically linked to late-onset (>60 years) familial and sporadic AD, which accounts for most AD cases. It has a gene-dose effect of increasing the risk and lowering the age of onset of the disease.<sup>282</sup> All well-conducted genome-wide association studies on late-onset AD from different populations around the world have identified ApoE4 as the top late-onset AD gene with extremely high confidence.<sup>283</sup> Remarkably, the lifetime risk estimate of developing AD for individuals with two copies of the ApoE4 allele (~2% of the population) is ~60% by the age of 85, and for those with one copy of the ApoE4 allele (~25% of the population) ~30%. In comparison, the lifetime risk of AD for those with two copies of the ApoE3 allele is ~10% by the

---

<sup>280</sup> <http://curealz.org/projects/alzheimer%E2%80%99s-genome-project%E2%84%A2>, <https://www.niagads.org/adsp/content/home>, <http://www.alzforum.org/news/community-news/alzheimers-whole-genome-data-now-available-nih>.

<sup>281</sup> <http://pittsburghquarterly.com/pq-health-science/pq-science/item/527-can-venture-capital-help-cure-alzheimer-s-disease.html>.

<sup>282</sup> Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science*. 1993 Aug 13;261(5123):921-3; <http://www.sciencemag.org/content/261/5123/921.full.pdf>. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, Pericak-Vance MA, Risch N, van Duijn CM. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. *J Am Med Assoc*. 1997;278:1349-56; <http://www.ncbi.nlm.nih.gov/pubmed/9343467>.

<sup>283</sup> Bertram L, Lill CM, Tanzi RE. The genetics of Alzheimer disease: back to the future. *Neuron*. 2010 Oct 21;68(2):270-81; [http://www.cell.com/neuron/pdf/S0896-6273\(10\)00837-8.pdf](http://www.cell.com/neuron/pdf/S0896-6273(10)00837-8.pdf).

age of 85. Between 40% and 80% of people with AD possess at least one ApoE4 allele.<sup>284</sup> Thus, ApoE4 should be considered a major gene, with semi-dominant inheritance, for late-onset AD.<sup>285</sup>

Apolipoprotein E enhances proteolytic break-down of A $\beta$  peptide, both within and between cells. The isoform ApoE4 is not as effective as the others at promoting these reactions, resulting in increased vulnerability to AD in individuals with that gene variation.<sup>286</sup> (ApoE4 is associated with higher levels of Vitamin D, which may help explain its prevalence despite its seeming complicity in various diseases or disorders.<sup>287</sup>) There is also evidence that the ApoE2 allele may serve a protective role in AD<sup>288</sup> – patients with two copies of ApoE4 have 25 times higher risk of AD than patients with two copies of ApoE2 (who have the lowest risk of AD).<sup>289</sup>

There are also deterministic genes that directly cause AD, guaranteeing that anyone who inherits one will develop the disorder. These genes, which are estimated to account for less than 5% of Alzheimer's cases, cause familial early-onset forms in which symptoms usually develop between a person's early 40s and mid-50s. Although the genes that cause "familial Alzheimer's" are rare, their discovery has provided important clues that help our understanding of Alzheimer's. Familial Alzheimer's disease is inherited in an autosomal dominant fashion, mostly attributable to mutations in one of three genes: those encoding amyloid precursor protein (APP), presenilin 1, and presenilin 2.<sup>290</sup> Most mutations in the APP and presenilin genes increase the production of

---

<sup>284</sup> Mahley RW, Weisgraber KH, Huang Y. Apolipoprotein E4: a causative factor and therapeutic target in neuropathology, including Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2006 Apr 11;103(15):5644-51; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1414631/>.

<sup>285</sup> Genin E, *et al.* APOE and Alzheimer disease: A major gene with semi-dominant inheritance. *Mol Psychiatry*. 2011 Sep;16(9):903-7; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3162068/>.

<sup>286</sup> Jiang Q, Lee CY, Mandrekar S, Wilkinson B, Cramer P, Zelcer N, Mann K, Lamb B, Willson TM, Collins JL, Richardson JC, Smith JD, Comery TA, Riddell D, Holtzman DM, Tontonoz P, Landreth GE. ApoE promotes the proteolytic degradation of A $\beta$ . *Neuron*. 2008 Jun 12;58(5):681-93; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2493297/>.

<sup>287</sup> Huebbe P, Nebel A, Siegert S, Moehring J, Boesch-Saadatmandi C, Most E, Pallauf J, Egert S, Müller MJ, Schreiber S, Nöthlings U, Rimbach G. APOE  $\epsilon$ 4 is associated with higher vitamin D levels in targeted replacement mice and humans. *FASEB J*. 2011 Sep;25(9):3262-70; <http://www.fasebj.org/content/25/9/3262.long>.

<sup>288</sup> Corder EH, Saunders AM, Risch NJ, Strittmatter WJ, Schmechel DE, Gaskell PC Jr, Rimmler JB, Locke PA, Conneally PM, Schmechel KE, *et al.* Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. *Nat Genet*. 1994 Jun;7(2):180-4; <http://www.ncbi.nlm.nih.gov/pubmed/7920638>.

<sup>289</sup> Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, Pericak-Vance MA, Risch N, van Duijn CM. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA*. 1997 Oct 22-29;278(16):1349-56; <http://www.ncbi.nlm.nih.gov/pubmed/9343467>.

<sup>290</sup> Waring SC, Rosenberg RN. Genome-wide association studies in Alzheimer disease. *Arch Neurol*. 2008 Mar;65(3):329-34; [http://archinte.jamanetwork.com/data/Journals/NEUR/7530/nmr70026\\_329\\_334.pdf](http://archinte.jamanetwork.com/data/Journals/NEUR/7530/nmr70026_329_334.pdf).

A $\beta$ <sub>42</sub>, the main component of senile plaques.<sup>291</sup> Some of the mutations merely alter the ratio between A $\beta$ <sub>42</sub> and the other major forms – particularly A $\beta$ <sub>40</sub> – without increasing A $\beta$ <sub>42</sub> levels,<sup>292</sup> so presenilin mutations may cause disease even if they lower the total amount of A $\beta$  produced.

Interestingly, studies of prospective AD patients having these genes suggest that the pathologies commonly associated with AD actually begin long before AD symptoms appear. For example, concentrations of A $\beta$ <sub>42</sub> in the cerebrospinal fluid (CSF) begin to decline 25 years before expected symptom onset. Fibrillar A $\beta$  deposition, increased concentrations of tau protein in the CSF, and an increase in brain atrophy were also detected 15 years early, and cerebral hypometabolism and impaired episodic memory were observed 10 years early.<sup>293</sup>

Epigenetic mechanisms may also play a role in AD pathogenesis.<sup>294</sup> Studies on human postmortem brain samples, peripheral leukocytes, and transgenic animal models have shown that aging and AD are associated with epigenetic dysregulation at various levels, including abnormal DNA methylation and histone modifications.<sup>295</sup> Although it is unclear whether the epigenetic changes observed in AD represent a cause or a consequence of the disease, twin studies support the notion that epigenetic mechanisms modulate AD risk.<sup>296</sup> Interestingly, pharmacologically inhibiting DNA methylation in the hippocampus after a learning task will impair memory

---

<sup>291</sup> Selkoe DJ. Translating cell biology into therapeutic advances in Alzheimer's disease. *Nature*. 1999 Jun 24;399(6738 Suppl):A23-31; <http://azolla.fc.ul.pt/aulas/BiologiaCelular/docs/Alzheimer.pdf>.

<sup>292</sup> Borchelt DR, Thinakaran G, Eckman CB, Lee MK, Davenport F, Ratovitsky T, Prada CM, Kim G, Seekins S, Yager D, Slunt HH, Wang R, Seeger M, Levey AI, Gandy SE, Copeland NG, Jenkins NA, Price DL, Younkin SG, Sisodia SS. Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1-42/1-40 ratio *in vitro* and *in vivo*. *Neuron*. 1996 Nov;17(5):1005-13; <http://www.sciencedirect.com/science/article/pii/S0896627300802305>. Shioi J, Georgakopoulos A, Mehta P, Kouchi Z, Litterst CM, Baki L, Robakis NK. FAD mutants unable to increase neurotoxic Abeta 42 suggest that mutation effects on neurodegeneration may be independent of effects on Abeta. *J Neurochem*. 2007 May;101(3):674-81; <http://onlinelibrary.wiley.com/doi/10.1111/j.1471-4159.2006.04391.x/full>.

<sup>293</sup> Bateman RJ, Xiong C, Benzinger TL, Fagan AM, Goate A, Fox NC, Marcus DS, Cairns NJ, Xie X, Blazey TM, Holtzman DM, Santacruz A, Buckles V, Oliver A, Moulder K, Aisen PS, Ghetti B, Klunk WE, McDade E, Martins RN, Masters CL, Mayeux R, Ringman JM, Rossor MN, Schofield PR, Sperling RA, Salloway S, Morris JC; Dominantly Inherited Alzheimer Network. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N Engl J Med*. 2012 Aug 30;367(9):795-804; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3474597/>.

<sup>294</sup> Day JJ, Sweatt JD. Epigenetic mechanisms in cognition. *Neuron*. 2011 Jun 9;70(5):813-29; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3118503/>.

<sup>295</sup> Chouliaras L, Rutten BP, Kenis G, Peerbooms O, Visser PJ, Verhey F, van Os J, Steinbusch HW, van den Hove DL. Epigenetic regulation in the pathophysiology of Alzheimer's disease. *Prog Neurobiol*. 2010;90:498-510; <http://www.ncbi.nlm.nih.gov/pubmed/20097254>.

<sup>296</sup> Chouliaras L, Rutten BP, Kenis G, Peerbooms O, Visser PJ, Verhey F, van Os J, Steinbusch HW, van den Hove DL. Epigenetic regulation in the pathophysiology of Alzheimer's disease. *Prog Neurobiol*. 2010;90:498-510; <http://www.ncbi.nlm.nih.gov/pubmed/20097254>.

consolidation in mice.<sup>297</sup> Promoting histone acetylation improves learning and memory in a mouse model of AD and increases learning-related gene expression in aged wild type mice,<sup>298</sup> suggesting epigenetic regulation of learning and memory.

Another recent study that looked at epigenetic signatures found that neuronal plasticity processes involved with learning and memory are dampened early in the onset of AD, while immune and inflammatory pathways are activated.<sup>299</sup>

Finally, a possible genetic source of excess beta amyloid (A $\beta$ ) has recently been discovered.<sup>300</sup> Amyloid precursor protein (APP), an integral membrane protein concentrated in the synapses of neurons, is best known as the precursor molecule whose proteolysis generates A $\beta$  (Section 2.4.1). APP is an ancient and highly conserved protein important in synaptic formation and repair, and in molecular transport inside neurons. In humans, the gene for APP is located on Chromosome 21 and contains 18 exons spanning 290 kilobases. Having an extra copy of the APP gene can cause rare “familial” Alzheimer’s disease, wherein the APP duplication can be passed on genetically and is present in all the cells of a patient’s body. By contrast, “sporadic” Alzheimer’s disease, which constitutes around 95% of AD cases, has been thought not to show any difference in the number of APP genes found in tissue samples, including whole brain. But the early studies that reported this were conducted without adequate appreciation of the possibility of brain “mosaicism” (i.e., when cells with varied DNA are present in the same person), and thus single neurons were not investigated.

A 2015 study<sup>301</sup> used five different types of experiments to examine the DNA content of single neurons for possible mosaicism. The researchers found that individual neurons from people with Alzheimer’s disease contained more DNA – on average, hundreds of millions of DNA base pairs

<sup>297</sup> Day JJ, Sweatt JD. Epigenetic mechanisms in cognition. *Neuron*. 2011 Jun 9;70(5):813-29; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3118503/>.

<sup>298</sup> Fischer A, Sananbenesi F, Wang X, Dobbin M, Tsai LH. Recovery of learning and memory is associated with chromatin remodelling. *Nature*. 2007 May 10;447(7141):178-82; <http://www.ncbi.nlm.nih.gov/pubmed/17468743>. Peleg S, Sananbenesi F, Zovoilis A, Burkhardt S, Bahari-Javan S, Agis-Balboa RC, Cota P, Wittnam JL, Gogol-Doering A, Opitz L, Salinas-Riester G, Dettenhofer M, Kang H, Farinelli L, Chen W, Fischer A. Altered histone acetylation is associated with age-dependent memory impairment in mice. *Science*. 2010 May 7;328(5979):753-56; <http://science.sciencemag.org/content/328/5979/753.long>.

<sup>299</sup> Gjonjeska E, Pfenning AR, Mathys H, Quon G, Kundaje A, Tsai LH, Kellis M. Conserved epigenomic signals in mice and humans reveal immune basis of Alzheimer’s disease. *Nature*. 2015 Feb 19;518(7539):365-9; <http://www.nature.com/nature/journal/v518/n7539/full/nature14252.html>.

<sup>300</sup> Bushman DM, Kaeser GE, Siddoway B, Westra JW, Rivera RR, Rehen SK, Yung YC, Chun J. Genomic mosaicism with increased amyloid precursor protein (APP) gene copy number in single neurons from sporadic Alzheimer’s disease brains. *Elife*. 2015 Feb 4;4; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4337608/>.

<sup>301</sup> Bushman DM, Kaeser GE, Siddoway B, Westra JW, Rivera RR, Rehen SK, Yung YC, Chun J. Genomic mosaicism with increased amyloid precursor protein (APP) gene copy number in single neurons from sporadic Alzheimer’s disease brains. *Elife*. 2015 Feb 4;4; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4337608/>.

more – and also more copies of the APP gene, with some neurons containing up to 12 copies. Cortical nuclei of Alzheimer’s brains displayed increased copy numbers (~3.8-4 copies) that are significantly higher than for control samples (~1.7-2.2 copies), with increased frequencies of high copy number nuclei (6 or more copies) primarily occurring in prefrontal cortex samples of AD brains. All these extra copies of the APP gene could cause overexpression of APP in all affected cells in the brain, leading, in turn, to overproduction of beta amyloid. The mosaicism itself could be caused (1) by a variety of environmental factors known to produce genetic damage, (2) by physical trauma, or even (3) by cellular aging.

## 2.4.6 Does Mercury Contribute to Alzheimer's Disease?

Mercury (Hg) has been used commercially and medically for centuries. In the past it was a common constituent of many medications. It is still sometimes found in hospitals in thermometers and blood-pressure cuffs and commercially in batteries, switches, and fluorescent light bulbs. Today, the exposure of the general human population comes from three major sources: fish consumption, dental amalgams, and vaccines. Each has its own characteristic form of mercury and distinctive toxicologic profile and clinical symptoms. Dental "silver" amalgams emit mercury vapor that is inhaled and absorbed into the bloodstream. Dentists and patients with amalgam fillings are exposed to this form of mercury. Liquid metallic mercury (quicksilver) still finds its way into homes, causing a risk of poisoning from the vapor and creating major cleanup costs. Humans are also exposed to two distinct but related organic forms, methyl mercury ( $\text{CH}_3\text{Hg}^+$ ) and ethyl mercury ( $\text{CH}_3\text{CH}_2\text{Hg}^+$ ). Fish are the main if not the only source of methyl mercury, since it is no longer used as a fungicide. In many countries, vaccinated babies are exposed to ethyl mercury which is the active ingredient of the preservative thimerosal used in vaccines.

While various forms of acute and chronic mercury poisoning have been known for centuries,<sup>302</sup> it has been alleged that the pathology of Alzheimer's disease (AD) might be caused or exacerbated by inorganic mercury.<sup>303</sup> While there appears to be no direct correlation between amalgam-filling status and AD,<sup>304</sup> amalgam exposure is certainly toxic for nerve cells *in vitro*.<sup>305</sup> One study found mercury blood levels in AD patients to be double those of controls and triple the control levels for early-onset AD cases, unrelated to the patient's dental amalgam status, and blood Hg levels also correlated with A $\beta$  levels in cerebrospinal fluid.<sup>306</sup> A few other studies confirmed elevated Hg concentrations in AD patient blood plasma.<sup>307</sup> As for brain tissue, mercury levels at autopsy are

---

<sup>302</sup> Rafati-Rahimzadeh M, Rafati-Rahimzadeh M, Kazemi S, Moghadamnia AA. Current approaches of the management of mercury poisoning: need of the hour. *Daru*. 2014 Jun 2;22:46; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4055906/>.

<sup>303</sup> Mutter J, Curth A, Naumann J, Deth R, Walach H. Does inorganic mercury play a role in Alzheimer's disease? A systematic review and an integrated molecular mechanism. *J Alzheimers Dis*. 2010;22(2):357-74; [http://www.alzheimer.org.ve/media/uploads/cyclope\\_old/adjuntos/Publicacioneninglesestudio.pdf](http://www.alzheimer.org.ve/media/uploads/cyclope_old/adjuntos/Publicacioneninglesestudio.pdf).

<sup>304</sup> Saxe SR, Wekstein MW, Kryscio RJ, Henry RG, Cornett CR, Snowdon DA, Grant FT, Schmitt FA, Donegan SJ, Wekstein DR, Ehmann WD, Markesbery WR. Alzheimer's disease, dental amalgam and mercury. *J Am Dent Assoc*. 1999 Feb;130(2):191-9; <http://www.ncbi.nlm.nih.gov/pubmed/10036842>.

<sup>305</sup> Lobner D, Asrari M. Neurotoxicity of dental amalgam is mediated by zinc. *J Dent Res*. 2003 Mar;82(3):243-6; <http://www.ncbi.nlm.nih.gov/pubmed/12598557>.

<sup>306</sup> Hock C, Drasch G, Golombowski S, Müller-Spahn F, Willershausen-Zönnchen B, Schwarz P, Hock U, Growdon JH, Nitsch RM. Increased blood mercury levels in patients with Alzheimer's disease. *J Neural Transm*. 1998;105(1):59-68; <http://www.ncbi.nlm.nih.gov/pubmed/9588761>.

<sup>307</sup> Basun H, Forssell LG, Wetterberg L, Winblad B. Metals and trace elements in plasma and cerebrospinal fluid in normal aging and Alzheimer's disease. *J Neural Transm Park Dis Dement Sect*. 1991;3(4):231-58;

usually higher in the grey matter, cerebellum, thalamus, putamen, and upper parietal and occipital lobes of AD brains than control brains,<sup>308</sup> and similarly in the amygdala and nucleus basalis of Meynert (nbM),<sup>309</sup> with the half-life of inorganic mercury in the brain probably on the order of several years to several decades.<sup>310</sup> Subcellular organelles, particularly microsomes (cell protein “factories”), can have elevated mercury levels in AD brain cells.<sup>311</sup>

The ability of Hg to increase amyloid A $\beta$  levels has been studied *in vitro* and *in vivo*, and the suggested mechanisms include an increased production, a reduced degradation, and/or a diminished brain clearance of the peptide. Olivieri *et al.*<sup>312</sup> reported increased secretion of both A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub> when neuroblastoma cells were exposed to 50  $\mu$ g/dL of inorganic Hg concomitant with reactive oxygen species (ROS) overproduction. A study conducted in aggregating brain-cell cultures of fetal rat telencephalon showed that methylHg (non-cytotoxic concentrations/10-50 days) produced increased APP levels accompanied by ROS production and glia activation.<sup>313</sup> Rat pheochromocytoma (PC12) cells exposed to both inorganic and organic (methylHg) mercury (10-1000 nM) showed a dose-dependent overproduction of A $\beta$ <sub>1-40</sub> (probably by an increase in APP levels) and a reduction in A $\beta$  degradation by neprilysin (NEP).<sup>314</sup> An increase in A $\beta$ <sub>1-42</sub> levels was observed in differentiated SH-SY5Y neuroblastoma cells exposed to

<http://www.ncbi.nlm.nih.gov/pubmed/1772577>. Gerhardsson L, Lundh T, Minthon L, Londos E. Metal concentrations in plasma and cerebrospinal fluid in patients with Alzheimer’s disease. *Dement Geriatr Cogn Disord*. 2008;25(6):508-15; <http://www.ncbi.nlm.nih.gov/pubmed/18463412/>.

<sup>308</sup> Ehmann WD, Markesbery WR, Alauddin M, Hossain TI, Brubaker EH. Brain trace elements in Alzheimer’s disease. *Neurotoxicology*. 1986 Spring;7(1):195-206; <http://www.ncbi.nlm.nih.gov/pubmed/3714121>.

<sup>309</sup> Thompson CM, Markesbery WR, Ehmann WD, Mao YX, Vance DE. Regional brain trace-element studies in Alzheimer’s disease. *Neurotoxicology*. 1988 Spring;9(1):1-7; <http://www.ncbi.nlm.nih.gov/pubmed/3393299>.

<sup>310</sup> Rooney JP. The retention time of inorganic mercury in the brain--a systematic review of the evidence. *Toxicol Appl Pharmacol*. 2014 Feb 1;274(3):425-35; <http://www.ncbi.nlm.nih.gov/pubmed/24368178>.

<sup>311</sup> Wenstrup D, Ehmann WD, Markesbery WR. Trace element imbalances in isolated subcellular fractions of Alzheimer’s disease brains. *Brain Res*. 1990 Nov 12;533(1):125-31; <http://www.ncbi.nlm.nih.gov/pubmed/2085723>.

<sup>312</sup> Olivieri G, Brack C, Müller-Spahn F, Stähelin HB, Herrmann M, Renard P, Brockhaus M, Hock C. Mercury induces cell cytotoxicity and oxidative stress and increases beta-amyloid secretion and tau phosphorylation in SHSY5Y neuroblastoma cells. *J Neurochem*. 2000 Jan;74(1):231-6; <http://www.ncbi.nlm.nih.gov/pubmed/10617124/>.

<sup>313</sup> Monnet-Tschudi F, Zurich MG, Boschat C, Corbaz A, Honegger P. Involvement of environmental mercury and lead in the etiology of neurodegenerative diseases. *Rev Environ Health*. 2006 Apr-Jun;21(2):105-17; <http://www.ncbi.nlm.nih.gov/pubmed/16898674/>.

<sup>314</sup> Song JW, Choi BS. Mercury induced the Accumulation of Amyloid Beta (A $\beta$ ) in PC12 Cells: The Role of Production and Degradation of A $\beta$ . *Toxicol Res*. 2013 Dec 31;29(4):235-40; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3936175/>.

Hg (10 and 20  $\mu\text{M}$ ) along with reduced activity of the A $\beta$ -degrading enzyme NEP.<sup>315</sup> The metals Zn, Cu and Fe show the highest potential to cause A $\beta$  aggregation, but Hg has no important effect.<sup>316</sup> Regarding *in vivo* studies, oral administration of 20-2000  $\mu\text{g}/\text{kg}/\text{day}/4$  weeks of methylHg produced a dose-dependent increase in A $\beta_{1-42}$  in the hippocampus of male rats, no significant changes in APP or NEP protein levels, and reduced brain expression of the LRP1 receptor (aka. apolipoprotein E receptor) which was positively correlated with increased A $\beta$  levels in the hippocampus and reduced levels in the CSF, suggesting a reduced clearance of the pathogenic peptide from the brain.<sup>317</sup>

Beyond the effects on A $\beta$  protein, *in vitro* studies have also found that mercury may interrupt polymerization of microtubules,<sup>318</sup> induce mitochondrial dysfunction,<sup>319</sup> change mitochondrial structure inducing a stress response in astrocytes,<sup>320</sup> and interfere with cell maturation<sup>321</sup> and other aspects of cell functioning including DNA repair,<sup>322</sup> intracellular calcium ( $\text{Ca}^{2+}$ )

---

<sup>315</sup> Chin-Chan M, Segovia J, Quintanar L, Arcos-López T, Hersh LB, Chow KM, Rodgers DW, Quintanilla-Vega B. Mercury Reduces the Enzymatic Activity of Neprilysin in Differentiated SH-SY5Y Cells. *Toxicol Sci.* 2015 May;145(1):128-37; <http://www.ncbi.nlm.nih.gov/pubmed/25673500>.

<sup>316</sup> Atwood CS, Moir RD, Huang X, Scarpa RC, Bacarra NM, Romano DM, Hartshorn MA, Tanzi RE, Bush AI. Dramatic aggregation of Alzheimer abeta by Cu(II) is induced by conditions representing physiological acidosis. *J Biol Chem.* 1998 May 22;273(21):12817-26; <http://www.jbc.org/content/273/21/12817.long>.

<sup>317</sup> Kim DK, Park JD, Choi BS. Mercury-induced amyloid-beta (A $\beta$ ) accumulation in the brain is mediated by disruption of A $\beta$  transport. *J Toxicol Sci.* 2014 Aug;39(4):625-35; [https://www.jstage.jst.go.jp/article/jts/39/4/39\\_625/pdf](https://www.jstage.jst.go.jp/article/jts/39/4/39_625/pdf).

<sup>318</sup> Bonacker D, Stoiber T, Wang M, Böhm KJ, Prots I, Unger E, Thier R, Bolt HM, Degen GH. Genotoxicity of inorganic mercury salts based on disturbed microtubule function. *Arch Toxicol.* 2004 Oct;78(10):575-83; <http://www.ncbi.nlm.nih.gov/pubmed/15205888>. Stoiber T, Bonacker D, Böhm KJ, Bolt HM, Thier R, Degen GH, Unger E. Disturbed microtubule function and induction of micronuclei by chelate complexes of mercury(II). *Mutat Res.* 2004 Oct 10;563(2):97-106; <http://www.ncbi.nlm.nih.gov/pubmed/15364276>.

<sup>319</sup> Carocci A, Rovito N, Sinicropi MS, Genchi G. Mercury toxicity and neurodegenerative effects. *Rev Environ Contam Toxicol.* 2014;229:1-18; <http://www.ncbi.nlm.nih.gov/pubmed/24515807>.

<sup>320</sup> Brawer JR, McCarthy GF, Gornitsky M, Frankel D, Mehindate K, Schipper HM. Mercuric chloride induces a stress response in cultured astrocytes characterized by mitochondrial uptake of iron. *Neurotoxicology.* 1998 Dec;19(6):767-76; <http://www.ncbi.nlm.nih.gov/pubmed/9863766>.

<sup>321</sup> Monnet-Tschudi F. Induction of apoptosis by mercury compounds depends on maturation and is not associated with microglial activation. *J Neurosci Res.* 1998 Aug 1;53(3):361-7; <http://www.ncbi.nlm.nih.gov/pubmed/9698164>.

<sup>322</sup> Palkiewicz P, Zwiers H, Lorscheider FL. ADP-ribosylation of brain neuronal proteins is altered by *in vitro* and *in vivo* exposure to inorganic mercury. *J Neurochem.* 1994 May;62(5):2049-52; <http://www.ncbi.nlm.nih.gov/pubmed/8158153>.

concentrations,<sup>323</sup> selenium metabolism,<sup>324</sup> neuroimmune signaling,<sup>325</sup> glutathione level (GSH bound to mercury does not function as an antioxidant),<sup>326</sup> and the linkage and structure of microtubules.<sup>327</sup> Mercury further disturbs the interaction between tubulin and GTP<sup>328</sup> and interferes with membrane structures, leading to axonal degeneration and neurofibrillary aggregates.<sup>329</sup>

It is fairly well-established that variations in apolipoprotein E or ApoE, a cholesterol-carrying blood protein, are related to the age of onset of AD.<sup>330</sup> In humans, ApoE comes in three different forms, ApoE2, ApoE3 and ApoE4 (Section 2.4.5). ApoE3 is the most common form in humans. The rarest is ApoE2, which seems to offer significant protection from Alzheimer's disease. Inheritance of ApoE4 is correlated with cerebral amyloid angiopathy, tauopathies, dementia with Lewy bodies, Parkinson's disease, multiple sclerosis, and a higher incidence (and significantly earlier onset) of Alzheimer's disease.<sup>331</sup> While the molecular basis for the correlation between

---

<sup>323</sup> Xu F, Farkas S, Kortbeek S, Zhang FX, Chen L, Zamponi GW, Syed NI. Mercury-induced toxicity of rat cortical neurons is mediated through N-Methyl-D-Aspartate receptors. *Mol Brain*. 2012 Sep 14;5:30; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3462706/>.

<sup>324</sup> Gailer J. Probing the bioinorganic chemistry of toxic metals in the mammalian bloodstream to advance human health. *J Inorg Biochem*. 2012 Mar;108:128-32; <http://www.ncbi.nlm.nih.gov/pubmed/22209021>.

<sup>325</sup> Thomas Curtis J, Chen Y, Buck DJ, Davis RL. Chronic inorganic mercury exposure induces sex-specific changes in central TNF $\alpha$  expression: importance in autism? *Neurosci Lett*. 2011 Oct 17;504(1):40-4; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3443965/>.

<sup>326</sup> Lee YW, Ha MS, Kim YK. Role of reactive oxygen species and glutathione in inorganic mercury-induced injury in human glioma cells. *Neurochem Res*. 2001 Nov;26(11):1187-93; <http://www.ncbi.nlm.nih.gov/pubmed/11874199>.

<sup>327</sup> Stoiber T, Degen GH, Bolt HM, Unger E. Interaction of mercury(II) with the microtubule cytoskeleton in IMR-32 neuroblastoma cells. *Toxicol Lett*. 2004 Jun 15;151(1):99-104; <http://www.ncbi.nlm.nih.gov/pubmed/15177645>.

<sup>328</sup> Khatoon S, Campbell SR, Haley BE, Slevin JT. Aberrant guanosine triphosphate-beta-tubulin interaction in Alzheimer's disease. *Ann Neurol*. 1989 Aug;26(2):210-5; <http://www.ncbi.nlm.nih.gov/pubmed/2774507>. Pendergrass JC, Haley BE, Vimy MJ, Winfield SA, Lorscheider FL. Mercury vapor inhalation inhibits binding of GTP to tubulin in rat brain: similarity to a molecular lesion in Alzheimer diseased brain. *Neurotoxicology*. 1997;18(2):315-24; <http://www.ncbi.nlm.nih.gov/pubmed/9291481>.

<sup>329</sup> Leong CC, Syed NI, Lorscheider FL. Retrograde degeneration of neurite membrane structural integrity of nerve growth cones following *in vitro* exposure to mercury. *Neuroreport*. 2001 Mar 26;12(4):733-7; <http://www.ncbi.nlm.nih.gov/pubmed/11277574>.

<sup>330</sup> Shi J, Han P, Kuniyoshi SM. Cognitive impairment in neurological diseases: lessons from apolipoprotein E. *J Alzheimers Dis*. 2014;38(1):1-9; <http://www.ncbi.nlm.nih.gov/pubmed/23948926>.

<sup>331</sup> Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science*. 1993 Aug 13;261(5123):921-3; <http://www.ncbi.nlm.nih.gov/pubmed/8346443/>.

ApoE4 and Alzheimer's disease is not firmly established, a unique feature of ApoE4, termed "domain interaction", may play a role. Studies have shown that arginine at position 112 causes amino acid side chain reorientation within the protein that promotes N- and C-terminal interaction via a unique salt bridge.<sup>332</sup> Domain interaction in ApoE4 induces a more compact structure with the arginine at position 112, increasing the "molten globule"-like properties of this isoform.<sup>333</sup>

But the "mercury argument" for Alzheimer's disease focuses on the ApoE cysteine substitutions, not the arginines. Cysteine is an amino acid with a sulfhydryl or "thiol" group (-SH) that strongly binds mercury ions.<sup>334</sup> The genetic differences between ApoE alleles involve substitutions between arginine and cysteine at positions 112 and 158 of the proteins (table, at right). All the other amino acids in the ApoE protein are identical. The protective form (ApoE2) has two cysteines at those positions, the common form (ApoE3) has one

ApoE <sup>2</sup> protein	<i>cys</i>	<i>cys</i>
ApoE <sup>3</sup> protein	<i>cys</i>	<i>arg</i>
ApoE <sup>4</sup> protein	<i>arg</i>	<i>arg</i>
	112	158

The Genetic ApoE Grid: Percent of US Population and Mean Age of Onset				Alzheimer's Risk vs Cysteine Residues at Positions 112 and 158			
		Maternal ApoE Allele			Maternal ApoE Allele		
		ApoE <sup>2</sup>	ApoE <sup>3</sup>	ApoE <sup>4</sup>	ApoE <sup>2</sup>	ApoE <sup>3</sup>	ApoE <sup>4</sup>
Paternal ApoE Allele	ApoE <sup>2</sup>	2 / 2 ( < 0.05% ) unknown onset age	2 / 3 ( ≈5.5% ) >90 onset age	2 / 4 ( ≈2.5% ) 80-90 onset age	4	3	2
	ApoE <sup>3</sup>	3 / 2 ( ≈5.5% ) >90 onset age	3 / 3 ( ≈60% ) 80-90 onset age	3 / 4 ( ≈10.5% ) 70-80 onset age	3	2	1
	ApoE <sup>4</sup>	4 / 2 ( ≈2.5% ) 80-90 onset age	4 / 3 ( ≈10.5% ) 70-80 onset age	4 / 4 ( ≈2% ) <70 onset age	2	1	0

cysteine and one arginine, and the increased-risk form (ApoE4) has two arginines. Cysteine can bind mercury but arginine cannot. Hence the cellular production of ApoE2, and ApoE3 to a lesser extent, could in principle carry mercury out of the brain neurons and into the cerebrospinal fluid, dumping it into the body for potential excretion. The number of cysteine residues in ApoE correlates perfectly with the observed age of AD onset and AD risks (charts, at left).<sup>335</sup>

In other words, ApoE4 may be a risk factor for AD because this protein does not have sulfhydryl groups to scavenge heavy metals like Hg, whereas ApoE2 has four SH groups and thus the

<sup>332</sup> Dong LM, Weisgraber KH. Human apolipoprotein E4 domain interaction. Arginine 61 and glutamic acid 255 interact to direct the preference for very low density lipoproteins. J Biol Chem. 1996 Aug 9;271(32):19053-7; <http://www.jbc.org/content/271/32/19053.long>. Mahley RW, Weisgraber KH, Huang Y. Apolipoprotein E: structure determines function, from atherosclerosis to Alzheimer's disease to AIDS. J Lipid Res. 2009 Apr;50 Suppl:S183-8; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2674716/>.

<sup>333</sup> "Molten globule" signifies an intermediate partially-folded protein state ([https://en.wikipedia.org/wiki/Molten\\_globule](https://en.wikipedia.org/wiki/Molten_globule)). Morrow JA, Hatters DM, Lu B, Hochtl P, Oberg KA, Rupp B, Weisgraber KH. Apolipoprotein E4 forms a molten globule. A potential basis for its association with disease. J Biol Chem. 2002 Dec 27;277(52):50380-5; <http://www.jbc.org/content/277/52/50380.long>.

<sup>334</sup> "Thiol"; <https://en.wikipedia.org/wiki/Thiol>

<sup>335</sup> Fowkes SW. Mercury toxicity and Alzheimer's disease. Smart Life News 2001;8(2); <http://ceri.com/alz72.htm>.

maximum ability to reduce the metal toxicity in the brain.<sup>336</sup> This factor alone could make ApoE2 a protective genotype for AD development.<sup>337</sup> Others have also observed a greater occurrence of the risky ApoE4 allele in patients with presumptive Hg-related neuropsychiatric symptoms with an elevated Hg body burden.<sup>338</sup>

It is widely accepted that Hg disrupts human brain development, produces cognitive and motor disabilities,<sup>339</sup> and produces memory loss and cognitive alterations in adults,<sup>340</sup> though a recent study found that serum Hg levels were not directly related to abnormal cognition in AD patients.<sup>341</sup> Limited evidence exists for an association of mercury amalgam dental fillings with multiple sclerosis,<sup>342</sup> but there are few studies on this issue for either Alzheimer's or Parkinson's diseases.<sup>343</sup> Better designed studies are needed, particularly for investigation of neurodegenerative diseases, in order to overcome the reticence of the mainstream medical community to more fully embrace the mercury theory of AD causation.<sup>344</sup>

---

<sup>336</sup> Mutter J, Naumann J, Sadaghiani C, Schneider R, Walach H. Alzheimer disease: mercury as pathogenetic factor and apolipoprotein E as a moderator. *Neuro Endocrinol Lett.* 2004 Oct;25(5):331-9; <http://www.ncbi.nlm.nih.gov/pubmed/15580166>.

<sup>337</sup> Suri S, Heise V, Trachtenberg AJ, Mackay CE. The forgotten APOE allele: a review of the evidence and suggested mechanisms for the protective effect of APOE ε2. *Neurosci Biobehav Rev.* 2013 Dec;37(10 Pt 2):2878-86; <http://www.ncbi.nlm.nih.gov/pubmed/24183852/>.

<sup>338</sup> Godfrey ME, Wojcik DP, Krone CA. Apolipoprotein E genotyping as a potential biomarker for mercury neurotoxicity. *J Alzheimers Dis.* 2003 Jun;5(3):189-95; <http://www.ncbi.nlm.nih.gov/pubmed/12897404>.

<sup>339</sup> Johansson C, Castoldi AF, Onishchenko N, Manzo L, Vahter M, Ceccatelli S. Neurobehavioural and molecular changes induced by methylmercury exposure during development. *Neurotox Res.* 2007 Apr;11(3-4):241-60; <http://www.ncbi.nlm.nih.gov/pubmed/17449462/>.

<sup>340</sup> Wojcik DP, Godfrey ME, Christie D, Haley BE. Mercury toxicity presenting as chronic fatigue, memory impairment and depression: diagnosis, treatment, susceptibility, and outcomes in a New Zealand general practice setting (1994-2006). *Neuro Endocrinol Lett.* 2006 Aug;27(4):415-23; <http://www.ncbi.nlm.nih.gov/pubmed/16891999/>. Chang JW, Pai MC, Chen HL, Guo HR, Su HJ, Lee CC. Cognitive function and blood methylmercury in adults living near a deserted chloralkali factory. *Environ Res.* 2008 Nov;108(3):334-9; <http://www.ncbi.nlm.nih.gov/pubmed/18675410/>.

<sup>341</sup> Park JH, Lee DW, Park KS, Joung H. Serum trace metal levels in Alzheimer's disease and normal control groups. *Am J Alzheimers Dis Other Demen.* 2014 Feb;29(1):76-83; <http://www.ncbi.nlm.nih.gov/pubmed/24164932>.

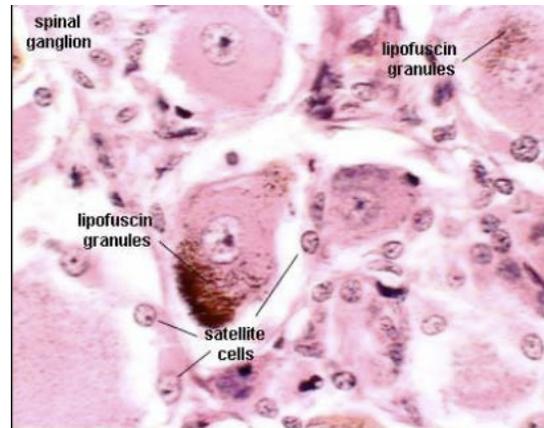
<sup>342</sup> Aminzadeh KK, Etminan M. Dental amalgam and multiple sclerosis: a systematic review and meta-analysis. *J Public Health Dent.* 2007 Winter;67(1):64-6; <http://www.ncbi.nlm.nih.gov/pubmed/17436982>.

<sup>343</sup> Bates MN. Mercury amalgam dental fillings: an epidemiologic assessment. *Int J Hyg Environ Health.* 2006 Jul;209(4):309-16; <http://www.ncbi.nlm.nih.gov/pubmed/16448848>.

<sup>344</sup> Clarkson TW, Magos L, Myers GJ. The toxicology of mercury – current exposures and clinical manifestations. *N Engl J Med.* 2003 Oct 30;349(18):1731-7; <http://www.dentalmercury.com/pdf/papers/Clarkson%20NEJM%20MeHg%20exposure.pdf>.

## 2.4.7 Lipofuscin Hypothesis of Alzheimer's Disease

Yellow-brown insoluble age-pigment lysosomal granules called "lipofuscin" collect in many of our cells. The accumulation of such lipochromes starts as early in life as 11 years old and rises with age,<sup>345</sup> activity level,<sup>346</sup> and caloric intake,<sup>347</sup> and varies with cell type.<sup>348</sup> Clumps of these yellow-brown autofluorescent granules – typically 1-3 microns in diameter – may occupy from 20% of brainstem neuron volume at age 20 to as much as 50% of cell volume by age 90.<sup>349</sup> Lipofuscin concentrations as high as 75% have been reported in Purkinje neurons of rats subjected to protein malnutrition.<sup>350</sup>



Lipofuscin granules are composed of lipid-containing residues of lysosomal digestion, usually arranged around the cell nucleus. Lipofuscin is likely the product of the oxidation of unsaturated fatty acids (e.g., lipid peroxidation products derived from malondialdehyde and glycation products)<sup>351</sup> and may be symptomatic of membrane damage or damage to mitochondria and

<sup>345</sup> Terman A, Brunk UT. Lipofuscin: mechanisms of formation and increase with age. *APMIS*. 1998 Feb;106(2):265-76; <http://www.ncbi.nlm.nih.gov/pubmed/9531959>.

<sup>346</sup> Basson AB, Terblanche SE, Oelofsen W. A comparative study on the effects of ageing and training on the levels of lipofuscin in various tissues of the rat. *Comp Biochem Physiol A Comp Physiol*. 1982;71(3):369-74; <http://www.ncbi.nlm.nih.gov/pubmed/6120780>.

<sup>347</sup> Moore WA, Davey VA, Weindruch R, Walford R, Ivy GO. The effect of caloric restriction on lipofuscin accumulation in mouse brain with age. *Gerontology*. 1995;41 Suppl 2:173-85; <http://www.ncbi.nlm.nih.gov/pubmed/8821330>.

<sup>348</sup> Harman D. Lipofuscin and ceroid formation: the cellular recycling system. *Adv Exp Med Biol*. 1989;266:3-15; <http://www.ncbi.nlm.nih.gov/pubmed/2486157>. Brunk UT, Jones CB, Sohal RS. A novel hypothesis of lipofuscinogenesis and cellular aging based on interactions between oxidative stress and autophagocytosis. *Mutat Res*. 1992 Sep;275(3-6):395-403; <http://www.ncbi.nlm.nih.gov/pubmed/1383780>.

<sup>349</sup> West CD. A quantitative study of lipofuscin accumulation with age in normals and individuals with Down's syndrome, phenylketonuria, progeria and transneuronal atrophy. *J Comp Neurol*. 1979 Jul 1;186(1):109-16; <http://www.ncbi.nlm.nih.gov/pubmed/156741>.

<sup>350</sup> James TJ, Sharma SP. Regional and lobular variation in neuronal lipofuscinosis in rat cerebellum: influence of age and protein malnourishment. *Gerontology*. 1995;41 Suppl 2:213-28; <http://www.ncbi.nlm.nih.gov/pubmed/8821333>.

<sup>351</sup> Horie K, Miyata T, Yasuda T, Takeda A, Yasuda Y, Maeda K, Sobue G, Kurokawa K. Immunohistochemical localization of advanced glycation end products, pentosidine, and

lysosomes. Aside from a large lipid content and crosslinked protein residues, lipofuscin is also known to contain sugars and metals such as mercury, aluminum, iron, copper and zinc.<sup>352</sup> Lipofuscin appears not to be readily naturally degradable.<sup>353</sup>

Lipofuscin, which means “dark fat” and is also known as “age pigment” or “lipopigment”, is currently defined operationally rather than structurally.<sup>354</sup> Its biochemical composition varies in different animal species, in different brain structures, and at different ages,<sup>355</sup> which suggests that there is not just one but many types of lipofuscin with similar tinctorial characteristics (e.g., autofluorescence).

In early studies, brain cell lipofuscin was thought to not be associated with mental<sup>356</sup> or motor<sup>357</sup> abnormalities or other detrimental cellular function.<sup>358</sup> However, more recently, abnormal accumulation of lipofuscin has been associated with a group of diseases of neurodegenerative disorder type called lipofuscinoses (Section 6.2.6), e.g., hereditary ceroid lipofuscinosis<sup>359</sup> or

carboxymethyllysine in lipofuscin pigments of Alzheimer’s disease and aged neurons. *Biochem Biophys Res Commun.* 1997 Jul 18;236(2):327-32; <http://www.ncbi.nlm.nih.gov/pubmed/9240434>.

<sup>352</sup> Terman A, Brunk UT. Lipofuscin. *Int J Biochem Cell Biol.* 2004 Aug;36(8):1400-4; <http://www.ncbi.nlm.nih.gov/pubmed/15147719>.

<sup>353</sup> Terman, A, Brunk, UT. On the degradability and exocytosis of ceroid/lipofuscin in cultured rat cardiac myocytes. *Mech Ageing Dev.* 1998 Jan 30;100(2):145-56; <http://www.ncbi.nlm.nih.gov/pubmed/9541135>. Brunk UT, Terman A. Lipofuscin: mechanisms of age-related accumulation and influence on cell function. *Free Radic Biol Med.* 2002 Sep 1;33(5):611-9; <http://www.ncbi.nlm.nih.gov/pubmed/12208347>.

<sup>354</sup> Brunk UT, Terman A. Lipofuscin: mechanisms of age-related accumulation and influence on cell function. *Free Radic Biol Med.* 2002 Sep 1;33(5):611-9; <http://www.ncbi.nlm.nih.gov/pubmed/12208347>. Gray DA, Woulfe J. Lipofuscin and aging: a matter of toxic waste. *Sci Aging Knowledge Environ.* 2005 Feb 2;2005(5):re1; <http://www.ncbi.nlm.nih.gov/pubmed/15689603>.

<sup>355</sup> Benavides SH, Monserrat AJ, Farina S, Porta EA. Sequential histochemical studies of neuronal lipofuscin in human cerebral cortex from the first to the ninth decade of life. *Arch Gerontol Geriatr.* 2002 May-Jun;34(3):219-31; <http://www.ncbi.nlm.nih.gov/pubmed/14764325>.

<sup>356</sup> West C. A quantitative study of lipofuscin accumulation with age in normals and individuals with Down’s syndrome, phenylketonuria, progeria and transneuronal atrophy. *J Comp Neurol.* 1979 Jul 1;186(1):109-16; <http://www.ncbi.nlm.nih.gov/pubmed/156741>. Drach LM, Bohl J, Goebel HH. The lipofuscin content of the inferior olivary nucleus in Alzheimer’s disease. *Dementia.* 1994 Sep-Oct;5(5):234-9; <http://www.ncbi.nlm.nih.gov/pubmed/7951678>.

<sup>357</sup> McHolm GB, Aguilar MJ, Norris FH. Lipofuscin in amyotrophic lateral sclerosis. *Arch Neurol.* 1984 Nov;41(11):1187-8; <http://www.ncbi.nlm.nih.gov/pubmed/6487103>.

<sup>358</sup> Davies I, Fotheringham A, Roberts C. The effect of lipofuscin on cellular function. *Mech Ageing Dev.* 1983 Nov-Dec;23(3-4):347-56; <http://www.ncbi.nlm.nih.gov/pubmed/6656318>.

<sup>359</sup> Shotelersuk V, Gahl WA (1998) Hermansky-Pudlak syndrome: models for intracellular vesicle formation. *Mol Genet Metab.* 1998 Oct;65(2):85-96; <http://www.ncbi.nlm.nih.gov/pubmed/9787100>.

neuronal ceroid-lipofuscinosis (NCL) disease (e.g., Batten disease<sup>360</sup>), which have been found to lead to premature death, with the ceroid apparently pathological only in neurons.<sup>361</sup>

Pathological accumulation of lipofuscin has also been implicated in Parkinson's disease,<sup>362</sup> autism,<sup>363</sup> and amyotrophic lateral sclerosis,<sup>364</sup> and more broadly in age-related macular degeneration,<sup>365</sup> acromegaly,<sup>366</sup> chronic obstructive pulmonary disease,<sup>367</sup> metabolic myopathy,<sup>368</sup> and melanosis coli<sup>369</sup> (caused by an accumulation of lipofuscin in the colon). Neuromelanin, a related neuronal pigment, has been only lightly studied in comparison to lipofuscin.<sup>370</sup>

---

<sup>360</sup> Elleder, M, Drahota, Z, Lisá V, Mares V, Mandys V, Müller J, Palmer DN. Tissue culture loading test with storage granules from animal models of neuronal ceroid-lipofuscinosis (Batten disease): testing their lysosomal degradability by normal and Batten cells. *Am J Med Genet.* 1995 Jun 5;57(2):213-21; <http://www.ncbi.nlm.nih.gov/pubmed/7668332>.

<sup>361</sup> Kida E, Golabek AA, Wisniewski KE. Cellular pathology and pathogenic aspects of neuronal ceroid lipofuscinoses. *Adv Genet.* 2001;45:35-68; <http://www.ncbi.nlm.nih.gov/pubmed/11332776>.

<sup>362</sup> Ulfig N. Altered lipofuscin pigmentation in the basal nucleus (Meynert) in Parkinson's disease. *Neurosci Res.* 1989 Jun;6(5):456-62; <http://www.ncbi.nlm.nih.gov/pubmed/2771203>.

<sup>363</sup> López-Hurtado E, Prieto JJ. A microscopic study of language-related cortex in autism. *Am J Biochem Biotechnol.* 2008;4:130-45; <http://docsdrive.com/pdfs/sciencepublications/ajbbsp/2008/130-145.pdf>.

<sup>364</sup> Bajaj NP, Al-Sarraj ST, Anderson V, Kibble M, Leigh N, Miller CC. Cyclin-dependent kinase-5 is associated with lipofuscin in motor neurones in amyotrophic lateral sclerosis. *Neurosci Lett.* 1998 Mar 27;245(1):45-8; <http://www.ncbi.nlm.nih.gov/pubmed/9596352>.

<sup>365</sup> Wolf G. Lipofuscin and macular degeneration. *Nutr Rev.* 2003 Oct;61(10):342-6; <http://www.ncbi.nlm.nih.gov/pubmed/14604266>. Novak JZ. Age-related macular degeneration (AMD): pathogenesis and therapy. *Pharmacol Rep.* 2006 May-Jun;58(3):353-63; [http://www.if-pan.krakow.pl/pjp/pdf/2006/3\\_353.pdf](http://www.if-pan.krakow.pl/pjp/pdf/2006/3_353.pdf).

<sup>366</sup> Khaleeli AA, Levy RD, Edwards RH, McPhail G, Mills KR, Round JM, Betteridge DJ. The neuromuscular features of acromegaly: a clinical and pathological study. *J Neurol Neurosurg Psychiatry.* 1984 Sep;47(9):1009-15; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1028006/>.

<sup>367</sup> Allaire J, Maltais F, LeBlanc P, Simard PM, Whittom F, Doyon JF, Simard C, Jobin J. Lipofuscin accumulation in the vastus lateralis muscle in patients with chronic obstructive pulmonary disease. *Muscle Nerve.* 2002 Mar;25(3):383-9; <http://www.ncbi.nlm.nih.gov/pubmed/11870715>.

<sup>368</sup> Feeney EJ, Austin S, Chien YH, Mandel H, Schoser B, Prater S, Hwu WL, Ralston E, Kishnani PS, Raben N. The value of muscle biopsies in Pompe disease: identifying lipofuscin inclusions in juvenile- and adult-onset patients. *Acta Neuropathol Commun.* 2014 Jan 2;2:2; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3892035/>.

<sup>369</sup> Freeman HJ. "Melanosis" in the small and large intestine. *World J Gastroenterol.* 2008 Jul 21;14(27):4296-9; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2731179/>.

<sup>370</sup> Some neurons also contain a pigment granule called neuromelanin, a brownish-black substance that is a byproduct of the synthesis of catecholamines in dopamine metabolism. At least one study found that AD brains produce less of this intraneuronal pigment than normal brains. Reyes MG, Faraldi F, Rydman R,

Interestingly, pathological accumulation of lipofuscin has also been implicated in Alzheimer's disease, starting with experiments and discussions in the 1980s,<sup>371</sup> the 1990s,<sup>372</sup> and the 2000s.<sup>373</sup> By 2011, the above evidence led Giaccone *et al.*<sup>374</sup> to propose a "lipofuscin hypothesis of Alzheimer's disease," which theorizes that the release of lipofuscin into the extracellular space following the death of neurons could substantially contribute to the formation of A $\beta$  senile plaques in Alzheimer's disease.

According to this hypothesis, following the death of neurons during aging (due to several unrelated causes), lipofuscin is set free in the neuropil where it cannot be rapidly degraded due to its biochemical characteristics. Therefore, the fate of this waste product may be to linger in the extracellular milieu, giving rise to a focal impairment in the tissue that may represent the starting point of the senile plaque. Unlike previous theories concerning the possible involvement of lipofuscin in the pathogenesis of AD,<sup>375</sup> Giaccone's hypothesis does not require that intraneuronal

Wang CC. Decreased nigral neuromelanin in Alzheimer's disease. *Neurol Res.* 2003 Mar;25(2):179-82; <http://www.ncbi.nlm.nih.gov/pubmed/12635519>.

<sup>371</sup> Dowson JH. Neuronal lipofuscin accumulation in ageing and Alzheimer dementia: a pathogenic mechanism? *Br J Psychiatry.* 1982 Feb;140:142-8; <http://www.ncbi.nlm.nih.gov/pubmed/7074296>. Mann DM, Yates PO, Marcyniuk B. Changes in nerve cells of the nucleus basalis of Meynert in Alzheimer's disease and their relationship to ageing and to the accumulation of lipofuscin pigment. *Mech Ageing Dev.* 1984 Apr-May;25(1-2):189-204; <http://www.ncbi.nlm.nih.gov/pubmed/6202988>. Amano N, Matsuishi T, Suzuki K, Yokoi S, Saitoh A, Yagishita S. Ultrastructural study of the neuronal lipofuscin – an autopsy case of familial Alzheimer's disease. *No To Shinkei.* 1984 Jul;36(7):657-63, in Japanese; <http://www.ncbi.nlm.nih.gov/pubmed/6487435>.

<sup>372</sup> Stojanovic A, Roher AE, Ball MJ. Quantitative analysis of lipofuscin and neurofibrillary tangles in the hippocampal neurons of Alzheimer disease brains. *Dementia.* 1994 Sep-Oct;5(5):229-33; <http://www.ncbi.nlm.nih.gov/pubmed/7951677>. Drach LM, Bohl J, Wach S, Schlote W, Goebel HH. Reduced intraneuronal lipofuscin content in dementia with Lewy bodies compared with Alzheimer's disease and controls. *Dement Geriatr Cogn Disord.* 1998 Jan-Feb;9(1):1-5; <http://www.ncbi.nlm.nih.gov/pubmed/9469257>.

<sup>373</sup> D'Andrea MR, Nagele RG, Gumula NA, Reiser PA, Polkovitch DA, Hertzog BM, Andrade-Gordon P. Lipofuscin and Abeta42 exhibit distinct distribution patterns in normal and Alzheimer's disease brains. *Neurosci Lett.* 2002 Apr 19;323(1):45-9; <http://www.ncbi.nlm.nih.gov/pubmed/11911987>. Mountjoy CQ, Dowson JH, Harrington C, Cairns MR, Wilton-Cox H. Characteristics of neuronal lipofuscin in the superior temporal gyrus in Alzheimer's disease do not differ from non-diseased controls: a comparison with disease-related changes in the superior frontal gyrus. *Acta Neuropathol.* 2005 May;109(5):490-6; <http://www.ncbi.nlm.nih.gov/pubmed/15759127>. Double KL, Dedov VN, Fedorow H, Kettle E, Halliday GM, Garner R, Brunk UT. The comparative biology of neuromelanin and lipofuscin in the human brain. *Cell Mol Life Sci.* 2008 Jun;65(11):1669-82; <http://www.ncbi.nlm.nih.gov/pubmed/18278576>.

<sup>374</sup> Giaccone G, Orsi L, Cupidi C, Tagliavini F. Lipofuscin hypothesis of Alzheimer's disease. *Dement Geriatr Cogn Dis Extra.* 2011 Jan;1(1):292-6; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3235942/>.

<sup>375</sup> Double KL, Dedov VN, Fedorow H, Kettle E, Halliday GM, Garner R, Brunk UT. The comparative biology of neuromelanin and lipofuscin in the human brain. *Cell Mol Life Sci.* 2008 Jun;65(11):1669-82; <http://www.ncbi.nlm.nih.gov/pubmed/18278576>.

lipofuscin is intrinsically harmful to neuronal cells, only that it may become so when it is released into the extracellular space.

While there are differences in the tinctorial properties and structures of lipofuscin and the amyloid of senile plaques, lipofuscin contains A $\beta$  and its precursor molecule.<sup>376</sup> Its relocation from the intra- to the extracellular compartment (together with the intervention of microglia, astrocytes and a robust neuroinflammatory response) could greatly modify its morphological, tinctorial and biochemical characteristics. This scenario would also reverse the most commonly held concept that the A $\beta$  peptide self-polymerizes over years to form senile plaques. In Giaccone's view, the lipofuscin released into the extracellular space may act as a source of A $\beta$  oligomers for a prolonged period of time.

Lipofuscin might provide the missing link between the factors that are known to be involved in the pathogenesis of AD (such as oxidative stress, mitochondrial dysfunction and the activation of innate immune responses)<sup>377</sup> and the senile plaques that represent its earliest microscopically visible structural alterations. Specifically, the rate of lipofuscin formation is closely related to oxidative stress.<sup>378</sup> Mitochondrial autophagocytosis is believed to be a major contributor to lipofuscin formation,<sup>379</sup> and mitochondria are the remnants of ancient bacterial intruders that have become symbionts of eukaryotic cells.<sup>380</sup> The hydrophobic and insoluble characteristics of lipofuscin correspond closely to those of substances that are most effective in inducing an innate immune response.<sup>381</sup> Finally, A $\beta$  deposition in AD occurs not only in the neuropil but also in vessel walls, and this distribution is consistent with the release of debris or waste products that follow the perivascular spaces when they are extruded from the cellular compartment.

---

<sup>376</sup> Bancher C, Grundke-Iqbal I, Iqbal K, Kim KS, Wisniewski HM. Immunoreactivity of neuronal lipofuscin with monoclonal antibodies to the amyloid beta-protein. *Neurobiol Aging*. 1989 Mar-Apr;10(2):125-32; <http://www.ncbi.nlm.nih.gov/pubmed/2657463>. Gómez-Ramos P, Asunción Morán M. Ultrastructural localization of intraneuronal Abeta-peptide in Alzheimer disease brains. *J Alzheimers Dis*. 2007 Mar;11(1):53-9; <http://www.ncbi.nlm.nih.gov/pubmed/17361035>.

<sup>377</sup> Castellani RJ, Lee H, Zhu X, Perry G, Smith MA. Alzheimer disease pathology as a host response. *J Neuropathol Exp Neurol*. 2008 Jun;67(6):523-31; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2763411/>.

<sup>378</sup> Brunk UT, Terman A. Lipofuscin: mechanisms of age-related accumulation and influence on cell function. *Free Radic Biol Med*. 2002 Sep 1;33(5):611-9; <http://www.ncbi.nlm.nih.gov/pubmed/12208347>.

<sup>379</sup> Gray DA, Woulfe J. Lipofuscin and aging: a matter of toxic waste. *Sci Aging Knowledge Environ*. 2005 Feb 2;2005(5):re1; <http://www.ncbi.nlm.nih.gov/pubmed/15689603>.

<sup>380</sup> Rotte C, Henze K, Muller M, Martin W. Origins of hydrogenosomes and mitochondria. *Curr Opin Microbiol*. 2000 Oct;3(5):481-6; [http://www.molevol.hhu.de/fileadmin/redaktion/Fakultaeten/Mathematisch-Naturwissenschaftliche\\_Fakultaet/Biologie/Institute/Molekulare\\_Evolution/Dokumente/Rotte\\_Curr\\_Opin\\_Microbiol\\_2000.pdf](http://www.molevol.hhu.de/fileadmin/redaktion/Fakultaeten/Mathematisch-Naturwissenschaftliche_Fakultaet/Biologie/Institute/Molekulare_Evolution/Dokumente/Rotte_Curr_Opin_Microbiol_2000.pdf).

<sup>381</sup> Seong SY, Matzinger P. Hydrophobicity: an ancient damage-associated molecular pattern that initiates innate immune responses. *Nat Rev Immunol*. 2004 Jun;4(6):469-78; <http://www.ncbi.nlm.nih.gov/pubmed/15173835>.

The aforementioned “lipofuscin hypothesis of AD” considers A $\beta$  deposition as a downstream phenomenon that is critical and essential but not an absolutely and invariably causative event in determining the onset of AD. It could be that in sporadic AD, A $\beta$  is more than an epiphenomenon because it is tightly linked to the release of lipofuscin in the neuropil. But that scenario differs when A $\beta$  deposition is induced artificially, e.g., in the transgenic mouse model of AD where large quantities of A $\beta$  do not induce significant neuronal degeneration.<sup>382</sup>

Lipofuscin is a matrix that recapitulates the insults and damage a neuron receives during the life of an individual. It seems likely that its composition and characteristics are influenced by various genetic and environmental factors that may reinforce or weaken each other and, in combination with factors that alter the chances of age-related neuronal death, may modulate the overall individual risk of developing AD.

As yet, no drug is known to eliminate intracellular lipofuscin from neurons. The drug piracetam<sup>383</sup> and grape seed flavanols<sup>384</sup> prevent ethanol-induced lipofuscin formation in rat neurons. The acetylhomocysteine thiolactone, a free radical scavenger drug able to activate the superoxide dismutase, shows a lytic effect on neuronal lipofuscin.<sup>385</sup> Centrophenoxine reduces lipofuscin deposits (studied both biochemically and histochemically), indicating that the drug inhibits lipofuscin accumulation by elevating the activity of the antioxidant enzymes.<sup>386</sup> Naturally-occurring lipofuscinolytic bacteria have also been argued to exist<sup>387</sup> but have not yet been isolated.

---

<sup>382</sup> Duyckaerts C, Potier M-C, Delatour B. Alzheimer disease models and human neuropathology: similarities and differences. *Acta Neuropathol.* 2008 Jan;115(1):5-38; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2100431/>.

<sup>383</sup> Paula-Barbosa MM, Brandão F, Pinho MC, Andrade JP, Madeira MD, Cadete-Leite A. The effects of piracetam on lipofuscin of the rat cerebellar and hippocampal neurons after long-term alcohol treatment and withdrawal: a quantitative study. *Alcohol Clin Exp Res.* 1991 Oct;15(5):834-8; <http://www.ncbi.nlm.nih.gov/pubmed/1755517>.

<sup>384</sup> de Freitas V, da Silva Porto P, Assunção M, Cadete-Leite A, Andrade JP, Paula-Barbosa MM. Flavonoids from grape seeds prevent increased alcohol-induced neuronal lipofuscin formation. *Alcohol Alcohol.* 2004 Jul-Aug;39(4):303-11; <http://alcalc.oxfordjournals.org/content/39/4/303.long>.

<sup>385</sup> Aloj Totáro E, Pisanti FA, Continillo A, Liberatori E. Morphological evaluation of the lipofuscinolytic effect of acetylhomocysteine thiolactone. *Arch Gerontol Geriatr.* 1985 Apr;4(1):67-72; <http://www.ncbi.nlm.nih.gov/pubmed/4004411>.

<sup>386</sup> Roy D, Pathak DN, Singh R. Effect of centrophenoxine on the antioxidative enzymes in various regions of the aging rat brain. *Exp Gerontol.* 1983;18(3):185-97; <https://www.ncbi.nlm.nih.gov/pubmed/6416880>.

<sup>387</sup> de Grey AD, Alvarez PJ, Brady RO, Cuervo AM, Jerome WG, McCarty PL, Nixon RA, Rittmann BE, Sparrow JR. Medical bioremediation: prospects for the application of microbial catabolic diversity to aging and several major age-related diseases. *Ageing Res Rev.* 2005 Aug;4(3):315-38; [https://www.researchgate.net/profile/W\\_Jerome/publication/7705795\\_Medical\\_bioremediation\\_prospects\\_for\\_the\\_application\\_of\\_microbial\\_catabolic\\_diversity\\_to\\_aging\\_and\\_several\\_major\\_age-related\\_diseases/links/004635268741f62fab000000.pdf](https://www.researchgate.net/profile/W_Jerome/publication/7705795_Medical_bioremediation_prospects_for_the_application_of_microbial_catabolic_diversity_to_aging_and_several_major_age-related_diseases/links/004635268741f62fab000000.pdf).

Clinical trials using stem cells to replace lysosomal enzyme deficiencies in human patients with neuronal ceroid lipofuscinosis have shown no evidence of success.<sup>388</sup> However, in one study a tetrahydropyridoethers-class small molecule administered to monkeys caused retinal pigment epithelial cells to significantly release lipofuscin, and macrophages that had taken up lipofuscin were found in 4 eyes.<sup>389</sup> Four classes of therapeutics are now being investigated as potential treatments for inhibition of lipofuscin formation in the retina: direct inhibitors of key visual cycle enzymes, RBP4 antagonists, primary amine-containing aldehyde traps, and deuterated analogs of vitamin A.<sup>390</sup>

---

<sup>388</sup> Selden NR, Al-Uzri A, Huhn SL, Koch TK, Sikora DM, Nguyen-Driver MD, Guillaume DJ, Koh JL, Gultekin SH, Anderson JC, Vogel H, Sutcliffe TL, Jacobs Y, Steiner RD. Central nervous system stem cell transplantation for children with neuronal ceroid lipofuscinosis. *J Neurosurg Pediatr.* 2013 Jun;11(6):643-52; <http://www.ncbi.nlm.nih.gov/pubmed/23581634>.

<sup>389</sup> Julien S, Schraermeyer U. Lipofuscin can be eliminated from the retinal pigment epithelium of monkeys. *Neurobiol Aging.* 2012 Oct;33(10):2390-7; <http://www.ncbi.nlm.nih.gov/pubmed/22244091>.

<sup>390</sup> Petrukhin K. Pharmacological inhibition of lipofuscin accumulation in the retina as a therapeutic strategy for dry AMD treatment. *Drug Discov Today Ther Strateg.* 2013;10(1):e11-e20; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4140438/>

## 2.4.8 A Synoptic View of the Etiology of Alzheimer's Disease

As noted at the start of Section 2.4, the precise cause of Alzheimer's disease has not yet been firmly established. We have reviewed amyloid A $\beta$  (Section 2.4.1) and tau protein (Section 2.4.2) oligomerization and aggregation, along with many other molecular pathologies (Section 2.4.3), natural aging (Section 2.4.4), genetics (Section 2.4.5), mercury poisoning (Section 2.4.6), and lipofuscin (Section 2.4.7) – each representing a piece of the overall puzzle of AD. But what is the ultimate cause of AD, the farthest-upstream “first mover” that leads to pathology? If we could identify and eliminate this cause, we might have a preventative treatment for Alzheimer's disease.

In this Section, we present a synoptic perspective that attempts to combine current knowledge with the best recent thinking of AD theorists to create a coherent picture of the upstream etiology of late-onset Alzheimer's disease. The perspective presented here draws heavily from the model of AD recently developed by the Lahiri group at the Indiana University School of Medicine in 2011 and 2014.<sup>391</sup> This view is almost certainly neither complete nor the final answer, but the framework is at least self-consistent and appears to encompass most of the known observables of AD.

The onset of aging affects pathways that control the processing and degradation of abnormal proteins, including A $\beta$ . It is therefore hypothesized that the most upstream cause of Alzheimer's disease is the natural process of aging and its disorganizing effects on cellular protein homeostasis, aka. “proteostasis”. Cellular proteostasis requires maintaining the proper balance between competing processes of protein production (proteosynthesis) and protein degradation (proteolysis). If cells produce too much or too little of a given protein, or degrade and remove too much or too little of a given protein, problems can develop. As we age, proteostasis begins to fail, eventually allowing AD or other potentially lethal degenerative conditions to develop.

Age-related decline in overall proteolytic activity has been observed in almost all organisms studied, and specific age-related defects in the different proteolytic systems have been reported.<sup>392</sup> The molecular basis of this age-related decline seems to offer a plausible explanation for the late-onset observed in many neurodegenerative diseases.<sup>393</sup>

---

<sup>391</sup> Bailey JA, Maloney B, Ge YW, Lahiri DK. Functional activity of the novel Alzheimer's amyloid  $\beta$ -peptide interacting domain (A $\beta$ ID) in the APP and BACE1 promoter sequences and implications in activating apoptotic genes and in amyloidogenesis. *Gene*. 2011 Nov 15;488(1-2):13-22; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3372404/>. Perez FP, Bose D, Maloney B, Nho K, Shah K, Lahiri DK. Late-onset Alzheimer's disease, heating up and foxed by several proteins: pathomolecular effects of the aging process. *J Alzheimers Dis*. 2014;40(1):1-17; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4126605/>.

<sup>392</sup> Martinez-Vicente M, Sovak G, Cuervo AM. Protein degradation and aging. *Exp Gerontol*. 2005 Aug-Sep;40(8-9):622-33; <http://www.einstein.yu.edu/cuervo/images/EGMartinez-Vicente.pdf>.

<sup>393</sup> Levine B, Klionsky DJ. Development by self-digestion: molecular mechanisms and biological functions of autophagy. *Dev Cell*. 2004 Apr;6(4):463-77; [http://www.cell.com/developmental-cell/pdf/S1534-5807\(04\)00099-1.pdf](http://www.cell.com/developmental-cell/pdf/S1534-5807(04)00099-1.pdf).

One of the earliest events in the aging process – the collapse of proteostasis – is believed to be led by a sharp decline of the Heat Shock Response<sup>394</sup> as observed in cell cultures and animal studies.<sup>395</sup> In humans, a slow attenuation of the Heat Shock Response begins after the third or fourth decade of life.<sup>396</sup> (This attenuation can also be enhanced by environmental stressors.) After the onset of the attenuation of the Heat Shock Response, abnormal protein accumulation slowly occurs<sup>397</sup> including extracellular A $\beta$  accumulation in plaques after the age of 30 years.<sup>398</sup> (A $\beta$  is produced naturally throughout the human body and serves dozens of normal physiological functions; see **Appendix A**.) Following a couple of decades of excess A $\beta$  accumulation, the clinical signs of dementia most commonly begin to be observed when individuals are over 50 years of age.<sup>399</sup>

---

<sup>394</sup> **Heat Shock Response** is the cellular response to “heat shock” that includes the transcriptional up-regulation of genes encoding **heat shock proteins** (HSPs) as part of the cell’s internal repair mechanism. HSPs are also called “stress-proteins” because, in addition to excessive heat they also respond to excessive cold and oxygen deprivation by activating several cascade pathways. (The appearance of the word “heat” in the name HSP is in part an historical accident and can be somewhat misleading to the uninitiated.) HSPs are also present in cells under perfectly normal conditions, serving, for example, as molecular chaperones which ensure that the cell’s proteins are folded into the right shape and are present in the right place at the right time. Such HSPs can help new or misfolded proteins to fold into their correct three-dimensional conformations, which is essential for their function, while other HSPs can shuttle proteins from one compartment to another inside the cell, and target old or terminally misfolded proteins to proteases for degradation. HSPs are also believed to play a role in the presentation of pieces of proteins (or peptides) on the cell surface to help the immune system recognize diseased cells.

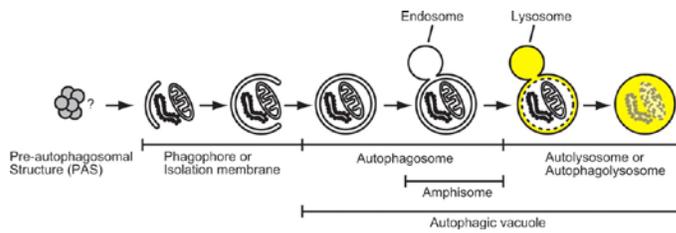
<sup>395</sup> Locke M, Tanguay RM. Diminished heat shock response in the aged myocardium. *Cell Stress Chaperones*. 1996;1:251-260; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC376462/>. Verbeke P, Fonager J, Clark BF, Rattan SI. Heat shock response and ageing: mechanisms and applications. *Cell Biol Int*. 2001;25:845-857; <http://www.ncbi.nlm.nih.gov/pubmed/11518492>. Ben-Zvi A, Miller EA, Morimoto RI. Collapse of proteostasis represents an early molecular event in *Caenorhabditis elegans* aging. *Proc Natl Acad Sci U S A*. 2009;106:14914-9; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2736453/>. Volovik Y, Maman M, Dubnikov T, Bejerano-Sagie M, Joyce D, Kapernick EA, Cohen E, Dillin A. Temporal requirements of heat shock factor-1 for longevity assurance. *Aging Cell*. 2012;11:491-9; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4349560/>.

<sup>396</sup> Jurivich DA, Qiu L, Welk JF. Attenuated stress responses in young and old human lymphocytes. *Mech Ageing Dev*. 1997;94:233-249; <http://www.ncbi.nlm.nih.gov/pubmed/9147375>.

<sup>397</sup> Verbeke P, Fonager J, Clark BF, Rattan SI. Heat shock response and ageing: mechanisms and applications. *Cell Biol Int*. 2001;25:845-857; <http://www.ncbi.nlm.nih.gov/pubmed/11518492>.

<sup>398</sup> Nistor M, Don M, Parekh M, Sarsoza F, Goodus M, Lopez GE, Kawas C, Leverenz J, Doran E, Lott IT, Hill M, Head E. Alpha- and beta-secretase activity as a function of age and beta-amyloid in Down syndrome and normal brain. *Neurobiol Aging*. 2007;28:1493-1506; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3375834/>.

<sup>399</sup> Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, Iwatsubo T, Jack CR, Jr, Kaye J, Montine TJ, Park DC, Reiman EM, Rowe CC, Siemers E, Stern Y, Yaffe K, Carrillo MC, Thies B, Morrison-Bogorad M, Wagster MV, Phelps CH. Toward defining the preclinical stages of Alzheimer’s disease: recommendations from the National Institute on Aging-Alzheimer’s Association workgroups on



Returning to the causal sequence: The initial Heat Shock Response attenuation leads to decreased degradation of A $\beta$ , facilitated by proteasome<sup>400</sup> dysfunction, decreased production of molecular chaperones<sup>401</sup>, and dysfunction of

chaperone-mediated autophagy,<sup>402</sup> all of which produces increased intracellular A $\beta$ . These increased intracellular A $\beta$  levels activate inefficient compensatory autophagy systems involving autophagic vacuoles or phagosomes<sup>403</sup> (image, above)<sup>404</sup>. The increase in autophagy cannot be sustained and the excess intracellular A $\beta$  is released into the extracellular space from vacuoles accumulating in the cytoplasm. This extracellular A $\beta$ , now tagged with ubiquitin,<sup>405</sup> can form

diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 2011;7:280-292; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3220946/>.

<sup>400</sup> **Proteasomes** are protein complexes inside human cells, whose main function is to degrade unneeded or damaged proteins by proteolysis, a chemical reaction that breaks peptide bonds. See: <https://en.wikipedia.org/wiki/Proteasome>.

<sup>401</sup> **Chaperones** are proteins that assist the covalent folding or unfolding and the assembly or disassembly of other macromolecular structures. Chaperones are concerned primarily with protein folding. One major function is to prevent both newly synthesized polypeptide chains and assembled subunits from aggregating into nonfunctional structures. Thus many (but not all) chaperones are heat shock proteins because the tendency to aggregate increases as proteins are "denatured" (destruction of native folded structure) by stress. Some chaperone systems work as foldases, supporting the folding of proteins in an ATP-dependent manner, while other chaperones work as holdases, binding folding intermediates to prevent their aggregation. Others are involved in transport across membranes, assisting in protein degradation, bacterial adhesin activity, and in responding to diseases linked to protein aggregation. See: [https://en.wikipedia.org/wiki/Chaperone\\_\(protein\)](https://en.wikipedia.org/wiki/Chaperone_(protein)).

<sup>402</sup> **Chaperone-mediated autophagy** refers to the chaperone-dependent selection of soluble cytosolic proteins that are then targeted to lysosomes and directly translocated across the lysosome membrane for degradation. (A lysosome is a spherical membrane-bound cell organelle found in most animal cells, usually containing more than 50 different enzymes capable of breaking down virtually all kinds of biomolecules including proteins, nucleic acids, carbohydrates, lipids, and most cellular debris.) The unique features of this type of autophagy are the selectivity on the proteins that are degraded by this pathway and the direct shuttling of these proteins across the lysosomal membrane without the requirement for the formation of additional vesicles. See: [https://en.wikipedia.org/wiki/Chaperone-mediated\\_autophagy](https://en.wikipedia.org/wiki/Chaperone-mediated_autophagy).

<sup>403</sup> A **phagosome** is a vesicle formed around a particle absorbed by phagocytosis. The vacuole is formed by the fusion of the cell membrane around the particle. A phagosome is a cellular compartment in which, e.g., pathogenic microorganisms can be killed and digested. Phagosomes fuse with lysosomes in their maturation process, forming phagolysosomes. See: <https://en.wikipedia.org/wiki/Phagosome>.

<sup>404</sup> Mizushima N. Autophagy: process and function. *Genes Dev.* 2007 Nov 15;21(22):2861-73; <http://genesdev.cshlp.org/content/21/22/2861.long>.

<sup>405</sup> **Ubiquitin** is a small (8.5 kDa) regulatory protein found in almost all human tissues. Ubiquitination is a post-translational modification (an addition to a protein after it has been made) wherein ubiquitin is

toxic oligomers, which in turn can produce reactive oxygen species. Keep in mind that this is a slow cascade of biochemical events that typically takes years, even decades, to fully unfold.

The increasing concentration of toxic A $\beta$  oligomers activates several “backup” systems, including those activated by multifunctional nuclear transcription factors such as FoxO3<sup>406</sup> (and other FoxO family members) which attempt to ameliorate the problem by converting the toxic oligomers into non-toxic neuritic plaques, thus preventing neurodegeneration that might otherwise occur in the presence of these A $\beta$  oligomers. FOXO3, among its multiple functions, catalyzes formation of amyloid plaque from A $\beta$ .<sup>407</sup> Extracellular non-plaque A $\beta$  is also cleared by enzymes such as insulin-degrading enzyme. These ameliorative processes explain the temporary lag between plaque formation and clinical AD symptoms.

Unfortunately, the continuing aging-related failing proteostasis eventually begins to affect the “backup” systems as well. After sufficient dysfunction has accumulated, the attenuation of, e.g., FoxO3, is initiated. Inflammatory/oxidative stress from increasing amounts of reactive oxygen species directs FoxO3 toward the nucleus, an apoptotic pathway that can ultimately trigger cell suicide. This redirection of FoxO3 reduces the production of amyloid plaque, allowing greater formation of toxic extracellular A $\beta$  oligomers and interfering with enzymatic clearance of extracellular A $\beta$ , permitting plaque buildup even though overall plaque catalysis has been somewhat reduced. The combination of neuronal cell death and extracellular events finally leads to clinical AD symptoms.

The cognitive symptoms of clinical AD are first driven by synaptic failure.<sup>408</sup> In mild AD, there is a reduction of the presynaptic vesicle protein synaptophysin.<sup>409</sup> With advancing disease, synapses are disproportionately lost relative to neurons, and this loss is the best correlate with

attached to a substrate protein, usually tagging it for degradation via the proteasome. The addition of ubiquitin can affect proteins in other ways as well, e.g., alter their cellular location, affect their activity, or promote or prevent protein interactions. See: <https://en.wikipedia.org/wiki/Ubiquitin>.

<sup>406</sup> **FoxO3** (Forkhead box O3 protein) is a human protein, encoded by the *FoxO3* gene, that belongs to the O subclass of the forkhead family of transcription factors which are characterized by a distinct forkhead DNA-binding domain. This protein likely functions as a trigger for apoptosis through upregulation of genes necessary for cell death or downregulation of anti-apoptotic proteins, and it is thought that FoxO3 is also involved in protection from oxidative stress by upregulating antioxidants such as catalase and MnSOD. FOXO3 also regulates neural stem cell homeostasis (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2775802/>). A variant of FoxO3 has been shown to be associated with longevity in humans. See: <https://en.wikipedia.org/wiki/FOXO3>.

<sup>407</sup> Perez FP, Bose D, Maloney B, Nho K, Shah K, Lahiri DK. Late-onset Alzheimer’s disease, heating up and foxed by several proteins: pathomolecular effects of the aging process. *J Alzheimers Dis.* 2014;40(1):1-17; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4126605/>.

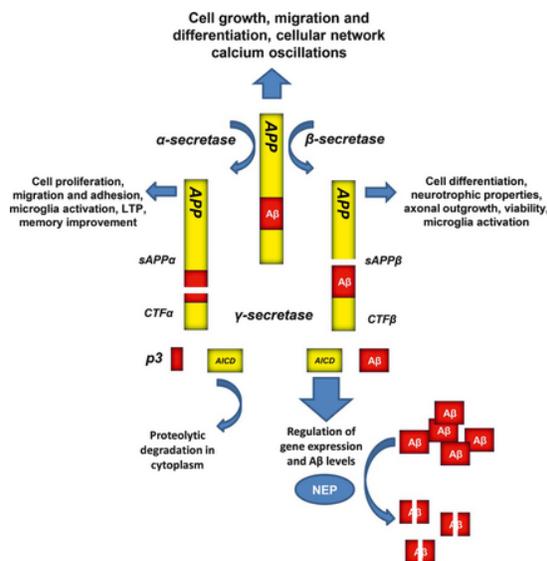
<sup>408</sup> Selkoe DJ. Alzheimer’s disease is a synaptic failure. *Science.* 2002 Oct 25;298(5594):789-91; <http://www.columbia.edu/cu/biology/faculty/kelley/city-seminar/papers/Selkoe.pdf>.

<sup>409</sup> Masliah E, Mallory M, Alford M, DeTeresa R, Hansen LA, McKeel DW, Jr, Morris JC. Altered expression of synaptic proteins occurs early during progression of Alzheimer’s disease. *Neurology.* 2001 Jan 9;56(1):127-9; <http://www.neurology.org/content/56/1/127.long>.

dementia.<sup>410</sup> A $\beta$  oligomers mediate synaptic dysfunction by binding and self-association to structures (e.g., membrane receptors, mitochondria) in a manner that leads to synaptic dysfunction and memory impairment.<sup>411</sup>

While A $\beta$  accumulation is the earliest event directly in the development of AD, this alone is not sufficient to produce the clinical AD syndrome. The cognitive decline of AD occurs only in the setting of synaptic dysfunction and/or additional neurodegeneration, including paired helical filaments (PHFs), aka. neurofibrillary tangles, from tau protein, accompanied by neuronal loss.<sup>412</sup> Evidence also suggests that additional factors, such as cognitive reserve, white matter alterations, dopaminergic depletion, cerebrovascular disease, and Lewy bodies, alter the relationship between the neuropathological and clinical manifestations of AD.<sup>413</sup>

There may also exist one or more multifactorial feedback loops that can trigger excessive intracellular A $\beta$  production, greatly speeding the patient's decline into clinical manifestations of Alzheimer's disease, as described in the next three paragraphs.



To briefly review the source of A $\beta$ , the neuron transmembrane protein called amyloid precursor protein (APP) is normally processed along two alternative pathways (image, left): first, a predominant (>90%) **non-amyloidogenic pathway** (on the left) in which APP is successively cleaved by  $\alpha$ -secretase and  $\gamma$ -secretase, which precludes production of A $\beta$ ; and, second, a minor **amyloidogenic pathway** (on the right) in which APP is cleaved by  $\beta$ - and  $\gamma$ -secretases releasing A $\beta$  peptide. Soluble, extracellular domains of APP are also released following the actions of  $\alpha$ -secretase (sAPP $\alpha$ ) and  $\beta$ -secretase (sAPP $\beta$ ). The various metabolites of APP, including A $\beta$  (see **Appendix A**), all have distinct physiological roles. In both catabolic

<sup>410</sup> Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann Neurol.* 1991 Oct;30(4):572-580; <http://www.ncbi.nlm.nih.gov/pubmed/1789684>.

<sup>411</sup> Puzzo D, Privitera L, Leznik E, Fa M, Staniszewski A, Palmeri A, Arancio O. Picomolar amyloid-beta positively modulates synaptic plasticity and memory in hippocampus. *J Neurosci.* 2008;28:14537-14545; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2673049/>.

<sup>412</sup> Filipcik P, Zilka N, Bugos O, Kucerak J, Koson P, Novak P, Novak M. First transgenic rat model developing progressive cortical neurofibrillary tangles. *Neurobiol Aging.* 2012;33:1448-1456; <http://www.ncbi.nlm.nih.gov/pubmed/21196063>.

<sup>413</sup> Perez FP, Bose D, Maloney B, Nho K, Shah K, Lahiri DK. Late-onset Alzheimer's disease, heating up and foxed by several proteins: pathomolecular effects of the aging process. *J Alzheimers Dis.* 2014;40(1):1-17; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4126605/>.

pathways, the  $\gamma$ -secretase-mediated intramembrane cleavage of APP, after  $\alpha$ - or  $\beta$ -secretase action, releases the APP intracellular domain (AICD), which is able to regulate transcription of several genes, including APP itself, the  $\beta$ -secretase enzyme (aka. BACE1)<sup>414</sup> and the A $\beta$ -degrading enzyme neprilysin (NEP).<sup>415</sup>

Bailey *et al.*<sup>416</sup> suggest that the A $\beta$  peptide may regulate its own production through feedback on its precursor protein and BACE1, leading to amyloidogenesis in Alzheimer's disease, with the A $\beta$  peptide functioning as a transcription factor<sup>417</sup> or co-factor inside the cell nucleus. In addition to other its functions (**Appendix A**), the A $\beta$  peptide directs normal apoptosis as well as regulating its own production through feedback on its precursor, APP, and the  $\beta$ -secretase enzyme, which would have pathological consequences relevant to AD. In this cytotoxic model, normal cytoprotective activity of A $\beta$ , such as protection against metal-induced oxidative stress,<sup>418</sup> eventually results in increasing A $\beta$  levels crossing a pathogenic concentration threshold, thus pushing production of APP and BACE1 to pathological levels and initiating a positive feedback loop. Higher levels of BACE1 protein would then favor greater production of A $\beta$ , and higher levels of APP protein would in turn provide more substrate. This combination would result in greater A $\beta$  production, which would then stimulate the production of proapoptotic proteins, further increasing the rate of cell death. Thus, A $\beta$ 's proposed activity as either transcription factor or co-factor would lead to an accumulation of excess A $\beta$  as toxic extracellular amyloid plaque, which is the hallmark of late-onset AD. In this view, A $\beta$  has a cytoprotective function, but under conditions of slowly growing stress – as occurs progressively during aging – the cell eventually crosses a threshold and A $\beta$  production accelerates up to the maximum runaway limits of the available substrate. A similar runaway feedback loop has already been proposed by another research group in cases of familial AD.<sup>419</sup>

---

<sup>414</sup> “BACE1” is the acronym for Beta-site APP Cleaving Enzyme 1.

<sup>415</sup> Octave JN, Pierrot N, Ferao Santos S, Nalivaeva NN, Turner AJ. From synaptic spines to nuclear signaling: nuclear and synaptic actions of the amyloid precursor protein. *J Neurochem*. 2013 Jul;126(2):183-90; <http://onlinelibrary.wiley.com/doi/10.1111/jnc.12239/full>.

<sup>416</sup> Bailey JA, Maloney B, Ge YW, Lahiri DK. Functional activity of the novel Alzheimer's amyloid  $\beta$ -peptide interacting domain (A $\beta$ ID) in the APP and BACE1 promoter sequences and implications in activating apoptotic genes and in amyloidogenesis. *Gene*. 2011 Nov 15;488(1-2):13-22; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3372404/>.

<sup>417</sup> A **transcription factor** is a protein that binds to specific DNA sequences, thereby controlling the rate of transcription of genetic information from DNA to messenger RNA, which in turn can instruct ribosomes to synthesize specific proteins.

<sup>418</sup> e.g., oxidative stress can induce uptake of extracellular A $\beta$  peptide into neuronal cells. Bailey JA, Maloney B, Ge YW, Lahiri DK. Functional activity of the novel Alzheimer's amyloid  $\beta$ -peptide interacting domain (A $\beta$ ID) in the APP and BACE1 promoter sequences and implications in activating apoptotic genes and in amyloidogenesis. *Gene*. 2011 Nov 15;488(1-2):13-22; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3372404/>.

<sup>419</sup> “We describe a positive loop that exists between the secretases that are responsible for the generation of the amyloid- $\beta$  component of Alzheimer disease. According to our hypothesis, in familial Alzheimer disease, the primary overproduction of amyloid- $\beta$  can induce BACE1 transcription and drive a further increase of amyloid- $\beta$  precursor protein processing and resultant amyloid- $\beta$  production. In sporadic

Oxidative stress alone may likewise lead to A $\beta$  overproduction. Both amyloid deposits and soluble A $\beta$  seem to drive the accumulation of reactive oxygen species.<sup>420</sup> Oxidative stress is itself able to induce the increased generation of A $\beta$  species and A $\beta$ PP processing.<sup>421</sup> And yet another possible feedback loop may be operative: incomplete degradation of proteins that reduces the degradative ability of the proteasome, causing protein recycling ability to decline further. This could produce highly amyloidogenic fragments that rapidly induce the aggregation of full-length protein; the aggregates, in turn, reduce proteasome activity, leading to further accumulation of fragments, “creating a vicious cycle of cytotoxicity”.<sup>422</sup> Other related “vicious cycles” in Alzheimer’s disease have also been identified.<sup>423</sup>

Similar considerations may apply to tau protein. The production of neurofibrillary tangles from hyperphosphorylated tau protein could be aging-related. As with A $\beta$ , described above, the phosphorylation of tau can be linked to the altered protein turnover and failing proteolysis that occurs during aging. For example, the detection of ubiquitin immunoreactivity in tau inclusions

Alzheimer disease, many factors, among them oxidative stress and inflammation, with consequent induction of presenilins and BACE1, would activate a loop and proceed with the generation of amyloid- $\beta$  and its signaling role onto BACE1 transcription. According to our hypothesis, in familial AD the primary overproduction of A $\beta_{42}$  can induce BACE1 transcription, and determine a further increase of A $\beta$ PP processing and of amyloid production. In sporadic AD, one of many causal factors, such as oxidative stress and inflammation, can determine a primary induction of PS1/Pen2 and of BACE1, and the loop proceeds with the generation of A $\beta_{42}$  and its signaling to BACE1 transcription.” Tabaton M, Zhu X, Perry G, Smith MA, Giliberto L. Signaling effect of amyloid-beta(42) on the processing of AbetaPP. *Exp Neurol*. 2010 Jan;221(1):18-25; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2812589/>.

<sup>420</sup> Behl C. Oxidative stress in Alzheimer’s disease: implications for prevention and therapy. *Subcell Biochem*. 2005;38:65-78; <http://www.ncbi.nlm.nih.gov/pubmed/15709473/>. Praticò D. Oxidative stress hypothesis in Alzheimer’s disease: a reappraisal. *Trends Pharmacol Sci*. 2008 Dec;29(12):609-15; <http://www.ncbi.nlm.nih.gov/pubmed/18838179/>.

<sup>421</sup> Paola D, Domenicotti C, Nitti M, Vitali A, Borghi R, Cottalasso D, Zaccheo D, Odetti P, Strocchi P, Marinari UM, Tabaton M, Pronzato MA. Oxidative stress induces increase in intracellular amyloid beta-protein production and selective activation of betaI and betaII PKCs in NT2 cells. *Biochem Biophys Res Commun*. 2000 Feb 16;268(2):642-6; <http://www.ncbi.nlm.nih.gov/pubmed/10679257/>. Tamagno E, Parola M, Bardini P, Piccini A, Borghi R, Guglielmotto M, Santoro G, Davit A, Danni O, Smith MA, Perry G, Tabaton M. Beta-site APP cleaving enzyme up-regulation induced by 4-hydroxynonenal is mediated by stress-activated protein kinases pathways. *J Neurochem*. 2005 Feb;92(3):628-36; <http://onlinelibrary.wiley.com/doi/10.1111/j.1471-4159.2004.02895.x/epdf>. Tong Y, Zhou W, Fung V, Christensen MA, Qing H, Sun X, Song W. Oxidative stress potentiates BACE1 gene expression and Abeta generation. *J Neural Transm*. 2005 Mar;112(3):455-69; <http://www.ncbi.nlm.nih.gov/pubmed/15614428>.

<sup>422</sup> Liu CW, Giasson BI, Lewis KA, Lee VM, Demartino GN, Thomas PJ. A precipitating role for truncated alpha-synuclein and the proteasome in alpha-synuclein aggregation: implications for pathogenesis of Parkinson disease. *J Biol Chem*. 2005 Jun 17;280(24):22670-8; <http://www.jbc.org/content/280/24/22670.long>.

<sup>423</sup> Standridge JB. Vicious cycles within the neuropathophysiologic mechanisms of Alzheimer’s disease. *Curr Alzheimer Res*. 2006 Apr;3(2):95-108; <http://www.ncbi.nlm.nih.gov/pubmed/16611010>.

has been interpreted as a failure of the ubiquitin-proteasome system to proteolytically degrade excess tau.<sup>424</sup> Experimental proteasomal inhibition causes an accumulation of particularly hyperphosphorylated tau species<sup>425</sup> and a disruption of neuritic transport,<sup>426</sup> and the experimental inhibition of autophagy in neurons results in 3-fold accumulation of phosphomimic tau over wild type tau, indicating that both autophagic and proteasomal pathways are responsible for the clearance of phosphorylated tau species.<sup>427</sup> Aging-related failure of the autophagic and proteasomal degradation pathways would result in the slow accumulation of excessive hyperphosphorylated tau, leading to increased production of neurofibrillary tangles.

A large number of different kinases (enzymes that add phosphate) and phosphatases (enzymes that remove phosphate) have been shown to regulate tau phosphorylation, and an imbalance in tau kinase and phosphatase activity is believed to result in tau hyperphosphorylation in disease.<sup>428</sup> But it may be mainly declining protein phosphatase activity, rather than activation of kinases, that underlies aging-related neurofilament hyperphosphorylation and can best account for rising neurofilament phosphorylation in the maturing brain, potentially compounding similar changes associated with adult-onset neurodegenerative diseases.<sup>429</sup> Aging-related failure of phosphatase function would decrease the rate of tau dephosphorylation, increasing the hyperphosphorylated tau concentration in neurons and again leading to increased production of neurofibrillary tangles.

In particular, the phosphatase enzymes PP2A and PP2B are both present in human brain tissue and have the ability to dephosphorylate the abnormal (pathological) phosphorylation sites on the tau protein,<sup>430</sup> but PP2A seems to be the major tau phosphatase in the brain.<sup>431</sup> Aging-related

---

<sup>424</sup> Bancher C, Brunner C, Lassmann H, Budka H, Jellinger K, Wiche G, Seitelberger F, Grundke-Iqbal I, Iqbal K, Wisniewski HM Accumulation of abnormally phosphorylated tau precedes the formation of neurofibrillary tangles in Alzheimer's disease. *Brain Res.* 1989 Jan 16; 477(1-2):90-9. <https://www.ncbi.nlm.nih.gov/pubmed/2495152/>.

<sup>425</sup> Shimura H, Schwartz D, Gygi SP, Kosik KS CHIP-Hsc70 complex ubiquitinates phosphorylated tau and enhances cell survival. *J Biol Chem.* 2004 Feb 6; 279(6):4869-76. <https://www.ncbi.nlm.nih.gov/pubmed/14612456/>.

<sup>426</sup> Agholme L, Nath S, Domert J, Marcusson J, Kågedal K, Hallbeck M. Proteasome inhibition induces stress kinase dependent transport deficits – implications for Alzheimer's disease. *Mol Cell Neurosci.* 2014 Jan; 58:29-39. <https://www.ncbi.nlm.nih.gov/pubmed/24270002/>.

<sup>427</sup> Rodríguez-Martín T, Cuchillo-Ibáñez I, Noble W, Nyenya F, Anderton BH, Hanger DP Tau phosphorylation affects its axonal transport and degradation. *Neurobiol Aging.* 2013 Sep; 34(9):2146-57. <https://www.ncbi.nlm.nih.gov/pubmed/23601672/>.

<sup>428</sup> Brion JP. Neurofibrillary tangles and Alzheimer's disease. *Eur Neurol.* 1998 Oct;40(3):130-40; <http://www.ncbi.nlm.nih.gov/pubmed/9748670>.

<sup>429</sup> Veeranna, Yang DS, Lee JH, Vinod KY, Stavrides P, Amin ND, Pant HC, Nixon RA. Declining phosphatases underlie aging-related hyperphosphorylation of neurofilaments. *Neurobiol Aging.* 2011 Nov;32(11):2016-29; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2891331/>.

<sup>430</sup> Matsuo ES, Shin RW, Billingsley ML, Van deVoorde A, O'Connor M, Trojanowski JQ, Lee VM. Biopsy-derived adult human brain tau is phosphorylated at many of the same sites as Alzheimer's disease

reduction in both the expression<sup>432</sup> and activity<sup>433</sup> of PP2A has been observed in AD brains. PP2A activity is also inhibited by PP2A I1 and PP2A I2 – endogenous inhibitors of PP2A that are elevated in the aging AD brain.<sup>434</sup> These age-related changes, along with increased demethylation of Leu 309 and increased phosphorylation of Tyr 307 on the PP2AC subunit, contribute to the lowering PP2A activity in AD.<sup>435</sup>

Like A $\beta$  (see **Appendix A**), normal tau protein might have a variety of microtubule-independent physiological functions, many of which may not yet be known. A few of these functions could include: the facilitation or enhancement of excitatory neurotransmission by regulating the distribution of synaptic activity-related signaling molecules<sup>436</sup> and acting as a reversible transport

paired helical filament tau. *Neuron*. 1994 Oct;13(4):989-1002;  
<https://www.ncbi.nlm.nih.gov/pubmed/7946342>.

<sup>431</sup> Goedert M, Jakes R, Qi Z, Wang JH, Cohen P. Protein phosphatase 2A is the major enzyme in brain that dephosphorylates tau protein phosphorylated by proline-directed protein kinases or cyclic AMP-dependent protein kinase. *J Neurochem*. 1995 Dec;65(6):2804-7; <https://www.ncbi.nlm.nih.gov/pubmed/7595582>. Kins S, Cramer A, Evans DR, Hemmings BA, Nitsch RM, Gotz J. Reduced protein phosphatase 2A activity induces hyperphosphorylation and altered compartmentalization of tau in transgenic mice. *J Biol Chem*. 2001 Oct 12;276(41):38193-200;  
<http://www.jbc.org/content/early/2001/07/25/jbc.M102621200.long>.

<sup>432</sup> Vogelsberg-Ragaglia V, Schuck T, Trojanowski JQ, Lee VM. PP2A mRNA expression is quantitatively decreased in Alzheimer's disease hippocampus. *Exp Neurol*. 2001 Apr;168(2):402-12;  
<http://www.ncbi.nlm.nih.gov/pubmed/11259128>. Jiang CH, Tsien JZ, Schultz PG, Hu Y. The effects of aging on gene expression in the hypothalamus and cortex of mice. *Proc Natl Acad Sci U S A*. 2001;98:1930-1934; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC29359/>. Loring JF, Wen X, Lee JM, Seilhamer J, Somogyi R. A gene expression profile of Alzheimer's disease. *DNA Cell Biol*. 2001 Nov;20(11):683-95; <https://www.ncbi.nlm.nih.gov/pubmed/11788046>.

<sup>433</sup> Gong CX, Shaikh S, Wang JZ, Zaidi T, Grundke-Iqbal I, Iqbal K. Phosphatase activity toward abnormally phosphorylated tau: decrease in Alzheimer disease brain. *J Neurochem*. 1995 Aug;65(2):732-8; <https://www.ncbi.nlm.nih.gov/pubmed/7616230>. Tomobe K, Shinozuka T, Kawashima T, Kawashima-Ohya Y, Nomura Y. Age-related changes of forkhead transcription factor FOXO1 in the liver of senescence-accelerated mouse SAMP8. *Arch Gerontol Geriatr*. 2013 Nov-Dec;57(3):417-22;  
<http://www.ncbi.nlm.nih.gov/pubmed/23806789>. Liu G, Chen M, Mi N, Yang W, Li X, Wang P, Yin N, Li Y, Yue F, Chan P, Yu S. Increased oligomerization and phosphorylation of  $\alpha$ -synuclein are associated with decreased activity of glucocerebrosidase and protein phosphatase 2A in aging monkey brains. *Neurobiol Aging*. 2015 Sep;36(9):2649-59; <http://www.ncbi.nlm.nih.gov/pubmed/26149921>.

<sup>434</sup> Tanimukai H, Grundke-Iqbal I, Iqbal K Up-regulation of inhibitors of protein phosphatase-2A in Alzheimer's disease. *Am J Pathol*. 2005 Jun;166(6):1761-71.  
<http://www.ncbi.nlm.nih.gov/pubmed/15920161/>.

<sup>435</sup> Liu R, Wang JZ Protein phosphatase 2A in Alzheimer's disease. *Pathophysiology*. 2009 Oct;16(4):273-7. <http://www.ncbi.nlm.nih.gov/pubmed/19278841/>.

<sup>436</sup> Morris M, Maeda S, Vossel K, Mucke L. The many faces of tau. *Neuron*. 2011 May 12;70(3):410-26;  
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3319390/>.

block for vesicles and organelles,<sup>437</sup> excitotoxic signaling in dendritic spines,<sup>438</sup> the induction of long-term depression in the hippocampus,<sup>439</sup> the ability to bind DNA (for as-yet unidentified purposes),<sup>440</sup> and the induction of apoptosis.<sup>441</sup>

One of the final consequences of the disturbance of proteostasis by protein oxidation and impairment of the proteasomal system may be, for example, the accumulation of highly crosslinked undegradable aggregates such as lipofuscin (Section 2.4.7).<sup>442</sup>

To briefly summarize our synoptic view of AD: The natural process of aging<sup>443</sup> – which is already measurable as early as 30 years of age<sup>444</sup> – slowly reduces the effectiveness of the biochemical pathways that control the normal processing and degradation of proteins, leading to a failure of proteostasis and subsequently to the accumulation of excess A $\beta$ , tau protein, and a cascade of pathological biochemical events, manifesting eventually in the clinical symptoms of Alzheimer's disease.

---

<sup>437</sup> Thies E, Mandelkow EM. Missorting of tau in neurons causes degeneration of synapses that can be rescued by the kinase MARK2/Par-1. *J Neurosci*. 2007 Mar 14;27(11):2896-907; <http://www.jneurosci.org/content/27/11/2896.long>.

<sup>438</sup> Haass C, Mandelkow E. Fyn-tau-amyloid: a toxic triad. *Cell*. 2010 Aug 6;142(3):356-8; <http://www.sciencedirect.com/science/article/pii/S0092867410008366>.

<sup>439</sup> Kimura T, Whitcomb DJ, Jo J, Regan P, Piers T, Heo S, Brown C, Hashikawa T, Murayama M, Seok H, Sotiropoulos I, Kim E, Collingridge GL, Takashima A, Cho K. Microtubule-associated protein tau is essential for long-term depression in the hippocampus. *Philos Trans R Soc Lond B Biol Sci*. 2013 Dec 2;369(1633):20130144; <http://www.ncbi.nlm.nih.gov/pubmed/24298146>.

<sup>440</sup> Qi H, Cantrelle FX, Benhelli-Mokrani H, Smet-Nocca C, Buée L, Lippens G, Bonnefoy E, Galas MC, Landrieu I. Nuclear magnetic resonance spectroscopy characterization of interaction of Tau with DNA and its regulation by phosphorylation. *Biochemistry*. 2015 Feb 24;54(7):1525-33; <http://www.ncbi.nlm.nih.gov/pubmed/25623359>.

<sup>441</sup> Spears W, Furgerson M, Sweetnam JM, Evans P, Gearing M, Fechtmeier M, Furukawa R, Hirano bodies differentially modulate cell death induced by tau and the amyloid precursor protein intracellular domain. *BMC Neurosci*. 2014 Jun 14;15:74; <http://www.ncbi.nlm.nih.gov/pubmed/24929931>.

<sup>442</sup> Höhn A, Grune T. Lipofuscin: formation, effects and role of macroautophagy. *Redox Biol*. 2013 Jan 19;1:140-4; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3757681/>.

<sup>443</sup> Currais A, Goldberg J, Farrokhi C, Chang M, Prior M, Dargusch R, Daugherty D, Armando A, Quehenberger O, Maher P, Schubert D. A comprehensive multiomics approach toward understanding the relationship between aging and dementia. *Aging (Albany NY)*. 2015 Nov;7(11):937-55; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4694064/>.

<sup>444</sup> Belsky DW, Caspi A, Houts R, Cohen HJ, Corcoran DL, Danese A, Harrington H, Israel S, Levine ME, Schaefer JD, Sugden K, Williams B, Yashin AI, Poulton R, Moffitt TE. Quantification of biological aging in young adults. *Proc Natl Acad Sci U S A*. 2015 Jul 28;112(30):E4104-10; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4522793/>.

### Chapter 3. Conventional Treatments for Alzheimer’s Disease

Alzheimer’s disease is a complex neurodegenerative disorder that seems to involve interactions among multiple genetic, epigenetic, and environmental factors and pathways (Section 2.4). This multifactoriality contributes to the heterogeneity of patient populations and makes it difficult to test drugs in clinical trials without pre-selecting appropriate patient groups and matching them up with the most suitable drugs.<sup>445</sup>

There is currently no known cure for Alzheimer’s disease. Available treatments offer relatively small symptomatic benefit and remain essentially palliative in nature. According to a 2015 survey of the neuropharmaceuticals industry,<sup>446</sup> **treatments** for Alzheimer’s that have a big impact “**are unlikely anytime soon.**” Notes the survey:

“Industry bet big on injectable medicines to prevent or reverse Alzheimer’s by attacking the buildup of plaques in the brain – and failed.”

“What’s next, a lower-risk approach: targeting Alzheimer’s symptoms but **not trying to reverse the disease.**”

A 2014 study in the journal *Alzheimer’s Research & Therapy*<sup>447</sup> reported that AD clinical trials have been limited, investigative agents do not pass from phase to phase readily and few make it to regulatory review, coming in at a lower rate compared to other therapeutic areas. The study authors examined Clinicaltrials.gov for AD trials from 2002 to 2012 to autopsy the drug development process for AD drugs. They found that in the study period, sponsors completed 413 AD trials, with only 83 proceeding to Stage III. **The overall failure rate was 99.6 percent.**

Current treatment approaches can be divided into pharmaceutical, psychosocial and caregiving. Only the first of these is relevant to the current discussion. Accordingly, Section 3.1 summarizes existing pharmaceutical treatments. Research directions for future mainstream pharmaceutical treatments (including drugs, genetic engineering, stem cells, and even conventional nanotechnology) are summarized in Section 3.2.

---

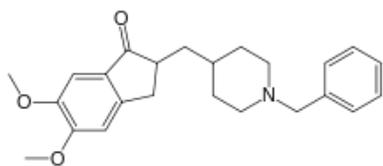
<sup>445</sup> Huang Y, Mucke L. Alzheimer mechanisms and therapeutic strategies. *Cell*. 2012;148(6):1204-22; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3319071/>.

<sup>446</sup> Matthew Herper, “Brain Boom – Health Care 2025,” *Forbes*, Vol. 195, 2 Mar 2015, pp. 76-83; <http://www.forbes.com/sites/matthewherper/2015/02/11/brain-boom-the-drug-companies-bringing-neuroscience-back-from-the-brink/>.

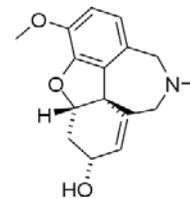
<sup>447</sup> Cummings JL, Morstorf T, Zhong K. Alzheimer’s disease drug-development pipeline: few candidates, frequent failures. *Alzheimers Res Ther*. 2014 Jul 3;6(4):37; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4095696/>.

### 3.1 Current Pharmaceutical Treatments for Alzheimer's Disease

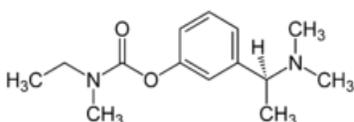
Five medications are currently employed to treat the cognitive problems of Alzheimer's disease.



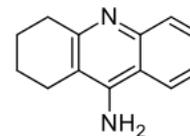
The first four FDA-approved drugs are acetylcholinesterase inhibitors – **donepezil** (aka. Aricept; image, left), **galantamine** (aka. Razadyne, Nivalin, Reminyl, Lycoremime; image, right), **rivastigmine** (aka. Exelon; image, left



below), and **tacrine** (aka. Cognex; image, right below)<sup>448</sup>. (Huperzine A, a reversible acetylcholinesterase inhibitor, is promising but requires further evidence before its use can be recommended.<sup>449</sup>) Reduction in the activity of the cholinergic neurons is a well-known feature of Alzheimer's disease.<sup>450</sup> Acetylcholinesterase inhibitors are employed to reduce the rate at which acetylcholine (ACh) is broken down, thereby increasing the concentration of ACh in the brain and combating the loss of ACh caused by the death of cholinergic neurons.<sup>451</sup> There is evidence for the efficacy of these medications in mild to moderate Alzheimer's



disease,<sup>452</sup> and some evidence for their use in the advanced stage. Only donepezil (trade name: Aricept) is approved for treatment of advanced AD dementia.<sup>453</sup>



<sup>448</sup> Tacrine was the prototypical cholinesterase inhibitor for the treatment of Alzheimer's disease, but in 2013 its use was discontinued in the US due to concerns over safety.

<http://www.livertox.nih.gov/Tacrine.htm>.

<sup>449</sup> Li J, Wu HM, Zhou RL, Liu GJ, Dong BR. Huperzine A for Alzheimer's disease. Cochrane Database Syst Rev. 2008 Apr 16;(2):CD005592; <http://www.ncbi.nlm.nih.gov/pubmed/18425924>.

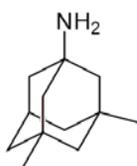
<sup>450</sup> Geula C, Mesulam MM. Cholinesterases and the pathology of Alzheimer disease. Alzheimer Dis Assoc Disord. 1995;9 Suppl 2:23-8; <http://www.ncbi.nlm.nih.gov/pubmed/8534419>.

<sup>451</sup> Stahl SM. The new cholinesterase inhibitors for Alzheimer's disease, part 2: illustrating their mechanisms of action. J Clin Psychiatry. 2000 Nov;61(11):813-4; <http://www.ncbi.nlm.nih.gov/pubmed/11105732>.

<sup>452</sup> Birks J. Cholinesterase inhibitors for Alzheimer's disease. Cochrane Database Syst Rev. 2006 Jan 25;(1):CD005593; <http://www.ncbi.nlm.nih.gov/pubmed/16437532>. Birks J, Grimley Evans J, Iakovidou V, Tsolaki M, Holt FE. Rivastigmine for Alzheimer's disease. Cochrane Database Syst Rev. 2009 Apr 15;(2):CD001191; <http://www.ncbi.nlm.nih.gov/pubmed/19370562>.

<sup>453</sup> Birks J, Harvey RJ. Donepezil for dementia due to Alzheimer's disease. Cochrane Database Syst Rev. 2006 Jan 25;(1):CD001190; <http://www.ncbi.nlm.nih.gov/pubmed/16437430>.

The use of these drugs in mild cognitive impairment has not shown any effect in delaying the onset of AD.<sup>454</sup> The most common side effects are nausea and vomiting, both of which are linked to cholinergic excess. These side effects arise in approximately 10-20% of users, are mild to moderate in severity, and can be managed by slowly adjusting medication doses. Less common secondary effects include muscle cramps, decreased heart rate (bradycardia), decreased appetite and weight, and increased gastric acid production.<sup>455</sup>



The fifth approved drug, **memantine** (aka. Namenda, Axura, Akatinol, Ebixa, Abixa, Memox), is an NMDA receptor antagonist (image, left).<sup>456</sup> Here's how it works. Glutamate is a useful excitatory neurotransmitter of the nervous system, although excessive amounts in the brain can lead to cell death through a process called excitotoxicity which consists of the overstimulation of glutamate receptors.<sup>457</sup> Excitotoxicity occurs not only in Alzheimer's disease, but also in other neurological diseases such as Parkinson's disease and multiple sclerosis. Memantine is a noncompetitive NMDA receptor antagonist first used as an anti-influenza agent.<sup>458</sup> It acts on the glutamatergic system by blocking NMDA receptors and inhibiting their overstimulation by glutamate. Memantine has been shown to be modestly efficacious in the treatment of moderate to severe Alzheimer's disease. Its effects in the initial stages of AD are unknown.<sup>459</sup> Reported adverse events with memantine are infrequent and mild, including hallucinations, confusion, dizziness, headache and fatigue. The combination of memantine and donepezil (aka. Namzaric) has been shown to be "of statistically significant but clinically marginal effectiveness".<sup>460</sup>

<sup>454</sup> Raschetti R, Albanese E, Vanacore N, Maggini M. Cholinesterase inhibitors in mild cognitive impairment: a systematic review of randomised trials. *PLoS Medicine*. 2007;4(11):e338; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2082649/>.

<sup>455</sup> Birks J. Cholinesterase inhibitors for Alzheimer's disease. *Cochrane Database Syst Rev*. 2006 Jan 25;(1):CD005593; <http://www.ncbi.nlm.nih.gov/pubmed/16437532>.

<sup>456</sup> Pohanka M. Cholinesterases, a target of pharmacology and toxicology. *Biomedical Papers of the Medical Faculty of the University Palacký, Olomouc, Czech Republic*. 2011;155(3):219-29; <http://biomed.papers.upol.cz/pdfs/bio/2011/03/02.pdf>.

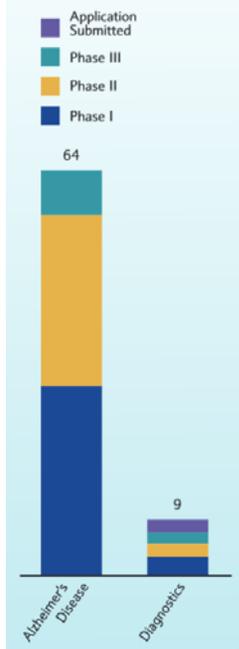
<sup>457</sup> BCI-838, a prodrug that is converted to its active form (BCI-632) after being metabolized in the body, blocks glutamate receptors and is being tested by Brain Cells Inc. as a possible Alzheimer's drug. Kim SH, Steele JW, Lee SW, Clemenson GD, Carter TA, Treuner K, Gadiant R, Wedel P, Glabe C, Barlow C, Ehrlich ME, Gage FH, Gandy S. Proneurogenic Group II mGluR antagonist improves learning and reduces anxiety in Alzheimer A $\beta$  oligomer mouse. *Mol Psychiatry*. 2014 Nov;19(11):1235-42; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4217144/>.

<sup>458</sup> Lipton SA. Paradigm shift in neuroprotection by NMDA receptor blockade: memantine and beyond. *Nat Rev Drug Discov*. 2006 Feb;5(2):160-70; <http://www.ncbi.nlm.nih.gov/pubmed/16424917>.

<sup>459</sup> Areosa Sastre A, McShane R, Sherriff F. Memantine for Dementia. *Cochrane Database Syst Rev*. 2004 Oct 18;(4):CD003154; <http://www.ncbi.nlm.nih.gov/pubmed/15495043>.

<sup>460</sup> Raina P, Santaguida P, Ismaila A, Patterson C, Cowan D, Levine M, Booker L, Oremus M. Effectiveness of cholinesterase inhibitors and memantine for treating dementia: evidence review for a clinical practice guideline. *Ann Intern Med*. 2008 Mar 4;148(5):379-97; <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.511.985&rep=rep1&type=pdf>.

#### Medicines and Diagnostics In Development for Alzheimer's Disease



However, the benefit from the use of any of these five approved drugs is small.<sup>461</sup> So far, no medication has been clearly shown to significantly delay or halt the progression of the disease. A few related drugs are in trials, such as **encenicline** (aka. EVP-6124), an  $\alpha 7$  nicotinic acetylcholine receptor partial agonist under investigation for the symptomatic treatment of AD.<sup>462</sup> Typical good results appear to be a slowing of the natural 3-9 year course of the disease by perhaps 6 months, or up to 12 months in the rarest cases. Encapsulated cells that release NGF (nerve growth factor) have also been implanted in the basal forebrain of AD patients in an attempt to increase acetylcholine levels in the brain.<sup>463</sup>

**Antipsychotic drugs** (e.g., aripiprazole (Abilify, Aripiprex), clozapine (Clozaril), haloperidol (Haldol (US, UK), Aloperidin, Bioperidolo, Brotopon, Dozic, Duraperidol (Germany), Einalon S, Eukystol, Halosten, Keselan, Linton, Peluces, Serenace, Sigaperidol), olanzapine (Zyprexa, Zypadhera, Lanzek, Symbyax), quetiapine (Seroquel), risperidone (Risperdal, Risdon, Sizodon), and ziprasidone (Geodon, Zeldox, Zipwell)) have proven modestly useful in reducing aggression and psychosis in Alzheimer's disease with behavioral problems, but are also associated with serious adverse effects such as stroke, movement difficulties, or cognitive decline, that recommend against their routine employment.<sup>464</sup> When used in the long-term, such drugs have been shown to associate with increased mortality.<sup>465</sup>

<sup>461</sup> Birks J, Harvey RJ. Donepezil for dementia due to Alzheimer's disease. *Cochrane Database Syst Rev.* 2006 Jan 25;(1):CD001190; <http://www.ncbi.nlm.nih.gov/pubmed/16437430>. Commission de la transparence. Drugs for Alzheimer's disease: best avoided. No therapeutic advantage. *Prescrire Int.* 2012 Jun;21(128):150; <http://www.ncbi.nlm.nih.gov/pubmed/22822592>.

<sup>462</sup> Deardorff WJ, Shobassy A, Grossberg GT. Safety and clinical effects of EVP-6124 in subjects with Alzheimer's disease currently or previously receiving an acetylcholinesterase inhibitor medication. *Expert Rev Neurother.* 2015 Jan;15(1):7-17; <http://www.ncbi.nlm.nih.gov/pubmed/25495510>.

<sup>463</sup> Karami A, Eyjolfssdottir H, Vijayaraghavan S, Lind G, Almqvist P, Kadir A, Linderöth B, Andreasen N, Blennow K, Wall A, Westman E, Ferreira D, Kristoffersen Wiberg M, Wahlund LO, Seiger Å, Nordberg A, Wahlberg L, Darreh-Shori T, Eriksdotter M. Changes in CSF cholinergic biomarkers in response to cell therapy with NGF in patients with Alzheimer's disease. *Alzheimers Dement.* 2015 Feb 9. pii: S1552-5260(15)00007-2; <http://www.ncbi.nlm.nih.gov/pubmed/25676388>.

<sup>464</sup> Sink KM, Holden KF, Yaffe K. Pharmacological treatment of neuropsychiatric symptoms of dementia: a review of the evidence. *JAMA.* 2005 Feb 2;293(5):596-608; <http://archpedi.jamanetwork.com/article.aspx?articleid=200265>.

<sup>465</sup> Ballard C, Hanney ML, Theodoulou M, Douglas S, McShane R, Kossakowski K, Gill R, Juszczak E, Yu LM, Jacoby R. The dementia antipsychotic withdrawal trial (DART-AD): long-term follow-up of a randomised placebo-controlled trial. *Lancet Neurol.* 2009 Feb;8(2):151-7; <http://www.familymed.med.ualberta.ca/Library/Documents/Withdrawanti-psychoticTherapyPaper.pdf>.

Other medications often used as near-palliatives for Alzheimer's disease include:

**antidepressant drugs** for low mood and irritability (e.g., citalopram (Celexa, Cipramil, Citalopram, and 39 others<sup>466</sup>), fluoxetine (Prozac, Sarafem, and 478 others<sup>467</sup>), paroxetine (Paxil, Aropax, Brisdelle, Deroxat, Pexeva, Paxtine, Paraxyl, Sereupin, Seroxat), sertraline (Zoloft, Lustral, Daxid, Deprax, Altruline, Besitran, Eleval, Emergen, Gladem, Implicane, Sedoran, Sealdin, Serivo, Lowfin, Stimuloton, Serimel, Seretral, Tresleen, Sertralin Bluefish), trazodone (Desyrel, Depyrel, Mesyrel, Molipaxin, Oleptro, Trazodil, Trazorel, Trialodine, Trittico), and nortriptyline (Sensoval, Aventyl, Pamelor, Norpress, Allegron, Noritren, Nortrilen));

**anxiolytic drugs**<sup>468</sup> for anxiety, restlessness, verbally disruptive behavior and resistance (e.g., lorazepam (Ativan, Orfidal, and 74 others<sup>469</sup>), oxazepam (Serax and 21 others<sup>470</sup>), alprazolam (Xanax), clonazepam (Klonopin), and temazepam (Restoril, Normison));

**sleeping pills**<sup>471</sup> (e.g., zaleplon (Sonata, Starnoc, Andante), zolpidem (Ambien, Ambien CR, Intermezzo, Stilnox, Stilnoct, Sublinox, Hypnogen, Zonadin, Sanval, Zolsana, Zolfresh), and chloral hydrate); and

**nootropics** or “smart drugs” (e.g., piracetam (Breinox, Dinagen, Lucetam, Nootropil, Nootropyl, Oikamid), aniracetam (Draganon, Sarpul, Ampamet, Memodrin, Referan, Pergamid), pramiracetam (Remen, Neupramir, Pramistar), cerebrolysin,<sup>472</sup> oxyracetam (ISF 2522),

---

<sup>466</sup> [http://en.wikipedia.org/wiki/Citalopram#Brand\\_names](http://en.wikipedia.org/wiki/Citalopram#Brand_names)

<sup>467</sup> <http://www.drugs.com/international/fluoxetine.html>

<sup>468</sup> But note: Taking these benzodiazepine-based anxiolytics for more than 3 months is associated with up to 51% increase in Alzheimer's disease, rising up to 100% for 6 months of use. Billioti de Gage S, Moride Y, Ducruet T, Kurth T, Verdoux H, Tournier M, Pariente A, Bégaud B. Benzodiazepine use and risk of Alzheimer's disease: case-control study. *BMJ*. 2014 Sep 9;349:g5205; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159609/>.

<sup>469</sup> <http://en.wikipedia.org/wiki/Lorazepam#History>

<sup>470</sup> <http://en.wikipedia.org/wiki/Oxazepam>

<sup>471</sup> But note: People taking anticholinergic sleeping medications (e.g., at least 4 mg/day diphenhydramine – Benadryl, Unisom, Sominex, ZzzQuil, Nytol) for more than three years have increased risk of dementia. High intake of anticholinergic drugs increases dementia risk by 54% compared with no use, and the risk of Alzheimer's rises by 63%. Gray SL, Anderson ML, Dublin S, Hanlon JT, Hubbard R, Walker R, Yu O, Crane PK, Larson EB. Cumulative use of strong anticholinergics and incident dementia: a prospective cohort study. *JAMA Intern Med*. 2015 Mar 1;175(3):401-7; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4358759/>.

<sup>472</sup> Wei ZH, He QB, Wang H, Su BH, Chen HZ. Meta-analysis: the efficacy of nootropic agent Cerebrolysin in the treatment of Alzheimer's disease. *J Neural Transm*. 2007;114(5):629-34; <http://www.ncbi.nlm.nih.gov/pubmed/17318304>.

coluracetam (BCI-540, MKC-231), nimodopine, dihydroergicristine, and noopept (GVS-111, N-phenylacetyl-L-prolylglycine ethyl ester)).

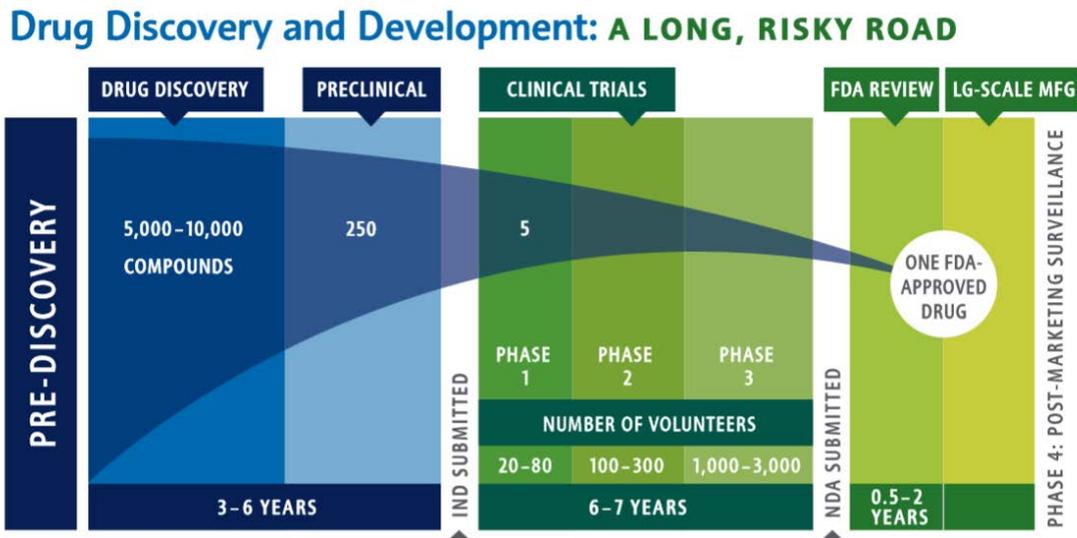
In summary: No current treatments stop or reverse the progression of AD, though some may temporarily improve symptoms.<sup>473</sup>

---

<sup>473</sup> “Dementia Fact sheet N°362,” World Health Organization, Mar 2015;  
<http://www.who.int/mediacentre/factsheets/fs362/en/>.

### 3.2 Future Conventional Treatments for Alzheimer’s Disease

As of Nov 2014, the safety and efficacy of more than 400 pharmaceutical treatments had been or were being investigated in over 1,500 clinical trials worldwide, and approximately a quarter of these compounds had entered in Phase III trials, the last step prior to review by regulatory agencies.<sup>474</sup> In early 2015, Pharmaceutical Research and Manufacturers of America estimated that 73 Alzheimer’s drugs were in development.<sup>475</sup> (See also Appendix B.)



Vast sums of money have been spent attacking Alzheimer’s disease, largely without success. Between 1998 and 2011, 101 drugs aimed at treating the disease failed. For every one medicine that got developed, 34 didn’t even get that far. For example, in 2008, Myriad Genetics (MYGN) pulled the plug on the experimental drug **Flurizan** after Phase III clinical trials found the drug didn’t improve cognitive functioning in an 18-month study of 1,684 patients. Myriad spent about \$200 million developing Flurizan. In 2010, Eli Lilly (LLY) halted work on its Alzheimer’s treatment, **semagacestat**, after the drug was found to be ineffective and actually made patients worse. Another drug called **bapineuzumab**, co-developed by Irish drugmaker Elan and Wyeth, failed to halt the mental decline of Alzheimer’s patients. In 2012, Pfizer and Johnson & Johnson said they were ending development of an intravenous formulation of bapineuzumab after Phase III trials “showed no treatment effect on either cognitive or functional outcomes...biomarker analyses indicated that bapineuzumab engaged its target, but had no benefit.” Johnson & Johnson reportedly took a \$700M charge for the failure, and Elan wrote off more than \$100M for the

<sup>474</sup> “Clinical Trials. Found 1504 studies with search of: Alzheimer,” US National Institutes of Health, 30 Oct 2014; <http://www.clinicaltrials.gov/ct2/results?term=alzheimer>.

<sup>475</sup> Jonathan Berr, “Will a cure for Alzheimer’s ever be found?” CBS Moneywatch, 9 Jan 2015; <http://www.cbsnews.com/news/will-a-cure-for-alzheimers-ever-be-found/>.

value of its stake in bapineuzumab.<sup>476</sup> In late 2014, Roche Holding pulled the plug on a Phase III trial of its Alzheimer's drug candidate **gantenerumab** because it was ineffective in patients with early stages of the memory-robbing disease, although gantenerumab is still being evaluated as a treatment for the later stages of Alzheimer's.

One major issue that has been highlighted by the failures of so many AD clinical trials is the design of the trials themselves. It is now generally accepted that a large number of the clinical trials of AD treatments may have failed because the patients were too far advanced in the disease process to see any clinical effect from a potential therapeutic. Amyloid deposition in AD is now known to begin many years before the appearance of cognitive symptoms and the ultimate diagnosis of dementia.<sup>477</sup> Much drug development in AD is now beginning to focus on the targeting of patients at the very early stages of the disease, before the appearance of obvious dementia, especially in groups with familial AD. Another recurring problem is that drugs tested in mouse models often perform much differently when applied to humans.

The most prominent conventional therapeutic intervention strategies for future AD treatment, many already in clinical trials (along with many failures), are summarized in the Sections that follow.

---

<sup>476</sup> Bertha Coombs, "The race for next-generation Alzheimer's drugs," CNBC, 5 Sep 2013; <http://www.cnbc.com/id/101011893>.

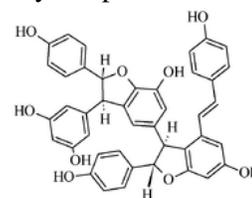
<sup>477</sup> Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, Iwatsubo T, Jack CR Jr, Kaye J, Montine TJ, Park DC, Reiman EM, Rowe CC, Siemers E, Stern Y, Yaffe K, Carrillo MC, Thies B, Morrison-Bogorad M, Wagster MV, Phelps CH. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. 2011 May;7(3):280-92; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3220946/>.

### 3.2.1 Reduce Amyloid- $\beta$ Production

There are currently three main therapeutic intervention strategies aimed at amyloid-beta or A $\beta$ , the notional primary villain in Alzheimer's disease: reducing A $\beta$  production (discussed below), preventing A $\beta$  aggregation (Section 3.4.2), and facilitating A $\beta$  clearance (Section 3.4.3).

As noted in Section 2.4.1, A $\beta$  monomer is initially produced by the sequential cleavage of the protein APP via the protease enzymes  $\beta$ -secretase and  $\gamma$ -secretase. ( $\alpha$ -secretase cleavage precludes A $\beta$  formation by cleaving within the A $\beta$  peptide sequence, thus making A $\beta$  formation impossible; it is considered to be part of the non-amyloidogenic pathway in APP processing.) Thus one popular strategy is to modify or interfere with the  $\beta$ - and  $\gamma$ -secretases so that they make less of the toxic amyloid protein.<sup>478</sup>

**$\beta$ -secretase inhibitors** work by blocking the enzyme that initially cleaves amyloid precursor protein to produce A $\beta$ . Recently developed inhibitors are potent, selective, can cross the blood-brain barrier, and can inhibit production of A $\beta$  in the brains of experimental animals. Continuous administration was shown to rescue age-related cognitive decline in transgenic AD mice, and by 2012 a few inhibitors were in early stage human trials.<sup>479</sup> For example, Merck is currently testing a new drug called **MK-8931** in Phase II trials, which cut the levels of beta amyloid protein in cerebrospinal fluid by up to 92% in Phase I trials.<sup>480</sup> In Sep 2014 Eli Lilly and AstraZeneca began Phase II/III trials on **AZD3293** (aka. LY3314814), an oral  $\beta$ -secretase inhibitor, that is planned to recruit 1,500 patients and end in May 2019.<sup>481</sup> Another pre-trial study found that a resveratrol-derived trimer molecule called **miyabenol C** (image, right) potently inhibits  $\beta$ -secretase activity without altering *in vitro* or *in vivo* concentrations of the enzyme.<sup>482</sup> One potential problem is that  $\beta$ -secretase (aka. BACE) is



<sup>478</sup> MacLeod R, Hillert EK, Cameron RT, Baillie GS. The role and therapeutic targeting of  $\alpha$ -,  $\beta$ - and  $\gamma$ -secretase in Alzheimer's disease. *Future Sci. OA* 14 Apr 2015; <http://www.future-science.com/doi/pdf/10.4155/fso.15.9>

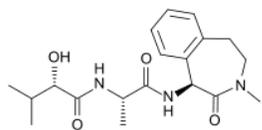
<sup>479</sup> Ghosh AK, Brindisi M, Tang J. Developing  $\beta$ -secretase inhibitors for treatment of Alzheimer's disease. *J Neurochem.* 2012 Jan;120 Suppl 1:71-83; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3276244/>.

<sup>480</sup> Menting KW, Claassen JA.  $\beta$ -secretase inhibitor; a promising novel therapeutic drug in Alzheimer's disease. *Front Aging Neurosci.* 2014 Jul 21;6:165; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4104928/>.

<sup>481</sup> <http://www.news-medical.net/news/20141201/Eli-Lilly-AstraZeneca-begin-AZD3293-Phase-III-study-for-treatment-of-early-Alzheimers-disease.aspx>.

<sup>482</sup> Hu J, Lin T, Gao Y, Xu J, Jiang C, Wang G, Bu G, Xu H, Chen H, Zhang YW. The Resveratrol Trimer Miyabenol C Inhibits  $\beta$ -Secretase Activity and  $\beta$ -Amyloid Generation. *PLoS One.* 2015 Jan 28;10(1):e0115973; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4309453/>.

an aspartic-acid protease important in the formation of myelin sheaths in peripheral nerve cells<sup>483</sup> and is also necessary for the proper function of muscle spindles.<sup>484</sup> So simply inhibiting all BACE activity or removing all BACE might have unwanted side effects on human health.



**$\gamma$ -secretase inhibitors** work by blocking the enzyme that performs the final cleavage step to produce A $\beta$ . **Semagacestat** (aka. LY450139; image, left) is a small-molecule  $\gamma$ -secretase inhibitor (technically, a noncompetitive enzyme inhibitor with an allosteric binding site) that was developed as a potential treatment for Alzheimer's disease.

However, a Phase III trial<sup>485</sup> found that as compared with placebo, "semagacestat did not improve cognitive status, patients receiving the higher dose had significant worsening of functional ability, [and] semagacestat was associated with more adverse events including skin cancers and infections." These results led Eli Lilly to halt further development of the drug.<sup>486</sup> Another gamma-secretase inhibition compound (**LY-411575**)<sup>487</sup> "reduces soluble A $\beta$  levels and rescues the neuronal dysfunction" in mouse AD models,<sup>488</sup> but is not yet in clinical trials. Some early work suggested that the cancer drug **imatinib** (aka. Gleevec) can bind to gamma-secretase activating protein (GSAP) which selectively increases the production and accumulation of neurotoxic beta-amyloid plaques,<sup>489</sup> but confirmation has not been forthcoming.<sup>490</sup>

<sup>483</sup> Willem M, Garratt AN, Novak B, Citron M, Kaufmann S, Rittger A, DeStrooper B, Saftig P, Birchmeier C, Haass C. Control of peripheral nerve myelination by the beta-secretase BACE1. *Science*. 2006 Oct 27;314(5799):664-6; <http://www.sciencemag.org/content/314/5799/664.long>.

<sup>484</sup> Cheret C, Willem M, Fricker FR, Wende H, Wulf-Goldenberg A, Tahirovic S, Nave KA, Saftig P, Haass C, Garratt AN, Bennett DL, Birchmeier C. Bace1 and Neuregulin-1 cooperate to control formation and maintenance of muscle spindles. *EMBO J*. 2013 Jul 17;32(14):2015-28; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3715864/>.

<sup>485</sup> Doody RS, *et al.* A Phase 3 trial of semagacestat for treatment of Alzheimer's disease. *N Engl J Med*. 2013 Jul 25;369(4):341-50; [http://departmentofmedicine.com/ger/documents/semagacestat\\_alzheimers\\_treatment.pdf](http://departmentofmedicine.com/ger/documents/semagacestat_alzheimers_treatment.pdf).

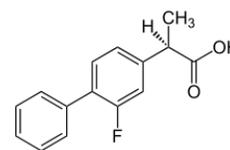
<sup>486</sup> <https://investor.lilly.com/releasedetail.cfm?releaseid=499794>

<sup>487</sup> Lanz TA, Hosley JD, Adams WJ, Merchant KM. Studies of A $\beta$  pharmacodynamics in the brain, cerebrospinal fluid, and plasma in young (plaque-free) Tg2576 mice using the  $\gamma$ -secretase inhibitor N2-[(2S)-2-(3,5-difluorophenyl)-2-hydroxyethanoyl]-N1-[(7S)-5-methyl-6-oxo-6,7-dihydro-5H-dibenzo[b,d]azepin-7-yl]-L-alaninamide (LY-411575) *J Pharmacol Exp Ther*. 2004;309(1):49-55; <http://www.ncbi.nlm.nih.gov/pubmed/14718585>.

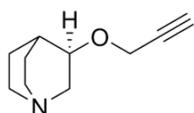
<sup>488</sup> Busche MA, Chen X, Henning HA, Reichwald J, Staufenbiel M, Sakmann B, Konnerth A. Critical role of soluble amyloid- $\beta$  for early hippocampal hyperactivity in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci USA*. 2012 May 29; 109(22): 8740-8745; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3365221/>.

<sup>489</sup> Netzer WJ, Dou F, Cai D, Veach D, Jean S, Li Y, Bornmann WG, Clarkson B, Xu H, Greengard P. Gleevec inhibits beta-amyloid production but not Notch cleavage. *Proc Natl Acad Sci U S A*. 2003 Oct 14;100(21):12444-9; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC218777/>.

**$\gamma$ -secretase modulators** target the  $\gamma$ -secretase enzyme and work by shifting the cleavage sites to favor production of shorter and less toxic species of A $\beta$ . The best-known in this class was **Tarenflurbil** (aka. MPC-7869, R-flubiprofen; image, right), a promising selective A $\beta_{42}$  lowering agent that was believed to reduce the production of the toxic amyloid beta in favor of shorter forms of the peptide. But in 2009 a Phase III trial<sup>491</sup> concluded that “tarenflurbil did not slow cognitive decline or the loss of activities of daily living in patients with mild AD,” causing Myriad Genetics to cease further development of the drug.<sup>492</sup> Other possibly useful  $\gamma$ -secretase modulators continue to be investigated<sup>493</sup> but have not yet advanced to clinical trials.



**$\alpha$ -secretase activators** are theorized to work by increasing  $\alpha$ -secretase cleavage in order to reduce A $\beta$  production, and also by increasing production of sAPP $\alpha$ , a potentially neuroprotective form of the APP. Several metalloprotease disintegrins (ADAM10 and ADAM17) are major  $\alpha$ -secretases. Up-regulation of an  $\alpha$ -secretase, ADAM10 in an APP transgenic mouse, increased sAPP $\alpha$ , decreased A $\beta$  production, reduced plaque formation, and alleviated cognitive deficits.<sup>494</sup> One example of such activity is the psoriasis drug **acitretin**, now being investigated for AD applications.<sup>495</sup> Another pathway of stimulating  $\alpha$ -secretase activity is stimulation of the muscarine-1 receptors with M1-agonists: the M1-agonist **talsaclidine** (image, left) has been shown to decrease CFS-levels of A $\beta_{42}$  in a clinical trial.<sup>496</sup>



<sup>490</sup> “GSAP Revisited: Does It Really Play a Role in Processing A $\beta$ ?” AlzForum, 3 Jan 2014; <http://www.alzforum.org/news/research-news/gsap-revisited-does-it-really-play-role-processing-av>.

<sup>491</sup> Green RC, Schneider LS, Amato DA, Beelen AP, Wilcock G, Swabb EA, Zavitz KH; Tarenflurbil Phase 3 Study Group. Effect of tarenflurbil on cognitive decline and activities of daily living in patients with mild Alzheimer disease: a randomized controlled trial. JAMA. 2009 Dec 16;302(23):2557-64; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2902875/>.

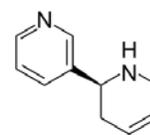
<sup>492</sup> “Myriad Genetics Reports Results of U.S. Phase 3 Trial of Flurizan™ in Alzheimer’s Disease,” <http://web.archive.org/web/20080730012308/http://www.myriad.com/news/release/1170283>.

<sup>493</sup> For example: Takeo K, Tanimura S, Shinoda T, Osawa S, Zahariev IK, Takegami N, Ishizuka-Katsura Y, Shinya N, Takagi-Niidome S, Tominaga A, Ohsawa N, Kimura-Someya T, Shirouzu M, Yokoshima S, Yokoyama S, Fukuyama T, Tomita T, Iwatsubo T. Allosteric regulation of  $\gamma$ -secretase activity by a phenylimidazole-type  $\gamma$ -secretase modulator. Proc Natl Acad Sci U S A. 2014 Jul 22;111(29):10544-9; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4115564/>. Flesch D, Ness J, Lamers C, Dehm F, Popella S, Steri R, Ogorek I, Hieke M, Dannhardt G, Werz O, Weggen S, Schubert-Zsilavecz M. SAR-studies of  $\gamma$ -secretase modulators with PPAR $\gamma$ -agonistic and 5-lipoxygenase-inhibitory activity for Alzheimer’s disease. Bioorg Med Chem Lett. 2015 Feb 15;25(4):841-6; <http://www.ncbi.nlm.nih.gov/pubmed/25575659>.

<sup>494</sup> Fahrenholz F, Postina R. Alpha-secretase activation – an approach to Alzheimer’s disease therapy. Neurodegener Dis. 2006;3(4-5):255-261; <http://www.ncbi.nlm.nih.gov/pubmed/17047365>.

<sup>495</sup> Endres K, Fahrenholz F, Lotz J, Hiemke C, Teipel S, Lieb K, Tüscher O, Fellgiebel A. Increased CSF APPs- $\alpha$  levels in patients with Alzheimer disease treated with acitretin. Neurology. 2014 Nov 18;83(21):1930-5; <http://www.ncbi.nlm.nih.gov/pubmed/25344383>.

Finally, **anatabine** (aka. CigRx; image, right), an alkaloid derived from tobacco plants and tomatoes, reduces A $\beta$  production<sup>497</sup> and has anti-inflammatory activity<sup>498</sup> in AD mice. The antisense oligonucleotide **OL-1** against the amyloid- $\beta$  protein precursor (A $\beta$ PP) can decrease A $\beta$ PP expression and amyloid- $\beta$  protein (A $\beta$ ) production. This antisense molecule rapidly crosses the blood-brain barrier, reverses learning and memory impairments, reduces oxidative stress, and restores brain-to-blood efflux of A $\beta$  in AD mice.<sup>499</sup> Note that OL-1 doesn't target symptoms. Its molecules bind to messenger RNA, allowing certain genes to be "turned off." Here, the blockage of RNA prevents extra amyloid from being produced by targeting its precursor protein and making less of it.




---

<sup>496</sup> Hock C, Maddalena A, Raschig A, Müller-Spahn F, Eschweiler G, Hager K, Heuser I, Hampel H, Müller-Thomsen T, Oertel W, Wienrich M, Signorell A, Gonzalez-Agosti C, Nitsch RM. Treatment with the selective muscarinic m1 agonist talsaclidine decreases cerebrospinal fluid levels of A beta 42 in patients with Alzheimer's disease. *Amyloid*. 2003 Mar; 10(1):1-6; <http://www.ncbi.nlm.nih.gov/pubmed/12762134>.

<sup>497</sup> Paris D, Beaulieu-Abdelahad D, Bachmeier C, Reed J, Ait-Ghezala G, Bishop A, Chao J, Mathura V, Crawford F, Mullan M. Anatabine lowers Alzheimer's A $\beta$  production *in vitro* and *in vivo*. *Eur J Pharmacol*. 2011 Nov 30;670(2-3):384-91; <http://www.ncbi.nlm.nih.gov/pubmed/21958873>.

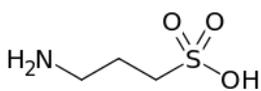
<sup>498</sup> Paris D, Beaulieu-Abdelahad D, Abdullah L, Bachmeier C, Ait-Ghezala G, Reed J, Verma M, Crawford F, Mullan M. Anti-inflammatory activity of anatabine via inhibition of STAT3 phosphorylation. *Eur J Pharmacol*. 2013 Jan 5;698(1-3):145-53; <http://www.ncbi.nlm.nih.gov/pubmed/23178521>.

<sup>499</sup> Farr SA, Erickson MA, Niehoff ML, Banks WA, Morley JE. Central and peripheral administration of antisense oligonucleotide targeting amyloid- $\beta$  protein precursor improves learning and memory and reduces neuroinflammatory cytokines in Tg2576 (A $\beta$ PPswe) mice. *J Alzheimers Dis*. 2014;40(4):1005-16; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4136536/>.

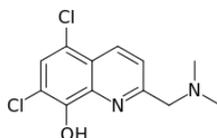
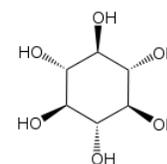
### 3.2.2 Prevent Amyloid- $\beta$ Aggregation

Another anti-A $\beta$  therapeutic intervention strategy is aimed at preventing A $\beta$  aggregation, although this approach has not yet had much success.

For example, **homotaurine** (3-amino-1-propanesulfonic acid, aka. Alzhemed, Tramiprosate; image, left) was designed as an anti-amyloid therapy.<sup>500</sup> *In vitro*, Alzhemed was shown to preferentially bind soluble A $\beta$ , inhibit A $\beta$  aggregation and fibrillogenesis, and inhibit A $\beta$  neurotoxicity.<sup>501</sup> However, Phase III clinical trials failed to show cognitive improvement compared to placebo.<sup>502</sup>



Another promising candidate, **ELND005** (aka. AZD-103, scyllo-Inositol; image, right) breaks down neurotoxic fibrils, allowing amyloid peptides to clear the body rather than form amyloid plaques. Phase I trials produced encouraging results by Aug 2007, but a Phase II trial involving 353 patients ended in 2011 with the conclusion that “primary clinical efficacy outcomes were not significant”.<sup>503</sup>



**PBT2** (aka. 8-hydroxy quinoline; image, left) is a metal-protein attenuating compound that affects the Cu<sup>2+</sup>-mediated and Zn<sup>2+</sup>-mediated toxic oligomerization of A $\beta$  seen in Alzheimer’s disease. It removes copper and

<sup>500</sup> Gauthier S, Aisen PS, Ferris SH, Saumier D, Duong A, Haine D, Garceau D, Suhy J, Oh J, Lau W, Sampalis J. Effect of tramiprosate in patients with mild-to-moderate Alzheimer’s disease: exploratory analyses of the MRI sub-group of the Alphase study. *J Nutr Health Aging*. 2009 Jun;13(6):550-7; <http://www.ncbi.nlm.nih.gov/pubmed/19536424>.

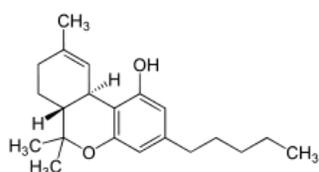
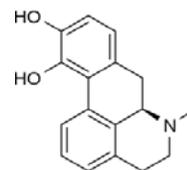
<sup>501</sup> Gervais F, Paquette J, Morissette C, Krzywkowski P, Yu M, Azzi M, Lacombe D, Kong X, Aman A, Laurin J, Szarek WA, Tremblay P. Targeting soluble Abeta peptide with Tramiprosate for the treatment of brain amyloidosis. *Neurobiol Aging*. 2007 Apr;28(4):537-47; <http://www.ncbi.nlm.nih.gov/pubmed/16675063>.

<sup>502</sup> Michele G. Sullivan, “Tramiprosate Falls Short in Phase III Alzheimer’s Trial : Unusually large placebo effect could be a recurring problem in studies that allow concomitant medications ,” *Clinical Psychiatry News*, 1 Nov 2007; [http://www.ecardiologynews.com/fileadmin/content\\_pdf/cpn/archive\\_pdf/vol35iss11/70720\\_main.pdf](http://www.ecardiologynews.com/fileadmin/content_pdf/cpn/archive_pdf/vol35iss11/70720_main.pdf). Aisen PS, Gauthier S, Ferris SH. Tramiprosate in mild-to-moderate Alzheimer’s disease – a randomized, double-blind, placebo-controlled, multi-centre study (the Alphase Study) *Arch Med Sci*. 2011;7:102-11; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3258678/>.

<sup>503</sup> Salloway S, Sperling R, Keren R, Porsteinsson AP, van Dyck CH, Tariot PN, Gilman S, Arnold D, Abushakra S, Hernandez C, Crans G, Liang E, Quinn G, Bairu M, Pastrak A, Cedarbaum JM; ELND005-AD201 Investigators. A phase 2 randomized trial of ELND005, scyllo-inositol, in mild to moderate Alzheimer disease. *Neurology*. 2011 Sep 27;77(13):1253-62; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3179648/>.

zinc from cerebrospinal fluid, which are held to be necessary catalysts for amyloid beta aggregation. While initial trials seemed favorable,<sup>504</sup> a recently-completed Phase II trial<sup>505</sup> found that the drug “did not meet its primary endpoint of a statistically significant reduction in the levels of beta-amyloid plaques in the brains of prodromal/mild Alzheimer’s disease patients....we looked for an impact on the insoluble plaques but did not see it differ significantly from the placebo.”

**Apomorphine** (image, right) has been reported to be an inhibitor of A $\beta$  aggregation.<sup>506</sup> These molecules were found to interfere with A $\beta$ <sub>1-40</sub> fibrillization as determined by transmission electron microscopy, Thioflavin T fluorescence and velocity sedimentation analytical ultracentrifugation studies. Using electron microscopy, time-dependent studies demonstrate that apomorphine and its derivatives promote the oligomerization of A $\beta$  but inhibit its fibrillization. No clinical trials have yet begun for this compound.



Another interesting possibility is the cannabinoids. For instance, **THC** (image, left), one of the active ingredients in marijuana, competitively inhibits the enzyme acetylcholinesterase (AChE) as well as prevents AChE-induced amyloid  $\beta$ -peptide (A $\beta$ ) aggregation, the key pathological marker of Alzheimer’s disease.<sup>507</sup>

Note the authors: “Compared to currently approved drugs prescribed for the treatment of Alzheimer’s disease, THC is a considerably superior inhibitor of A $\beta$  aggregation.” But there are no clinical trials for efficacy against AD yet.

**MRZ-99030** was recently identified as a dipeptide that modulates A $\beta$ <sub>1-42</sub> aggregation by triggering a non-amyloidogenic aggregation pathway, thereby reducing the amount of intermediate toxic soluble oligomeric A $\beta$  species and ameliorating cognitive deficits in rats

<sup>504</sup> “Study Evaluating the Safety, Tolerability and Efficacy of PBT2 in Patients with Early Alzheimer’s Disease” (Clinical Trial). US National Institutes of Health. 13 Jan 2008; <http://clinicaltrials.gov/ct2/show/NCT00471211>. Faux NG, Ritchie CW, Gunn A, Rembach A, Tsatsanis A, Bedo J, Harrison J, Lannfelt L, Blennow K, Zetterberg H, Ingelsson M, Masters CL, Tanzi RE, Cummings JL, Herd CM, Bush AI. PBT2 rapidly improves cognition in Alzheimer’s Disease: additional phase II analyses. *J Alzheimers Dis.* 2010;20(2):509-16; <http://iospress.metapress.com/content/303w6x1163m61274/fulltext.pdf>.

<sup>505</sup> “Prana Biotechnology announces preliminary results of Phase 2 IMAGINE trial of PBT2 in Alzheimer’s disease,” PR Newswire, 31 Mar 2014; <http://www.prnewswire.com/news-releases/prana-biotechnology-announces-preliminary-results-of-phase-2-imagine-trial-of-pbt2-in-alzheimers-disease-253173581.html>.

<sup>506</sup> Lashuel HA, Hartley DM, Balakhaneh D, Aggarwal A, Teichberg S, Callaway DJ. New class of inhibitors of amyloid-beta fibril formation. Implications for the mechanism of pathogenesis in Alzheimer’s disease. *J Biol Chem.* 2002 Nov 8;277(45):42881-90; <http://www.jbc.org/content/277/45/42881.long>.

<sup>507</sup> Eubanks LM1, Rogers CJ, Beuscher AE 4th, Koob GF, Olson AJ, Dickerson TJ, Janda KD. A molecular link between the active component of marijuana and Alzheimer’s disease pathology. *Mol Pharm.* 2006 Nov-Dec;3(6):773-7; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2562334/>.

injected with A $\beta$ <sub>1-42</sub>.<sup>508</sup> Other synthetic  **$\alpha$ -sheet peptides** have been designed that can target toxic A $\beta$  oligomers and inhibit their aggregation into amyloid fibrils.<sup>509</sup>

Recent studies have also revealed that once A $\beta$ <sub>42</sub> fibrils are generated, their surfaces effectively catalyze the formation of neurotoxic A $\beta$  oligomers – but a **molecular chaperone**, a human Brichos domain, can specifically inhibit this catalytic cycle and limit human A $\beta$ <sub>42</sub> toxicity.<sup>510</sup>

Another possible approach just beginning to be explored is the use of **disaggregases** such as engineered variants of heat shock protein Hsp104.<sup>511</sup> Natural Hsp104 solubilizes disordered aggregates and amyloid, but has limited activity against human neurodegenerative disease proteins. However, engineered variants of Hsp104 have yielded large gains in protective activity against deleterious misfolding of  $\alpha$ -synuclein, TDP-43, FUS, and TAF15 (proteins connected with Parkinson's disease, ALS, and frontotemporal dementia),<sup>512</sup> but so far no luck with the approach on disaggregating A $\beta$  or tau in connection with Alzheimer's disease.

Finally, a 2010 study reported that long-term **EMF exposure** directly associated with cell phone use (918 MHz; 0.25 Watts/kg) provides cognitive benefits in mice, apparently by reducing brain amyloid-beta deposition through A $\beta$  anti-aggregation actions and increased brain temperature during exposure periods.<sup>513</sup>

---

<sup>508</sup> Rammes G, Gravius A, Ruitenbergh M, Wegener N, Chambon C, Sroka-Saidi K, Jeggo R, Staniaszek L, Spanswick D, O'Hare E, Palmer P, Kim EM, Bywalez W, Egger V, Parsons CG. MRZ-99030 - A novel modulator of A $\beta$  aggregation: II - Reversal of A $\beta$  oligomer-induced deficits in long-term potentiation (LTP) and cognitive performance in rats and mice. *Neuropharmacology*. 2015 May;92:170-82; <http://www.ncbi.nlm.nih.gov/pubmed/25637092>.

<sup>509</sup> Hopping G, Kellock J, Barnwal RP, Law P, Bryers J, Varani G, Caughey B, Daggett V. Designed  $\alpha$ -sheet peptides inhibit amyloid formation by targeting toxic oligomers. *Elife*. 2014 Jul 15;3:e01681; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4091096/>.

<sup>510</sup> Cohen SI, Arosio P, Presto J, Kurudenkandy FR, Biverstål H, Dolfe L, Dunning C, Yang X, Frohm B, Vendruscolo M, Johansson J, Dobson CM, Fisahn A, Knowles TP, Linse S. A molecular chaperone breaks the catalytic cycle that generates toxic A $\beta$  oligomers. *Nat Struct Mol Biol*. 2015 Mar;22(3):207-13; <http://www.ncbi.nlm.nih.gov/pubmed/25686087>.

<sup>511</sup> Jackrel ME, Shorter J. Reversing deleterious protein aggregation with re-engineered protein disaggregases. *Cell Cycle*. 2014;13(9):1379-83; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4050135/>. Jackrel ME, DeSantis ME, Martinez BA, Castellano LM, Stewart RM, Caldwell KA, Caldwell GA, Shorter J. Potentiated Hsp104 variants antagonize diverse proteotoxic misfolding events. *Cell*. 2014 Jan 16;156(1-2):170-82; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3909490/>.

<sup>512</sup> Jackrel ME, Shorter J. Engineering enhanced protein disaggregases for neurodegenerative disease. *Prion*. 2015;9(2):90-109; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4601286/>.

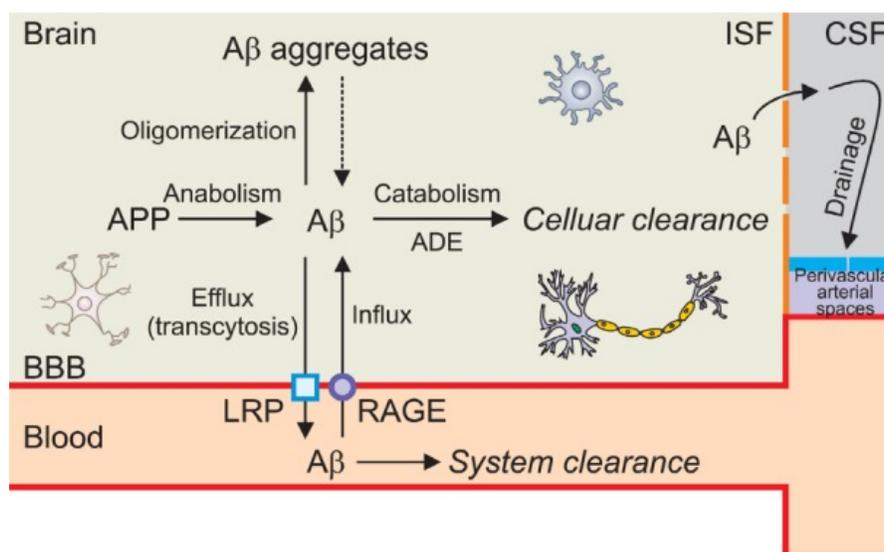
<sup>513</sup> Arendash GW, Sanchez-Ramos J, Mori T, Mamcarz M, Lin X, Runfeldt M, Wang L, Zhang G, Sava V, Tan J, Cao C. Electromagnetic field treatment protects against and reverses cognitive impairment in Alzheimer's disease mice. *J Alzheimers Dis*. 2010;19(1):191-210; <http://www.ncbi.nlm.nih.gov/pubmed/20061638>. Arendash GW. Transcranial electromagnetic treatment against Alzheimer's disease: why it has the potential to trump Alzheimer's disease drug development. *J Alzheimers Dis*. 2012;32(2):243-66; <http://www.ncbi.nlm.nih.gov/pubmed/22810103>.

### 3.2.3 Facilitate Amyloid- $\beta$ Clearance

The third anti-A $\beta$  therapeutic intervention strategy is aimed at facilitating A $\beta$  clearance from the brain. The most common late-onset form of Alzheimer's disease is characterized by an overall impairment in A $\beta$  clearance.<sup>514</sup>

The clearance of A $\beta$  from the brain is accomplished by several mechanisms which include non-enzymatic and enzymatic pathways (**Figure 8**). Nonenzymatic pathways include interstitial fluid drainage, uptake by microglial phagocytosis, and transport across the blood vessel walls into the circulation. Multiple A $\beta$ -degrading enzymes (ADE) implicated in the clearance process have been identified, including neprilysin, insulin-degrading enzyme, matrix metalloproteinase-9, glutamate carboxypeptidase II, and others.<sup>515</sup> Any of these avenues can in theory be exploited to develop a therapeutic strategy for enhanced A $\beta$  clearance from the brain.

*Figure 8. Schematic of A $\beta$  clearance pathways. The blood-brain barrier (BBB) separates circulating blood from the brain interstitial fluid (ISF) in the central nervous system (CNS). APP: Amyloid precursor protein, ADE: Amyloid- $\beta$  degradation enzyme, LRP: Low-density lipoprotein receptor-related protein, RAGE: Receptor for advanced glycation end product, CSF: Cerebrospinal fluid. (schematic from Yoon and Jo, 2012)<sup>510</sup>*



<sup>514</sup> Mawuenyega KG, Sigurdson W, Ovod V, Munsell L, Kasten T, Morris JC, Yarasheski KE, Bateman RJ. Decreased clearance of CNS beta-amyloid in Alzheimer's disease. *Science*. 2010 Dec 24;330(6012):1774; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3073454/>.

<sup>515</sup> Yoon SS, Jo SA. Mechanisms of Amyloid- $\beta$  Peptide Clearance: Potential Therapeutic Targets for Alzheimer's Disease. *Biomol Ther (Seoul)*. 2012 May;20(3):245-55; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3794520/>.

To date, there has probably been insufficient attention paid to the possibility of Alzheimer's therapeutics employing **A $\beta$ -cleaving proteases**.<sup>516</sup> Most known A $\beta$ -degrading proteases are zinc metalloproteases, e.g., neprilysin (Section 2.4.1). Because neprilysin is thought to be the rate-limiting step in amyloid beta degradation,<sup>517</sup> it has been considered a potential therapeutic target; compounds such as the peptide hormone **somatostatin** have been identified that increase the enzyme's activity level.<sup>518</sup>

Three functionally related serine proteases are also implicated in A $\beta$  degradation (plasmin, uPA, and tPA), and one cysteine protease (CatB) has been specifically implicated in the degradation of A $\beta$  *in vivo*. There is some evidence that altered A $\beta$  degradation may be operative in a very large number of AD cases.<sup>519</sup> One recent study describes the development of a novel drug by Wyeth, called **PAZ-417**, that promotes plasmin-mediated A $\beta$  degradation and is effective in lowering A $\beta$  levels and reversing memory defects in animal models.<sup>520</sup> PAZ-417 has undergone three Phase I trials for safety but its current status is unknown. Perhaps the most amusing approach involves **snake venom** from Russell's viper, one of the most dangerous snakes in Southeast Asia. Small synthetic peptides templated from RVV-V (aka. coagulation factor V activator, a component of Russell's viper venom) shred the A $\beta$  aggregates into non-toxic monomers after a specific stretch of the synthetic peptide binds to a segment of A $\beta$  and initiates the process.<sup>521</sup>

Phase III trials are starting on **TTP488** (aka. PF-04494700), a novel, small-molecule, orally active antagonist of RAGE (Receptor for Advanced Glycation Endproducts) that has slowed cognitive decline in patients with mild to moderate Alzheimer's disease, exhibiting increased

<sup>516</sup> Leissring MA. The AbetaCs of Abeta-cleaving proteases. J Biol Chem. 2008 Oct 31;283(44):29645-9; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2662051/>.

<sup>517</sup> Iwata N, Tsubuki S, Takaki Y, Watanabe K, Sekiguchi M, Hosoki E, Kawashima-Morishima M, Lee HJ, Hama E, Sekine-Aizawa Y, Saido TC. Identification of the major Abeta1-42-degrading catabolic pathway in brain parenchyma: suppression leads to biochemical and pathological deposition. Nat Med. 2000 Feb;6(2):143-50; <http://www.ncbi.nlm.nih.gov/pubmed/10655101>.

<sup>518</sup> Iwata N, Higuchi M, Saido TC. Metabolism of amyloid-beta peptide and Alzheimer's disease. Pharmacol Ther. 2005 Nov;108(2):129-48; <http://www.ncbi.nlm.nih.gov/pubmed/16112736>.

<sup>519</sup> Jiang Q, Lee CY, Mandrekar S, Wilkinson B, Cramer P, Zelcer N, Mann K, Lamb B, Willson TM, Collins JL, Richardson JC, Smith JD, Comery TA, Riddell D, Holtzman DM, Tontonoz P, Landreth GE. ApoE promotes the proteolytic degradation of Abeta. Neuron. 2008 Jun 12;58(5):681-93; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2493297/>.

<sup>520</sup> Jacobsen JS, Comery TA, Martone RL, Elokda H, Crandall DL, Oganessian A, Aschmies S, Kirksey Y, Gonzales C, Xu J, Zhou H, Atchison K, Wagner E, Zaleska MM, Das I, Arias RL, Bard J, Riddell D, Gardell SJ, Abou-Gharbia M, Robichaud A, Magolda R, Vlasuk GP, Bjornsson T, Reinhart PH, Pangalos MN. Enhanced clearance of Abeta in brain by sustaining the plasmin proteolysis cascade. Proc Natl Acad Sci U S A. 2008 Jun 24;105(25):8754-9; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2438386/>.

<sup>521</sup> Bhattacharjee P, Bhattacharyya D. Factor V activator from Daboia russelli russelli venom destabilizes  $\beta$ -amyloid aggregate, the hallmark of Alzheimer disease. J Biol Chem. 2013 Oct 18;288(42):30559-70; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3798527/>.



placebo-patients and non-antibody responders worsened, these antibody-responders showed a degree of stability in cognitive levels as assessed by the neuropsychological test battery (although not by other measures), and had lower levels of the protein tau in their cerebrospinal fluid. These results may suggest reduced disease activity in the antibody-responder group. Autopsies found that immunization resulted in clearance of amyloid plaques, but did not prevent progressive neurodegeneration.<sup>528</sup> Recent news on Betabloc seems more encouraging.<sup>529</sup> Second-generation active A $\beta$  vaccines (CAD106 (Novartis), ACC-001 (Pfizer),<sup>530</sup> and Affitope AD02 (AFFiRiS AG)) have been developed and are under clinical testing, although the trials of ACC-001 were suspended in 2008 for safety reasons.<sup>531</sup> Plasmid-based nonviral A $\beta$ <sub>42</sub> DNA vaccines have also shown some success in mouse models.<sup>532</sup>

The second approach is **passive immunotherapy** (monoclonal antibodies), an infused beta amyloid antibody or “passive vaccine” approach that does not invoke the immune system and would require regular infusions to maintain the artificial antibody levels. The most advanced such candidate is **bapineuzumab** (aka. AAB-001; chemical formula C<sub>6466</sub>H<sub>10018</sub>N<sub>1734</sub>O<sub>2026</sub>S<sub>44</sub>), a monoclonal antibody designed as essentially identical to the natural antibody triggered by the earlier active AN-1792 vaccine. Bapineuzumab was once believed to hold great potential for the treatment of AD and had reached Phase III trials, but in 2012 it failed to produce significant improvements in patients in two major trials.<sup>533</sup> On 6 Aug 2012, Pfizer and Johnson & Johnson said they were “ending development of an intravenous formulation” of bapineuzumab, and that Phase III trials “showed no treatment effect on either cognitive or functional outcomes. Biomarker analyses indicated that bapineuzumab engaged its target, but had no benefit.”<sup>534</sup> Another drug called **solanezumab** (chemical formula C<sub>6396</sub>H<sub>9922</sub>N<sub>1712</sub>O<sub>1996</sub>S<sub>42</sub>), a humanized monoclonal antibody funded by Eli Lilly, preferentially binds soluble forms of amyloid and in preclinical studies promoted its clearance from the brain, but in Phase III trials failed to improve

---

<sup>528</sup> Holmes C, Boche D, Wilkinson D, Yadegarfar G, Hopkins V, Bayer A, Jones RW, Bullock R, Love S, Neal JW, Zotova E, Nicoll JA. Long-term effects of Abeta42 immunisation in Alzheimer’s disease: follow-up of a randomised, placebo-controlled phase I trial. *Lancet*. 2008 Jul 19;372(9634):216-23; [http://www.thelancet.com/pdfs/journals/lancet/PIIS0140-6736\(08\)61075-2.pdf](http://www.thelancet.com/pdfs/journals/lancet/PIIS0140-6736(08)61075-2.pdf).

<sup>529</sup> <http://www.dailymail.co.uk/news/article-10539/Cure-Alzheimers-closer.html>

<sup>530</sup> “Study Evaluating ACC-001 in Mild to Moderate Alzheimers Disease Subjects,” Clinical Trial. US National Institutes of Health. 11 Mar 2008; <http://www.clinicaltrials.gov/ct/show/NCT00498602>. “Study Evaluating Safety, Tolerability, and Immunogenicity of ACC-001 in Subjects with Alzheimer’s Disease,” US National Institutes of Health, Jan 2013; <http://clinicaltrials.gov/ct2/show/NCT00479557>.

<sup>531</sup> “Alzheimer’s Disease Vaccine Trial Suspended on Safety Concern,” Medpage Today, 18 Apr 2008; <http://www.medpagetoday.com/MeetingCoverage/AAN/tb/9165>.

<sup>532</sup> Okura Y, Miyakoshi A, Kohyama K, Park IK, Staufenbiel M, Matsumoto Y. Nonviral Abeta DNA vaccine therapy against Alzheimer’s disease: long-term effects and safety. *Proc Natl Acad Sci U S A*. 2006 Jun 20;103(25):9619-24; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1480456/>.

<sup>533</sup> <http://www.reuters.com/article/2012/08/07/us-pfizer-alzheimers-idUSBRE8751F120120807>.

<sup>534</sup> <http://www.alzforum.org/therapeutics/bapineuzumab>.

cognition or functional ability.<sup>535</sup> New passive anti-A $\beta$  immunotherapies – **gantenerumab** (chemical formula C<sub>6496</sub>H<sub>10072</sub>N<sub>1740</sub>O<sub>2024</sub>S<sub>42</sub>) and **crenezumab** (chemical formula C<sub>6442</sub>H<sub>9966</sub>N<sub>1706</sub>O<sub>2018</sub>S<sub>40</sub>) – have also been developed and are being subjected to clinical testing.<sup>536</sup> Another monoclonal antibody, **BAN2401**, has 1000 times stronger binding preference for A $\beta$  protofibrils than for A $\beta$  monomers and is currently in Phase IIb trials.<sup>537</sup> **Intravenous immunoglobulin** (IVIG) – natural antibodies to amyloid beta – once looked promising, but Phase II<sup>538</sup> and Phase III trials found no evidence that the progression of AD had been slowed.<sup>539</sup>

**Aducanumab** (aka. BIIB037; chemical formula C<sub>6472</sub>H<sub>10028</sub>N<sub>1740</sub>O<sub>2014</sub>S<sub>46</sub>) may be the most promising passive immunotherapy drug currently in development, at Biogen Idec.<sup>540</sup> BIIB037 is a fully human IgG1 monoclonal antibody against a conformational epitope found on A $\beta$ . It was originally derived by the biotech company Neurimmune in Schlieren, Switzerland, from healthy, aged donors who were cognitively normal. The rationale was that these donors' immune systems had successfully resisted Alzheimer's disease and that the operative antibodies could be turned into therapeutics by a process called "reverse translational medicine." BIIB037 binds aggregated forms of A $\beta$ , not monomer. In the brain, BIIB037 preferentially binds parenchymal over vascular amyloid. In Mar 2015, Biogen announced its decision to skip Phase II and move into Phase III clinical trials based on interim data suggesting A $\beta$  lowering and a cognitive benefit. The company plans to initiate enrollment later in 2015 and to release results in 2016.<sup>541</sup>

---

<sup>535</sup> Doody RS, Thomas RG, Farlow M, Iwatsubo T, Vellas B, Joffe S, Kieburtz K, Raman R, Sun X, Aisen PS, Siemers E, Liu-Seifert H, Mohs R; Alzheimer's Disease Cooperative Study Steering Committee; Solanezumab Study Group. Phase 3 trials of solanezumab for mild-to-moderate Alzheimer's disease. *N Engl J Med*. 2014 Jan 23;370(4):311-21; [www.nejm.org/doi/full/10.1056/NEJMoa1312889](http://www.nejm.org/doi/full/10.1056/NEJMoa1312889).

<sup>536</sup> A Phase III clinical study of gantenerumab was stopped on 19 Dec 2014 after disappointing results; <http://www.roche.com/media/store/releases/med-cor-2014-12-19b.htm>. Phase II studies of crenezumab showed mildly positive results in 2014; <http://www.roche.com/investors/updates/inv-update-2014-07-16.htm>.

<sup>537</sup> Lannfelt L, Möller C, Basun H, Osswald G, Sehlin D, Satlin A, Logovinsky V, Gellerfors P. Perspectives on future Alzheimer therapies: amyloid- $\beta$  protofibrils – a new target for immunotherapy with BAN2401 in Alzheimer's disease. *Alzheimers Res Ther*. 2014 Mar 24;6(2):16; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4054967/>.

<sup>538</sup> Dodel R, Rominger A, Bartenstein P, Barkhof F, Blennow K, Förster S, Winter Y, Bach JP, Popp J, Alferink J, Wiltfang J, Buerger K, Otto M, Antuono P, Jacoby M, Richter R, Stevens J, Melamed I, Goldstein J, Haag S, Wietek S, Farlow M, Jessen F. Intravenous immunoglobulin for treatment of mild-to-moderate Alzheimer's disease: a phase 2, randomised, double-blind, placebo-controlled, dose-finding trial. *Lancet Neurol*. 2013 Mar;12(3):233-43; [http://www.octapharma.com/fileadmin/user\\_upload/temp/Dodel\\_2013.pdf](http://www.octapharma.com/fileadmin/user_upload/temp/Dodel_2013.pdf).

<sup>539</sup> Loeffler DA. Intravenous immunoglobulin and Alzheimer's disease: what now? *J Neuroinflammation*. 2013 Jun 5;10:70; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3720252/>.

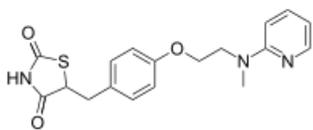
<sup>540</sup> <http://www.alzforum.org/therapeutics/aducanumab>.

<sup>541</sup> <http://biogen.newshq.businesswire.com/press-release/corporate/biogen-idec-presents-positive-interim-results-phase-1b-study-investigational>.

Reviewing the inflammation that accompanied the human trials of active AN-1792 vaccine that successfully cleared A $\beta$ , several researchers sought to activate microglial activity using drugs already known to be safe for humans. They decided on a combination of **glatiramer acetate** (aka. Copaxone), an approved drug for multiple sclerosis that acts as a decoy for errant immune-system attacks, and **Protollin**, an adjuvant that stimulates innate immunity. Given as a nasal mist, the combination reduced amyloid beta in mouse brains by 83% compared to controls.<sup>542</sup> Apparently the mist has not yet been tested in humans.

Immunotherapy is a strategy being studied by most pharmaceutical companies, with at least 6 mechanisms under current investigation: First, direct disassembly of plaques by conformation-selective antibodies; second, antibody-induced activation of microglial cells and phagocytosis of pathologic protein deposits; third, non-complement mediated phagocytosis activation of microglial cells; fourth, neutralization of toxic soluble oligomers; fifth, a shift in equilibrium toward efflux of specific proteins from the brain, creating a peripheral sink by clearance of circulating A $\beta$  cell-mediated immune responses; and sixth, immunoglobulin M (IgM)-mediated hydrolysis.

Four other rather unusual approaches for clearing soluble and insoluble amyloid have been tried.



First, recent studies suggest an association between insulin resistance and AD (fat cell sensitivity to insulin can decline with aging). In early clinical trials, a certain insulin sensitizer called **rosiglitazone** (aka. Avandia; image, left) improved cognition in a subset of AD patients.<sup>543</sup> *In vitro*, beneficial effects of rosiglitazone on primary cortical rat neurons were demonstrated.<sup>544</sup> Initial research suggested intranasal insulin, increasing insulin levels in the brain with minimal insulin increase in the rest of the body, might also be utilized.<sup>545</sup> Preclinical studies show that insulin clears soluble beta-amyloid from the brain within minutes

<sup>542</sup> Frenkel D, Maron R, Burt DS, Weiner HL. Nasal vaccination with a proteasome-based adjuvant and glatiramer acetate clears beta-amyloid in a mouse model of Alzheimer disease. *J Clin Invest.* 2005 Sep;115(9):2423-33; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1184038/>.

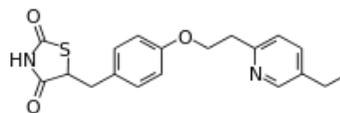
<sup>543</sup> Risner ME, Saunders AM, Altman JF, Ormandy GC, Craft S, Foley IM, Zvartau-Hind ME, Hosford DA, Roses AD; Rosiglitazone in Alzheimer's disease study group. Efficacy of rosiglitazone in a genetically defined population with mild-to-moderate Alzheimer's disease. *Pharmacogenomics J.* 2006 Jul-Aug;6(4):246-54; <http://www.ncbi.nlm.nih.gov/pubmed/16446752>.

<sup>544</sup> Brodbeck J, Balestra ME, Saunders AM, Roses AD, Mahley RW, Huang Y. Rosiglitazone increases dendritic spine density and rescues spine loss caused by apolipoprotein E4 in primary cortical neurons. *Proc Natl Acad Sci U S A.* 2008 Jan 29;105(4):1343-6; <http://www.ncbi.nlm.nih.gov/pubmed/18212130>.

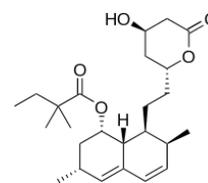
<sup>545</sup> Freiherr J, Hallschmid M, Frey WH 2nd, Br nner YF, Chapman CD, H lscher C, Craft S, De Felice FG, Benedict C. Intranasal insulin as a treatment for Alzheimer's disease: a review of basic research and clinical evidence. *CNS Drugs.* 2013 Jul;27(7):505-14; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3709085/>.

after a systemic injection in diabetic transgenic mice modeling AD.<sup>546</sup> Although approved by the FDA in 1999, rosiglitazone has been the subject of over 13,000 lawsuits due to alleged adverse side effects, which has dramatically reduced its use in the US, suspended its use in Europe, and caused it to be withdrawn entirely in the UK, New Zealand, South Africa, and India.<sup>547</sup>

Researchers believe that during the disease process, abnormal proteins cause brain cells to become insulin resistant and then die. A body of evidence suggests that this may be reversed with already-FDA-approved drugs used in the treatment of diabetes. If successful, these drugs, now on the market, can be immediately administered to Alzheimer's patients. For example, **pioglitazone** (aka. Actos; image, right),<sup>548</sup> a drug approved for the treatment of type 2 diabetes, is being tested in an Alzheimer's Association trial for efficacy in delaying the onset of mild cognitive impairment (MCI).<sup>549</sup> The study was recruiting participants in 2015.



Second, there have been reports of an association between antecedent statin use and decreased tau neurofibrillary tangle burden at autopsy.<sup>550</sup> It was hypothesized that **simvastatin** (aka. Zocor; image, right), a statin, might stimulate brain vascular endothelial cells to create a “beta-amyloid ejector”.<sup>551</sup> The use of this statin could have a causal relationship to decreased development of Alzheimer's disease. However, a clinical trial involving 406 patients found that simvastatin had no benefit on the progression of symptoms in individuals with mild to moderate AD despite significant lowering of cholesterol.<sup>552</sup>



<sup>546</sup> Vandal M, White PJ, Tremblay C, St-Amour I, Chevrier G, Emond V, Lefrançois D, Virgili J, Planel E, Giguere Y, Marette A, Calon F. Insulin reverses the high-fat diet-induced increase in brain A $\beta$  and improves memory in an animal model of Alzheimer disease. *Diabetes*. 2014 Dec;63(12):4291-301; <http://www.ncbi.nlm.nih.gov/pubmed/25008180>.

<sup>547</sup> <http://en.wikipedia.org/wiki/Rosiglitazone>

<sup>548</sup> <http://en.wikipedia.org/wiki/Pioglitazone>

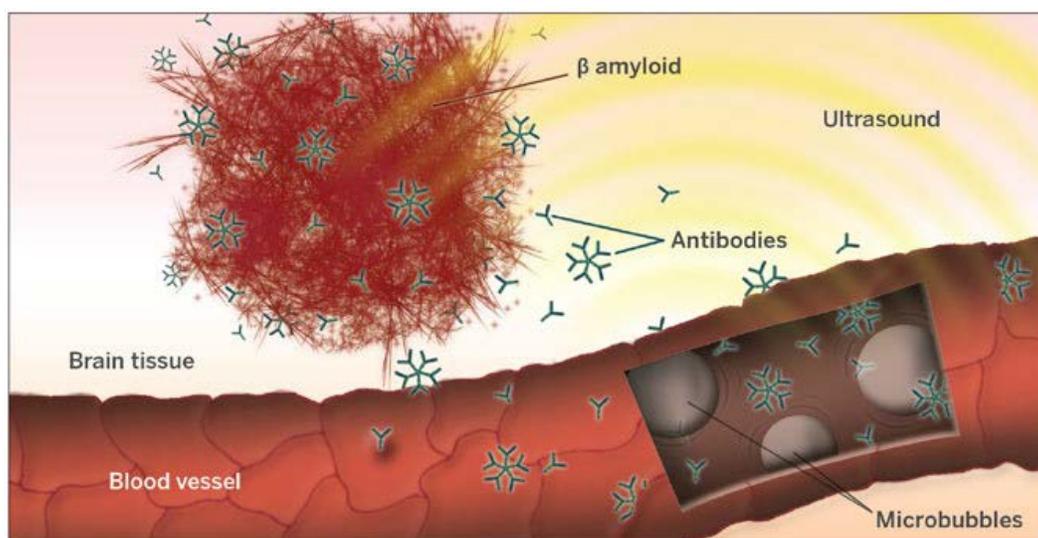
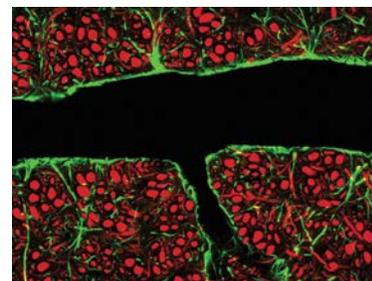
<sup>549</sup> Geldmacher DS, Fritsch T, McClendon MJ, Landreth G. A randomized pilot clinical trial of the safety of pioglitazone in treatment of patients with Alzheimer disease. *Arch Neurol*. 2011 Jan;68(1):45-50; <http://www.ncbi.nlm.nih.gov/pubmed/20837824>.

<sup>550</sup> Li G, Larson EB, Sonnen JA, Shofer JB, Petrie EC, Schantz A, Peskind ER, Raskind MA, Breitner JC, Montine TJ. Statin therapy is associated with reduced neuropathologic changes of Alzheimer disease. *Neurology*. 2007 Aug 28;69(9):878-85; <http://www.ncbi.nlm.nih.gov/pubmed/17724290>.

<sup>551</sup> Whitfield JF. The road to LOAD: late-onset Alzheimer's disease and a possible way to block it. *Expert Opin Ther Targets*. 2007 Oct;11(10):1257-60; <http://www.ncbi.nlm.nih.gov/pubmed/17907956>.

<sup>552</sup> Sano M, Bell KL, Galasko D, Galvin JE, Thomas RG, van Dyck CH, Aisen PS. A randomized, double-blind, placebo-controlled trial of simvastatin to treat Alzheimer disease. *Neurology*. 2011 Aug 9;77(6):556-63; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3149154/>.

Third, there is ultrasound.<sup>553</sup> Note that the blood-brain barrier (BBB), a tightly packed layer of cells that lines the brain's blood vessels (Section 4.3.1; image, right; green = astrocytes, red = neurons), protects it from infections, toxins, and other threats but makes the organ frustratingly hard to treat. A strategy that combines ultrasound with microscopic bloodborne bubbles can briefly open the barrier, in theory giving drugs or the immune system better access to the brain. Microbubbles injected into the blood vibrate under ultrasound, temporarily forcing apart the cells lining the blood-brain barrier. This could allow amyloid-fighting antibodies to slip into brain tissue or rouse microglia to clean up the protein (image, below).

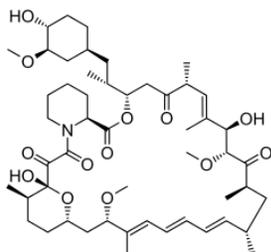


Researchers tested the ultrasound strategy in a mouse model of Alzheimer's.<sup>554</sup> After injecting these animals with a solution of microscopic bubbles, they scanned an ultrasound beam in a zigzag pattern across each animal's entire skull, rather than focusing on discrete areas as others have done. After 6-8 weekly treatments, the team tested the rodents on three different memory tasks. Alzheimer's mice in the control group, which received microbubble injections but no stimulation, showed no improvement. Mice whose blood-brain barriers had been made permeable by ultrasound saw full restoration of memory in all three tasks. The team also found a two- to fivefold reduction in different types of  $\beta$ -amyloid plaques in the brain tissue of the treated group, and much more  $A\beta$  protein in the microglia of treated animals. Human trials are scheduled for 2017.

<sup>553</sup> <http://news.sciencemag.org/biology/2015/03/ultrasound-therapies-target-brain-cancers-and-alzheimer-s-disease>

<sup>554</sup> Leinenga G, Götz J. Scanning ultrasound removes amyloid- $\beta$  and restores memory in an Alzheimer's disease mouse model. *Sci Transl Med.* 2015 Mar 11;7(278):278ra33; <http://www.ncbi.nlm.nih.gov/pubmed/25761889>.

Biochemically controlled modulation of tight junction components at the BBB could also enhance the clearance of A $\beta$  from the brain. In a mouse model of AD, plasma A $\beta_{1-40}$  levels were significantly increased, brain A $\beta_{1-40}$  levels were decreased, and cognitive function was enhanced when both claudin-5 and occludin were suppressed using siRNAs directed against claudin-5 and occludin transcripts.<sup>555</sup> The presence of A $\beta$  can cause a transient down-regulation of claudin-5 and occludin, allowing for its own paracellular clearance across the BBB.



Fourth, there is some evidence that the FDA-approved immunosuppressant drug **rapamycin** (aka. sirolimus, rapamune; image, left) may increase autophagy and thus facilitate the clearance of aggregation-prone proteins such as A $\beta$ , pathological prion protein, and  $\alpha$ -synuclein.<sup>556</sup> Multiple studies have reported that rapamycin can reduce A $\beta_{42}$  levels *in vivo* and block or delay AD progression in a transgenic mouse model of the disease.<sup>557</sup> At least one company, Navitor Pharmaceuticals, is investigating rapamycin as a possible treatment alternative for Alzheimer's.<sup>558</sup> However, adverse side effects of rapamycin have also been reported, including lung toxicity in some patients, increased risk of cancer and type 2 diabetes, and of course reduced immune activity against bacterial pathogens.

More recently, injections of the interleukin signaling molecule IL-33 seem to mobilize microglia to digest A $\beta$  plaques and to reduce inflammation in AD mouse models,<sup>559</sup> with clinical trials to test human toxicity of IL-33 apparently soon to begin.<sup>560</sup>

<sup>555</sup> Keaney J, Walsh DM, O'Malley T, Hudson N, Crosbie DE, Loftus T, Sheehan F, McDaid J, Humphries MM, Callanan JJ, Brett FM, Farrell MA, Humphries P, Campbell M. Autoregulated paracellular clearance of amyloid- $\beta$  across the blood-brain barrier. *Sci Adv.* 2015 Sep 4;1(8):e1500472; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4610013/>.

<sup>556</sup> Caccamo A, Majumder S, Richardson A, Strong R, Oddo S. Molecular interplay between mammalian target of rapamycin (mTOR), amyloid-beta, and Tau: effects on cognitive impairments. *J Biol Chem.* 2010 Apr 23;285(17):13107-20; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2857107/>. Cai Z, Yan LJ. Rapamycin, Autophagy, and Alzheimer's Disease. *J Biochem Pharmacol Res.* 2013 Jun;1(2):84-90; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3697866/>.

<sup>557</sup> Spilman P, Podlutskaya N, Hart MJ, Debnath J, Gorostiza O, Bredesen D, Richardson A, Strong R, Galvan V. Inhibition of mTOR by rapamycin abolishes cognitive deficits and reduces amyloid-beta levels in a mouse model of Alzheimer's disease. *PLoS One.* 2010 Apr 1;5(4):e9979; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2848616/>. Richardson A, Galvan V, Lin AL, Oddo S. How longevity research can lead to therapies for Alzheimer's disease: The rapamycin story. *Exp Gerontol.* 2015 Aug;68:51-8; <http://www.sciencedirect.com/science/article/pii/S0531556514003490>.

<sup>558</sup> Navitor, Inc. "Disease Applications," 2015; <http://www.navitorpharma.com/drugdevelopment/applications.html>.

<sup>559</sup> Fu AK, Hung KW, Yuen MY, Zhou X, Mak DS, Chan IC, Cheung TH, Zhang B, Fu WY, Liew FY, Ip NY. IL-33 ameliorates Alzheimer's disease-like pathology and cognitive decline. *Proc Natl Acad Sci U S A.* 2016 May 10;113(19):E2705-13; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4868478/>.

<sup>560</sup> "Protein research offers 'promising' potential treatment of Alzheimer's disease," Univ. of Glasgow, 19 Apr 2016; [http://www.gla.ac.uk/news/headline\\_456223\\_en.html](http://www.gla.ac.uk/news/headline_456223_en.html).

### 3.2.4 Reduce Pathological Tau Production

Over the last 20 years there have been more than 100 drugs developed to target beta-amyloid protein, but all of these drugs have failed. While this failure could be attributed to several factors, including the quality of the drugs or the timeliness in which they were delivered, there is also speculation that these drugs could simply be failing because they're focused on the wrong protein.<sup>561</sup> Like A $\beta$ , tau protein can also clump together and block cells from getting the nutrients that they need. Similarly, when tau becomes problematic in one part of the brain, a chain reaction occurs that causes more tau to go bad.

Historically, interest in tau as a clinical target had been muted, partly because tau pathology seems to occur downstream of A $\beta$  (making it uncertain whether tau-directed therapeutics would prevent A $\beta$ -induced impairments) and partly because tau is posttranslationally modified in Alzheimer's disease (making it unclear which modifications should be targeted). Nevertheless, reducing tau has been shown to block some A $\beta$ -induced cognitive impairments in mouse models,<sup>562</sup> and researchers are beginning to work on other ways of lowering tau expression.

One obvious target is to seek tau **kinase inhibitors**<sup>563</sup> or tau **phosphatase activators**<sup>564</sup> to indirectly halt tau hyperphosphorylation, recognized as leading to pathological aggregation of the protein. However, difficulties in finding specific inhibitors/activators with adequate safety profiles have resulted in a paucity of new drugs in this area.<sup>565</sup> **Davunetide** (see Section 3.2.9) has been described as a tau hyperphosphorylation inhibitor as well as an inhibitor of caspase 3 activation<sup>566</sup> that has been successfully evaluated in both *in vitro* and *in vivo* AD models.

The intriguing link between phosphorylation and tau pathology has provided the impetus to examine the role of kinase inhibitors as potential therapeutics targeting tau, since kinases induce the hyperphosphorylation of tau. Despite the lack of clear insight into the "best" target kinase,

---

<sup>561</sup> <http://www.bioprocessonline.com/doc/has-alzheimer-s-research-been-focused-on-wrong-protein-0001>.

<sup>562</sup> Roberson ED, Scarce-Lavie K, Palop JJ, Yan F, Cheng IH, Wu T, Gerstein H, Yu GQ, Mucke L. Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. *Science*. 2007 May 4;316(5825):750-4; <http://www.sciencemag.org/content/316/5825/750.full>.

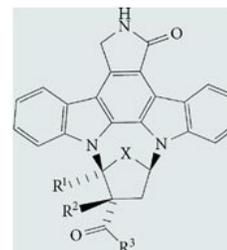
<sup>563</sup> Medina M, Avila J. Glycogen synthase kinase-3 (GSK-3) inhibitors for the treatment of Alzheimer's disease. *Curr Pharm Des*. 2010;16(25):2790-8; <http://www.ncbi.nlm.nih.gov/pubmed/20698823/>.

<sup>564</sup> Rudrabhatla P, Pant HC. Role of protein phosphatase 2A in Alzheimer's disease. *Curr Alzheimer Res*. 2011 Sep;8(6):623-32; <http://www.ncbi.nlm.nih.gov/pubmed/21605044/>.

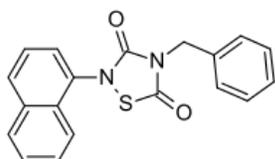
<sup>565</sup> Navarrete LP, Pérez P, Morales I, Maccioni RB. Novel drugs affecting tau behavior in the treatment of Alzheimer's disease and tauopathies. *Curr Alzheimer Res*. 2011 Sep;8(6):678-85; <http://www.ncbi.nlm.nih.gov/pubmed/21605038/>.

<sup>566</sup> Idan-Feldman A, Ostritsky R, Gozes I. Tau and caspase 3 as targets for neuroprotection. *Int J Alzheimers Dis*. 2012;2012:493670; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3369463/>.

efforts are underway to develop cyclin-dependent kinase (CDK5) inhibitors, glycogen synthase kinase (GSK3 $\beta$ ) inhibitors, and other tau kinase inhibitors. One study that used a mixed kinase inhibitor, **SRN-003-556** (aka. indolocarbazole; image, right), targeting ERK2/CDC2, GSK3 $\beta$ , PKA, and PKC, showed some efficacy in a tau mouse model.<sup>567</sup>



**Lithium chloride**, a dual action GSK3 inhibitor, also reduced tau phosphorylation, tau accumulation, and axonal degeneration in tau mice.<sup>568</sup>



**Tideglusib** (aka. NP-12, NP031112; image, left) is a non-ATP competitive inhibitor of GSK3 that has entered clinical trials for AD. Tideglusib had disease-modifying effects when administered to transgenic mice that develop both tau and amyloid pathology.<sup>569</sup> Pilot trials for tideglusib in AD showed good tolerance except for some moderate, asymptomatic, fully reversible increases in liver enzymes,<sup>570</sup> but a Phase II trial in progressive supranuclear palsy patients found no clinical efficacy relative to placebo.<sup>571</sup>

<sup>567</sup> Hinners I, Hill A, Otto U, Michalsky A, Mack TG, Striggow F. Tau kinase inhibitors protect hippocampal synapses despite of insoluble tau accumulation. *Mol Cell Neurosci*. 2008;37(3):559-567; <http://www.ncbi.nlm.nih.gov/pubmed/18201898>.

<sup>568</sup> Nakashima H, Ishihara T, Suguimoto P, Yokota O, Oshima E, Kugo A, Terada S, Hamamura T, Trojanowski JQ, Lee VM, Kuroda S. Chronic lithium treatment decreases tau lesions by promoting ubiquitination in a mouse model of tauopathies. *Acta Neuropathol (Berl)*. 2005;110(6):547-556; <http://www.ncbi.nlm.nih.gov/pubmed/16228182>.

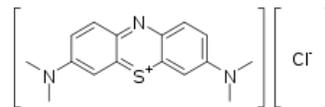
<sup>569</sup> Serenó L, Coma M, Rodríguez M, Sánchez-Ferrer P, Sánchez MB, Gich I, Agulló JM, Pérez M, Avila J, Guardia-Laguarta C, Clarimón J, Lleó A, Gómez-Isla T. A novel GSK-3 $\beta$  inhibitor reduces Alzheimer's pathology and rescues neuronal loss *in vivo*. *Neurobiol Dis*. 2009 Sep;35(3):359-67; <http://www.ncbi.nlm.nih.gov/pubmed/19523516/>.

<sup>570</sup> del Ser T, Steinwachs KC, Gertz HJ, Andrés MV, Gómez-Carrillo B, Medina M, Vericat JA, Redondo P, Fleet D, León T. Treatment of Alzheimer's disease with the GSK-3 inhibitor tideglusib: a pilot study. *J Alzheimers Dis*. 2013;33(1):205-15; <http://www.ncbi.nlm.nih.gov/pubmed/22936007>.

<sup>571</sup> Tolosa E, Litvan I, Höglinger GU, Burn D, Lees A, Andrés MV, Gómez-Carrillo B, León T, Del Ser T; TAUIROS Investigators. A phase 2 trial of the GSK-3 inhibitor tideglusib in progressive supranuclear palsy. *Mov Disord*. 2014 Apr;29(4):470-8; <http://www.ncbi.nlm.nih.gov/pubmed/24532007>.

### 3.2.5 Prevent Pathological Tau Aggregation

The aggregation of tau is toxic to cells. Multiple compounds have been identified through cell culture or *in vitro* screens as tau aggregation inhibitors.<sup>572</sup> In 2008, a clinical trial showed positive results in modifying the course of the disease in mild to moderate AD using **methylthioninium chloride** (aka. Rember, TRX-015, MTC, methylthionine, methylene blue; image, right), a phenothiazine drug that inhibits tau aggregation<sup>573</sup> and dissolves paired helical filaments from AD brain tissue that make up neurofibrillary tangles.<sup>574</sup> This 2008 trial of 321 people with mild Alzheimer's disease in the United Kingdom and Singapore found that taking the drug 3 times a day over a period of 50 weeks slows down the development of Alzheimer's disease by about 81%.<sup>575</sup> Work with methylthioninium chloride showed that bioavailability of methylthioninium from the gut was affected by feeding and by stomach acidity, leading to unexpectedly variable dosing.<sup>576</sup> A Phase II trial completed in 2015 was apparently the first clinical trial that has attempted to directly target the hallmark



<sup>572</sup> Khlistunova I, Biernat J, Wang Y, Pickhardt M, von Bergen M, Gazova Z, Mandelkow E, Mandelkow EM. Inducible expression of Tau repeat domain in cell models of tauopathy: aggregation is toxic to cells but can be reversed by inhibitor drugs. *J Biol Chem.* 2006 Jan 13;281(2):1205-14; <http://www.ncbi.nlm.nih.gov/pubmed/16246844>.

<sup>573</sup> Wischik CM, Bentham P, Wischik DJ, Seng KM. Tau aggregation inhibitor (TAI) therapy with Rember™ arrests disease progression in mild and moderate Alzheimer's disease over 50 weeks. *Alzheimer's & Dementia.* 2008;4(4):T167; <http://www.alzheimersanddementia.com/article/S1552-5260%2808%2900598-0/abstract>. Harrington CR. Methylthioninium chloride (MTC) acts as a tau aggregation inhibitor (TAI) in a cellular model and reverses tau pathology in transgenic mouse models of Alzheimer's disease. *Alzheimer's & Dementia.* 2008;4(4):T120-1; <http://dx.doi.org/10.1016%2Fj.jalz.2008.05.259>.

<sup>574</sup> Wischik CM, Edwards PC, Lai RYK, Roth M, Harrington CR. Selective inhibition of Alzheimer disease-like tau aggregation by phenothiazines. *Proc Natl Acad Sci U S A.* 1996 Oct 1;93(20):11213-8; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC38310/>.

<sup>575</sup> Emma Wilkinson, "Alzheimer's drug 'halts' decline," *BBC News*, 29 Jul 2008; <http://news.bbc.co.uk/2/hi/health/7525115.stm>.

<sup>576</sup> Baddeley TC, McCaffrey J, Storey JM, Cheung JK, Melis V, Horsley D, Harrington CR, Wischik CM. Complex disposition of methylthioninium redox forms determines efficacy in tau aggregation inhibitor therapy for Alzheimer's disease. *J Pharmacol Exp Ther.* 2015 Jan;352(1):110-8; <http://www.ncbi.nlm.nih.gov/pubmed/25320049>.

neurofibrillary tangle pathology of AD.<sup>577</sup> A new stabilized formulation, the prodrug LMTX (aka. TRx0237), is currently in Phase III trials by TauRx Pharmaceuticals.<sup>578</sup>

---

<sup>577</sup> Wischik CM, Staff RT, Wischik DJ, Bentham P, Murray AD, Storey JMD, Kook KA, Harrington CR. Tau aggregation inhibitor therapy: an exploratory phase 2 study in mild or moderate Alzheimer's disease. *J Alzheimer Dis.* 2015; 44(2):705-20; <http://core.ac.uk/download/pdf/29151100.pdf>.

<sup>578</sup> Wischik CM, Harrington CR, Storey JM. Tau-aggregation inhibitor therapy for Alzheimer's disease. *Biochem Pharmacol.* 2014 Apr 15;88(4):529-39; <http://www.sciencedirect.com/science/article/pii/S0006295213007612>. See also: <https://www.clinicaltrials.gov/ct2/archive/NCT01689233>.

### 3.2.6 Facilitate Pathological Tau Clearance

As with A $\beta$  (Section 3.2.3), **active immunization** is being investigated for tau pathologies. For example, an active vaccine, **AADvac1**, was investigated in rat models of AD and induced a robust protective humoral immune response, with antibodies discriminating between pathological and physiological tau.<sup>579</sup> Active immunization targeting crucial domains of Alzheimer tau eliminated tau aggregation and neurofibrillary pathology. AADvac1 has already entered Phase I clinical trials.

**Passive immunization** is also being pursued for AD tauopathies. Intraneuronal accumulation of abnormally hyperphosphorylated tau in the brain is a histopathological hallmark of Alzheimer's disease. One study involving mice treated with tau antibodies **43D** and **77E9** demonstrated that passive immunization targeting of normal tau can effectively clear the hyperphosphorylated tau protein and possibly also reduce A $\beta$  pathology from the brain.<sup>580</sup> In another study,<sup>581</sup> a single dose of **tau oligomer-specific monoclonal antibody** (TOMA), administered either intravenously or intracerebroventricularly for passive immunization in mice, was sufficient to reverse both locomotor and memory deficits in a mouse model of tauopathy for 60 days, coincident with rapid reduction of tau oligomers (but not phosphorylated NFTs or monomeric tau). These findings provide the first direct evidence in support of a critical role for tau oligomers in Alzheimer's disease progression and validate tau oligomers as a target for the treatment of AD and other neurodegenerative tauopathies.

Since hyperphosphorylated tau accumulates as paired helical filaments that in turn aggregate into neurofibrillary tangles inside nerve cell bodies, a drug that could inhibit or reverse hyperphosphorylation might reduce the tangle formation in AD. For example, **gracilins** are sponge-derived diterpenoid compounds that can reduce tau hyperphosphorylation; A $\beta$ <sub>42</sub> and hyperphosphorylated tau levels decreased after treatment using two neuroblastoma cellular models.<sup>582</sup>

---

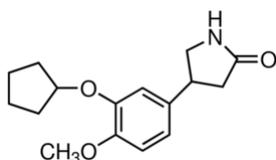
<sup>579</sup> Kontseikova E, Zilka N, Kovacech B, Novak P, Novak M. First-in-man tau vaccine targeting structural determinants essential for pathological tau-tau interaction reduces tau oligomerisation and neurofibrillary degeneration in an Alzheimer's disease model. *Alzheimers Res Ther.* 2014 Aug 1;6(4):44; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4255368/>.

<sup>580</sup> Dai CL, Chen X, Kazim SF, Liu F, Gong CX, Grundke-Iqbal I, Iqbal K. Passive immunization targeting the N-terminal projection domain of tau decreases tau pathology and improves cognition in a transgenic mouse model of Alzheimer disease and tauopathies. *J Neural Transm (Vienna).* 2015 Apr;122(4):607-17; <http://www.ncbi.nlm.nih.gov/pubmed/25233799>.

<sup>581</sup> Castillo-Carranza DL1, Sengupta U, Guerrero-Muñoz MJ, Lasagna-Reeves CA, Gerson JE, Singh G, Estes DM, Barrett AD, Dineley KT, Jackson GR, Kaye R. Passive immunization with Tau oligomer monoclonal antibody reverses tauopathy phenotypes without affecting hyperphosphorylated neurofibrillary tangles. *J Neurosci.* 2014 Mar 19;34(12):4260-72; <http://www.jneurosci.org/content/34/12/4260.long>.

<sup>582</sup> Leirós M, Alonso E, Rateb ME, Houssen WE, Ebel R, Jaspars M, Alfonso A, Botana LM. Gracilins: Spongionella-derived promising compounds for Alzheimer disease. *Neuropharmacology.* 2015 Jun;93:285-93; <http://www.ncbi.nlm.nih.gov/pubmed/25724081>.

Increasing proteasome activity is another approach to accelerated clearance of aggregated tau. Using a genetically engineered mouse model of tauopathy, it was found that proteasome activity slowed down as levels of abnormal tau increased, and that treating the mice at the early stages of



tauopathy with the drug **rolipram** (a phosphodiesterase-4 inhibitor; image, left) increased proteasome activity and led to lower levels of aggregated tau and improvements in cognitive performance.<sup>583</sup> The drug worked exclusively during the early stages of degeneration. Treating the mice at later stages of the disease was not effective.

Several intracellular proteins have been implicated as key molecules that regulate the aggregation and folding of tau (e.g., tau chaperones) or potentially mediate clearance of the misfolded and aggregated tau. The ubiquitin ligase carboxyl terminus of **heat shock cognate70-interacting protein** (CHIP) can polyubiquitinate tau and may play a crucial role in preventing accumulation of phospho-tau and neurofibrillary tangles.<sup>584</sup> Such data suggest that modulation of CHIP and the ubiquitin proteasome system could alter tau pathology. Finally, heat shock proteins have been suggested as possible modifiers of tau pathology. **HSP90 inhibitors** that induce a heat shock response can reduce tau phosphorylation at certain sites and are currently being tested in humans as anti-cancer agents.<sup>585</sup>

---

<sup>583</sup> Myeku N, Clelland CL, Emrani S, Kukushkin NV, Yu WH, Goldberg AL, Duff KE. Tau-driven 26S proteasome impairment and cognitive dysfunction can be prevented early in disease by activating cAMP-PKA signaling. *Nat Med.* 2016 Jan;22(1):46-53; <http://www.ncbi.nlm.nih.gov/pubmed/26692334>.

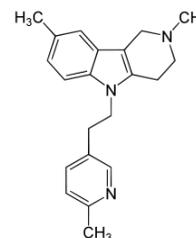
<sup>584</sup> Dickey CA, Yue M, Lin WL, Dickson DW, Dunmore JH, Lee WC, Zehr C, West G, Cao S, Clark AM, Caldwell GA, Caldwell KA, Eckman C, Patterson C, Hutton M, Petrucelli L. Deletion of the ubiquitin ligase CHIP leads to the accumulation, but not the aggregation, of both endogenous phospho- and caspase-3-cleaved tau species. *J Neurosci.* 2006;26(26):6985-96; <http://wormshack.ua.edu/pdf/jneuroscimayo.pdf>.

<sup>585</sup> Dickey CA, Dunmore J, Lu B, Wang JW, Lee WC, Kamal A, Burrows F, Eckman C, Hutton M, Petrucelli L. HSP induction mediates selective clearance of tau phosphorylated at proline-directed Ser/Thr sites but not KXGS (MARK) sites. *Faseb J.* 2006;20(6):753-5; <http://www.fasebj.org/content/early/2006/04/01/fj.05-5343fje.long>.

### 3.2.7 Control Inflammation

Since it is believed that neurons can die via inflammatory responses to A $\beta$  and tau aggregation, it is hypothesized that blocking inflammation may slow the progression of AD. A large, randomized trial of non-steroidal anti-inflammatory drugs (NSAIDs) showed that asymptomatic individuals treated with conventional NSAIDs such as naproxen indeed experienced reduced AD incidence, but only after 2 to 3 years.<sup>586</sup> In contrast, NSAIDs actually had adverse effects in later stages of AD pathogenesis.

In 2008, a clinical trial showed positive results in modifying the course of disease in mild to moderate AD with **dimebon** (aka. Latrepirdine, “Brain Cell Apoptosis Inhibitor”; image, right), an antihistamine.<sup>587</sup> However, the consecutive Phase III trial of dimebon failed to show positive effects in the primary and secondary endpoints.<sup>588</sup>



The powerful anti-inflammatory agent **Etanercept** (aka. Enbrel; chemical formula C<sub>2224</sub>H<sub>3475</sub>N<sub>621</sub>O<sub>698</sub>S<sub>36</sub>) is being studied for Alzheimer’s disease.<sup>589</sup> However, this use is controversial<sup>590</sup> and Amgen, the manufacturer of Enbrel, has disavowed this application of its product.<sup>591</sup>

<sup>586</sup> Breitner JC, Baker LD, Montine TJ, Meinert CL, Lyketsos CG, Ashe KH, Brandt J, Craft S, Evans DE, Green RC, Ismail MS, Martin BK, Mullan MJ, Sabbagh M, Tariot PN; ADAPT Research Group. Extended results of the Alzheimer’s disease anti-inflammatory prevention trial. *Alzheimers Dement*. 2011 Jul;7(4):402-11; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3149804/>.

<sup>587</sup> Doody RS, GavriloVA SI, Sano M, Thomas RG, Aisen PS, Bachurin SO, Seely L, Hung D. Effect of dimebon on cognition, activities of daily living, behaviour, and global function in patients with mild-to-moderate Alzheimer’s disease: a randomised, double-blind, placebo-controlled study. *Lancet*. 2008;372(9634):207-15; <http://www.ncbi.nlm.nih.gov/pubmed/18640457>.

<sup>588</sup> Bezprozvanny I. The rise and fall of Dimebon. *Drug News & Perspectives*. 2010 Oct;23(8):518-23; <http://www.ncbi.nlm.nih.gov/pubmed/21031168>.

<sup>589</sup> Tobinick E. Perispinal etanercept for treatment of Alzheimer’s disease. *Curr Alzheimer Res*. 2007 Dec;4(5):550-2; <http://www.ncbi.nlm.nih.gov/pubmed/18220520>. Tobinick E. Deciphering the physiology underlying the rapid clinical effects of perispinal etanercept in Alzheimer’s disease. *Curr Alzheimer Res*. 2012 Jan;9(1):99-109; <http://www.ncbi.nlm.nih.gov/pubmed/22191562>.

<sup>590</sup> <http://www.sciencebasedmedicine.org/enbrel-for-stroke-and-alzheimers/>.

<sup>591</sup> “Amgen Statement on Alzheimer’s Case Study,” 2015; [http://www.amgen.com/media/rapid\\_cognitive\\_improvement.html](http://www.amgen.com/media/rapid_cognitive_improvement.html).

A new anti-inflammatory microglial modulator drug, **CHF5074**, was tested on 94 patients having mild cognitive impairment.<sup>592</sup> Biomarkers of neuroinflammation were reduced but there was no effect on neuropsychological performance except among ApoE4 carriers.

In early 2015, GliaCure completed a Phase I trial<sup>593</sup> of **GC021109**, a drug intended to reduce both inflammation and A $\beta$  in neurons. GC021109 reportedly binds the microglial P2Y6 receptor. P2Y6 signaling is thought to be involved in shifting the phenotype of microglia (which tend to surround amyloid plaques) from patrolling to phagocytic.<sup>594</sup>

A 2016 study<sup>595</sup> found that a tyrosine kinase inhibitor (**GW2580**) inhibits the colony-stimulating factor 1 receptor CSF1R in AD-model mice and blockades microglial proliferation, improves performance in memory and behavioral tasks, and prevents synaptic degeneration, “shifting the microglial inflammatory profile to an anti-inflammatory phenotype.” The healthy number of microglia needed to maintain normal immune function in the brain was maintained, suggesting the blocking of CSF1R only reduces excess microglia. The researchers are now looking for a safe and suitable drug version that could be tested in humans.

Microglia clear away misfolded proteins, produce trophic and regenerative factors, and regulate and terminate toxic inflammation. Recent studies point to a steady decline of these normal microglial functions in aging and in AD. In AD, microglia not only lose their capacity to clear A $\beta$  peptides but also develop a persistent proinflammatory phenotype that does not resolve, accelerating neuronal and synaptic injury.<sup>596</sup> A recent study at Stanford University found that selectively blocking the prostaglandin E2 (PGE2) receptor protein called EP2 (found on microglia cells that manage inflammation and anti-inflammatory responses) restores microglial chemotaxis and A $\beta$  clearance, suppresses toxic inflammation, increases cytoprotective insulin-like growth

<sup>592</sup> Ross J, Sharma S, Winston J, Nunez M, Bottini G, Franceschi M, Scarpini E, Frigerio E, Fiorentini F, Fernandez M, Sivilia S, Giardino L, Calza L, Norris D, Cicirello H, Casula D, Imbimbo BP. CHF5074 reduces biomarkers of neuroinflammation in patients with mild cognitive impairment: a 12-week, double-blind, placebo-controlled study. *Curr Alzheimer Res.* 2013 Sep;10(7):742-53; <http://www.ncbi.nlm.nih.gov/pubmed/23968157>.

<sup>593</sup> <https://clinicaltrials.gov/ct2/show/NCT02254369?term=GC+021109&rank=1>, <http://www.alzforum.org/therapeutics/gc-021109>, <http://gliacure.com/gliacure-enters-first-in-human-clinical-trial-in-alzheimers-disease/>.

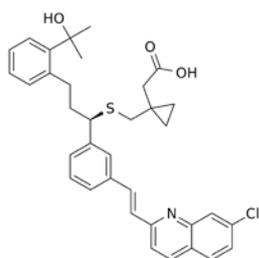
<sup>594</sup> Koizumi S, Shigemoto-Mogami Y, Nasu-Tada K, Shinozaki Y, Ohsawa K, Tsuda M, Joshi BV, Jacobson KA, Kohsaka S, Inoue K. UDP acting at P2Y6 receptors is a mediator of microglial phagocytosis. *Nature.* 2007 Apr 26;446(7139):1091-5; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3464483/>.

<sup>595</sup> Olmos-Alonso A, Schettters ST, Sri S, Askew K, Mancuso R, Vargas-Caballero M, Holscher C, Perry VH, Gomez-Nicola D. Pharmacological targeting of CSF1R inhibits microglial proliferation and prevents the progression of Alzheimer’s-like pathology. *Brain.* 2016 Mar;139(Pt 3):891-907; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4766375/>.

<sup>596</sup> Fiala M, Lin J, Ringman J, Kermani-Arab V, Tsao G, Patel A, Lossinsky AS, Graves MC, Gustavson A, Sayre J, Sofroni E, Suarez T, Chiappelli F, Bernard G. Ineffective phagocytosis of amyloid-beta by macrophages of Alzheimer’s disease patients. *J Alzheimers Dis.* 2005 Jun;7(3):221-32; discussion 255-62; <http://www.ncbi.nlm.nih.gov/pubmed/16006665>.

factor 1 (IGF1) signaling, prevents synaptic injury and memory deficits, and reverses memory decline in a mouse AD model.<sup>597</sup> Thus an EP2 inhibitor drug (e.g., analogous to **TG6-10-1**)<sup>598</sup> might be useful in AD treatment. Stanford researchers are hoping to create a compound which only blocks microglial EP2 to prevent unnecessary side effects.<sup>599</sup>

Increasing inflammatory response might also help in some cases. One study suggests that the anti-inflammatory interleukin cytokine IL-10 could be responsible for the immune system failing to clear beta amyloid plaques from the brain, with mice having low levels of IL-10 performing cognitively better in terms of learning and memory tests and with the IL-10 signaling pathway abnormally elevated in Alzheimer's disease patient brains.<sup>600</sup> Drugs targeting the down-



regulation or blocking of IL-10 could be the key to restoring an Alzheimer disease patients' immune system to normal and having it rid beta amyloid plaque on its own. Similarly, reducing levels of the inflammatory cytokine (signaling molecule) p40 lowers the A $\beta$  plaque load. Intracerebroventricular delivery of antibodies to p40 significantly reduce the concentration of soluble A $\beta$  species and reverse cognitive deficits in aged AD mice.<sup>601</sup>

Finally, an FDA-approved asthma drug called **montelukast** (aka. Singulair; image, left)<sup>602</sup> has been shown to improve memory and learning, increase the rate of new neuron growth, reduce brain

<sup>597</sup> Johansson JU, Woodling NS, Wang Q, Panchal M, Liang X, Trueba-Saiz A, Brown HD, Mhatre SD, Loui T, Andreasson KI. Prostaglandin signaling suppresses beneficial microglial function in Alzheimer's disease models. *J Clin Invest.* 2015 Jan;125(1):350-64; <http://www.jci.org/articles/view/77487>.

<sup>598</sup> Jiang J, Quan Y, Ganesh T, Pouliot WA, Dudek FE, Dingleline R. Inhibition of the prostaglandin receptor EP2 following status epilepticus reduces delayed mortality and brain inflammation. *Proc Natl Acad Sci U S A.* 2013 Feb 26;110(9):3591-6; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3587237/>. Rojas A, Ganesh T, Lelutiu N, Gueorguieva P, Dingleline R. Inhibition of the prostaglandin EP2 receptor is neuroprotective and accelerates functional recovery in a rat model of organophosphorus induced status epilepticus. *Neuropharmacology.* 2015 Feb 3;93C:15-27; <http://www.ncbi.nlm.nih.gov/pubmed/25656476>.

<sup>599</sup> <http://www.telegraph.co.uk/news/science/science-news/11280504/Has-Stanford-University-found-a-cure-for-Alzheimers-disease.html>. See also: <http://med.stanford.edu/news/all-news/2014/12/blocking-receptor-in-brains-immune-cells-counters-alzheimers.html>.

<sup>600</sup> Guillot-Sestier MV, Doty KR, Gate D, Rodriguez J Jr, Leung BP, Rezai-Zadeh K, Town T. IL10 deficiency rebalances innate immunity to mitigate Alzheimer-like pathology. *Neuron.* 2015 Feb 4;85(3):534-48; <http://www.ncbi.nlm.nih.gov/pubmed/25619654>.

<sup>601</sup> Vom Berg J, Prokop S, Miller KR, Obst J, Kälin RE, Lopategui-Cabezas I, Wegner A, Mair F, Schipke CG, Peters O, Winter Y, Becher B, Heppner FL. Inhibition of IL-12/IL-23 signaling reduces Alzheimer's disease-like pathology and cognitive decline. *Nat Med.* 2012 Dec;18(12):1812-9; <http://www.ncbi.nlm.nih.gov/pubmed/23178247>.

<sup>602</sup> <https://en.wikipedia.org/wiki/Montelukast>. "Old rat brains rejuvenated and new neurons grown by asthma drug," *Daily News*, 23 Oct 2015; <https://www.newscientist.com/article/dn28384-old-rat-brains-rejuvenated-and-new-neurons-grown-by-asthma-drug/>.

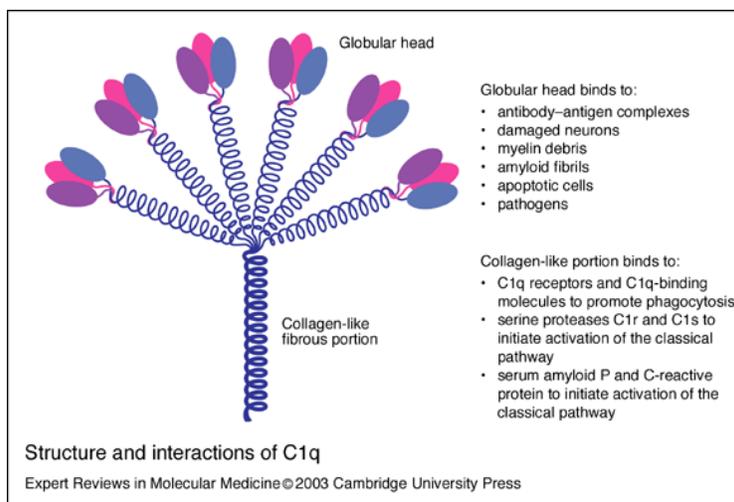
inflammation, and generally “rejuvenate” the brains of older rats.<sup>603</sup> The team also found that the blood-brain barrier – which stops infectious agents from reaching the brain and which weakens in old age – became stronger in old rats following the 6-week drug treatment regimen. The drug, technically known as a “leukotriene receptor antagonist,” had no effect on young animals, probably because it targets inflammation associated with age and disease – possibly including Alzheimer’s disease and Huntington’s disease, among other conditions.

---

<sup>603</sup> Marschallinger J, Schäffner I, Klein B, Gelfert R, Rivera FJ, Illes S, Grassner L, Janssen M, Rotheneichner P, Schmuckermair C, Coras R, Boccazzi M, Chishti M, Lagler FB, Renic M, Bauer HC, Singewald N, Blümcke I, Bogdahn U, Couillard-Despres S, Lie DC, Abbracchio MP, Aigner L. Structural and functional rejuvenation of the aged brain by an approved anti-asthmatic drug. *Nat Commun.* 2015 Oct 27;6:8466; <http://www.nature.com/ncomms/2015/151027/ncomms9466/full/ncomms9466.html>.

### 3.2.8 Modulate Complement Cascade

As noted in Section 2.4.4, C1q (image, below), a protein component of complement factor C1 known as the initiator of the “classical pathway” of the complement system in the human immune response, may be implicated in Alzheimer’s disease. Dysregulation of the complement cascade, either by changes in receptor expression, enhanced activation of different complement pathways or imbalances between complement factor production and complement cascade inhibitors may all contribute to the involvement of complement in AD.<sup>604</sup>



A 2013 study of brain tissue in mice of varying ages, as well as postmortem samples of a 2-month-old human infant and an elderly person, found that C1q exponentially increases in the aging brain, creating as much as a 300-fold buildup that concentrates around the brain’s synapses.<sup>605</sup>

Barres, the principal study author, believes that amyloid buildup is a symptom of AD rather than the cause, and that the complement turns on first and starts to kill synapses. If true, then blocking this complement cascade could treat Alzheimer’s. Barres is so confident about his findings that he is already developing a drug to target the complement system in the brain. In 2011, he co-founded a company, Annexon Bioscience,<sup>606</sup> which has been working on creating a drug that

<sup>604</sup> Crehan H, Hardy J, Pocock J. Microglia, Alzheimer’s disease, and complement. *Int J Alzheimers Dis.* 2012;2012:983640; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3432348/>.

<sup>605</sup> Stephan AH, Madison DV, Mateos JM, Fraser DA, Lovelett EA, Coutellier L, Kim L, Tsai HH, Huang EJ, Rowitch DH, Berns DS, Tenner AJ, Shamloo M, Barres BA. A dramatic increase of C1q protein in the CNS during normal aging. *J Neurosci.* 2013 Aug 14;33(33):13460-74; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3742932/>.

<sup>606</sup> <http://annexonbio.com>.

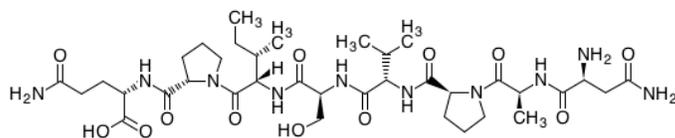
binds and inhibits the C1q protein. Their leading candidate is **ANX005**, a humanized monoclonal antibody that inhibits early components of the classical complement cascade, currently in preclinical development.<sup>607</sup>

---

<sup>607</sup> “Attacking all angles of Alzheimer’s,” Biotech Primer Weekly, 17 Mar 2016; <http://weekly.biotechprimer.com/attacking-all-angles-of-alzheimers/>.

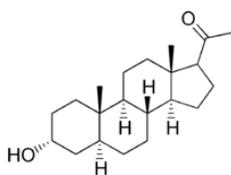
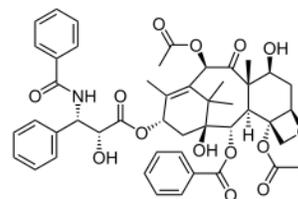
### 3.2.9 Neuroprotective Agents

**AL-108** (aka. NAP, Davunetide; image, below) is the intranasal formulation of NAP, a peptide of eight amino acids (NAPVSIQ). Phase IIa clinical results have recently shown that AL-108 has a positive impact on memory function in patients with amnesic mild cognitive impairment (aMCI), a precursor to Alzheimer's disease.<sup>608</sup> The clinical development of AL-108 has been based on extensive studies showing pre-clinical efficacy for NAP which has demonstrated potent



neuroprotective activity *in vitro* and *in vivo*. Its mechanism of action is thought to center on the modulation of microtubule stability in the face of outside damage.

Recent experiments have also demonstrated that anti-cancer microtubule (MT)-stabilizing drugs like **paclitaxel** (image, right) can rescue tau-induced behavioral decline and hallmark neuron pathologies.<sup>609</sup> Various other classes of MT-stabilizing agents are known.<sup>610</sup>



**Allopregnanolone** (aka. ALLO, AP $\alpha$ , 3 $\alpha$ ,5 $\alpha$ -THP; image, left) has been identified as a potential neuroprotective drug agent for AD. Brain levels of neurosteroids such as allopregnanolone decline in old age and in AD,<sup>611</sup> and allopregnanolone has been shown to aid the neurogenesis that reverses cognitive deficits in a mouse model of AD.<sup>612</sup> The molecule is being considered for development as a regenerative therapeutic for Alzheimer's

<sup>608</sup> Gozes I, Stewart A, Morimoto B, Fox A, Sutherland K, Schmeche D. Addressing Alzheimer's disease tangles: from NAP to AL-108. *Curr Alzheimer Res.* 2009 Oct;6(5):455-60; <http://www.ncbi.nlm.nih.gov/pubmed/19874271>.

<sup>609</sup> Erez H, Shemesh OA, Spira ME. Rescue of tau-induced synaptic transmission pathology by paclitaxel. *Front Cell Neurosci.* 2014 Feb 10;8:34; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3918585/>.

<sup>610</sup> Brunden KR, Trojanowski JQ, Smith AB 3rd, Lee VM, Ballatore C. Microtubule-stabilizing agents as potential therapeutics for neurodegenerative disease. *Bioorg Med Chem.* 2014 Sep 15;22(18):5040-9; <http://www.ncbi.nlm.nih.gov/pubmed/24433963>.

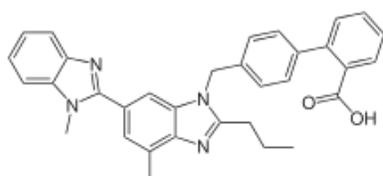
<sup>611</sup> Marx CE, Trost WT, Shampine LJ, Stevens RD, Hulette CM, Steffens DC, Ervin JF, Butterfield MI, Blazer DG, Massing MW, Lieberman JA. The neurosteroid allopregnanolone is reduced in prefrontal cortex in Alzheimer's disease. *Biol Psychiatry.* 2006 Dec 15;60(12):1287-94; <http://www.ncbi.nlm.nih.gov/pubmed/16997284>.

<sup>612</sup> Wang JM, Singh C, Liu L, Irwin RW, Chen S, Chung EJ, Thompson RF, Brinton RD. Allopregnanolone reverses neurogenic and cognitive deficits in mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A.* 2010 Apr 6;107(14):6498-503; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2851948/>.

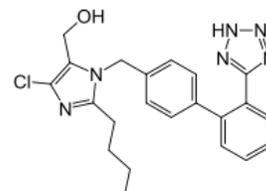
disease.<sup>613</sup>

Several early epidemiological studies had linked the female hormone **estrogen** to improved memory and possible delay or prevention of Alzheimer's disease in women. But a large, long-term clinical trial sponsored by the NIH found that women aged 65+ who took estrogen combined with another hormone, progestin, had twice the rate of dementia, including Alzheimer's disease, than those women not taking the hormones.<sup>614</sup> The hormone combination also did not protect against the development of mild cognitive impairment, a form of mental decline less severe than dementia. Similarly, treating older male AD patients with the hormone **testosterone** has small<sup>615</sup> to minimal<sup>616</sup> beneficial effects on cognition.

A retrospective analysis of five million patient records with the US Department of Veterans Affairs system found that different types of commonly used anti-hypertensive medications had very different AD outcomes. Patients taking angiotensin receptor blockers (ARBs) were 35%-40% less likely to develop AD than those using other anti-hypertensives.<sup>617</sup>



**Losartan** (aka. Cozaar; image, right), an angiotensin II (AngII) receptor blocker that selectively binds AngII type 1 (AT1) receptors, exerted some protective and restorative effects on hallmark symptoms of AD in a mouse AD model.<sup>618</sup>



<sup>613</sup> Irwin RW, Brinton RD. Allopregnanolone as regenerative therapeutic for Alzheimer's disease: translational development and clinical promise. *Prog Neurobiol.* 2014 Feb;113:40-55; <http://www.ncbi.nlm.nih.gov/pubmed/24044981>.

<sup>614</sup> Shumaker SA, Legault C, Rapp SR, Thal L, Wallace RB, Ockene JK, Hendrix SL, Jones BN 3rd, Assaf AR, Jackson RD, Kotchen JM, Wassertheil-Smoller S, Wactawski-Wende J; WHIMS Investigators. Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. *JAMA.* 2003 May 28;289(20):2651-62; <http://www.ncbi.nlm.nih.gov/pubmed/12771112>

<sup>615</sup> Cherrier MM, Matsumoto AM, Amory JK, Asthana S, Bremner W, Peskind ER, Raskind MA, Craft S. Testosterone improves spatial memory in men with Alzheimer disease and mild cognitive impairment. *Neurology.* 2005 Jun 28;64(12):2063-8; <http://www.ncbi.nlm.nih.gov/pubmed/15985573>.

<sup>616</sup> Lu PH, Masterman DA, Mulnard R, Cotman C, Miller B, Yaffe K, Reback E, Porter V, Swerdloff R, Cummings JL. Effects of testosterone on cognition and mood in male patients with mild Alzheimer disease and healthy elderly men. *Arch Neurol.* 2006 Feb;63(2):177-85; <http://www.ncbi.nlm.nih.gov/pubmed/16344336>.

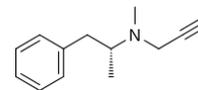
<sup>617</sup> Boston University, "Angiotensin receptor blockers are lower incidence, progression of Alzheimer's disease," 28 Jul 2008; <http://phys.org/news136426165.html>.

<sup>618</sup> Ongali B, Nicolakakis N, Tong XK, Aboukassim T, Papadopoulos P, Rosa-Neto P, Lecrux C, Imboden H, Hamel E. Angiotensin II type 1 receptor blocker losartan prevents and rescues cerebrovascular, neuropathological and cognitive deficits in an Alzheimer's disease model. *Neurobiol Dis.* 2014 Aug;68:126-36; <http://www.ncbi.nlm.nih.gov/pubmed/24807206>.

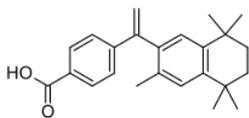
another AngII type 1 receptor blocker, showed similar benefits in human trials.<sup>619</sup>

Translocator protein (TSPO) ligands such as **Ro5-4864** have been found to be neuroprotective following a wide range of insults including peripheral nerve injury, traumatic brain injury, excitotoxic lesion, inflammatory insult, and may also be a promising target for the development of therapeutics for the treatment of AD.<sup>620</sup>

The increased activity of monoamine oxidase-B (MAO-B) in AD brains is suggested to cause oxidative damage, and MAO-B inhibitors have been reported to inhibit neuronal degeneration. **Selegiline** (aka. L- deprenyl; image, right), a selective MAO-B inhibitor, improves mental health scores in human patients but apparently has no impact on senile/neuritic plaques, neurofibrillary tangles, or A $\beta$  load.<sup>621</sup> Another antioxidative neuroprotector, **TFP5** (a 24-residue peptide), rescues AD pathology in mice; treated mice also display decreased inflammation, amyloid plaques, NFTs, cell death, and 2 months of extended life.<sup>622</sup> No results yet in humans.



Angiotensin IV analogs exhibit anti-dementia activity and promote synaptic growth and augmented connectivity in aging rats.<sup>623</sup> M3 Biotechnology<sup>624</sup> is developing **MM-201**, a “small molecule mimetic of a neurotrophic factor (growth factor) that is blood-brain barrier (BBB) permeant, potently neurotrophic and neuroprotective, and capable of reversing cognitive and motoneuron deficits in animal models of Alzheimer’s and Parkinson’s disease.”



Another possible neuroprotective agent called **bexarotene** (aka. BEXA, Targretin; image, left), already FDA-approved for anti-cancer uses, was

<sup>619</sup> Li W, Zhang JW, Lu F, Ma MM, Wang JQ, Suo AQ, Bai YY, Liu HQ. Effects of telmisartan on the level of A $\beta$ 1-42, interleukin-1 $\beta$ , tumor necrosis factor  $\alpha$  and cognition in hypertensive patients with Alzheimer’s disease. *Zhonghua Yi Xue Za Zhi*. 2012 Oct 23;92(39):2743-6, in Chinese; <http://www.ncbi.nlm.nih.gov/pubmed/23290159>.

<sup>620</sup> Barron AM, Garcia-Segura LM, Caruso D, Jayaraman A, Lee JW, Melcangi RC, Pike CJ. Ligand for translocator protein reverses pathology in a mouse model of Alzheimer’s disease. *J Neurosci*. 2013 May 15; 33(20): 8891–8897; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3733563/>.

<sup>621</sup> Alafuzoff I, Helisalmi S, Heinonen EH, Reinikainen K, Hallikainen M, Soininen H, Koivisto K. Selegiline treatment and the extent of degenerative changes in brain tissue of patients with Alzheimer’s disease. *Eur J Clin Pharmacol*. 2000 Feb-Mar;55(11-12):815-9; <http://www.ncbi.nlm.nih.gov/pubmed/10805059>.

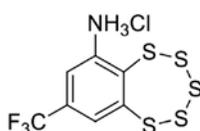
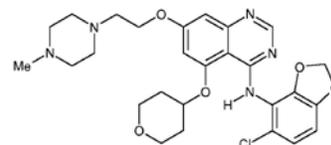
<sup>622</sup> Shukla V, Zheng YL, Mishra SK, Amin ND, Steiner J, Grant P, Kesavapany S, Pant HC. A truncated peptide from p35, a Cdk5 activator, prevents Alzheimer’s disease phenotypes in model mice. *FASEB J*. 2013 Jan;27(1):174-86; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3528323/>.

<sup>623</sup> McCoy AT, Benoist CC, Wright JW, Kawas LH, Bule-Ghogare JM, Zhu M, Appleyard SM, Wayman GA, Harding JW. Evaluation of metabolically stabilized angiotensin IV analogs as procognitive/antidementia agents. *J Pharmacol Exp Ther*. 2013 Jan;344(1):141-54; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3533412/>.

<sup>624</sup> <http://www.m3bio.com/>.

found in an initial study to rapidly clear soluble A $\beta$  from the brain, reduce neuritic plaque burden, reverse behavioral deficits, and improve cognition in mouse models.<sup>625</sup> But after several years of attempting to replicate these results without success, the drug now appears to have no beneficial effect on AD mice.<sup>626</sup>

**Saracatinib** (aka. AZD0530, an FDA-approved cancer drug; image, right) is hoped to work on AD patients by protecting neurons from the damage caused by the oligomeric form of the beta-amyloid protein; it is now in Phase IIa trials.<sup>627</sup> AZD0530 treatment also reduces microglial activation in APP/PS1 mice, and rescues tau phosphorylation and deposition abnormalities in APP/PS1/Tau transgenic mice.<sup>628</sup> Other already-approved off-purpose drugs currently being tested as possible treatments for Alzheimer's include **acamprosate calcium**, which is prescribed to reduce cravings and alleviate withdrawal symptoms in alcoholics, and **baclofen**, a medication used to treat multiple sclerosis. Another neuroprotective agent, **T-817MA**, is currently in a Phase II multi-center, randomized, double blind, placebo-controlled, parallel group study sponsored by Toyama Chemical Co.<sup>629</sup>



**TC-2153** (aka. benzopentathiepin 8-(trifluoromethyl)-1,2,3,4,5-benzopentathiepin-6-amine hydrochloride; image, left), an inhibitor of STEP (STriatal-Enriched protein tyrosine Phosphatase) neuron-specific phosphatase, has been found to improve cognitive function in 6- and 12-month old AD mice, but with no change in beta amyloid and phospho-tau levels.<sup>630</sup>

<sup>625</sup> Cramer PE, Cirrito JR, Wesson DW, Lee CY, Karlo JC, Zinn AE, Casali BT, Restivo JL, Goebel WD, James MJ, Brunden KR, Wilson DA, Landreth GE. ApoE-directed therapeutics rapidly clear  $\beta$ -amyloid and reverse deficits in AD mouse models. *Science*. 2012 Mar 23;335(6075):1503-6; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3651582/>.

<sup>626</sup> Balducci C, Paladini A, Micotti E, Tolomeo D, La Vitola P, Grigoli E, Richardson JC, Forloni G. The Continuing Failure of Bexarotene in Alzheimer's Disease Mice. *J Alzheimers Dis*. 2015;46(2):471-82; [https://www.researchgate.net/profile/Gianluigi\\_Forloni/publication/273702858\\_The\\_Continuing\\_Failure\\_of\\_Bexarotene\\_in\\_Alzheimers\\_Disease\\_Mice/links/5578362208aeb6d8c01ec51a.pdf](https://www.researchgate.net/profile/Gianluigi_Forloni/publication/273702858_The_Continuing_Failure_of_Bexarotene_in_Alzheimers_Disease_Mice/links/5578362208aeb6d8c01ec51a.pdf).

<sup>627</sup> Nygaard HB, van Dyck CH, Strittmatter SM. Fyn kinase inhibition as a novel therapy for Alzheimer's disease. *Alzheimers Res Ther*. 2014 Feb 5;6(1):8; <http://www.ncbi.nlm.nih.gov/pubmed/24495408>

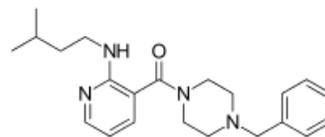
<sup>628</sup> Kaufman AC, Salazar SV, Haas LT, Yang J, Kostylev MA, Jeng AT, Robinson SA, Gunther EC, van Dyck CH, Nygaard HB, Strittmatter SM. Fyn inhibition rescues established memory and synapse loss in Alzheimer mice. *Ann Neurol*. 2015 Jun;77(6):953-71; <http://www.ncbi.nlm.nih.gov/pubmed/25707991>.

<sup>629</sup> <http://www.noblestudy.org/>; <https://clinicaltrials.gov/ct2/show/NCT02079909>. See also: <http://national.deseretnews.com/article/3853/new-alzheimers-drug-attempts-to-enter-treatment-landscape.html>.

<sup>630</sup> Xu J, Chatterjee M, Baguley TD, Brouillette J, Kurup P, Ghosh D, Kanyo J, Zhang Y, Seyb K, Ononenyi C, Foscue E, Anderson GM, Gresack J, Cuny GD, Glicksman MA, Greengard P, Lam TT, Tautz L, Nairn AC, Ellman JA, Lombroso PJ. Inhibitor of the tyrosine phosphatase STEP reverses cognitive deficits in a mouse model of Alzheimer's disease. *PLoS Biol*. 2014 Aug 5;12(8):e1001923; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4122355/>.

The pharmaceutical company GlaxoSmithKline is currently screening a million compounds, looking for additional STEP inhibitor drug candidates.<sup>631</sup> Interestingly, black tea also contains tyrosine phosphatase inhibitors.<sup>632</sup>

**NSI-189** (image, right) is an experimental drug being studied by Neuralstem, Inc. that has been shown to increase the hippocampal volume of adult mice by 20% and has been shown to stimulate neurogenesis of human hippocampal stem cells *in vitro* and *in vivo*.



A Phase Ib clinical trial for treating major depressive disorder in 24 patients started in 2012 and completed in July 2014.<sup>633</sup> The study found improvements in the patients' condition at doses of 40/80 mg/day but no significant improvements at a higher dosage of 120 mg/day. Neuralstem intends to pursue further clinical trials for this drug, targeting Alzheimer's and a variety of other neurological conditions. **NNI-362**, a drug under development by Neuronascent, "promoted the growth of new hippocampal neurons that not only migrated to the correct functional location but also differentiated and survived long enough to reverse previously observed cognitive declines" in a mouse model of AD.<sup>634</sup> Neuronascent is preparing for Phase I trials of NNI-362.

**GDF11** (aka. Growth Differentiation Factor 11 or Bone Morphogenic Protein 11/BMP-11), whose concentration in human blood decreases as we age, has been found to improve the cerebral vasculature and to enhance neurogenesis in aging mice and thus might be useful for treating age-related neurodegenerative conditions such as Alzheimer's disease.<sup>635</sup> The amylin receptor antagonist **AC253** neutralizes the depressant effects of A $\beta$ <sub>1-42</sub> on hippocampal long-term potentiation (i.e., fixation of long-term memories).<sup>636</sup>

<sup>631</sup> <http://www.newsweek.com/alzheimers-cure-worked-mice-compound-tc-2153-263171>.

<sup>632</sup> Ma J, Li Z, Xing S, Ho WT, Fu X, Zhao ZJ. Tea contains potent inhibitors of tyrosine phosphatase PTP1B. *Biochem Biophys Res Commun*. 2011 Apr 1;407(1):98-102; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3070786/>.

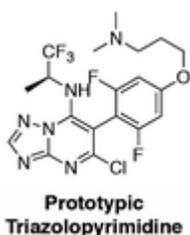
<sup>633</sup> <https://clinicaltrials.gov/ct2/show/NCT01310881>; <https://clinicaltrials.gov/ct2/show/NCT01520649>.

<sup>634</sup> "Attacking all angles of Alzheimer's," *Biotech Primer Weekly*, 17 Mar 2016; <http://weekly.biotechprimer.com/attacking-all-angles-of-alzheimers/>.

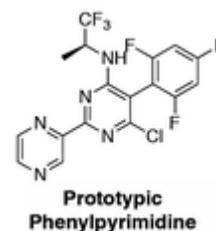
<sup>635</sup> Katsimparidi L, Litterman NK, Schein PA, Miller CM, Loffredo FS, Wojtkiewicz GR, Chen JW, Lee RT, Wagers AJ, Rubin LL. Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. *Science*. 2014 May 9;344(6184):630-4; <http://www.sciencemag.org/content/344/6184/630.full.pdf>.

<sup>636</sup> Kimura R, MacTavish D, Yang J, Westaway D, Jhamandas JH. Beta amyloid-induced depression of hippocampal long-term potentiation is mediated through the amylin receptor. *J Neurosci*. 2012 Nov 28;32(48):17401-6; <http://www.jneurosci.org/content/32/48/17401.long>.

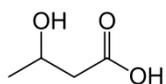
A drug developed by Astra Zeneca called **AZD05030**, which failed to treat solid tumors in previous studies, helped block damage triggered during the formation of amyloid-beta plaques.<sup>637</sup> The new drug seems to block the activation of the enzyme FYN (which leads to the loss of synaptic connections between brain cells, and later neurodegenerative disease). With this treatment, cells under bombardment by beta amyloid plaques show restored synaptic connections and reduced inflammation, and the animal's memory, which was lost during the course of the disease, comes back.



A neuroprotective approach to AD tauopathy is to deploy microtubule (MT) stabilizing drugs that can to some extent replace the lost activity of the departed stabilizing tau protein. **Epothilone D** is the only brain-penetrant MT-stabilizer to be evaluated in tau transgenic mice and in AD patients, but this natural product has potential deficiencies as a drug candidate including an intravenous route of administration and the inhibition of the P-glycoprotein (Pgp) transporter. Researchers have identified selected **triazolopyrimidines** (image, left) and **phenylpyrimidines** (image, right) that are orally bioavailable and brain-penetrant without disruption of Pgp function. Pharmacodynamic studies confirm that representative compounds from these series enhance MT-stabilization in the brains of wild-type mice.<sup>638</sup>



Glucose is the brain's principal energy substrate. AD brains exhibit a pathological decrease in their ability to use glucose, but ketone bodies can be an effective alternative energy substrate for the brain. Pursuing a ketogenic diet approach to neuroprotection, Accera Inc. first gave AD patients oral ketone bodies (i.e.,  **$\beta$ -hydroxybutyrate**; image, left) in a preliminary study.<sup>639</sup> Later, in a randomized double-blind placebo-controlled clinical study,<sup>640</sup> they gave other AD patients the oral ketogenic compound **AC-1202** (a medium-chain triglyceride composed of glycerin and caprylic acid) to see if the elevation of plasma ketone body levels would improve cognitive functioning. Plasma ketone levels did



<sup>637</sup> Kaufman AC, Salazar SV, Haas LT, Yang J, Kostylev MA, Jeng AT, Robinson SA, Gunther EC, van Dyck CH, Nygaard HB, Strittmatter SM. Fyn inhibition rescues established memory and synapse loss in Alzheimer mice. *Ann Neurol*. 2015 Jun;77(6):953-71; <http://www.ncbi.nlm.nih.gov/pubmed/25707991>.

<sup>638</sup> Lou K, Yao Y, Hoyer AT, James MJ, Cornec AS, Hyde E, Gay B, Lee VM, Trojanowski JQ, Smith AB 3rd, Brunden KR, Ballatore C. Brain-penetrant, orally bioavailable microtubule-stabilizing small molecules are potential candidate therapeutics for Alzheimer's disease and related tauopathies. *J Med Chem*. 2014 Jul 24;57(14):6116-27; <http://www.ncbi.nlm.nih.gov/pubmed/24992153>.

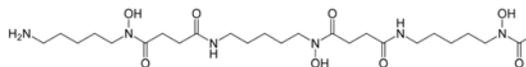
<sup>639</sup> Reger MA, Henderson ST, Hale C, Cholerton B, Baker LD, Watson GS, Hyde K, Chapman D, Craft S. Effects of beta-hydroxybutyrate on cognition in memory-impaired adults. *Neurobiol Aging*. 2004 Mar;25(3):311-4; <http://www.ncbi.nlm.nih.gov/pubmed/15123336>.

<sup>640</sup> Henderson ST, Vogel JL, Barr LJ, Garvin F, Jones JJ, Costantini LC. Study of the ketogenic agent AC-1202 in mild to moderate Alzheimer's disease: a randomized, double-blind, placebo-controlled, multicenter trial. *Nutr Metab (Lond)*. 2009 Aug 10;6:31; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2731764/>.

increase in both studies. Memory performance and cognitive function improved in AD patients lacking the ApoE4 genetic mutation (Section 2.4.5) but not in AD patients bearing the mutation.

Studies have suggested that people with Alzheimer's have higher iron levels in their brains, and that high iron might hasten the disease's onset. For example, mildly cognitively-impaired elderly patients were diagnosed with AD on average 3 months earlier for every extra ng/ml of ferritin (an iron-binding protein) found in their cerebrospinal fluid.<sup>641</sup> This doesn't prove that reducing iron levels will cut people's risk of Alzheimer's, but

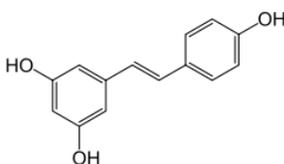
a 1991 trial of an iron-binding drug (**deferoxamine**; image, right)<sup>642</sup> that rids the body of some of its iron suggests it's a



hypothesis worth investigating. Deferoxamine halved the rate of Alzheimer's cognitive decline but was overlooked when the beta-amyloid theory of the disease became dominant. The drug gets into the brain and reduces levels of the metal there without disturbing blood levels too much.

Brain-derived neurotrophic factor (**BDNF**) has shown some neuroprotective effects in several animal models of AD,<sup>643</sup> but there are no human clinical trials yet even though "BDNF-based therapy is increasingly expected to ameliorate the symptoms of AD."<sup>644</sup>

**Resveratrol** (image, below), a naturally occurring phytochemical present in red wine, grapes, berries, chocolate and peanuts, has antioxidant and anti-inflammatory properties and facilitates non-amyloidogenic breakdown of the amyloid precursor protein (APP), promotes removal of neurotoxic amyloid beta (A $\beta$ ) peptides, reduces damage to neuronal cells via a variety of additional mechanisms, and has demonstrated neuroprotective effects in several *in vitro* and *in vivo* models of AD.<sup>645</sup>



<sup>641</sup> Ayton S, Faux NG, Bush AI; Alzheimer's Disease Neuroimaging Initiative. Ferritin levels in the cerebrospinal fluid predict Alzheimer's disease outcomes and are regulated by APOE. *Nat Commun.* 2015 May 19;6:6760; <http://www.ncbi.nlm.nih.gov/pubmed/25988319>.

<sup>642</sup> Crapper McLachlan DR, Dalton AJ, Kruck TP, Bell MY, Smith WL, Kalow W, Andrews DF. Intramuscular desferrioxamine in patients with Alzheimer's disease. *Lancet.* 1991 Jun 1;337(8753):1304-8; <http://www.ncbi.nlm.nih.gov/pubmed/1674295>.

<sup>643</sup> Nagahara AH, Merrill DA, Coppola G, Tsukada S, Schroeder BE, Shaked GM, Wang L, Blesch A, Kim A, Conner JM, Rockenstein E, Chao MV, Koo EH, Geschwind D, Masliah E, Chiba AA, Tuszynski MH. Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease. *Nat Med.* 2009 Mar;15(3):331-7; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2838375/>.

<sup>644</sup> Adachi N, Numakawa T, Richards M, Nakajima S, Kunugi H. New insight in expression, transport, and secretion of brain-derived neurotrophic factor: Implications in brain-related diseases. *World J Biol Chem.* 2014 Nov 26;5(4):409-28; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4243146/>.

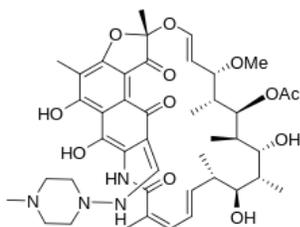
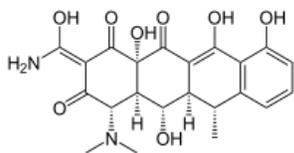
<sup>645</sup> Braidy N, Jugder BE, Poljak A, Jayasena T, Mansour H, Nabavi SM, Sachdev P, Grant R. Resveratrol as a potential therapeutic candidate for the treatment and management of Alzheimer's disease. *Curr Top Med Chem.* 2016;16(17):1951-60; <http://www.ncbi.nlm.nih.gov/pubmed/26845555>.

Finally, both hibernation and external cooling induce expression of a number of cold-shock proteins in the brain, including the RNA binding protein, **RBM3**.<sup>646</sup> In hibernating mammals, cooling induces loss of synaptic contacts which are reformed on rewarming, but in synaptically-compromised AD mice the capacity to regenerate synapses after cooling declines in parallel with the loss of induction of RBM3. AD mice lacking genes for RBM3 experience exacerbated synapse loss and accelerated disease. But enhanced expression of RBM3 in the hippocampus of AD mice prevents this deficit and restores the capacity for synapse reassembly after cooling, giving sustained synaptic protection, preventing behavioral deficits and neuronal loss, and significantly prolonging survival.

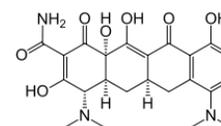
---

<sup>646</sup> Peretti D, Bastide A, Radford H, Verity N, Molloy C, Martin MG, Moreno JA, Steinert JR, Smith T, Dinsdale D, Willis AE, Mallucci GR. RBM3 mediates structural plasticity and protective effects of cooling in neurodegeneration. *Nature*. 2015 Feb 12;518(7538):236-9; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4338605/>.

### 3.2.10 Antibiotic and Antiviral Medications



Only two clinical trials have been done to investigate the efficacy of antibiotic therapy for AD. The first, in 1995, tested 410 AD patients with **cycloserine** over a 26-week treatment period; no difference from placebo was observed.<sup>647</sup> The second, completed in 2004, tested 101 AD patients with **doxycycline** (image, left top) and **rifampin** (image, left bottom) over a 3-month treatment period. The authors of the study indicated that these drugs were effective in delaying the progress of the disease, and “reduced cognitive worsening at 6 months of follow-up in patients with mild to moderate AD.”<sup>648</sup> A re-examination of the same data using “...AUC analysis of the pooled index showed significant treatment effect over the 12-month period”.<sup>649</sup> Several other studies using animal models of Alzheimer’s disease have found that **minocycline**<sup>650</sup> (image, right) and **doxycycline**<sup>651</sup> (image, left top) exert at



least some protective effect in preventing neuron death and slowing the onset of the disease.

<sup>647</sup> Fakouhi TD, Jhee SS, Sramek JJ, Benes C, Schwartz P, Hantsburger G, Herting R, Swabb EA, Cutler NR. Evaluation of cycloserine in the treatment of Alzheimer’s disease. *J Geriatr Psychiatry Neurol.* 1995 Oct;8(4):226-30; <http://www.ncbi.nlm.nih.gov/pubmed/8561836>.

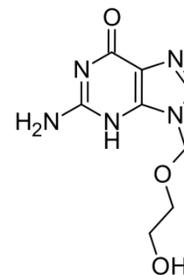
<sup>648</sup> Loeb MB, Molloy DW, Smieja M, Standish T, Goldsmith CH, Mahony J, Smith S, Borrie M, Decoteau E, Davidson W, McDougall A, Gnarp J, O’Donnell M, Chernesky M. A randomized, controlled trial of doxycycline and rifampin for patients with Alzheimer’s disease. *J Am Geriatr Soc.* 2004 Mar;52(3):381-7; <http://www.ncbi.nlm.nih.gov/pubmed/14962152>.

<sup>649</sup> Carusone SC, Goldsmith CH, Smieja M, Loeb M. Summary measures were a useful alternative for analyzing therapeutic clinical trial data. *J Clin Epidemiol.* 2006 Apr;59(4):387-92; <http://www.ncbi.nlm.nih.gov/pubmed/16549261>.

<sup>650</sup> Hunter CL, Quintero EM, Gilstrap L, Bhat NR, Granholm AC. Minocycline protects basal forebrain cholinergic neurons from mu p75-saporin immunotoxic lesioning. *Eur J Neurosci.* 2004 Jun;19(12):3305-16; <http://www.ncbi.nlm.nih.gov/pubmed/15217386>. Choi Y, Kim HS, Shin KY, Kim EM, Kim M, Kim HS, Park CH, Jeong YH, Yoo J, Lee JP, Chang KA, Kim S, Suh YH. Minocycline attenuates neuronal cell death and improves cognitive impairment in Alzheimer’s disease models. *Neuropsychopharmacology.* 2007 Nov;32(11):2393-404; <http://www.ncbi.nlm.nih.gov/pubmed/17406652>.

<sup>651</sup> Jankowsky JL1, Slunt HH, Gonzales V, Savonenko AV, Wen JC, Jenkins NA, Copeland NG, Younkin LH, Lester HA, Younkin SG, Borchelt DR. Persistent amyloidosis following suppression of Abeta production in a transgenic model of Alzheimer disease. *PLoS Med.* 2005 Dec;2(12):e355; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1283364/>. Khlistunova I, Biernat J, Wang Y, Pickhardt M, von Bergen M, Gazova Z, Mandelkow E, Mandelkow EM. Inducible expression of Tau repeat domain in cell models of tauopathy: aggregation is toxic to cells but can be reversed by inhibitor drugs. *J Biol Chem.* 2006 Jan 13;281(2):1205-14; <http://www.jbc.org/content/281/2/1205.long>.

The common herpes simplex virus HSV1 has been found to co-locate with amyloid plaques,<sup>652</sup> also causing the formation of A $\beta$  and abnormal tau,<sup>653</sup> which raises the possibility that AD could be treated or prevented with antiviral medication. It's already known that **memantine**, an FDA-approved AD drug, has antiviral properties.<sup>654</sup> A combination of the antiviral medication **acyclovir** (image, right) and sulfated fucans that is particularly effective against HSV1 has been proposed for testing on Alzheimer's with the aim of slowing or stopping disease progression.<sup>655</sup> Alternatively, **Lycium barbarum polysaccharides** are alleged both to possess antiviral properties and to ameliorate AD symptoms while enhancing neurogenesis, in a mouse model.<sup>656</sup>




---

<sup>652</sup> Wozniak MA, Mee AP, Itzhaki RF. Herpes simplex virus type 1 DNA is located within Alzheimer's disease amyloid plaques. *J Pathol.* 2009 Jan;217(1):131-8; <http://www.ncbi.nlm.nih.gov/pubmed/18973185>. Martin C, Solís L, Concha MI, Otth C. Herpes simplex virus tipo 1 como factor de riesgo asociado con la enfermedad de Alzheimer [Herpes Simplex Virus Type 1 as Risk Factor Associated to Alzheimer Disease]. *Revista Médica De Chile.* 2011 Jun;139(6):779-86, in Spanish; <http://www.ncbi.nlm.nih.gov/pubmed/22051760>.

<sup>653</sup> Wozniak MA, Frost AL, Itzhaki RF. The helicase-primase inhibitor BAY 57-1293 reduces the Alzheimer's disease-related molecules induced by herpes simplex virus type 1. *Antiviral Res.* 2013 Sep;99(3):401-4; <http://www.ncbi.nlm.nih.gov/pubmed/23867133>.

<sup>654</sup> Brison E, Jacomy H, Desforges M, Talbot PJ. Novel treatment with neuroprotective and antiviral properties against a neuroinvasive human respiratory virus. *J Virol.* 2014 Feb;88(3):1548-63; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3911624/>.

<sup>655</sup> Wozniak M, Bell T, Dénes Á, Falshaw R, Itzhaki R. Anti-HSV1 activity of brown algal polysaccharides and possible relevance to the treatment of Alzheimer's disease. *Int J Biol Macromol.* 2015 Mar;74:530-40; <http://www.ncbi.nlm.nih.gov/pubmed/25583021>.

<sup>656</sup> Cheng J, Zhou ZW, Sheng HP, He LJ, Fan XW, He ZX, Sun T, Zhang X, Zhao RJ, Gu L, Cao C, Zhou SF. An evidence-based update on the pharmacological activities and possible molecular targets of Lycium barbarum polysaccharides. *Drug Des Devel Ther.* 2014 Dec 17;9:33-78; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4277126/>.

### 3.2.11 Nanoparticle Therapies

In the last decade there has been increasing interest in the use of nano-drugs<sup>657</sup> and nanoparticles<sup>658</sup> (typically <100 nm in size) for the diagnosis and treatment of Alzheimer's disease. Nanoparticles enable the delivery of a great variety of drugs including anti-cancer drugs, analgesics, anti-Alzheimer's drugs, cardiovascular drugs, protease inhibitors, and various macromolecules into the brain after intravenous injection of animals. The mechanism of the nanoparticle-mediated drug transport across the blood-brain barrier (BBB)<sup>659</sup> appears to be receptor-mediated endocytosis followed by transcytosis into the brain or by drug release within the endothelial cells. Modification of the nanoparticle surface with covalently attached targeting ligands or by coating with certain surfactants that lead to the adsorption of specific plasma proteins after injection is necessary for this receptor-mediated uptake.<sup>660</sup> However, the scattered experimental reports of neuron damage caused by some types of nanoparticles are cautionary.<sup>661</sup>

Nanoparticles are most commonly employed as carriers for active drugs.<sup>662</sup> For example, PLGA-b-PEG nanoparticles loaded with selegiline (Section 3.2.9) appear promising for destabilizing the  $\beta$ -amyloid fibrils in Alzheimer's patients,<sup>663</sup> and albumin nanoparticles carrying tacrine (Section

<sup>657</sup> Ashraf GM, Tabrez S, Jabir NR, Firoz CK, Ahmad S, Hassan I, Alexiou A, Kamal MA. An Overview on Global Trends in Nanotechnological Approaches for Alzheimer Therapy. *Curr Drug Metab.* 2015;16(8):719-27; <http://www.ncbi.nlm.nih.gov/pubmed/26560324>.

<sup>658</sup> Sahni JK, Doggui S, Ali J, Baboota S, Dao L, Ramassamy C. Neurotherapeutic applications of nanoparticles in Alzheimer's disease. *J Control Release.* 2011 Jun 10;152(2):208-31; <http://www.ncbi.nlm.nih.gov/pubmed/21134407>.

<sup>659</sup> Gidwani M, Singh AV. Nanoparticle enabled drug delivery across the blood brain barrier: *in vivo* and *in vitro* models, opportunities and challenges. *Curr Pharm Biotechnol.* 2014;14(14):1201-12; <http://www.ncbi.nlm.nih.gov/pubmed/24809717>.

<sup>660</sup> Kreuter J. Drug delivery to the central nervous system by polymeric nanoparticles: what do we know? *Adv Drug Deliv Rev.* 2014 May;71:2-14; <http://www.ncbi.nlm.nih.gov/pubmed/23981489>.

<sup>661</sup> Chin-Chan M, Navarro-Yepes J, Quintanilla-Vega B. Environmental pollutants as risk factors for neurodegenerative disorders: Alzheimer and Parkinson diseases. *Front Cell Neurosci.* 2015 Apr 10;9:124; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4392704/>. Wilson CL, Natarajan V, Hayward SL, Khalimonchuk O, Kidambi S. Mitochondrial dysfunction and loss of glutamate uptake in primary astrocytes exposed to titanium dioxide nanoparticles. *Nanoscale.* 2015 Nov 28;7(44):18477-88; <http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1022&context=chemengtheses>.

<sup>662</sup> Sarvaiya J, Agrawal YK. Chitosan as a suitable nanocarrier material for anti-Alzheimer drug delivery. *Int J Biol Macromol.* 2015 Jan;72:454-65; <http://www.ncbi.nlm.nih.gov/pubmed/25199867>.

<sup>663</sup> Baysal I, Yabanoglu-Ciftci S, Tunc-Sarisozen Y, Ulubayram K, Ucar G. Interaction of selegiline-loaded PLGA-b-PEG nanoparticles with beta-amyloid fibrils. *J Neural Transm.* 2013 Jun;120(6):903-10; <http://www.ncbi.nlm.nih.gov/pubmed/23420173>.

3.1) may overcome some of the problems associated with that drug.<sup>664</sup> In some cases the drug itself may be fashioned into nanoparticles, with improved results.<sup>665</sup> The following is a brief summary of some of the more common research directions in the use of nanoparticles for AD.

**Monoclonal Antibodies.** (1) A monoclonal antibody against fibrillar human amyloid- $\beta_{42}$  that is surface-coated onto a functionalized phospholipid monolayer binds to cerebrovascular amyloid deposits in arterioles of AD mice after infusion into the external carotid artery.<sup>666</sup> (2) Intranasal administration of M13 bacteriophage – a filamentous nanometer-sized biological particle 900 nm in length and 6-10 nm wide, comprising a coat surrounding a single-stranded, closed circular DNA – used as a delivery vector of anti-beta amyloid antibody fragment into Alzheimer's APP transgenic mice enables *in vivo* targeting of beta amyloid plaques.<sup>667</sup> This large nanoparticle binds to  $\beta$ -amyloid and  $\alpha$ -synuclein proteins, leading to plaque disaggregation in models of Alzheimer's and Parkinson's disease.<sup>668</sup> (See phage company: <http://neurophage.com/about/>.)

**Chelators.** Nanoparticles conjugated to chelators show unique ability to cross the blood-brain barrier (BBB), chelate metals, and exit through the BBB with their corresponding complexed metal ions, possibly providing a safer and more effective means of reducing the metal load in neural tissue and attenuating the harmful effects of oxidative damage.<sup>669</sup> Nanoparticles – tiny, artificial or natural organic polymers – can transport metal chelating agents across the blood-brain barrier regardless of their size and hydrophilicity, reducing the oxidative toxicity of excess metals in an AD brain without inducing any such toxicity themselves.<sup>670</sup> One study employed lactoferrin (Lf), a natural iron binding protein (image, right) whose receptor is



<sup>664</sup> Luppi B, Bigucci F, Corace G, Delucca A, Cerchiara T, Sorrenti M, Catenacci L, Di Pietra AM, Zecchi V. Albumin nanoparticles carrying cyclodextrins for nasal delivery of the anti-Alzheimer drug tacrine. *Eur J Pharm Sci.* 2011 Nov 20;44(4):559-65; <http://www.ncbi.nlm.nih.gov/pubmed/22009109>.

<sup>665</sup> Cheng KK, Yeung CF, Ho SW, Chow SF, Chow AH, Baum L. Highly stabilized curcumin nanoparticles tested in an *in vitro* blood-brain barrier model and in Alzheimer's disease Tg2576 mice. *AAPS J.* 2013 Apr;15(2):324-36; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3675736/>.

<sup>666</sup> Poduslo JF, Hultman KL, Curran GL, Preboske GM, Chamberlain R, Marjańska M, Garwood M, Jack CR Jr, Wengenack TM. Targeting vascular amyloid in arterioles of Alzheimer disease transgenic mice with amyloid  $\beta$  protein antibody-coated nanoparticles. *J Neuropathol Exp Neurol.* 2011 Aug;70(8):653-61; <http://www.ncbi.nlm.nih.gov/pubmed/21760540>.

<sup>667</sup> Frenkel D, Solomon B. Filamentous phage as vector-mediated antibody delivery to the brain. *Proc Natl Acad Sci U S A.* 2002 Apr 16;99(8):5675-9; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC122830/>.

<sup>668</sup> Ksendzovsky A, Walbridge S, Saunders RC, Asthagiri AR, Heiss JD, Lonser RR. Convection-enhanced delivery of M13 bacteriophage to the brain. *J Neurosurg.* 2012 Aug;117(2):197-203; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3786326/>.

<sup>669</sup> Liu G, Men P, Perry G, Smith MA. Nanoparticle and iron chelators as a potential novel Alzheimer therapy. *Methods Mol Biol.* 2010;610:123-44; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2836834/>.

<sup>670</sup> Bonda DJ, Liu G, Men P, Perry G, Smith MA, Zhu X. Nanoparticle delivery of transition-metal chelators to the brain: Oxidative stress will never see it coming! *CNS Neurol Disord Drug Targets.* 2012 Feb;11(1):81-5; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4112585/>.

highly expressed in both respiratory epithelial cells and neurons, to facilitate the nose-to-brain drug delivery of neuroprotection peptides using an Lf-conjugated PEG-PCL (polyethyleneglycol–polycaprolactone) nanoparticle.<sup>671</sup>

**Anti-oxidants.** (1) One research group designed nitroxyl radical-containing nanoparticles possessing nitroxyl radical in the core that chemically scavenges reactive oxygen species, and then tested these nanoparticles for their potential neuroprotective role on A $\beta$ -induced cytotoxicity in human neuroblastoma SH-SY5Y cells, with somewhat positive results.<sup>672</sup> (2) Targeting metal-induced oxidative stress that can contribute to neuronal cell death in Alzheimer’s disease, another group prepared bioactive hybrid pegylated silica nanoparticles capable of working as host-carriers of potent antioxidants such as the natural flavonoid quercetin.<sup>673</sup> (3) Chronic administration of a carboxyfullerene (“buckyball”) mimic of superoxide dismutase to mice “rescued age-related cognitive impairment.”<sup>674</sup> (4) Low Level Laser Therapy has been demonstrated to mitigate amyloid- $\beta$  peptide induced oxidative stress and inflammation, a key driver of Alzheimer’s disease, but the major issue in moving this forward from cell cultures to live animals and potentially to human subjects is the shallow penetration depth of light through skin. A recent study used BRET-Qdots (Bioluminescence Resonance Energy Transfer to Quantum Dots) as an alternative internal near-infrared light source with good efficacy.<sup>675</sup>

**Amyloid Decoys.** Magnetic nanoparticles coated with heparin have been synthesized and demonstrated to bind with A $\beta$ .<sup>676</sup> These nanoparticles exhibited little toxicity to neuronal cells while at the same time effectively protecting them from A $\beta$ -induced cytotoxicity.

<sup>671</sup> Liu Z, Jiang M, Kang T, Miao D, Gu G, Song Q, Yao L, Hu Q, Tu Y, Pang Z, Chen H, Jiang X, Gao X, Chen J. Lactoferrin-modified PEG-co-PCL nanoparticles for enhanced brain delivery of NAP peptide following intranasal administration. *Biomaterials*. 2013 May;34(15):3870-81; <http://www.ncbi.nlm.nih.gov/pubmed/23453061>.

<sup>672</sup> Chonpathompikunlert P, Yoshitomi T, Han J, Toh K, Isoda H, Nagasaki Y. Chemical nanotherapy: nitroxyl radical-containing nanoparticle protects neuroblastoma SH-SY5Y cells from Abeta-induced oxidative stress. *Ther Deliv*. 2011 May;2(5):585-97; <http://www.ncbi.nlm.nih.gov/pubmed/22833976>.

<sup>673</sup> Nday CM, Halevas E, Jackson GE, Salifoglou A. Quercetin encapsulation in modified silica nanoparticles: potential use against Cu(II)-induced oxidative stress in neurodegeneration. *J Inorg Biochem*. 2015 Apr;145:51-64; <http://www.ncbi.nlm.nih.gov/pubmed/25634813>.

<sup>674</sup> Quick KL, Ali SS, Arch R, Xiong C, Wozniak D, Dugan LL. A carboxyfullerene SOD mimetic improves cognition and extends the lifespan of mice. *Neurobiol Aging*. 2008 Jan;29(1):117-28; <http://www.ncbi.nlm.nih.gov/pubmed/17079053>.

<sup>675</sup> Bungart BL, Dong L, Sobek D, Sun GY, Yao G, Lee JC. Nanoparticle-emitted light attenuates amyloid- $\beta$ -induced superoxide and inflammation in astrocytes. *Nanomedicine*. 2014 Jan;10(1):15-7; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3895489/>.

<sup>676</sup> Wang P, Kouyoumdjian H, Zhu DC, Huang X. Heparin nanoparticles for  $\beta$  amyloid binding and mitigation of  $\beta$  amyloid associated cytotoxicity. *Carbohydr Res*. 2015 Mar 20;405:110-4; <http://www.ncbi.nlm.nih.gov/pubmed/25498198>.

**Amyloid Aggregation Inhibitors.** (1) Gold nanoparticles were designed as a multifunctional A $\beta$  inhibitor, showing synergistic effects in inhibiting A $\beta$  aggregation, dissociating A $\beta$  fibrils and decreasing A $\beta$ -mediated peroxidase activity and A $\beta$ -induced cytotoxicity.<sup>677</sup> (2) A similar effect has been described for liposomes<sup>678</sup> and, more recently, for cationic polystyrene nanoparticles.<sup>679</sup> (3) Atomic force microscopy experiments have confirmed the inhibitory effect of the fullerene C<sub>60</sub> (“buckyball”) on A $\beta$ <sub>16-22</sub> fibrillation, and the larger fullerene C<sub>180</sub> nanoparticle displays an unexpected stronger inhibitory effect on the  $\beta$ -sheet formation of A $\beta$ <sub>16-22</sub> peptides.<sup>680</sup> (The inhibition of  $\beta$ -sheet formation has been considered a primary therapeutic strategy for AD.) Unfortunately, Linse *et al.*<sup>681</sup> have reported that nanoparticles such as copolymer particles, cerium oxide particles, quantum dots, and carbon nanotubes also enhance the possible appearance of a critical nucleus for fibril nucleation. The high surface-to-volume ratio of nanoparticles means that potentially high concentrations of protein may be adsorbed at the particle surface, enhancing the probability of partially unfolded proteins coming into frequent contact and promoting amyloid formation if that protein is suitable. Another study found that lower concentrations of magnetic nanoparticles inhibited amyloid fibrillation but higher concentrations enhanced fibrillation.<sup>682</sup>

**Amyloid Disaggregation.** (1) Near infrared radiation is absorbed by conductive polyaniline nanoparticles causing an increase of their temperature which induces the collapse of their thermosensitive polymer shell, triggering particle aggregation. This makes it possible to locally heat the nanoparticles and induce protein aggregates of neurodegenerative diseases (e.g. Alzheimer’s) to dissolve.<sup>683</sup> (2) Toxic aggregates of the protein  $\beta$ -amyloid (A $\beta$ ) involved in

<sup>677</sup> Gao N, Sun H, Dong K, Ren J, Qu X. Gold-nanoparticle-based multifunctional amyloid- $\beta$  inhibitor against Alzheimer’s disease. *Chemistry*. 2015 Jan 7;21(2):829-35; <http://www.ncbi.nlm.nih.gov/pubmed/25376633>.

<sup>678</sup> Bokvist M, Lindström F, Watts A, Gröbner G. Two types of Alzheimer’s beta-amyloid (1-40) peptide membrane interactions: aggregation preventing transmembrane anchoring versus accelerated surface fibril formation. *J Mol Biol*. 2004 Jan 23;335(4):1039-49; <http://www.ncbi.nlm.nih.gov/pubmed/14698298/>.

<sup>679</sup> Cabaleiro-Lago C, Quinlan-Pluck F, Lynch I, Dawson KA, Linse S. Dual effect of amino modified polystyrene nanoparticles on amyloid  $\beta$  protein fibrillation. *ACS Chem Neurosci*. 2010 Apr 21;1(4):279-87; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3368671/>.

<sup>680</sup> Xie L, Luo Y, Lin D, Xi W, Yang X, Wei G. The molecular mechanism of fullerene-inhibited aggregation of Alzheimer’s  $\beta$ -amyloid peptide fragment. *Nanoscale*. 2014 Aug 21;6(16):9752-62; <http://www.ncbi.nlm.nih.gov/pubmed/25004796>.

<sup>681</sup> Linse S, Cabaleiro-Lago C, Xue WF, Lynch I, Lindman S, Thulin E, Radford SE, Dawson KA. Nucleation of protein fibrillation by nanoparticles. *Proc Natl Acad Sci U S A*. 2007 May 22;104(21):8691-6; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1866183/>.

<sup>682</sup> Mahmoudi M, Quinlan-Pluck F, Monopoli MP, Sheibani S, Vali H, Dawson KA, Lynch I. Influence of the physiochemical properties of superparamagnetic iron oxide nanoparticles on amyloid  $\beta$  protein fibrillation in solution. *ACS Chem Neurosci*. 2013 Mar 20;4(3):475-85; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3605812/>.

<sup>683</sup> Abel SB, Molina MA, Rivarola CR, Kogan MJ, Barbero CA. Smart polyaniline nanoparticles with thermal and photothermal sensitivity. *Nanotechnology*. 2014 Dec 12;25(49):495602; <http://www.ncbi.nlm.nih.gov/pubmed/25407569>.

Alzheimer's disease can be destabilized upon electromagnetic irradiation of the peptide Cys-Leu-Pro-Phe-Phe-Asp (CLPFFD) adsorbed on gold nanospheres (AuNSs).<sup>684</sup> (3) Employing a cascade-targeting strategy for precise drug delivery, another research group developed a dual-functional nanoparticle drug delivery system loaded with  $\beta$ -sheet breaker peptide H102 (TQNP/H102). Two targeting peptides, TGN and QSH, were conjugated to the surface of the nanoparticles for blood-brain barrier transport and  $A\beta_{42}$  targeting, respectively. Spatial learning and memory among the AD model mice in the H102 treatment group were significantly improved compared with the AD control group.<sup>685</sup>

**Microglial Inhibitors.** The specific inhibition of microglial cells in tau-mutant AD mice by using fibrin  $\gamma_{377-395}$  peptide conjugated to iron oxide ( $\gamma$ - $Fe_2O_3$ ) nanoparticles of  $21 \pm 3.5$  nm diameter has been reported.<sup>686</sup>

**Neuroprotection.** At least one study has found neuroprotective potential in hydrated fullerene  $C_{60}$  "buckyball" ( $C_{60}HyFn$ ),<sup>687</sup> and injection of colloidal  $C_{60}$  apparently confers protection from  $A\beta$  toxicity in rat brains.<sup>688</sup> However, hydroxylated  $C_{60}$  can have toxic effects on brain cells.<sup>689</sup>

<sup>684</sup> Vera AM, Cárcamo JJ, Aliaga AE, Gómez-Jeria JS, Kogan MJ, Campos-Valette MM. Interaction of the CLPFFD peptide with gold nanospheres. A Raman, surface enhanced Raman scattering and theoretical study. *Spectrochim Acta A Mol Biomol Spectrosc.* 2015 Jan 5;134:251-6; <http://www.ncbi.nlm.nih.gov/pubmed/25022496>.

<sup>685</sup> Zhang C, Zheng X, Wan X, Shao X, Liu Q, Zhang Z, Zhang Q. The potential use of H102 peptide-loaded dual-functional nanoparticles in the treatment of Alzheimer's disease. *J Control Release.* 2014 Oct 28;192:317-24; <http://www.ncbi.nlm.nih.gov/pubmed/25102404>.

<sup>686</sup> Glat M, Skaat H, Menkes-Caspi N, Margel S, Stern EA. Age-dependent effects of microglial inhibition *in vivo* on Alzheimer's disease neuropathology using bioactive-conjugated iron oxide nanoparticles. *J Nanobiotechnology.* 2013 Sep 23;11:32; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3851539/>.

<sup>687</sup> Vorobyov V, Kaptsov V, Gordon R, Makarova E, Podolski I, Sengpiel F. Neuroprotective effects of hydrated fullerene c60: cortical and hippocampal EEG interplay in an amyloid-infused rat model of Alzheimer's disease. *J Alzheimers Dis.* 2015 Jan 1;45(1):217-33; <http://www.ncbi.nlm.nih.gov/pubmed/25589720>.

<sup>688</sup> Makarova EG, Gordon RY, Podolski IY. Fullerene C60 prevents neurotoxicity induced by intrahippocampal microinjection of amyloid-beta peptide. *J Nanosci Nanotechnol.* 2012 Jan;12(1):119-26; <http://www.ncbi.nlm.nih.gov/pubmed/22523954>.

<sup>689</sup> Yamada T, Nakaoka R, Sawada R, Matsuoka A, Tsuchiya T. Effects of intracerebral microinjection of hydroxylated-[60]fullerene on brain monoamine concentrations and locomotor behavior in rats. *J Nanosci Nanotechnol.* 2010 Jan;10(1):604-11; <http://www.ncbi.nlm.nih.gov/pubmed/20352899>.

### 3.2.12 Gene Therapies

One gene therapy approach to Alzheimer's disease would involve modifying or supplementing the genes in the patient's neurons with additional genes having some beneficial effect.<sup>690</sup> The history of gene therapy is not unblemished and progress with this approach is expected to be very cautious and slow.

Gene therapy experiments on neural cells are ongoing. For example, in one study a 19-nt oligonucleotide targeting BACE1, the key enzyme in amyloid beta protein (A $\beta$ ) production, was synthesized and introduced into the pSilenCircle vector to construct a short hairpin (shRNA) expression plasmid against the BACE1 gene, which vector was then transfected into C17.2 neural stem cells and primary neural stem cells, causing downregulation of the BACE1 gene and inducing a considerable reduction in A $\beta$  protein production.<sup>691</sup>

Animal studies in this area are plentiful. Most recently, recombinant lentiviral vectors were used to overexpress the glial cell-derived neurotrophic factor (GDNF) gene to induce neuroprotection in mouse models of AD.<sup>692</sup> A gene therapy-based approach with adeno-associated virus (AAV) demonstrated that IGF2 overexpression in the hippocampus of aged wild-type mice enhances memory and promotes dendritic spine formation.<sup>693</sup> A recombinant AAV vector expressing the human HIF-1 $\alpha$  gene (rAAV-HIF-1 $\alpha$ ) showed that rAAV-HIF-1 $\alpha$  represses hippocampal neuronal apoptosis induced by amyloid-beta protein.<sup>694</sup> It has also been suggested that gene therapy could be applied to mutant mitochondrial DNA.<sup>695</sup>

---

<sup>690</sup> Pardo J, Morel GR, Astiz M, Schwerdt JI, León ML, Rodríguez SS, Hereñú CB, Goya RG. Gene therapy and cell reprogramming for the aging brain: achievements and promise. *Curr Gene Ther.* 2014 Feb;14(1):24-34; <http://www.ncbi.nlm.nih.gov/pubmed/24450294>.

<sup>691</sup> Liu Z, Li S, Liang Z, Zhao Y, Zhang Y, Yang Y, Wang M, Li F. Targeting  $\beta$ -secretase with RNAi in neural stem cells for Alzheimer's disease therapy. *Neural Regen Res.* 2013 Nov 25;8(33):3095-106; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4158703/>.

<sup>692</sup> Revilla S, Ursulet S, Álvarez-López MJ, Castro-Freire M, Perpiñá U, García-Mesa Y, Bortolozzi A, Giménez-Llort L, Kaliman P, Cristòfol R, Sarkis C, Sanfeliu C. Lenti-GDNF gene therapy protects against Alzheimer's disease-like neuropathology in 3xTg-AD mice and MC65 cells. *CNS Neurosci Ther.* 2014 Nov;20(11):961-72; <http://www.ncbi.nlm.nih.gov/pubmed/25119316>.

<sup>693</sup> Pascual-Lucas M, Viana da Silva S, Di Scala M, Garcia-Barroso C, González-Aseguinolaza G, Mulle C, Alberini CM, Cuadrado-Tejedor M, Garcia-Osta A. Insulin-like growth factor 2 reverses memory and synaptic deficits in APP transgenic mice. *EMBO Mol Med.* 2014 Aug 6;6(10):1246-62; <http://www.ncbi.nlm.nih.gov/pubmed/25100745>.

<sup>694</sup> Chai X, Kong W, Liu L, Yu W, Zhang Z, Sun Y. A viral vector expressing hypoxia-inducible factor 1 alpha inhibits hippocampal neuronal apoptosis. *Neural Regen Res.* 2014 Jun 1;9(11):1145-53; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4146100/>.

<sup>695</sup> Leitão-Rocha A, Guedes-Dias P, Pinho BR, Oliveira JM. Trends in mitochondrial therapeutics for neurological disease. *Curr Med Chem.* 2015;22(20):2458-67;

Following an apparently successful gene therapy experiment to use lentivirus vectors to deliver nerve growth factor into the aged rhesus monkey brain,<sup>696</sup> there has been at least one clinical trial attempting to apply gene therapy to human AD. In 2010, Ceregene completed a Phase I trial “to assess the safety and tolerability of CERE-110 [Adeno-Associated Virus (AAV)-based vector-mediated delivery of Nerve Growth Factor (NGF)] in subjects with mild to moderate Alzheimer’s disease.”<sup>697</sup> After successful completion,<sup>698</sup> a Phase II follow-up on this work was launched but there were no formal updates after 2012.<sup>699</sup> By 2014, the company announced the successful completion of another Phase I study using a stereotactic (surgical) implantation method, which the researchers say supports moving to Phase II with a “multicenter, double-blind, sham-surgery-controlled trial.”<sup>700</sup> Apparently this work is continuing.

Over the last decade there have been nine gene therapy clinical trials for Parkinson’s, a disease whose pathology is felt by many to be related to Alzheimer’s, albeit in a more focused territory.<sup>701</sup> Each trial started with considerable optimism and yet none, to this point, have born sufficiently strong clinical evidence or found a clear path towards regulatory approval. And gene therapy for Alzheimer’s is probably a tougher nut to crack than for Parkinson’s.

Another possible gene therapy strategy is RNA silencing of APP and tau protein production.<sup>702</sup> Silencing has been shown in human neuroblastoma cells to “enhance synaptic activity, may

[https://www.researchgate.net/profile/Jorge\\_Oliveira11/publication/272193079\\_Trends\\_in\\_Mitochondrial\\_Therapeutics\\_for\\_Neurological\\_Disease/links/55f7089708aeafc8abf6c49c.pdf](https://www.researchgate.net/profile/Jorge_Oliveira11/publication/272193079_Trends_in_Mitochondrial_Therapeutics_for_Neurological_Disease/links/55f7089708aeafc8abf6c49c.pdf).

<sup>696</sup> Nagahara AH, Bernot T, Moseanko R, Brignolo L, Blesch A, Conner JM, Ramirez A, Gasmil M, Tuszynski MH. Long-term reversal of cholinergic neuronal decline in aged non-human primates by lentiviral NGF gene delivery. *Exp Neurol*. 2009 Jan;215(1):153-9; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2632603/>.

<sup>697</sup> <https://clinicaltrials.gov/ct2/show/NCT00087789>.

<sup>698</sup> Mandel RJ. CERE-110, an adeno-associated virus-based gene delivery vector expressing human nerve growth factor for the treatment of Alzheimer’s disease. *Curr Opin Mol Ther*. 2010 Apr;12(2):240-7; <http://www.ncbi.nlm.nih.gov/pubmed/20373268>.

<sup>699</sup> <https://clinicaltrials.gov/ct2/show/NCT00876863>.

<sup>700</sup> Rafii MS, Baumann TL, Bakay RA, Ostrove JM, Siffert J, Fleisher AS, Herzog CD, Barba D, Pay M, Salmon DP, Chu Y, Kordower JH, Bishop K, Keator D, Potkin S, Bartus RT. A phase 1 study of stereotactic gene delivery of AAV2-NGF for Alzheimer’s disease. *Alzheimers Dement*. 2014 Sep;10(5):571-81; <http://www.ncbi.nlm.nih.gov/pubmed/24411134>.

<sup>701</sup> Bartus RT, Weinberg MS, Samulski RJ. Parkinson’s disease gene therapy: success by design meets failure by efficacy. *Mol Ther*. 2014 Mar;22(3):487-97; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3944322/>.

<sup>702</sup> Manczak M, Reddy PH. RNA silencing of genes involved in Alzheimer’s disease enhances mitochondrial function and synaptic activity. *Biochim Biophys Acta*. 2013 Dec;1832(12):2368-78; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3830527/>.

improve mitochondrial maintenance and function, and may protect against toxicities of AD-related genes, [thus] may have therapeutic value for patients with AD.” Studies of RNA silencing of genes for APP,<sup>703</sup> tau,<sup>704</sup> PS1,<sup>705</sup> and BACE1<sup>706</sup> have reduced the mRNA levels of targeted genes. Silencing of APP in several studies using AD transgenic mouse models and AD-related genes resulted in reduced levels of A $\beta$  and the amelioration of cognitive deficits in the mice,<sup>707</sup> suggesting a promising therapeutic value for APP silencing in AD patients. However, there appear to have been no clinical trials testing this approach as of early 2016.

---

<sup>703</sup> Miller VM, Gouvion CM, Davidson BL, Paulson HL. Targeting Alzheimer’s disease genes with RNA interference: an efficient strategy for silencing mutant alleles. *Nucleic Acids Res.* 2004 Jan 30;32(2):661-8; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC373334/>. Rodríguez-Lebrón E, Gouvion CM, Moore SA, Davidson BL, Paulson HL. Allele-specific RNAi mitigates phenotypic progression in a transgenic model of Alzheimer’s disease. *Mol Ther.* 2009 Sep;17(9):1563-73; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2835271/>.

<sup>704</sup> Robeson RH, Dunckley T. High-content RNA interference assay: analysis of tau hyperphosphorylation as a generic paradigm. *Methods Mol Biol.* 2011;700:221-38; <http://www.ncbi.nlm.nih.gov/pubmed/21204037>. Miller VM, Gouvion CM, Davidson BL, Paulson HL. Targeting Alzheimer’s disease genes with RNA interference: an efficient strategy for silencing mutant alleles. *Nucleic Acids Res.* 2004 Jan 30;32(2):661-8; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC373334/>. Rodríguez-Lebrón E, Gouvion CM, Moore SA, Davidson BL, Paulson HL. Allele-specific RNAi mitigates phenotypic progression in a transgenic model of Alzheimer’s disease. *Mol Ther.* 2009 Sep;17(9):1563-73; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2835271/>.

<sup>705</sup> Kandimalla RJ, Wani WY, Binukumar BK, Gill KD. siRNA against presenilin 1 (PS1) down regulates amyloid  $\beta$ 42 production in IMR-32 cells. *J Biomed Sci.* 2012 Jan 3;19:2; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3282656/>.

<sup>706</sup> Sierant M, Kubiak K, Kazmierczak-Baranska J, Padaszynska A, Kuwabara T, Warashina M, Nacmias B, Sorbi S, Nawrot B. RNA interference in silencing of genes of Alzheimer’s disease in cellular and rat brain models. *Nucleic Acids Symp Ser (Oxf).* 2008;(52):41-2; <http://nass.oxfordjournals.org/content/52/1/41.long>. Chen S, Ge X, Chen Y, Lv N, Liu Z, Yuan W. Advances with RNA interference in Alzheimer’s disease research. *Drug Des Devel Ther.* 2013;7:117-25; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3582316/>.

<sup>707</sup> Choy RW, Cheng Z, Schekman R. Amyloid precursor protein (APP) traffics from the cell surface via endosomes for amyloid  $\beta$  (A $\beta$ ) production in the trans-Golgi network. *Proc Natl Acad Sci U S A.* 2012 Jul 24;109(30):E2077-82; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3409748/>. Finan GM, Okada H, Kim TW. BACE1 retrograde trafficking is uniquely regulated by the cytoplasmic domain of sortilin. *J Biol Chem.* 2011 Apr 8;286(14):12602-16; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3069461/>. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhali S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat Biotechnol.* 2011 Apr;29(4):341-5; <http://www.ncbi.nlm.nih.gov/pubmed/21423189>. Miller VM, Gouvion CM, Davidson BL, Paulson HL. Targeting Alzheimer’s disease genes with RNA interference: an efficient strategy for silencing mutant alleles. *Nucleic Acids Res.* 2004 Jan 30;32(2):661-8; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC373334/>. Rodríguez-Lebrón E, Gouvion CM, Moore SA, Davidson BL, Paulson HL. Allele-specific RNAi mitigates phenotypic progression in a transgenic model of Alzheimer’s disease. *Mol Ther.* 2009 Sep;17(9):1563-73; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2835271/>.

The most successful contender for biotech-based gene editing is probably the CRISPR approach.<sup>708</sup> CRISPR works well in everything from butterflies to monkeys, and has already been used to create extra-muscular beagle dogs and sheep, long-haired goats, and pigs immune to common diseases.<sup>709</sup> CRISPR gene editing has successfully treated a genetic disease (Duchenne muscular dystrophy)<sup>710</sup> inside a fully developed living adult mammal (i.e., a mouse).<sup>711</sup> CRISPR is being tried for *in vivo* gene editing and may work on a gene-by-gene basis.<sup>712</sup> At present these methods are still immature and produce incomplete gene alterations along with off-target effects on other genes.<sup>713</sup>

Nevertheless, Big Pharma is getting interested. As one example, Bayer and CRISPR Therapeutics (<http://crisprtx.com/>) have established a joint venture to use CRISPR/Cas9 gene-editing technology to develop therapies to treat blood disorders, blindness, and congenital heart disease. Bayer will sink \$300 million into R&D over the five-year pact and is taking a minority stake worth \$35 million in CRISPR Therapeutics.<sup>714</sup> Rival gene editing firms Editas Medicine (<http://www.editasmedicine.com/>) and Intellia Therapeutics (<http://www.intelliata.com/>) have also received significant funding. Competing variants of CRISPR such as the PITCh (Precise Integration into Target Chromosome) system<sup>715</sup> are under investigation too.

---

<sup>708</sup> [https://en.wikipedia.org/wiki/CRISPR#Genome\\_engineering](https://en.wikipedia.org/wiki/CRISPR#Genome_engineering).

<sup>709</sup> CRISPR will make 2016 the year of gene-edited organisms. New Scientist, 15 Dec 2015; <https://www.newscientist.com/article/mg22830522-200-crispr-will-make-2016-the-year-of-gene-edited-organisms/>.

<sup>710</sup> Wojtal D, Kemaladewi DU, Malam Z, Abdullah S, Wong TW, Hyatt E, Baghestani Z, Pereira S, Stavropoulos J, Mouly V, Mamchaoui K, Muntoni F, Voit T, Gonorazky HD, Dowling JJ, Wilson MD, Mendoza-Londono R, Ivakine EA, Cohn RD. Spell Checking Nature: Versatility of CRISPR/Cas9 for Developing Treatments for Inherited Disorders. *Am J Hum Genet*. 2016 Jan 7;98(1):90-101; <http://www.ncbi.nlm.nih.gov/pubmed/26686765>.

<sup>711</sup> Dockrill P. CRISPR gene editing tool used to treat genetic disease in an animal for the first time. *Science Alert*, 4 Jan 2016; <http://www.sciencealert.com/crispr-gene-editing-tool-used-to-treat-genetic-disease-in-an-animal-for-the-first-time>.

<sup>712</sup> Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. Genome engineering using the CRISPR-Cas9 system. *Nat Protoc*. 2013 Nov;8(11):2281-308; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3969860/>.

<sup>713</sup> Liang P, Xu Y, Zhang X, Ding C, Huang R, Zhang Z, Lv J, Xie X, Chen Y, Li Y, Sun Y, Bai Y, Songyang Z, Ma W, Zhou C, Huang J. CRISPR/Cas9-mediated gene editing in human trippronuclear zygotes. *Protein Cell*. 2015 May;6(5):363-72; <http://link.springer.com/content/pdf/10.1007%2Fs13238-015-0153-5.pdf>.

<sup>714</sup> Jarvis L. Gene Editing: Bayer and Crispr Therapeutics form CRISPR/Cas9 technology pact. *C&EN* 94: 9, 4 Jan 2016; <http://cen.acs.org/articles/94/i1/Gene-Editing-Bayer-Crispr-Therapeutics.html>.

<sup>715</sup> An alternative TALEN/CRISPR-mediated gene insertion technique described in detail. Hiroshima University, 18 Dec 2015; [http://www.hiroshima-u.ac.jp/news/show/lang/en/id/1939/dir\\_id/0](http://www.hiroshima-u.ac.jp/news/show/lang/en/id/1939/dir_id/0).

### 3.2.13 Telomere Extension Therapy

As noted earlier (Section 2.4.4) the progressive shortening of telomeres with age may be implicated in the onset of Alzheimer's disease. As telomere therapy advocate Michael Fossel points out,<sup>716</sup> changes in gene expression that define aging in our cells are affected by the changing telomere lengths as these cells divide. If we reset the telomere to the original length, we can often reset gene expression and end up with a cell that looks and acts like a young cell, as demonstrated in the lab both in human tissues and in animals such as mice and rats. Indeed, resetting telomere lengths in the aging rodent brain causes the animals to begin acting normally again with their brains returning to near-normal volume and function.

Notes Fossel: "Telomeres can be reset using telomerase, and using enzymes comprising hTERT (human telomerase reverse transcriptase) and hTERC (human telomerase RNA component). Both of these telomere length-extending enzymes can be delivered into the human brain, using either liposomes or viral vectors, much as has already been done in animal trials. There are at least two biotech projects currently aimed at human trials, one via standard FDA-sponsored research (Telocyte)<sup>717</sup> and the other using a faster and less formal 'offshore' approach (BioViva).<sup>718</sup> "

---

<sup>716</sup> Michael Fossel, "Reversing Alzheimer's by Lengthening Telomeres," Humanity+ Media, 8 Oct 2015; <http://hplusmagazine.com/2015/10/08/reversing-alzheimers-by-lengthening-telomeres/>.

<sup>717</sup> **Telocyte** home page: <http://www.telocyte.com/>. Brief company summary (founded Jan 2015): <https://gust.com/companies/telocyte-llc>. Fossel's blog: <http://www.michaelfossel.com/blog/?cat=28>. "Human trials will involve an injection of the telomerase gene, which will function temporarily, then become inactive. Before becoming inactive, however, it will relengthen telomeres within the microglia and reset gene expression. We anticipate that the initial treatment will require 2-3 injections over a period of a few weeks." <http://www.telocyte.com/alzheimers-therapy/alzheimers-therapy>. "Current investors are underwriting FDA-sponsored phase 0 through phase II human trials of telomerase therapy as a treatment for Alzheimer's disease. Although the current animal data is remarkable, the FDA may well require that we demonstrate additional safety trials before permitting first-in-human trials. This phase (0) typically requires that we treat animal models, such as mice or dogs, to support the ethical use of telomerase therapy in human trials. Phase I human trials (to look at safety parameters in humans) will follow immediately, once the FDA grants an IND permit. Phase II trials (to look at dosage parameters in humans) will follow thereafter. Based on current animal data, we anticipate that our human trials will be clear in demonstrating the efficacy of telomerase therapy in human volunteers." <http://www.telocyte.com/alzheimers-therapy/investor-sponsored-research>.

<sup>718</sup> **BioViva USA Inc.**, "Projects: Alzheimer's Disease," <http://www.bioviva-science.com/alzheimers/>. See also: "Alzheimer's Cure Clinical Trial," Maximum Life Foundation, 2014; <http://www.maxlife.org/BioViva.asp>.

On 15 Sep 2015, Liz Parrish, CEO and founder of BioViva, reportedly injected herself with a viral vector containing genes for both telomerase and FST in an "offshore" clinic in Colombia. See: Antonio Regalado, "A Tale of Do-It-Yourself Gene Therapy," MIT Technology Review, 14 Oct 2015; <http://www.technologyreview.com/news/542371/a-tale-of-do-it-yourself-gene-therapy/>. On 21 Apr 2016, the company announced tentative success; <http://bioviva-science.com/2016/04/21/first-gene-therapy-successful-against-human-aging/>.

The biggest knock on telomerase therapy is that it could potentially increase the risk of cancer,<sup>719</sup> since lengthening the telomeres of cancer cells also increases the ability of those cells to survive. Indeed, one of the most promising proposed traditional avenues for treating cancer is to use a telomerase *inhibitor*.<sup>720</sup> But Fossel argues that telomerase therapy will improve the body's ability to fight off cancer. "In most cases," he writes,<sup>721</sup> "telomerase should be enormously beneficial in preventing or curing most age-related diseases as well as significantly lowering the risk of cancer. If the patient already has a cancer, the result is less clear. The worst possible scenario is to have just enough telomerase to enable cancer cells to not only survive but to get worse over time, which is precisely what most cancer cells do." It may also be relevant that most people who are old enough to exhibit the earliest biochemical indicia of AD are also old enough to have bodies riddled with pre-cancerous microtumors.<sup>722</sup>

---

<sup>719</sup> "Radical lifespan extension: A chat with Aubrey de Grey," 11 Sep 2015; <https://gigaom.com/2015/09/11/aubrey-de-grey/>.

<sup>720</sup> Ruden M, Puri N. Novel anticancer therapeutics targeting telomerase. *Cancer Treat Rev.* 2013 Aug;39(5):444-56; <http://www.ncbi.nlm.nih.gov/pubmed/22841437>.

<sup>721</sup> Michael Fossel, *The Telomerase Revolution*, BenBella Books, 2015; <http://www.amazon.com/dp/194163169X>.

<sup>722</sup> Autopsies have shown that every person over 50 years old has microscopic tumors in their thyroid glands, and more than one-third of autopsied women aged 40-50 have *in situ* microtumors in their breasts. Folkman J, Kalluri R. Cancer without disease. *Nature.* 2004 Feb 26;427(6977):787; [http://health120years.com/Hamlet/Hamlet\\_Cancer-without-disease.pdf](http://health120years.com/Hamlet/Hamlet_Cancer-without-disease.pdf).

### 3.2.14 Stem Cell Transplantation

At least three possible approaches for using stem cells to treat Alzheimer's disease have been proposed:<sup>723</sup>

(1) Use stem cells to regenerate or literally regrow diseased parts of the brain. This is how people most commonly think of treating AD with stem cells. The problem with this approach is that the architecture of the brain is physically integral to memory so even if we could grow a fresh, young part of the brain to replace one ravaged by AD, there would be no memories there. It would be like wiping the plate clean, although in theory the person could be re-educated (e.g., Section 5.3.3) and make new memories going forward in life.

(2) Use stem cells such as mesenchymal stem cells (MSC) as healers of the existing brain parenchyma, rather than as rebuilding agents, e.g., with MSC healing rather than replacing neurons. MSC may be considered the natural doctors of the body and could have anti-inflammatory and other powers that might ameliorate AD.

(3) Use stem cells such as MSC as drug delivery agents. For example, instead of giving crenezumab or some other drug systemically, it is possible that stem cells could more effectively directly deliver the drug cell-to-cell within the brain.

Although some companies already claim to offer stem cell treatments for Alzheimer's disease,<sup>724</sup> these currently lie outside of the approved and carefully controlled process of clinical trials. No proven, safe, and effective stem cell treatments for this disease are yet available, and much work remains to be done before stem cell therapies will be clinically feasible for AD and related disorders in humans.<sup>725</sup>

Many different types of neurons in all parts of the brain are affected by Alzheimer's disease. This poses a complex problem if we want to replace the damaged brain cells. For example, one approach might be to transplant neural stem cells (aka. NSCs, a type of stem cell found in the brain) into the brain of an Alzheimer's patient in the hope that these cells would make new, healthy neurons. But even if healthy working NSCs were available and could be transplanted

---

<sup>723</sup> "Can stem cells be used to treat Alzheimer's Disease?" Knoepfler Lab Stem Cell Blog, 16 May 2012; <http://www.ipsell.com/2012/05/can-stem-cells-be-used-to-treat-alzheimers-disease/>.

<sup>724</sup> "Alzheimer's Stem Cell Therapy," StemGenex; <http://stemgenex.com/studies/alzheimers-stem-cell-studies/>. "Alzheimer's Breakthrough. Alzheimer's Treatment," Stem Cell of America (formerly Medra, Inc.); <http://Stemcellofamerica.com/lp/alzheimers-treatment/>.

<sup>725</sup> Kim SU, de Vellis J. Stem cell-based cell therapy in neurological diseases: a review. *J Neurosci Res*. 2009 Aug 1;87(10):2183-20; <http://www.ncbi.nlm.nih.gov/pubmed/19301431>. Tong LM, Fong H, Huang Y. Stem cell therapy for Alzheimer's disease and related disorders: current status and future perspectives. *Exp Mol Med*. 2015 Mar 13;47:e151; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4351411/>.

safely, they would have to achieve several difficult tasks before any therapeutic benefits might be seen:

- travel into the multiple areas of the brain where damage has occurred;
- differentiate into the many types of neurons needed to replace the damaged or lost cells;
- do this in a way that enables the new neurons to integrate effectively into the brain, making connections to replace the lost parts of a complex network;
  - ensure that intravenous injections of stem cells don't block capillaries, causing embolisms and damaging brain tissue; and
  - ensure that stem cells don't give rise to tumors,<sup>726</sup> or make the wrong kind of cells or connections in the brain, leading to side effects like chronic pain.

Some scientists are concerned that the brain might lack the ability to integrate new neurons properly once Alzheimer's has taken hold. Another concern is that the newly-arriving transplanted stem cells might themselves be damaged by the ongoing amyloid and tau protein tangle buildup in the brain, which would mean that a transplant could have only a temporary effect.<sup>727</sup> Despite these significant challenges, scientists have been actively engaged in research on stem cell transplants in mice and a few studies have demonstrated the ability to improve cognition in animal models.<sup>728</sup> NSCs, capable of self-renewal and of differentiation into functional neurons and glia, have been shown to repair damaged networks and reverse memory and learning deficits in animal studies, providing new hope for curing AD patients via cell transplantation.<sup>729</sup>

Beyond simply replacing neurons, NSCs may also benefit the brain in other ways, such as by modulating inflammation, stimulating remyelination, and supplying trophic support. For instance, human NSCs that over-express the human choline acetyltransferase gene have been transplanted into the brains of AD rats, fully recovering the learning and memory function of the AD animals while inducing elevated levels of acetylcholine in cerebrospinal fluid.<sup>730</sup>

---

<sup>726</sup> Borlongan CV. Recent preclinical evidence advancing cell therapy for Alzheimer's disease. *Exp Neurol*. 2012 Sep;237(1):142-6; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4082718/>.

<sup>727</sup> Fan X, Sun D, Tang X, Cai Y, Yin ZQ, Xu H. Stem-cell challenges in the treatment of Alzheimer's disease: a long way from bench to bedside. *Med Res Rev*. 2014 Sep;34(5):957-78; <http://www.ncbi.nlm.nih.gov/pubmed/24500883/>.

<sup>728</sup> Chang KA, Kim HJ, Joo Y, Ha S, Suh YH. The therapeutic effects of human adipose-derived stem cells in Alzheimer's disease mouse models. *Neurodegener Dis*. 2014;13(2-3):99-102; <http://www.ncbi.nlm.nih.gov/pubmed/24157626>.

<sup>729</sup> Li XY, Bao XJ, Wang RZ. Potential of neural stem cell-based therapies for Alzheimer's disease. *J Neurosci Res*. 2015 Sep;93(9):1313-24; <http://www.ncbi.nlm.nih.gov/pubmed/25601591>. McGinley LM, Sims E, Lunn JS, Kashlan ON, Chen KS, Bruno ES, Pacut CM, Hazel T, Johe K, Sakowski SA, Feldman EL. Human Cortical Neural Stem Cells Expressing Insulin-Like Growth Factor-I: A Novel Cellular Therapy for Alzheimer's Disease. *Stem Cells Transl Med*. 2016 Mar;5(3):379-91; <http://stemcellstm.alphamedpress.org/content/early/2016/01/07/sctm.2015-0103.full.pdf>.

<sup>730</sup> Park D, Lee HJ, Joo SS, Bae DK, Yang G, Yang YH, Lim I, Matsuo A, Tooyama I, Kim YB, Kim SU. Human neural stem cells over-expressing choline acetyltransferase restore cognition in rat model of

As recently as 2012, some researchers still seemed pessimistic:

Research on the potential use of stem cell transplantation for AD has lagged far behind that of many other neurodegenerative disorders, likely as a result of the widespread nature of AD pathology. For many disorders, stem cell-based therapies have aimed to replace missing or defective cells. Transplantation of mesencephalic fetal tissue and neural stem cells for Parkinson's disease (PD), for example, has aimed to replace dopaminergic neurons of the substantia nigra that degenerate in this disorder. Unfortunately, in AD multiple neuronal systems and neurotransmitter phenotypes are affected, making cell-replacement strategies an extremely challenging approach. For effective cell replacement strategies for AD, neural stem cells would first need to migrate to multiple areas of the brain and then differentiate and mature into multiple neuronal subtypes. These neurons would then also need to re-innervate appropriate targets and establish physiologically relevant afferent connectivity, in essence recapitulating much of the complex brain circuitry that develops *in utero*. Thus, **cell-replacement approaches seem unlikely to succeed** for a diffuse disorder such as AD.<sup>731</sup>

Nevertheless a slowly growing body of successful work, mostly using animal models, has created an active research community pursuing possible stem cell treatment approaches for AD:

(1) Treatment with neural stem cells genetically modified to express the A $\beta$ -degrading enzyme neprilysin has been shown to lower A $\beta$  deposits throughout mouse brain.<sup>732</sup>

(2) Implantation of human umbilical cord blood-derived mesenchymal stem cells has reversed disease-associated microglial neuroinflammation and reversed the cognitive decline associated with A $\beta$  deposition in AD mice.<sup>733</sup>

(3) Human stem cell-derived neurons can functionally integrate into rat hippocampal network and drive endogenous neuronal network activity.<sup>734</sup>

cognitive dysfunction. *Exp Neurol*. 2012 Apr;234(2):521-6;  
<http://www.ncbi.nlm.nih.gov/pubmed/22245157/>.

<sup>731</sup> Chen WW, Blurton-Jones M. Concise review: Can stem cells be used to treat or model Alzheimer's disease? *Stem Cells*. 2012 Dec;30(12):2612-8; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3508338/>.

<sup>732</sup> Blurton-Jones M, Spencer B, Michael S, Castello NA, Agazaryan AA, Davis JL, Müller FJ, Loring JF, Masliah E, LaFerla FM. Neural stem cells genetically-modified to express neprilysin reduce pathology in Alzheimer transgenic models. *Stem Cell Res Ther*. 2014 Apr 16;5(2):46;  
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4055090/>.

<sup>733</sup> Lee HJ, Lee JK, Lee H, Carter JE, Chang JW, Oh W, Yang YS, Suh JG, Lee BH, Jin HK, Bae JS. Human umbilical cord blood-derived mesenchymal stem cells improve neuropathology and cognitive impairment in an Alzheimer's disease mouse model through modulation of neuroinflammation. *Neurobiol Aging*. 2012 Mar;33(3):588-602; <http://www.ncbi.nlm.nih.gov/pubmed/20471717>.

<sup>734</sup> Piña-Crespo JC, Talantova M, Cho EG, Soussou W, Dolatabadi N, Ryan SD, Ambasadhan R, McKercher S, Deisseroth K, Lipton SA. High-frequency hippocampal oscillations activated by optogenetic stimulation of transplanted human ESC-derived neurons. *J Neurosci*. 2012 Nov 7;32(45):15837-42;  
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3513396/>.

(4) Autologous fibroblasts genetically engineered to express nerve growth factor transplanted into the forebrains of eight human patients with mild Alzheimer's disease in a Phase I clinical trial showed no adverse effects after 22 months and showed possible improvement in the rate of cognitive decline.<sup>735</sup>

(5) Stem cells grafted into the intact mouse/rat brains have been mostly incorporated into the host parenchyma and differentiate into functional neural lineages. Stem cells exhibited targeted migration towards the damaged regions of lesioned brain, where they engrafted, proliferated and matured into functional neurons. Intravenously administered neural precursor cells (NPCs) migrate into brain-damaged areas and induce functional recovery, with animal models of AD providing evidence that transplanted stem cells or NPCs survive, migrate, and differentiate into cholinergic neurons, astrocytes, and oligodendrocytes with some amelioration of learning/memory deficits.<sup>736</sup>

(6) Intravenous transplantation of bone marrow-derived mononuclear cells (BMMCs) in two different AD mouse models suppressed neuronal loss, restored memory impairment to almost the same level as in wild-type mice, and reduced A $\beta$  deposition in the brain.<sup>737</sup>

(7) Embryonic interneuron<sup>738</sup> progenitor cells that were transplanted into the hippocampal hilus of aged mice developed into mature interneurons that were functionally integrated into the hippocampal circuitry, and restored normal learning, memory and cognitive function in two widely used AD-related mouse models.<sup>739</sup>

<sup>735</sup> Tuszynski MH, Thal L, Pay M, Salmon DP, U HS, Bakay R, Patel P, Blesch A, Vahlsing HL, Ho G, Tong G, Potkin SG, Fallon J, Hansen L, Mufson EJ, Kordower JH, Gall C, Conner J. A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease. *Nat Med.* 2005 May;11(5):551-5; <http://www.ncbi.nlm.nih.gov/pubmed/15852017>.

<sup>736</sup> Abdel-Salam OM. Stem cell therapy for Alzheimer's disease. *CNS Neurol Disord Drug Targets.* 2011 Jun;10(4):459-85; <http://www.ncbi.nlm.nih.gov/pubmed/21495961>.

<sup>737</sup> Kanamaru T, Kamimura N, Yokota T, Nishimaki K, Iuchi K, Lee H, Takami S, Akashiba H, Shitaka Y, Ueda M, Katsura KI, Kimura K, Ohta S. Intravenous transplantation of bone marrow-derived mononuclear cells prevents memory impairment in transgenic mouse models of Alzheimer's disease. *Brain Res.* 2015 Feb 16. pii: S0006-8993(15)00111-0; <http://www.ncbi.nlm.nih.gov/pubmed/25698614>.

<sup>738</sup> About 20%-30% of the neurons in the neocortex are inhibitory **interneurons**, creating neural circuits that enable communication between sensory or motor neurons and the central nervous system. Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C. Interneurons of the neocortical inhibitory system. *Nat Rev Neurosci.* 2004 Oct;5(10):793-807; <http://www.ncbi.nlm.nih.gov/pubmed/15378039>.

<sup>739</sup> Tong LM, Djukic B, Arnold C, Gillespie AK, Yoon SY, Wang MM, Zhang O, Knoferle J, Rubenstein JL, Alvarez-Buylla A, Huang Y. Inhibitory interneuron progenitor transplantation restores normal learning and memory in ApoE4 knock-in mice without or with A $\beta$  accumulation. *J Neurosci.* 2014 Jul 16;34(29):9506-15; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4099537/>.

(8) A large body of evidence is emerging that cell therapy works by providing trophic or “chaperone” support to the injured tissue and brain, with angiogenesis and neurogenesis coupled in the brain.<sup>740</sup>

(9) Artificial stimulation of neurogenesis can be tried.<sup>741</sup>

By 2015, assessments had become more hopeful but were still cautionary in respect to results for human subjects, given that no animal model appears to develop true AD and given that animal models often lack the range of phenotypes found in typical human AD. According to one leading group, “the disappointing preclinical data has resulted in few clinical trials using NSCs against Alzheimer’s disease, [so] stem cell-based regenerative therapies need to be further developed preclinically before clinical applications to AD.”<sup>742</sup>

Nevertheless, a few clinical trials may be starting soon:

- The biotech company Stemedica International, Epalinges, Switzerland, led by Alexei Lukashev, is developing human adult mesenchymal stem cells to combat Alzheimer’s disease.<sup>743</sup> After injecting the cells into the bloodstream of 15-month-old APP/PS1 mice once per week for 10 weeks, the amyloid load in the hippocampus of treated mice dropped by one-third compared with untreated controls. Meanwhile, more microglia clustered around plaques, while the number of pro-inflammatory microglia shrank, suggesting that the stem cells somehow influence this balance. Human mesenchymal stem cells are currently in a Phase I/II clinical trial for stroke.<sup>744</sup> Lukashev said he is applying for FDA approval for a Phase II Alzheimer’s study to start in 2015.<sup>745</sup>
- The California Institute for Regenerative Medicine has also given out 3 awards for Alzheimer’s projects that are now applying for FDA approval for trials. Two academic

---

<sup>740</sup> Hess DC, Borlongan CV. Stem cells and neurological diseases. *Cell Prolif.* 2008 Feb;41 Suppl 1:94-114; <http://www.ncbi.nlm.nih.gov/pubmed/18181951>.

<sup>741</sup> Galvan V, Bredesen DE. Neurogenesis in the adult brain: implications for Alzheimer’s disease. *CNS Neurol Disord Drug Targets.* 2007 Oct;6(5):303-10; <http://www.ncbi.nlm.nih.gov/pubmed/18045158>.

<sup>742</sup> Nam H, Lee KH, Nam DH, Joo KM. Adult human neural stem cell therapeutics: Current developmental status and prospect. *World J Stem Cells.* 2015 Jan 26;7(1):126-36; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4300923/>.

<sup>743</sup> “Stemedica International Announces Pre-clinical Data of its Alzheimer’s Study,” 19 Aug 2014; <http://stemedica-intl.com/press-releases/2014/08/19/stemedica-international-s-announces-pre-clinical-data-alzheimers-study/>.

<sup>744</sup> “A Study of Allogeneic Mesenchymal Bone Marrow Cells in Subjects With Ischemic Stroke,” <https://clinicaltrials.gov/ct2/show/NCT01297413>.

<sup>745</sup> “Ready or Not: Stem Cell Therapies Poised to Enter Trials for Alzheimer’s,” *AlzForum*, 20 Nov 2014; <http://www.alzforum.org/news/conference-coverage/ready-or-not-stem-cell-therapies-poised-enter-trials-alzheimers>.

studies identified small neuroprotective molecules through screens of stem cells. The third, led by Alexandra Capela at the biotech company StemCells Inc., Newark, California, proposes to transplant neural stem cells into AD patients.<sup>746</sup>

- A 2014 trial of fetal stem cell injections in mice with Alzheimer's disease by Eva Feldman's group at the Alfred Taubman Medical Research Institute found that the AD mice injected with cells performed the three cognition tests just as well as healthy mice, while AD mice injected with saline solution flunked the tests. Feldman awaits word from the FDA about which large mammal to use in the next set of Alzheimer's tests.<sup>747</sup>
- MEDIPOST has made a request to the FDA on 28 Jun 2013 to conduct Phase I/IIa clinical trials employing 'NEUROSTEM-AD', a possible treatment agent for Alzheimer's disease using human umbilical cord blood derived mesenchymal stem cells. This clinical study claims to be "the first in the world to use stem cells".<sup>748</sup> MEDIPOST successfully conducted the Phase I clinical trial using 'NEUROSTEM-AD' between Nov 2010 and Dec 2011,<sup>749</sup> and has since been conducting additional preclinical studies for the next year to study the routes of administration and methods.<sup>750</sup> "Unlike the administration of NEUROSTEM-AD through surgery into the brain once, during the first clinical trial, in the I/IIa clinical trial they have changed the method to the administration into cerebrospinal fluid three times to improve the patient convenience. Once the approval for clinical trial for NEUROSTEM-AD is given, MEDIPOST will be investigating the efficacy and safety of this agent together with the Samsung Seoul Medical Center for the next 2 years."

Current research is also exploring a type of stem cell called induced pluripotent stem cells (iPSCs) to study Alzheimer's disease.<sup>751</sup> These lab-grown stem cells are made by "reprogramming" specialized cells such as skin cells. The resulting iPSCs can produce all the different types of cells in the body. This means they could act as a source of cells that are

---

<sup>746</sup> "Restoration of memory in Alzheimer's disease: a new paradigm using neural stem cell therapy," California Institute for Regenerative Medicine (CIRM), 2013; <https://www.cirm.ca.gov/our-progress/awards/restoration-memory-alzheimer%E2%80%99s-disease-new-paradigm-using-neural-stem-cell>.

<sup>747</sup> "UM researcher Eva Feldman takes on ALS, Alzheimer's," 1 Aug 2014; <http://www.crainsdetroit.com/article/20140801/BLOG007/140809996/um-researcher-eva-feldman-takes-on-als-alzheimers-and-the-daily>.

<sup>748</sup> "'NEUROSTEM-AD' the treatment for Alzheimer's disease requested for phase 1.2a clinical trials," MEDIPOST Co., Ltd., Seoul, South Korea, 28 Jun 2013; <http://www.medi-post.com/2013/06/8254/>.

<sup>749</sup> <https://clinicaltrials.gov/ct2/show/NCT01297218>.

<sup>750</sup> <https://clinicaltrials.gov/ct2/show/NCT01696591>, <https://clinicaltrials.gov/ct2/show/NCT01547689>, and <https://clinicaltrials.gov/ct2/show/NCT02054208>.

<sup>751</sup> "Alzheimer's disease: how could stem cells help?" EuroStemCell, 22 Jan 2014; <http://www.eurostemcell.org/factsheet/alzheimer%E2%80%99s-disease-how-could-stem-cells-help>.

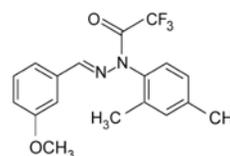
otherwise difficult to obtain, such as the neurons found in the brain. Scientists have used iPSC technology to grow neurons in the lab that show some of the key features of Alzheimer's disease. The lab-grown neurons release the beta amyloid protein that forms plaques in patients' brains. This gives scientists a valuable opportunity to study neurons similar to those affected by the disease in the brain, e.g., to gain a better understanding of how and why protein plaques and tangles are formed, and to search for and test new drugs. Many researchers believe that an important therapeutic role of iPSCs may be as a platform for drug discovery rather than for regeneration of diseased tissue.<sup>752</sup>

---

<sup>752</sup> Skalova S, Svadlakova T, Qureshi WM, Dev K, Mokry J. Induced Pluripotent Stem Cells and Their Use in Cardiac and Neural Regenerative Medicine. *Int J Mol Sci.* 2015 Feb 13;16(2):4043-67; <http://www.mdpi.com/1422-0067/16/2/4043/htm>.

### 3.2.15 Anti-Aging Drugs

If Alzheimer's disease is ultimately driven by the aging process, then perhaps drugs designed to combat aging might also prevent AD. One notable exploration of this possibility is currently underway by Salk Institute researchers. The Salk team has developed a drug candidate, called **J147** (image, right) that targets Alzheimer's major risk factor: old age.<sup>753</sup>



This group's latest work<sup>754</sup> shows that mice treated with J147 had better memory and cognition, healthier brain blood vessels, and other improved physiological features:

Previously, the team found that J147 could prevent and even reverse memory loss and Alzheimer's pathology in mice that have a version of the inherited form of Alzheimer's, the most commonly used mouse model. However, this form of the disease comprises only about 1 percent of Alzheimer's cases. For everyone else, old age is the primary risk factor. The team wanted to explore the effects of the drug candidate on a breed of mice that age rapidly and experience a version of dementia that more closely resembles the age-related human disorder.

In this latest work, the researchers used a comprehensive set of assays to measure the expression of all genes in the brain, as well as over 500 small molecules involved with metabolism in the brains and blood of three groups of the rapidly aging mice. The three groups of rapidly aging mice included one set that was young, one set that was old and one set that was old but fed J147 as they aged.

The old mice that received J147 performed better on memory and other tests for cognition and also displayed more robust motor movements. The mice treated with J147 also had fewer pathological signs of Alzheimer's in their brains. Importantly, because of the large amount of data collected on the three groups of mice, it was possible to demonstrate that many aspects of gene expression and metabolism in the old mice fed J147 were very similar to those of young animals. These included markers for increased energy metabolism, reduced brain inflammation and reduced levels of oxidized fatty acids in the brain. Another notable effect was that J147 prevented the leakage of blood from the microvessels in the brains of old mice. Damaged blood vessels are a common feature of aging in general, and in Alzheimer's, it is frequently much worse.

While these studies represent a new and exciting approach to Alzheimer's drug discovery and animal testing in the context of aging, the only way to demonstrate the clinical relevance of the

---

<sup>753</sup> Chen Q, Prior M, Dargusch R, Roberts A, Riek R, Eichmann C, Chiruta C, Akaishi T, Abe K, Maher P, Schubert D. A novel neurotrophic drug for cognitive enhancement and Alzheimer's disease. *PLoS One*. 2011;6(12):e27865; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3237323/>.

<sup>754</sup> Currais A, Goldberg J, Farrokhi C, Chang M, Prior M, Dargusch R, Daugherty D, Armando A, Quehenberger O, Maher P, Schubert D. A comprehensive multiomics approach toward understanding the relationship between aging and dementia. *Aging (Albany NY)*. 2015 Nov;7(11):937-55; <http://www.impactaging.com/papers/v7/n11/full/100838.html>.

work is to move J147 into human clinical trials for Alzheimer's disease. The team aims to begin human trials next year [in 2016].<sup>755</sup>

Interventions that can provably slow mammalian aging are rare but hardly nonexistent.<sup>756</sup> For example, one recent mouse study<sup>757</sup> found that supplementing diet with nicotinamide riboside countered senescence in adult neural, muscle, and melanocyte stem cells, improving mitochondrial function and suppressing dysfunction in a mouse model of muscular dystrophy. Nicotinamide riboside supplementation was found to extend the life span of wild-type mice fed the normal chow diet, joining a short list of compounds with this ability (SIRT1 activators, metformin, and rapamycin). Related clinical trials in humans are reportedly in the works.<sup>758</sup>

---

<sup>755</sup> "Experimental drug targeting Alzheimer's disease shows anti-aging effects," Salk News Release, 12 Nov 2015; [http://www.salk.edu/news/pressrelease\\_details.php?press\\_id=2130](http://www.salk.edu/news/pressrelease_details.php?press_id=2130).

<sup>756</sup> Guarente L. The resurgence of NAD<sup>+</sup>. *Science*. 2016 Jun 17;352(6292):1396-7; <http://science.sciencemag.org/content/352/6292/1396.full>.

<sup>757</sup> Zhang H, Ryu D, Wu Y, Gariani K, Wang X, Luan P, D'Amico D, Ropelle ER, Lutolf MP, Aebbersold R, Schoonjans K, Menzies KJ, Auwerx J. NAD<sup>+</sup> repletion improves mitochondrial and stem cell function and enhances life span in mice. *Science*. 2016 Jun 17;352(6292):1436-43; <http://science.sciencemag.org/content/352/6292/1436.full>.

<sup>758</sup> <http://gizmodo.com/a-promising-anti-aging-drug-will-soon-be-tried-in-human-1782363625>.

## Chapter 4. NEW APPROACH: Medical Nanorobotics

In this Chapter we propose and discuss a fundamentally new approach to preventing, arresting, and finally curing Alzheimer's disease, using the techniques of atomically precise manufacturing as exemplified by a nanofactory.

A nanofactory (Section 4.1) is a manufacturing system for building atomically precise products – in particular, microscopic **medical nanorobots** – in macroscale (e.g., kilogram) quantities. The medical nanorobots can then be used to treat Alzheimer's disease. Treatment dosages may typically run to several grams of nanorobots per Alzheimer's patient, and the robots can be extracted after usage and recycled if desired.

Our new approach to curing Alzheimer's disease using medical nanorobots may be summarized as the performance of four distinct tasks, as follows:

(1) First, use scanning probe microscopy (Section 4.1.1) to build atomically precise diamondoid structures using the methods of mechanosynthesis (Section 4.1.2).

(2) Second, use the aforementioned molecular tools to build a nanofactory (Section 4.1.3).

(3) Third, use the nanofactory to manufacture medical nanorobots (Sections 4.2 and 4.3) inexpensively and in therapeutic quantities, with these robots aimed at specific biological targets appropriate to the treatment of Alzheimer's disease in humans (Chapter 5).

(4) Fourth, open fee-for-service nanomedical clinics to offer nanorobotic treatments both to prospective Alzheimer's patients, on a preventative basis, and to existing Alzheimer's patients, on a curative basis.

## 4.1 Atomically Precise Manufacturing using Nanofactories

The nanofactory will be a high quality, extremely low cost, and very flexible manufacturing system in which products are built atom by atom – an atomically precise manufacturing system employing controlled molecular assembly. Nanofactories will enable the creation of fundamentally novel products having the intricate complexity and reliability currently found only in biological systems, but operating with greater speed, power, predictability, and, most importantly, working entirely under human engineering control at every step.

The principal inputs to a nanofactory may be simple hydrocarbon feedstock molecules such as natural gas or propane, along with water and small supplemental amounts of other simple molecules containing trace atoms of a few additional chemical elements needed to make useful products, such as oxygen, nitrogen, sulfur, or silicon. The nanofactory must also be provided with electrical power and a means for cooling the working unit.

The fastest and therefore preferred implementation pathway for this technology will employ scanning probe microscopy (Section 4.1.1) and the methods of mechanosynthesis (Section 4.1.2) to build the first nanofactory (Section 4.1.3). In the following discussion, please keep in mind that the diameters of individual atoms in covalently bonded molecules are approximately 76 pm for hydrogen, 146 pm for oxygen, 150 pm for nitrogen, and 154 pm for carbon,<sup>759</sup> where 1 picometer (pm) = 0.01 Angstrom (Å) = 0.001 nanometer =  $10^{-12}$  meter.

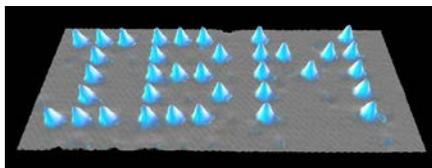
---

<sup>759</sup> [http://en.wikipedia.org/wiki/Atomic\\_radii\\_of\\_the\\_elements\\_\(data\\_page\)](http://en.wikipedia.org/wiki/Atomic_radii_of_the_elements_(data_page)).

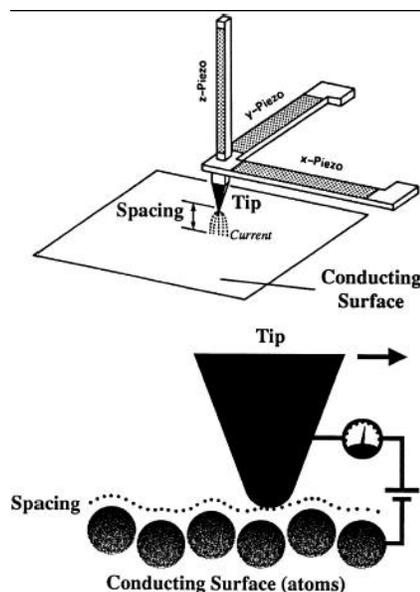
### 4.1.1 Scanning Probe Microscopy

A scanning probe microscope or “SPM” is an apparatus that can be used for building atomically precise structures atom by atom.

The first of the SPMs was the Scanning Tunneling Microscope (STM) developed in the late 1970s and early 1980s by Gerd Karl Binnig and Heinrich Rohrer at an IBM research lab in Zurich, Switzerland,<sup>760</sup> earning these scientists, along with Ernst Ruska, the 1986 Nobel Prize in Physics. The STM was initially used as an imaging device, capable of resolving individual atoms by recording the quantum tunneling current that occurs when an extremely sharp conductive probe tip (usually tungsten, nickel, gold, or platinum-iridium) is brought to within about one atomic diameter of an atom, and then adjusting the position of the tip to maintain a constant current as the tip is scanned over a bumpy atomic surface (at right). A height change as small as 100 pm can cause tunneling current to double. The tip is connected to an arm that is moved in three dimensions by stiff ceramic piezoelectric transducers that provide sub-nanometer positional control. If the tip is atomically sharp, then the tunneling current is



effectively confined to a region within ~100 pm of the point on the surface directly beneath the tip, thus the record of tip adjustments generates an atomic-scale topographic map of the surface. STM tips can scan samples at ~kHz frequencies, although slower scans are used for very rough surfaces and in some modern STMs the sample may be moved while the tip is held stationary. Perhaps the most iconic image is the classic 1989 picture of the IBM logo (image, left) spelled out with 35 xenon atoms arranged on a nickel surface.<sup>761</sup> These atoms were imaged by an STM tip after the individual atoms were laterally positioned using the same tip over a period of 22 hours in the lab. The work was done at very cold (cryogenic) temperatures to keep the atoms from moving around too much after being put in the proper places.



effectively confined to a region within ~100 pm of the point on the surface directly beneath the tip, thus the record of tip adjustments generates an atomic-scale topographic map of the surface. STM tips can scan samples at ~kHz frequencies, although slower scans are used for very rough surfaces and in some modern STMs

<sup>760</sup> Binnig G, Rohrer H. Scanning tunneling microscopy. *Helv Phys Acta*. 1982;55:726-735. Binnig G, Rohrer H, Gerber C, Weibel E. Surface studies by scanning tunneling microscopy. *Phys Rev Lett*. 1982;49:57-61 and *Phys Rev Lett*. 1983;50:120-123. Binnig G, Rohrer H. The Scanning Tunneling Microscope. *Sci Am*. 1985 Aug;253:50-56. Binnig G, Rohrer H. Scanning tunneling microscopy. *IBM J Res Develop*. 1986;30:355-369. Binnig G, Rohrer H. Scanning tunneling microscopy from birth to adolescence. *Rev Mod Phys* 1987 Jul;59:615-625.

<sup>761</sup> Eigler DM, Schweizer EK. Positioning Single Atoms with a Scanning Tunnelling Microscope. *Nature*. 1990 Apr 5;344:524-526; <http://www.nature.com/nature/journal/v344/n6266/abs/344524a0.html>.

STM technology has much improved over the last few decades. Instruments now regularly achieve resolutions of ~1 pm in the z direction (vertical) and ~10 pm in the xy (horizontal) plane which is better than atomic resolution.

A major limitation of the STM is that it only works with conducting materials such as metals or semiconductors, but not with insulators or biological structures such as DNA.<sup>762</sup> To remedy this situation, in 1986 Binnig, Quate and Gerber developed the Atomic Force Microscope (AFM)<sup>763</sup> which is sensitive directly to the forces between the tip and the sample, rather than to a tunneling current. An AFM can operate in at least three modes. In “attractive” or non-contact mode (NC-AFM or FM-AFM), the tip is held some tens of nanometers above the sample surface where it experiences the attractive combination of van der Waals, electrostatic, and magnetostatic forces. In “repulsive” or contact mode (C-AFM), the tip is pressed close enough to the surface to allow the electron clouds of tip and sample to overlap, generating a repulsive electrostatic force of ~10 nN, much like the stylus riding a groove in an old vinyl record player. There is also intermittent-contact mode (IC-AFM), which is sometimes called “tapping” mode. In any of these modes, a topographic map of the surface is generated by recording the up-and-down motions of the cantilever arm as the tip is scanned. These motions may be measured either by the deflection of a light spot reflected from a mirrored surface on the cantilever or by tiny changes in voltage generated by piezoelectric transducers attached to the moving cantilever arm. Typical laboratory AFM cantilevers have lengths of 100-400 microns, widths of 20-50 microns, and thicknesses between 0.4-3 microns. AFM tips may be positioned with ~10 pm precision, compressive loads as small as 1-10 pN of force are routinely measured,<sup>764</sup> and the tips may be operated even in liquids.<sup>765</sup>

The AFM is essentially a way to “touch” a molecule and to “feel” the shapes of the atoms comprising the molecule while the molecule is resting on a surface. In 2009, researchers at IBM Zurich used an AFM in constant-height non-contact mode in ultra-high vacuum (UHV) at cryogenic temperatures (5 K (kelvins), which is -268 °C or -451 °F) to scan an organic molecule that had been deposited either on a flat copper Cu(111) conductive surface, or on the same copper surface coated with a 2-monolayer thickness of insulating NaCl film.<sup>766</sup> Scan forces ranged from

---

<sup>762</sup> Lee GU, Chrisey LA, Colton RJ. Direct Measurement of the Forces Between Complementary Strands of DNA. *Science*. 1994 Nov 4;266:771-3. Boland T, Ratner BD. Direct measurement of hydrogen bonding in DNA nucleotide bases by atomic force microscopy. *Proc Natl Acad Sci USA*. 1995;92:5297-301.

<sup>763</sup> Binnig G, Quate CF, Gerber Ch. Atomic Force Microscopy. *Phys Rev Lett*. 1986 Mar 3;56:930-3.

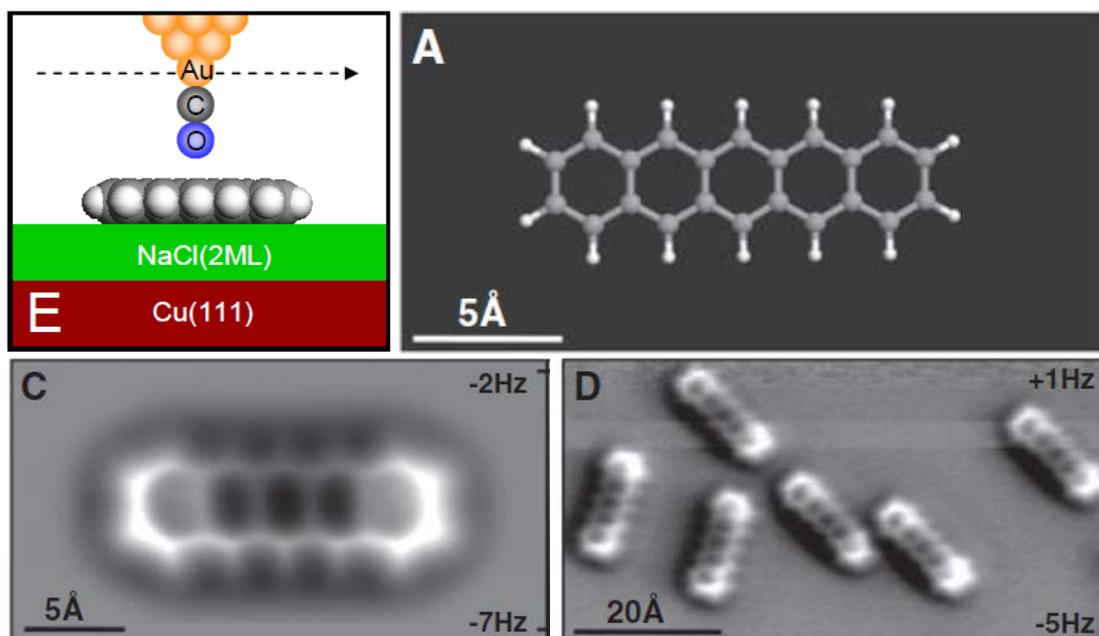
<sup>764</sup> Weisenhorn AL, Hansma PK, Albrecht TR, Quate CF. Forces in atomic force microscopy in air and water. *Appl Phys Lett*. 1989;54:2651-3. Chen CJ. *Introduction to Scanning Tunneling Microscopy*, Oxford University Press, Cambridge, 1993. Wiesendanger R. *Scanning Probe Microscopy and Spectroscopy: Methods and Applications*, Cambridge University Press, Cambridge, MA, 1994.

<sup>765</sup> Schaffer TE, Cleveland JP, Ohnesorge F, Walters DA, Hansma PK. Studies of vibrating atomic force microscope cantilevers in liquid. *J Appl Phys*. 1996;80:3622-7.

<sup>766</sup> Gross L, Mohn F, Moll N, Liljeroth P, Meyer G. The chemical structure of a molecule resolved by atomic force microscopy. *Science*. 2009 Aug 28;325(5944):1110-4;  
[https://www.researchgate.net/profile/Nikolaj\\_Moll/publication/256847086\\_Gross\\_et\\_al.\\_-2009\\_-](https://www.researchgate.net/profile/Nikolaj_Moll/publication/256847086_Gross_et_al._-2009_-)

0-100 pN. The first organic molecule they looked at was pentacene ( $C_{22}H_{14}$ ), a linear polycyclic hydrocarbon consisting of 5 fused benzene rings and 22 carbon atoms (**Figure 9**). By 2014 the Zurich group had imaged their largest molecule to date, a clover-shaped nanographene molecule with 22 fused benzene rings ( $C_{78}H_{36}$ ).<sup>767</sup>

*Figure 9. Top left: The AFM tip is gold atoms to which a single carbon monoxide (CO) molecule has been attached, making a very sharp tip. The pentacene molecule rests on the surface. Top right: The molecular structure of pentacene (gray = carbon, white = hydrogen). Bottom left: A single pentacene molecule on Cu(111), with all of its atoms clearly resolved. Bottom right: Again on Cu(111), six atomically-resolved pentacene molecules are in one image.*

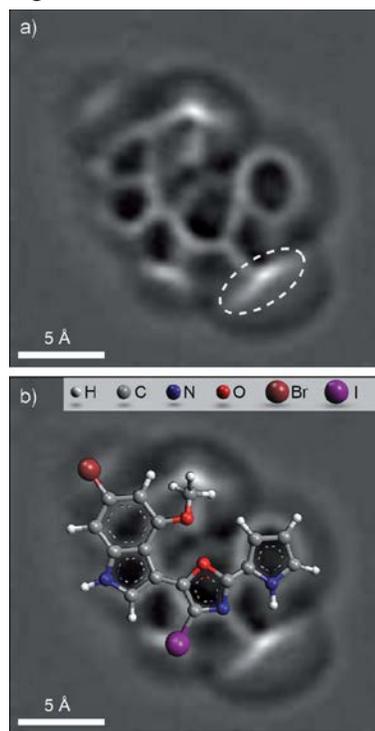


[The Chemical Structure of a Molecule Resolved by A Supplement/links/0deec5240013f7068700000.pdf](https://www.researchgate.net/publication/263707042_From_Perylene_to_a_22-Ring_Aromatic_Hydrocarbon_in_One-Pot/links/560522cc08aea25fce32191f.pdf).

<sup>767</sup> Schuler B, Collazos S, Gross L, Meyer G, Pérez D, Guitián E, Peña D. From perylene to a 22-ring aromatic hydrocarbon in one-pot. *Angew Chem Int Ed Engl.* 2014 Aug 18;53(34):9004-6; [https://www.researchgate.net/profile/Sara\\_Collazos/publication/263707042\\_From\\_Perylene\\_to\\_a\\_22-Ring\\_Aromatic\\_Hydrocarbon\\_in\\_One-Pot/links/560522cc08aea25fce32191f.pdf](https://www.researchgate.net/profile/Sara_Collazos/publication/263707042_From_Perylene_to_a_22-Ring_Aromatic_Hydrocarbon_in_One-Pot/links/560522cc08aea25fce32191f.pdf).

In similar manner, the IBM team used their AFM to distinguish, by “touch” alone, the carbon atom “bond order” – e.g., whether adjacent carbon atoms have single- (C–C), double- (C=C), or triple (C≡C) bonds – in various scanned individual organic molecules including polycyclic hydrocarbons and fullerenes.<sup>768</sup> Along with the charge distribution within individual surface-bound molecules,<sup>769</sup> the positioning of the sample molecule on the surface can be determined with atomic precision using AFM, including the deposited molecule’s lateral adsorption position, its adsorption height differences (with 3 pm precision), and the tilts of its molecular plane (to within 0.2°).<sup>770</sup>

In 2010, the Zurich team used the same technique (i.e., sample molecule deposited on a Cu(111) surface and scanned by a CO-functionalized AFM tip) to determine the exact pattern of atomic connectivity in a natural organic molecule of previously undetermined structure, a metabolite called cephalandole A.<sup>771</sup> By 2012, a larger collaboration of researchers<sup>772</sup> used a combination of the same atomic resolution AFM, along with Density-Functional Theory (DFT) quantum chemistry structure calculations and computer-aided structure elucidation (CASE), to solve the structure of the natural compound breitfussin A (image, right) with molecular formula



<sup>768</sup> Gross L, Mohn F, Moll N, Schuler B, Criado A, Guitián E, Peña D, Gourdon A, Meyer G. Bond-order discrimination by atomic force microscopy. *Science*. 2012 Sep 14;337(6100):1326-9; <http://science.sciencemag.org/content/sci/337/6100/1326.full.pdf>.

<sup>769</sup> Mohn F, Gross L, Moll N, Meyer G. Imaging the charge distribution within a single molecule. *Nat Nanotechnol*. 2012 Feb 26;7(4):227-31; [http://domino.watson.ibm.com/library/CyberDig.nsf/papers/C36A9B0FED78DA6F85257D20004D4871/\\$File/rz3873.pdf](http://domino.watson.ibm.com/library/CyberDig.nsf/papers/C36A9B0FED78DA6F85257D20004D4871/$File/rz3873.pdf).

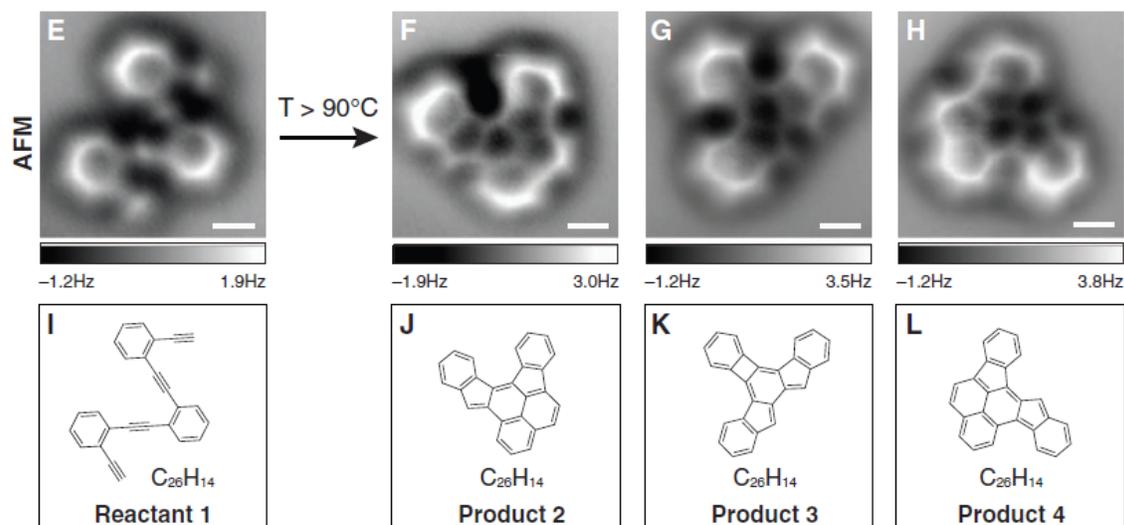
<sup>770</sup> Schuler B, Liu W, Tkatchenko A, Moll N, Meyer G, Mistry A, Fox D, Gross L. Adsorption geometry determination of single molecules by atomic force microscopy. *Phys Rev Lett*. 2013 Sep 5;111:106103.

<sup>771</sup> Gross L, Mohn F, Moll N, Meyer G, Ebel R, Abdel-Mageed WM, Jaspars M. Organic structure determination using atomic-resolution scanning probe microscopy. *Nat Chem*. 2010 Oct;2(10):821-5; <http://home.cc.umanitoba.ca/~hultin/chem2220/Support/CoolStuff/MolecularStructure/nchem.765.pdf>.

<sup>772</sup> Hanssen KØ, Schuler B, Williams AJ, Demissie TB, Hansen E, Andersen JH, Svenson J, Blinov K, Repisky M, Mohn F, Meyer G, Svendsen JS, Ruud K, Elyashberg M, Gross L, Jaspars M, Isaksson J. A combined atomic force microscopy and computational approach for the structural elucidation of breitfussin A and B: highly modified halogenated dipeptides from *Thuiaria breitfussi*. *Angew Chem Int Ed Engl*. 2012 Dec 3;51(49):12238-41; [https://www.researchgate.net/profile/Kenneth\\_Ruud/publication/232739017\\_A\\_Combined\\_Atomic\\_Force\\_Microscopy\\_and\\_Computational\\_Approach\\_for\\_the\\_Structural\\_Elucidation\\_of\\_Breitfussin\\_A\\_and\\_B\\_Highly\\_Modified\\_Halogenated\\_Dipeptides\\_from\\_Thuiaria\\_breitfussi/links/0912f50a775425b040000000.pdf](https://www.researchgate.net/profile/Kenneth_Ruud/publication/232739017_A_Combined_Atomic_Force_Microscopy_and_Computational_Approach_for_the_Structural_Elucidation_of_Breitfussin_A_and_B_Highly_Modified_Halogenated_Dipeptides_from_Thuiaria_breitfussi/links/0912f50a775425b040000000.pdf).

$C_{16}H_{11}N_3O_2BrI$  as confirmed by high-resolution mass spectrometry. Cephalandole A is a member of a chemical family of molecules that include sterols, polyhalogenated monoterpenes, and anthracenone derivatives. (The white encircled region marks a non-intrinsic molecule feature.) Remarkably, the AFM could determine all the connection positions of the cyclic systems as well as those of the substituent groups (MeO, Br, and I) – information that is difficult to obtain with other techniques.

AFM is now commonly used to record the changes in chemical structure that occur as an individual molecule undergoes a complex reaction on a surface. For example, Crommie's group at U.C. Berkeley used a cryogenic UHV non-contact AFM to track the transformations of an individual molecule of 1,2-bis((2-ethynylphenyl)ethynyl)benzene on a silver Ag(100) surface as it underwent a series of cyclization processes (image below; scale bar = 3 Å).<sup>773</sup> With the assistance of DFT-based quantum chemistry calculations, these bond-resolved single-molecule AFM images were sufficient to identify the structure of the original reactant and its successor product molecules.



<sup>773</sup> de Oteyza DG, Gorman P, Chen YC, Wickenburg S, Riss A, Mowbray DJ, Etkin G, Pedramrazi Z, Tsai HZ, Rubio A, Crommie MF, Fischer FR. Direct imaging of covalent bond structure in single-molecule chemical reactions. *Science*. 2013 Jun 21;340(6139):1434-7; [http://sitc.isoveradev.com/sites/default/files/research-papers/science-2013-de\\_oteyza-1434-7.pdf](http://sitc.isoveradev.com/sites/default/files/research-papers/science-2013-de_oteyza-1434-7.pdf).

### 4.1.2 Mechanosynthesis

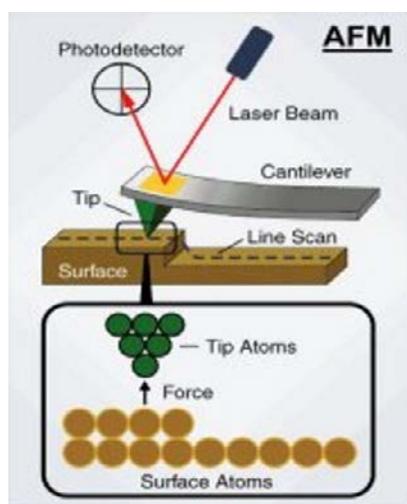
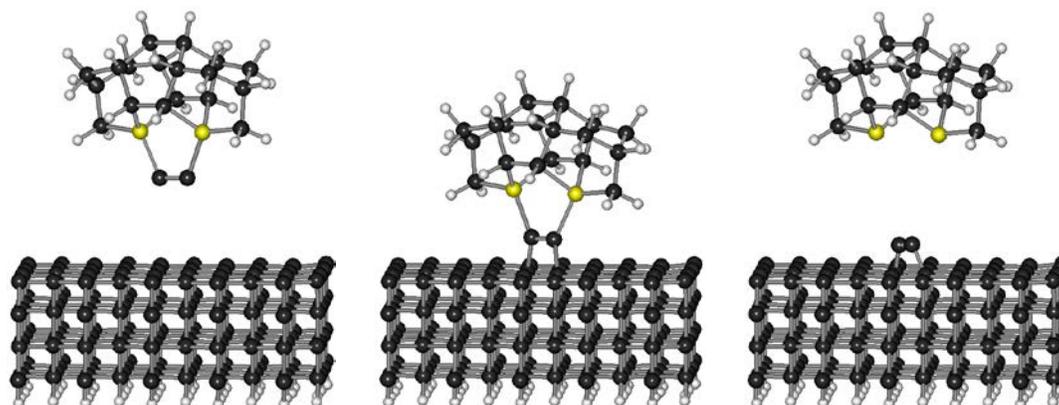
At the most primitive level of our new manufacturing process, atomically precise objects will be built atom by atom using “mechanosynthesis.” Mechanosynthesis, involving molecular positional fabrication, is the formation of covalent chemical bonds using precisely applied mechanical forces to build diamondoid<sup>774</sup> or other structures. Mechanosynthesis employs chemical reactions driven by the mechanically precise positioning of extremely reactive chemical species in an ultra-high vacuum environment. Mechanosynthesis may be automated via computer control, enabling programmable molecular positional fabrication.

Atomically precise fabrication involves holding feedstock atoms or molecules, and a growing nanoscale workpiece, in the proper relative positions and orientations so that when they touch they will chemically bond in the desired manner. In this process, a mechanosynthetic tool is brought up to the surface of a workpiece. One or more transfer atoms are added to, or removed from, the workpiece by the tool (**Figure 10**). Then the tool is withdrawn and recharged. This process is repeated until the workpiece (e.g., a growing nanopart) is completely fabricated to molecular precision with each atom in exactly the right place. Note that the transfer atoms are under positional control at all times to prevent unwanted side reactions from occurring. Side reactions are also prevented using proper reaction sequence design so that the interaction energetics help to avoid undesired pathological intermediate structures.

---

<sup>774</sup> Most diamondoids resemble ceramics. First and foremost, diamondoid materials include pure diamond, the crystalline allotrope of carbon. Among other exceptional properties, diamond has extreme hardness, high thermal conductivity, low frictional coefficient, chemical inertness, a wide electronic bandgap, and is the strongest and stiffest material presently known at ordinary pressures. Diamondoid materials also may include any stiff covalent solid that is similar to diamond in strength, chemical inertness, or other important material properties, and that possesses a dense three-dimensional network of bonds. Examples of such materials are carbon nanotubes and fullerenes, several strong covalent ceramics such as silicon carbide, silicon nitride, and boron nitride, and a few very stiff ionic ceramics such as sapphire (monocrystalline aluminum oxide) that can be covalently bonded to purely covalent structures such as diamond. Of course, large crystals of pure diamond are brittle and easily fractured. The intricate molecular structure of a diamondoid nanofactory macroscale product will more closely resemble a complex composite material, not a brittle solid crystal. Such atomically precise products, and the nanofactories that build them, should be extremely durable in normal use.

Figure 10. Three frames at the top show the DCB6Ge tooltip depositing two carbon atoms on a diamond surface. The tooltip is attached to a much larger tool handle structure (not shown) which is attached, in turn, to the macroscale tip of a laboratory-scale scanning probe microscope (e.g., see schematic, lower left, and image, lower right, of a UHV scanning probe microscope).



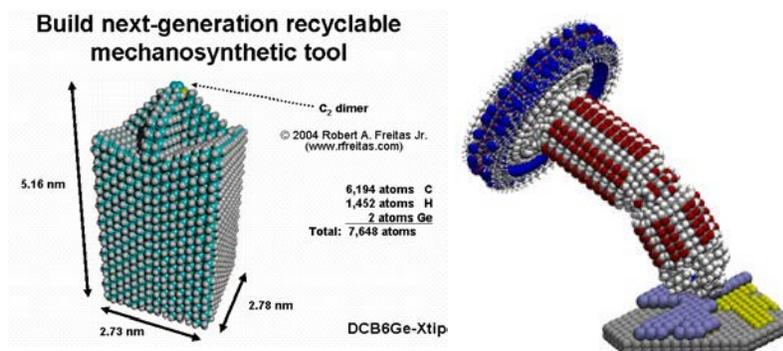
Mechanosynthesis has been extensively discussed in the theoretical literature since 1992,<sup>775</sup> was first demonstrated experimentally in 2003<sup>776</sup> and repeatedly in later years,<sup>777</sup> and the first U.S.

<sup>775</sup> Drexler KE. *Nanosystems: Molecular Machinery, Manufacturing, and Computation*, John Wiley & Sons, New York, 1992; Freitas RA Jr., Merkle RC. A minimal toolset for positional diamond mechanosynthesis. *J Comput Theor Nanosci.* 2008;5:760-861; <http://www.molecularassembler.com/Papers/MinToolset.pdf>

<sup>776</sup> Oyabu N, Custance O, Yi I, Sugawara Y, Morita S. Mechanical vertical manipulation of selected single atoms by soft nanoindentation using near contact atomic force microscopy. *Phys Rev Lett.* 2003 May 2;90(17):176102;

patent on mechanosynthesis was issued to Freitas in 2010.<sup>778</sup> Mechanosynthesis has not yet achieved widespread mainstream acceptance because historically it has proven experimentally challenging to accomplish, but it can be rapidly improved using cryogenic UHV scanning probe technology of the kind illustrated in **Figure 10**.

A scanning probe-based system would enable the fabrication of more precise, more easily rechargeable, and generally much improved mechanosynthetic tools. These more capable tools may include more stable handles of standardized dimensions, such as the rechargeable DCB6Ge dimer placement tool with the more reliable crossbar design (above, left),<sup>779</sup> or tools with more complex handles incorporating moving components (above, right).



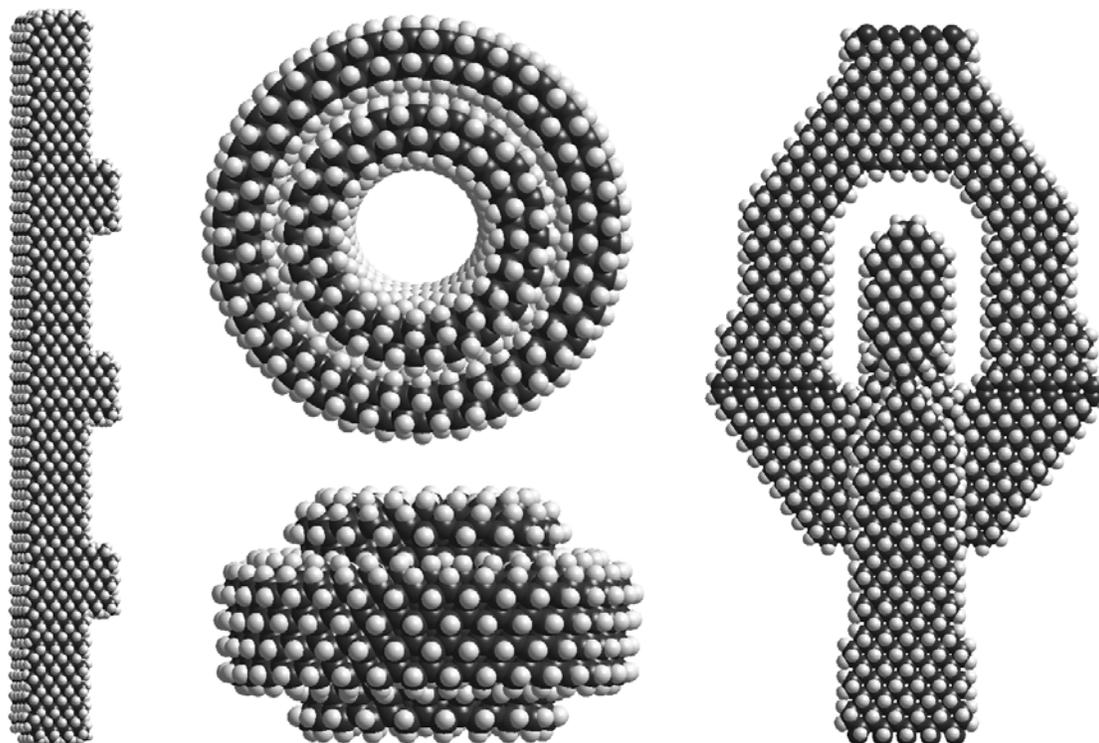
[https://www.researchgate.net/profile/Seizo\\_Morita/publication/10726137\\_Mechanical\\_vertical\\_manipulation\\_of\\_selected\\_single\\_atoms\\_by\\_soft\\_nanoindentation\\_using\\_near\\_contact\\_atomic\\_force\\_microscopy/links/0912f4fc41f5127627000000.pdf](https://www.researchgate.net/profile/Seizo_Morita/publication/10726137_Mechanical_vertical_manipulation_of_selected_single_atoms_by_soft_nanoindentation_using_near_contact_atomic_force_microscopy/links/0912f4fc41f5127627000000.pdf).

<sup>777</sup> Oyabu N, Custance O, Abe M, Moritabe S. Mechanical vertical manipulation of single atoms on the Ge(111)-c(2x8) surface by noncontact atomic force microscopy. Abstracts of Seventh International Conference on Non-Contact Atomic Force Microscopy, Seattle, Washington, USA, 12-15 Sep 2004, p. 34; <http://www.engr.washington.edu/epp/afm/abstracts/15Oyabu2.pdf>. Sugimoto Y, Pou P, Custance O, Jelinek P, Abe M, Perez R, Morita S. Complex patterning by vertical interchange atom manipulation using atomic force microscopy. *Science*. 2008;322(5900):413-417; <http://www.sciencemag.org/cgi/content/full/322/5900/413>. Kawai S, Foster AS, Canova FF, Onodera H, Kitamura S, Meyer E. Atom manipulation on an insulating surface at room temperature. *Nat Commun*. 2014 Jul 15;5:4403; <http://viesti.physics.aalto.fi/~asf/publications/Nature%20Comm%20manip.pdf>.

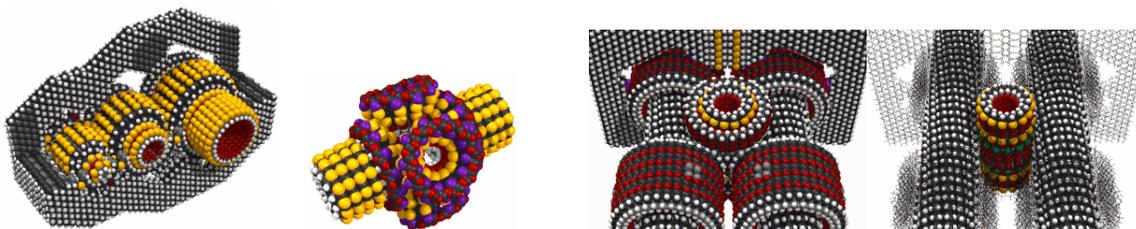
<sup>778</sup> Freitas RA Jr. Simple Tool for Positional Diamond Mechanosynthesis, and its Method of Manufacture. U.S. Patent 7,687,146, 30 Mar 2010.

<sup>779</sup> Peng J, Freitas RA Jr., Merkle RC, Von Ehr JR, Randall JN, Skidmore GD. Theoretical analysis of diamond mechanosynthesis. Part III. Positional C<sub>2</sub> deposition on diamond C(110) surface using Si/Ge/Sn-based dimer placement tools. *J Comput Theor Nanosci*. 2006 Feb;3(1):28-41; <http://www.MolecularAssembler.com/Papers/JCTNPengFeb06.pdf>.

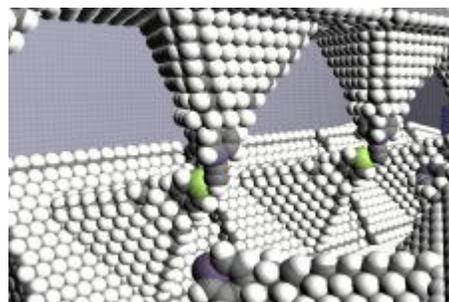
Later systems will incorporate more complex components such as the all-hydrocarbon diamond logic rod (below, left) for use in mechanical nanocomputers, the hydrocarbon bearing (below, center), the diamond universal joint (below, right), and related mechanical devices. The end result of this iterative development process will be a mature set of efficient, positionally controlled mechanosynthetic tools that can reliably build molecularly precise diamondoid structures – including more mechanosynthetic tools.



Once mechanosynthetic tooltips are developed for a few additional element types, a still wider variety of nanomachines can be fabricated incorporating atoms other than hydrogen, carbon and germanium (e.g., silicon, oxygen, nitrogen, and sulfur). Examples of these more varied diamondoid nanomachines include the speed reduction gear (below, left), in which the train of gears reduces the speed from the high-speed one on the left to the half-speed one on the right, and the differential gear (below, center) that smoothly converts mechanical rotation in one direction into mechanical rotation in the opposite direction. The largest publically reported molecular machine model that has been simulated using molecular dynamics is the worm drive assembly (below, pair at right), consisting of 11 separate components and over 25,000 atoms. The two tubular worm gears progress in opposite directions, converting rotary into linear motion.



Using computer-automated tooltips performing positionally-controlled mechanosynthesis in lengthy programmed sequences of reaction steps, we will be able to fabricate simple diamondoid nanomechanical parts such as bearings, gears, struts, springs, logic rods and casings, to atomic precision. Early tools will rapidly progress from single tools manipulated by laboratory scanning-probe-like mechanisms, to more complex multitip tools and jigs which the simple tools could initially fabricate, one at a time. In a factory production line (below), individual mechanosynthetic tooltips can be affixed to rigid moving support structures and guided through repeated contact events with workpieces, recharging stations, and other similarly-affixed opposable tooltips. These “molecular mills” can then perform repetitive fabrication steps using simple, efficient mechanisms in the manner of a production line. Such production lines can, in principle, be operated at very high speeds – with positionally constrained mechanosynthetic encounters possibly occurring at up to megahertz frequencies.<sup>780</sup>

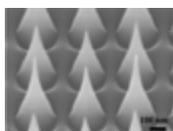


---

<sup>780</sup> Drexler KE. *Nanosystems: Molecular Machinery, Manufacturing, and Computation*, John Wiley & Sons, New York, 1992.

### 4.1.3 Nanofactories: Manufacturing Medical Nanorobots

The goal of molecular nanotechnology is to develop a manufacturing technology able to inexpensively manufacture most arrangements of atoms that can be specified in molecular detail – including complex arrangements involving millions or billions of atoms per product object. This will provide the ultimate manufacturing technology in terms of precision, flexibility, and low cost. To be practical, atomically precise manufacturing must also be able to assemble very large numbers of atomically identical product structures very quickly. Two central technical objectives thus form the core of our current strategy for atomically precise manufacturing: (1) programmable positional assembly including fabrication of diamondoid structures using molecular feedstock, as discussed above, and (2) massive parallelization of all fabrication and assembly processes, as briefly discussed below.



Conceptually, nanofactory systems capable of massively parallel fabrication<sup>781</sup> might employ, at the lowest level, large arrays of mechanosynthesis-enabled scanning probe tips all building similar diamondoid product structures in unison, superficially similar to the highly-uniform, well-aligned ultrasharp silicon nanotips (image, left) fabricated at a surface density of  $\sim 10^9$  tips/cm<sup>2</sup> in 2012.<sup>782</sup>

Analogous approaches are found in present-day larger-scale systems. For example, simple mechanical ciliary arrays consisting of 10,000 independent microactuators on a 1 cm<sup>2</sup> chip have been made at the Cornell National Nanofabrication Laboratory for microscale parts transport applications, and similarly at IBM for mechanical data storage applications.<sup>783</sup> Active probe arrays of 10,000 independently-actuated microscope tips have been developed by Mirkin's group at Northwestern University for dip-pen nanolithography (DPN) using DNA-based "ink".<sup>784</sup>

---

<sup>781</sup> Freitas RA Jr., Merkle RC. Kinematic Self-Replicating Machines. Landes Bioscience, Georgetown, TX, 2004, Section 5.7 "Massively Parallel Molecular Manufacturing"; <http://www.MolecularAssembler.com/KSRM/5.7.htm>.

<sup>782</sup> Wu CC, Ou KL, Tseng CL. Fabrication and characterization of well-aligned and ultra-sharp silicon nanotip array. *Nanoscale Res Lett.* 2012 Feb 13;7(1):120; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3292956/>.

<sup>783</sup> "1000 Tips for Ultrahigh-Density Data Storage," IBM News, Zurich Research Lab, 11 Oct 1999, <http://www.zurich.ibm.com/news/99/millipede.html>. IBM Research: Millipede, <http://domino.research.ibm.com/Comm/bios.nsf/pages/millipede.html>. Vettiger P, Cross G, Despont M, Drechsler U, Duerig U, Gotsmann B, Haeblerle W, Lantz M, Rothuizen H, Stutz R, Binnig G. The Millipede – nanotechnology entering data storage. Technical Report, IBM Zurich Research Lab; [http://domino.research.ibm.com/Comm/bios.nsf/pages/millipede.html/\\$FILE/pv7201-preprint.pdf](http://domino.research.ibm.com/Comm/bios.nsf/pages/millipede.html/$FILE/pv7201-preprint.pdf); also published in: Vettiger P, Cross G, Despont M, *et al.* The Millipede – nanotechnology entering data storage. *IEEE Trans. Nanotechnol.* 2002 Jun;1:39-55.

<sup>784</sup> Hong S, Mirkin CA. A nanoplotter with both parallel and serial writing capabilities. *Science* 2000 Jun 9;288:1808-11; <http://www.nanotechnology.northwestern.edu/press/Science%20.Vol288.9june2000.pdf>. Zhang M, Bullen D, Ryu KS, Liu C, Hong S, Chung S, Mirkin C. Passive and active probes for dip pen nanolithography. First IEEE Conference on Nanotechnology, 28-30 Oct 2001, Maui, HI;

Almost any desired 2D shape can be drawn with DPN using 10 tips in concert. A million-tip DPN array has been fabricated by the Micro Nano Technology Research Group at the University of Illinois,<sup>785</sup> and another microcantilever array manufactured by Protiveris Corp. has millions of interdigitated cantilevers on a single chip.<sup>786</sup> Alternatively, Martel's group at École Polytechnique Montreal has investigated using fleets of independently mobile wireless instrumented microrobot manipulators called NanoWalkers to collectively form a nanofactory system that might be used for positional manufacturing operations.<sup>787</sup> A decade ago, Zyvex Corp. (<http://www.zyvexlabs.com>) received a \$25 million, five-year, National Institute of Standards and Technology (NIST) contract to develop prototype microscale assemblers using microelectromechanical systems.<sup>788</sup>

At the end of a carefully focused development program, analogous work will lead to the design and fabrication of numerous production lines comprising a nanofactory, both for diamondoid mechanosynthesis and for component assembly operations. Ultimately, atomically precise macroscale products – including components of additional nanofactories – could be manufactured in desktop-size nanofactories efficiently designed for this purpose. The nanofactory system will

<http://mass.micro.uiuc.edu/publications/papers/64.pdf>. Bullen D, Zhang M, Liu C. Thermal-mechanical optimization of thermally actuated cantilever arrays. Smart Electronics, MEMS, and Nanotechnology Conference (4700), SPIE's 9th Annual International Symposium on Smart Structures and Materials, 17-21 Mar 2002, San Diego, CA; <http://mass.micro.uiuc.edu/publications/papers/70.pdf>. Zhang M, Bullen D, Chung SW, Hong S, Ryu KS, Fan Z, Mirkin CA, Liu C. A MEMS nanoplotter with high-density parallel dip-pen nanolithography probe arrays. Nanotechnology 2002 Apr;13:212-217; <http://mass.micro.uiuc.edu/publications/papers/72.pdf>. Wang X, Bullen D, Zou J, Ryu K, Liu C, Chung SW, Mirkin CA. Linear probe arrays for dip-pen nanolithography. Intl. Conf. on Micro & Nano Systems (ICMNS 2002), 11-14 Aug 2002, Kunming, China; <http://mass.micro.uiuc.edu/publications/papers/74.pdf>. Bullen D, Chung S, Wang X, Zou J, Liu C, Mirkin C. Development of parallel dip pen nanolithography probe arrays for high throughput nanolithography. (Invited) Symposium LL: Rapid Prototyping Technologies, Materials Research Society Fall Meeting, Boston, MA, Proceedings of the MRS, Vol. 758, 2-6 Dec 2002; <http://mass.micro.uiuc.edu/publications/papers/84.pdf>. Bullen D, Wang X, Zou J, Hong S, Chung S, Ryu K, Fan Z, Mirkin C, Liu C. Micromachined arrayed dip pen nanolithography probes for sub-100 nm direct chemistry patterning. Proc. 16th IEEE International Micro Electro Mechanical Systems Conference, MEMS 2003, Kyoto, Japan, 19-23 Jan 2003; <http://mass.micro.uiuc.edu/publications/papers/86.pdf>. Zou J, Bullen D, Wang X, Liu C, Mirkin CA. Conductivity-based contact sensing for probe arrays in dip-pen nanolithography. Appl Phys Lett. 2003;83:581.

<sup>785</sup> "MNTR Research Focus Slide Show: Passive Parallel DPN Array," 2006; <http://mass.micro.uiuc.edu/research/current/nanolithography/2006-focus-intro/slide11.html>

<sup>786</sup> "Microcantilever Arrays," Protiveris Corp., 2003; [http://www.protiveris.com/cantilever\\_tech/microcantileverarrays.html](http://www.protiveris.com/cantilever_tech/microcantileverarrays.html)

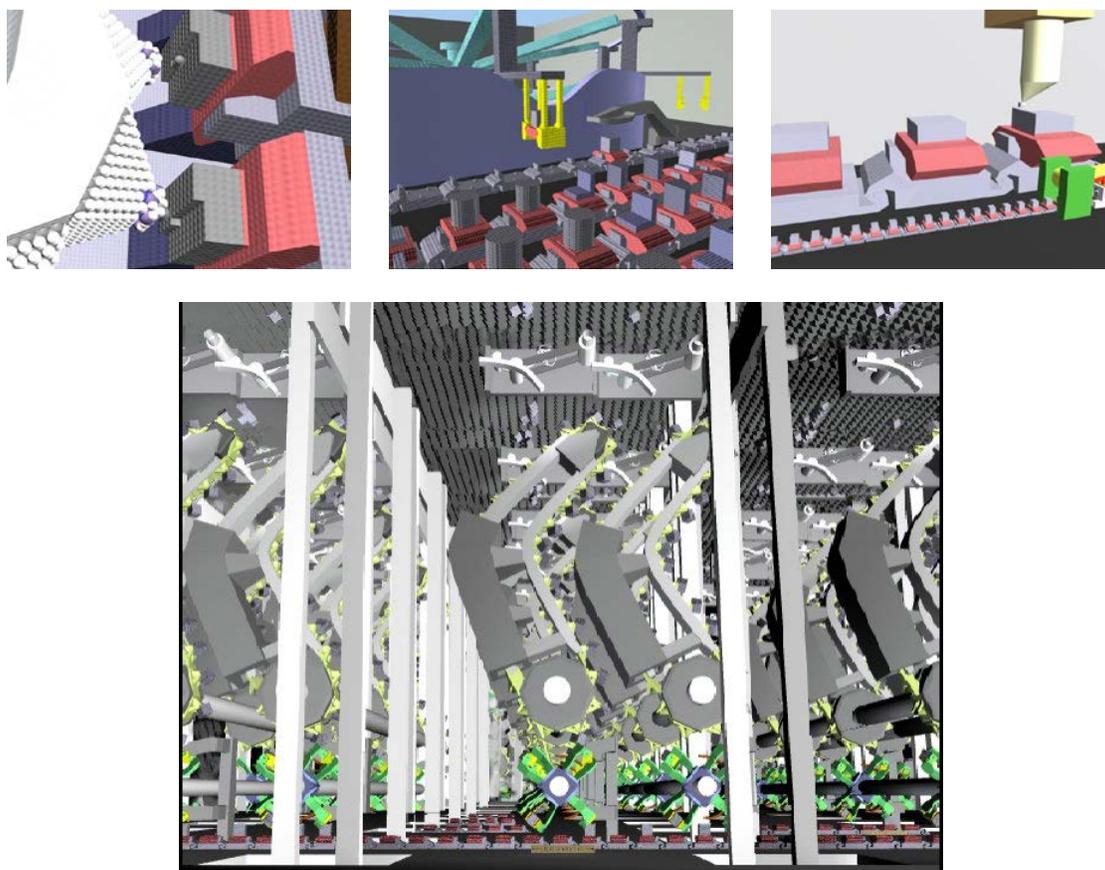
<sup>787</sup> Martel S, Hunter I. Nanofactories based on a fleet of scientific instruments configured as miniature autonomous robots. Proc. 3rd Intl Workshop on Microfactories; 16-18 Sep 2002; Minneapolis MN, pp. 97-100.

<sup>788</sup> Freitas RA Jr., Merkle RC. Kinematic Self-Replicating Machines. Landes Bioscience, Georgetown, TX, 2004, Section 4.20 "Zyvex Microscale Assemblers"; <http://www.MolecularAssembler.com/KSRM/4.20.htm>

include a progression of fabrication and assembly lines at several different physical scales, as conceptually illustrated in **Figure 12**, below.

In one conceivable design, at the smallest scale molecular mills could manipulate individual molecules to fabricate successively larger submicron-scale building blocks. These would be passed to larger block assemblers that assemble still larger microblocks, which would themselves be passed to even larger product assemblers that put together the final product. The microblocks could be placed in a specific pattern and sequence following construction blueprints created using modern “Design for Assembly” and “Design for Manufacturability” (DFM) philosophies. As plane after plane is completed, the product slowly extrudes outward through the surface of the nanofactory output platform.

*Figure 12. Assembly of nanoparts into larger components and product structures using mechanical manipulators at various size scales (e.g., perhaps  $0.01\ \mu\text{m}$ ,  $0.1\ \mu\text{m}$ ,  $1\ \mu\text{m}$ , and  $10\ \mu\text{m}$  in the four images below) on interconnected production lines inside a diamondoid nanofactory.<sup>789</sup>*

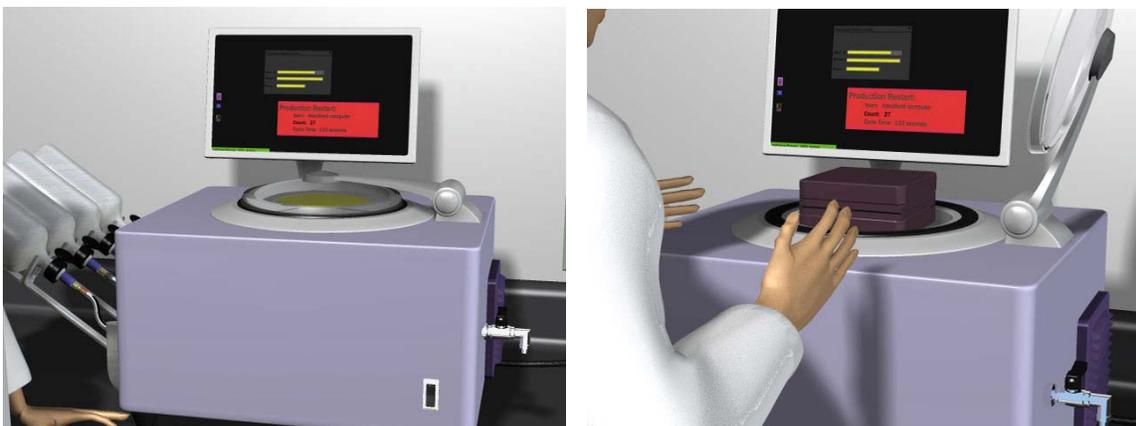


<sup>789</sup> John Burch nanofactory website; [http://www.lizardfire.com/html\\_nano/nano.html](http://www.lizardfire.com/html_nano/nano.html)

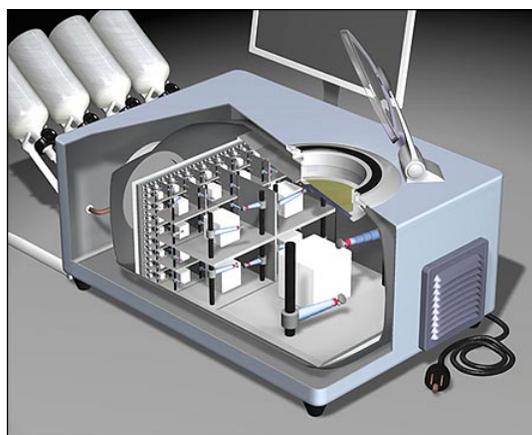
Of course, these images represent idealized conceptualizations of just one possible nanofactory architecture. Many other architectural approaches are readily conceived.<sup>790</sup>

As shown in the conceptual image of the desktop nanofactory below (**Figure 13**), the finished product in this example is a billion-CPU laptop supercomputer,<sup>791</sup> built to molecular precision all the way down to its constituent atoms. The laptop supercomputer product is emerging from the output port at the top of the nanofactory at the end of a production cycle.

*Figure 13. Conceptual vision of a desktop nanofactory appliance.*<sup>792</sup>



Rather than a laptop supercomputer, the nanofactory can be used to build medical nanorobots of modular design. The nanofactory for nanorobots would likely be a specialized type of limited-use nanofactory optimized for the fabrication and assembly of a small number of nanorobot modules that could be snapped together to make entire nanorobots at the targeted 1 kg/day initial production rate. The medical nanorobot factory might look something like the machine pictured in **Figure 13**, except that a sterile container of medical nanorobots might be emerging from the output platform at the top of the device instead of a folded laptop supercomputer.



<sup>790</sup> Robert A. Freitas Jr., Ralph C. Merkle, *Kinematic Self-Replicating Machines*, Landes Bioscience, Georgetown, TX, 2004; Section 4; <http://www.molecularassembler.com/KSRM/4.htm>.

<sup>791</sup> Merkle RC, Freitas RA Jr., Hogg T, Moore TE, Moses MS, Ryley J. Molecular mechanical computing systems. IMM Report No. 46, 29 March 2016; <http://www.imm.org/Reports/rep046.pdf>.

<sup>792</sup> John Burch nanofactory website; [http://www.lizardfire.com/html\\_nano/nano.html](http://www.lizardfire.com/html_nano/nano.html)

The end result of a dedicated nanofactory development program would be the creation of extremely clean, efficient, and inexpensive atomically precise manufacturing systems capable of producing macroscale quantities of atomically precise products. Nanofactories will make possible the manufacture of covalently-bonded products (e.g. nanomachines, including medical nanorobots) having the intricate complexity and reliability of biological systems combined with the greater speed, strength, power, and predictability of engineered mechanical systems.

## 4.2 Medical Nanorobots: Background

The early genesis of the concept of medical nanorobots, manufactured in nanofactories, sprang from the visionary idea that tiny nanomachines could be designed, manufactured, and introduced into the human body to perform cellular repairs at the molecular level. Although the medical application of nanotechnology was later championed in the popular writings of Drexler<sup>793</sup> in the 1980s and 1990s and in the technical writings of Freitas<sup>794</sup> in the 1990s and 2000s, the first scientist to voice the possibility was the late Nobel physicist Richard P. Feynman, who worked on the Manhattan Project at Los Alamos during World War II and later taught at Caltech for most of his professorial career.

In his prescient 1959 talk “There’s Plenty of Room at the Bottom,” Feynman proposed employing machine tools to make smaller machine tools, these to be used in turn to make still smaller machine tools, and so on all the way down to the atomic level.<sup>795</sup> He prophetically concluded that this is “a development which I think cannot be avoided.” After discussing his ideas with a colleague, Feynman offered the first known proposal for a medical nanorobotic procedure of any kind – in this instance, to cure heart disease: “A friend of mine (Albert R. Hibbs) suggests a very interesting possibility for relatively small machines. He says that, although it is a very wild idea, it would be interesting in surgery if you could swallow the surgeon. You put the mechanical surgeon inside the blood vessel and it goes into the heart and looks around. (Of course the information has to be fed out.) It finds out which valve is the faulty one and takes a little knife and slices it out. Other small machines might be permanently incorporated in the body to assist some inadequately functioning organ.” Later in his historic 1959 lecture, Feynman urges us to consider the possibility, in connection with microscopic biological cells, “that we can manufacture an object that maneuvers at that level!” The field had progressed far enough by 2007, half a century after Feynman’s speculations, to allow Martin Moskovits, Professor of Chemistry and Dean of Physical Science at UC Santa Barbara, to write<sup>796</sup> that “the notion of an ultra-small robot that can, for example, navigate the bloodstream performing microsurgery or

---

<sup>793</sup> Drexler KE. *Engines of Creation: The Coming Era of Nanotechnology*. Anchor Press/Doubleday, New York, 1986, Chapter 7 “Engines of Healing”; [http://e-drexler.com/d/06/00/EOC/EOC\\_Chapter\\_7.html](http://e-drexler.com/d/06/00/EOC/EOC_Chapter_7.html). Drexler KE, Peterson C, Pergamit G. *Unbounding the Future: The Nanotechnology Revolution*. William Morrow/Quill Books, New York, 1991, Chapter 10 “Nanomedicine”; [http://www.foresight.org/UTF/Unbound\\_LBW/chapt\\_10.html](http://www.foresight.org/UTF/Unbound_LBW/chapt_10.html).

<sup>794</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999; <http://www.nanomedicine.com/NMI.htm>. Freitas RA Jr. *Nanomedicine, Volume IIA: Biocompatibility*. Landes Bioscience, Georgetown, TX, 2003; <http://www.nanomedicine.com/NMIIA.htm>. Freitas RA Jr. “Technical Analyses of Types of Diamondoid Medical Nanorobots,” <http://nanomedicine.com/#NanorobotAnalyses>.

<sup>795</sup> Feynman RP. There’s plenty of room at the bottom. *Eng Sci (CalTech)* 1960;23:22-36; <http://www.zyvex.com/nanotech/feynman.html>.

<sup>796</sup> Moskovits M. Nanoassemblers: A likely threat? *Nanotech. Law & Bus.* 2007;4:187-195.

activating neurons so as to restore muscular activity, is not an unreasonable goal, and one that may be realized in the near future.”

Many questions arise when one first encounters the idea of micron-scale nanorobots, constructed of nanoscale components and operating inside the human body. At the most fundamental level, technical questions about the influence of quantum effects on molecular structures, friction and wear among nanomechanical components, radiation damage, other failure mechanisms, the influence of thermal noise on reliability, and the effects of Brownian bombardment on nanomachines have all been extensively discussed and resolved in the literature.<sup>797</sup> Self-assembled molecular motors consisting of just 50-100 atoms have been demonstrated experimentally since in the late 1990s.<sup>798</sup> Published discussions of technical issues of specific relevance to medical nanorobots include proposed methods for recognizing, sorting and pumping individual molecules,<sup>799</sup> theoretical designs for mechanical nanorobot sensors,<sup>800</sup> flexible hull surfaces,<sup>801</sup> power sources,<sup>802</sup> communications systems,<sup>803</sup> navigation systems,<sup>804</sup> manipulator mechanisms,<sup>805</sup> mobility mechanisms for travel through bloodstream, tissues and cells,<sup>806</sup>

---

<sup>797</sup> Drexler KE. *Nanosystems: Molecular Machinery, Manufacturing, and Computation*. John Wiley & Sons, New York, 1992. Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999; <http://www.nanomedicine.com/NMI/2.1.htm>.

<sup>798</sup> Kelly TR, De Silva H, Silva RA. Unidirectional rotary motion in a molecular system. *Nature*. 1999;401:150-152. Koumura N, Zijlstra RW, van Delden RA, *et al.* Light-driven monodirectional molecular rotor. *Nature* 1999;401:152-155. Huang TJ, Lu W, Tseng HR, *et al.* Molecular shuttle switching in closely packed Langmuir films, 11th Foresight Conf. Mol. Nanotech., San Francisco CA, 10-12 Oct 2003. Leigh DA, Wong JKY, Dehez F, *et al.* Unidirectional rotation in a mechanically interlocked molecular rotor. *Nature*. 2003;424:174-179. Browne WR, Feringa BL. Making molecular machines work. *Nat Nanotechnol*. 2006;1:25-35. Kay ER, Leigh DA, Zerbetto F. Synthetic molecular motors and mechanical machines. *Angew Chem Int Ed* 2007;46:72-191.

<sup>799</sup> Drexler KE. *Nanosystems: Molecular Machinery, Manufacturing, and Computation*. John Wiley & Sons, New York, 1992, Section 13.2. Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Chapter 3; <http://www.nanomedicine.com/NMI/3.1.htm>.

<sup>800</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Chapter 4; <http://www.nanomedicine.com/NMI/4.1.htm>.

<sup>801</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Chapter 5; <http://www.nanomedicine.com/NMI/5.1.htm>.

<sup>802</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Chapter 6; <http://www.nanomedicine.com/NMI/6.1.htm>.

<sup>803</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Chapter 7; <http://www.nanomedicine.com/NMI/7.1.htm>.

<sup>804</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Chapter 8; <http://www.nanomedicine.com/NMI/8.1.htm>.

<sup>805</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 9.3; <http://www.nanomedicine.com/NMI/9.3.htm>.

penetration of the blood-brain barrier (BBB) (see Section 4.3.1),<sup>807</sup> onboard clocks,<sup>808</sup> and nanocomputers,<sup>809</sup> along with the full panoply of nanorobot biocompatibility issues<sup>810</sup> (see Section 4.2.4).

The idea of placing semi-autonomous self-powered nanorobots inside of us might seem a bit odd, but the human body already teems with similar natural nanodevices. More than 40 trillion single-celled microbes swim through our colon, outnumbering our tissue cells almost ten to one.<sup>811</sup> Many bacteria move by whipping around a tiny tail, or flagellum, that is driven by a 30-nanometer biological ionic nanomotor powered by pH differences between the inside and the outside of the bacterial cell. Our bodies also maintain a population of more than a trillion motile biological nanodevices called fibroblasts and white cells such as neutrophils and lymphocytes, each measuring ~10 microns in size.<sup>812</sup> These beneficial natural nanorobots are constantly crawling around inside us, repairing damaged tissues, attacking invading microbes, and gathering up foreign particles and transporting them to various organs for disposal from the body.<sup>813</sup>

The greatest power of nanomedicine will begin to emerge as we learn to design and construct complete artificial nanorobots using nanometer-scale parts and subsystems such as diamondoid bearings and gears, nanomotors and molecular pumps, nanomanipulators, nanosensors, nanobatteries, and nanocomputers.

In the subsections below, we briefly describe three major classes of medical nanorobots – respirocytes (Section 4.2.1), microbivores (Section 4.2.2), and chromalloytes (Section 4.2.3) –

<sup>806</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 9.4; <http://www.nanomedicine.com/NMI/9.4.htm>.

<sup>807</sup> Freitas RA Jr. *Nanomedicine, Volume IIA: Biocompatibility*. Landes Bioscience, Georgetown, TX, 2003, Section 15.3.6.5; <http://www.nanomedicine.com/NMIIA/15.3.6.5.htm#p8>. See also: <http://nanotechweb.org/cws/article/tech/60813>, etc.

<sup>808</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 10.1; <http://www.nanomedicine.com/NMI/10.1.htm>.

<sup>809</sup> Drexler KE. *Nanosystems: Molecular Machinery, Manufacturing, and Computation*. John Wiley & Sons, New York, 1992, Chapter 12. Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 10.2; <http://www.nanomedicine.com/NMI/10.2.htm>.

<sup>810</sup> Freitas RA Jr. *Nanomedicine, Volume IIA: Biocompatibility*. Landes Bioscience, Georgetown, TX, 2003; <http://www.nanomedicine.com/NMIIA.htm>.

<sup>811</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 8.5.1; <http://www.nanomedicine.com/NMI/8.5.1.htm>.

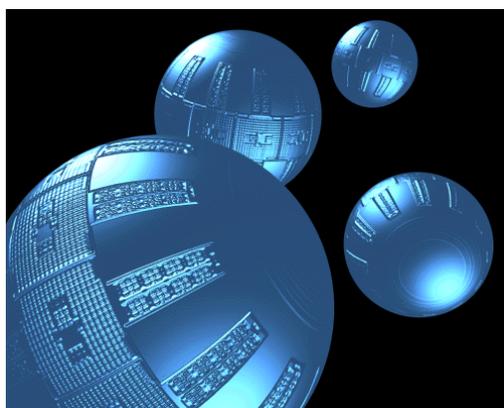
<sup>812</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 8.5.1; <http://www.nanomedicine.com/NMI/8.5.1.htm>.

<sup>813</sup> Freitas RA Jr. *Nanomedicine, Volume IIA: Biocompatibility*. Landes Bioscience, Georgetown, TX, 2003, Section 15.4.3.1; <http://www.nanomedicine.com/NMIIA/15.4.3.1.htm>.

then review major issues in nanorobot biocompatibility (Section 4.2.4) and enumerate some of the major advantages of nanorobots over other medical approaches and instrumentalities (Section 4.2.5). We close with a description of cell mills (Section 4.2.6) – a nanorobotically-enabled specialized nanofactory that can quickly and efficiently manufacture living biological cells as microscopic “replacement parts” for use in various medical therapeutic procedures.

## 4.2.1 Respirocyte-Class Nanorobots

The first theoretical design study of a medical nanorobot ever published in a peer-reviewed medical journal (by Freitas in 1998)<sup>814</sup> described an artificial mechanical red blood cell or “respirocyte” to be made of 18 billion precisely arranged atoms. The device was to be a bloodborne spherical 1-micron diamondoid 1000-atmosphere pressure vessel<sup>815</sup> with active pumping<sup>816</sup> powered by the oxidation of endogenous serum glucose,<sup>817</sup> able to deliver 236 times



more oxygen to the tissues per unit volume than natural red cells and to manage acidity caused by carbonic acid formation, all controlled by gas concentration sensors<sup>818</sup> and an onboard nanocomputer.<sup>819</sup> The basic operation of respirocytes (image, left; artwork by Forrest Bishop) is straightforward. These nanorobots would mimic the action of the natural hemoglobin(Hb)-filled red blood cells, while operating at 1000 atm vs. only 0.1-0.5 atm equivalent for natural Hb. In the tissues, oxygen will be pumped out of the device by the **molecular sorting rotors** (**Appendix C**) on one side. Carbon dioxide will be pumped into the

device by molecular sorting rotors on the other side, one molecule at a time. Half a minute later, when the respirocyte reaches the patient’s lungs in the normal course of the circulation of the blood, these same rotors reverse their direction of rotation, recharging the device with fresh oxygen and dumping the stored CO<sub>2</sub>, which diffuses into the lungs and can then be exhaled by the patient. Each rotor requires very little power, only ~0.03 pW to pump ~10<sup>6</sup> molecules/sec in continuous operation.

---

<sup>814</sup> Freitas RA Jr. Exploratory design in medical nanotechnology: A mechanical artificial red cell. *Artif Cells Blood Subst Immobil Biotech* 1998;26:411-430; <http://www.foresight.org/Nanomedicine/Respirocytes.html>.

<sup>815</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 10.3; <http://www.nanomedicine.com/NMI/10.3.htm>.

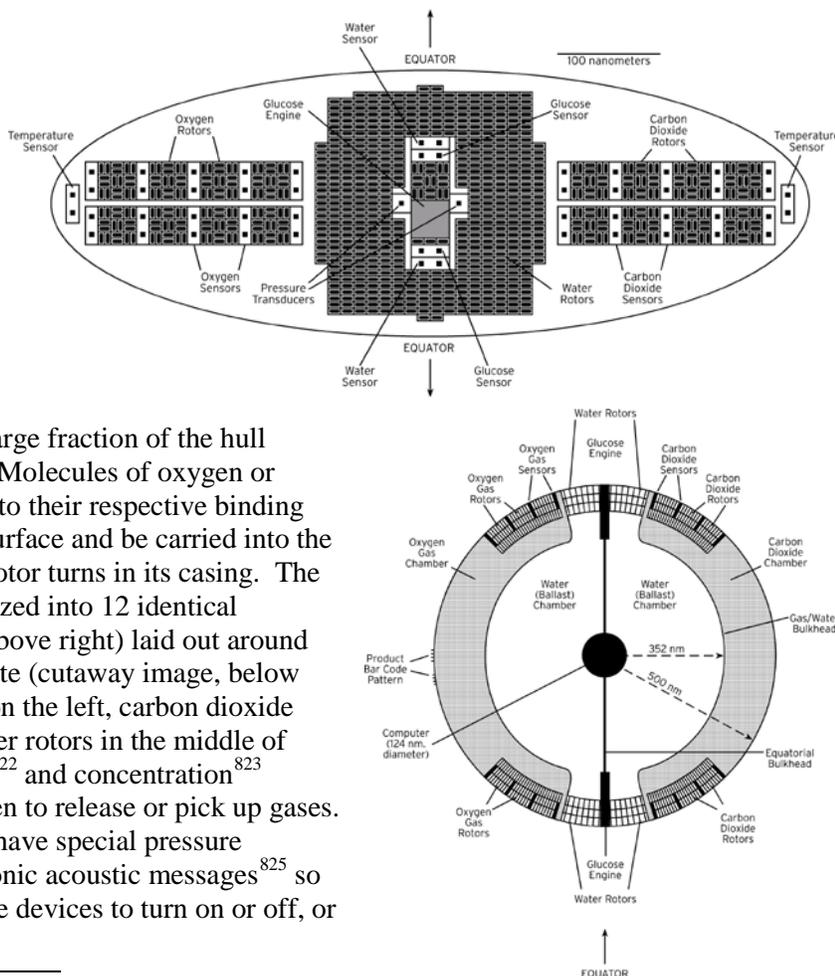
<sup>816</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 3.4.2; <http://www.nanomedicine.com/NMI/3.4.2.htm>.

<sup>817</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 6.3.4; <http://www.nanomedicine.com/NMI/6.3.4.htm>.

<sup>818</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 4.2.1; <http://www.nanomedicine.com/NMI/4.2.1.htm>.

<sup>819</sup> Drexler KE. *Nanosystems: Molecular Machinery, Manufacturing, and Computation*. John Wiley & Sons, New York, 1992, Chapter 12. Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 10.2; <http://www.nanomedicine.com/NMI/10.2.htm>.

In the exemplar respirocyte design,<sup>820</sup> onboard pressure tanks can hold up to 3 billion oxygen (O<sub>2</sub>) and carbon dioxide (CO<sub>2</sub>) molecules. Molecular sorting rotors<sup>821</sup> are arranged on the surface to load and unload gases from the pressurized tanks. Tens of thousands of these individual pumps cover a large fraction of the hull surface of the respirocyte. Molecules of oxygen or carbon dioxide may drift into their respective binding sites on the exterior rotor surface and be carried into the respirocyte interior as the rotor turns in its casing. The sorting rotor array is organized into 12 identical pumping stations (image, above right) laid out around the equator of the respirocyte (cutaway image, below right), with oxygen rotors on the left, carbon dioxide rotors on the right, and water rotors in the middle of each station. Temperature<sup>822</sup> and concentration<sup>823</sup> sensors tell the devices when to release or pick up gases. Each pumping station will have special pressure sensors<sup>824</sup> to receive ultrasonic acoustic messages<sup>825</sup> so the physician can (a) tell the devices to turn on or off, or



<sup>820</sup> Freitas RA Jr. Exploratory design in medical nanotechnology: A mechanical artificial red cell. *Artif Cells Blood Subst Immobil Biotech* 1998;26:411-430; <http://www.foresight.org/Nanomedicine/Respirocytes.html>.

<sup>821</sup> Drexler KE. *Nanosystems: Molecular Machinery, Manufacturing, and Computation*. John Wiley & Sons, New York, 1992, Section 13.2. Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 3.4.2; <http://www.nanomedicine.com/NMI/3.4.2.htm>. See also Appendix C.

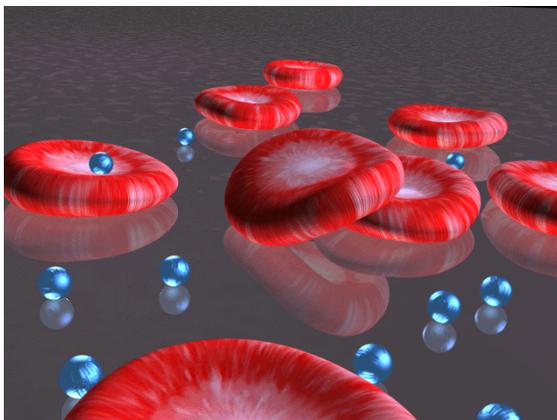
<sup>822</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 4.6; <http://www.nanomedicine.com/NMI/4.6.htm>.

<sup>823</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 4.2; <http://www.nanomedicine.com/NMI/4.2.htm>.

<sup>824</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 4.5; <http://www.nanomedicine.com/NMI/4.5.htm>.

<sup>825</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 7.2.2; <http://www.nanomedicine.com/NMI/7.2.2.htm>.

(b) change the operating parameters of the devices, while the nanorobots are inside a patient. The onboard nanocomputer enables complex device behaviors also remotely reprogrammable by the physician via externally applied ultrasound acoustic signals. Internal power will be transmitted mechanically or hydraulically using an appropriate working fluid, and can be distributed as required using rods and gear trains<sup>826</sup> or using pipes and mechanically operated valves, controlled by the nanocomputer. There is also a large internal void surrounding the nanocomputer which can be a vacuum, or can be filled with (or emptied of) water. This will allow the device to control its buoyancy very precisely and provides a crude but simple method for removing respirocytes from the body using a blood centrifuge, a future procedure now termed “nanapheresis”.<sup>827</sup>



A 5 cc therapeutic dose of 50% respirocyte saline suspension containing 5 trillion nanorobots would exactly replace the gas carrying capacity of the patient’s entire 5.4 liters of blood. If up to 1 liter of respirocyte suspension could safely be added to the human bloodstream,<sup>828</sup> this would keep a patient’s tissues safely oxygenated for up to 4 hours even if a heart attack caused the heart to stop beating, or if there was a complete absence of respiration or no external availability of oxygen. Primary medical applications of respirocytes might include emergency revival

of victims of carbon monoxide suffocation at the scene of a fire, rescue of drowning victims, and transfusable pre-oxygenated blood substitution. Respirocytes (image, left; artwork by Forrest Bishop) could serve as “instant blood” at an accident scene with no need for blood typing, and, thanks to the dramatically higher gas-transport efficiency of respirocytes over natural red cells, a mere 1 cm<sup>3</sup> infusion of the devices would provide the oxygen-carrying ability of a full liter of ordinary blood.

Larger doses of respirocytes could also: (1) be used as a temporary treatment for anemia and various lung and perinatal/neonatal disorders, (2) enhance tumor therapies and diagnostics and improve outcomes for cardiovascular, neurovascular, or other surgical procedures, (3) help prevent asphyxia and permit artificial breathing (e.g., underwater, high altitude, etc.), and (4) have many additional applications in sports, veterinary medicine, military science, and space exploration.

---

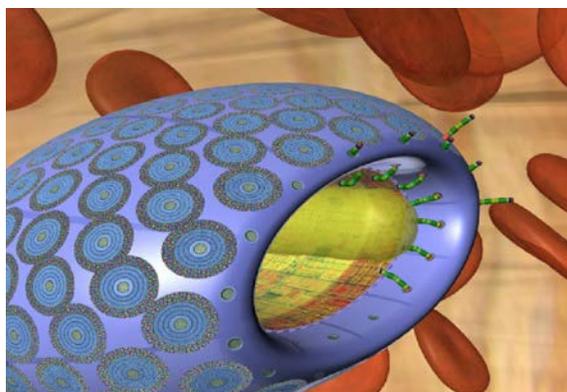
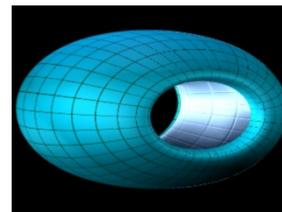
<sup>826</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 6.4.3.4; <http://www.nanomedicine.com/NMI/6.4.3.4.htm>.

<sup>827</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 10.3.6; <http://www.nanomedicine.com/NMI/10.3.6.htm>.

<sup>828</sup> Freitas RA Jr. Nanomedicine Vol. IIA: Biocompatibility. Landes Bioscience, Georgetown, TX, 2003, Section 15.6.2; <http://www.nanomedicine.com/NMI/15.6.2.htm>.

## 4.2.2 Microbivore-Class Nanorobots

Perhaps the most widely recognized form of disease is an attack on the human body by invading viruses, bacteria, protozoa, or other microscopic parasites. One general class of medical nanorobot can serve as the first-line nanomedical treatment for pathogen-related disease. Called a “microbivore”, this artificial nanorobotic white cell substitute, made of diamond and sapphire, would seek out and harmlessly digest unwanted bloodborne pathogens.<sup>829</sup> One main task of natural white cells is to phagocytose and kill microbial invaders in the bloodstream. Microbivore nanorobots would also perform the equivalent of phagocytosis and microbial killing, but would operate much faster, more reliably, and under human control.



The baseline microbivore (image, left; hull design, above; all microbivore artwork by Forrest Bishop) is designed as an oblate spheroidal nanomedical device measuring 3.4 microns in diameter along its major axis and 2.0 microns in diameter along its minor axis, consisting of 610 billion precisely arranged structural atoms in a gross geometric volume of 12.1 micron<sup>3</sup> and a dry mass of 12.2 picograms. This size helps to ensure that the nanorobot can safely pass through even the narrowest of human capillaries and other tight

spots in the spleen (e.g., the interendothelial splenofenestral slits)<sup>830</sup> and elsewhere in the human body.<sup>831</sup> The microbivore has a mouth with an iris-like door, called the ingestion port, where microbes are fed in to be digested. This port is large enough to internalize a single microbe from virtually any major bacteremic species in a single gulp. The microbivore also has a rear end, or exhaust port, where the completely digested remains of the pathogen are harmlessly expelled from the device. The rear door opens between the main body of the microbivore and a tail-cone structure. According to the scaling study by Freitas,<sup>832</sup> the device may consume up to 200 pW of continuous power (using bloodstream glucose and oxygen for energy) while completely digesting

<sup>829</sup> Freitas RA Jr. Microbivores: Artificial mechanical phagocytes using digest and discharge protocol. *J Evol Technol* 2005;14:1-52; <http://www.jetpress.org/volume14/freitas.html>.

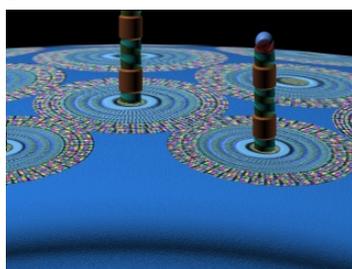
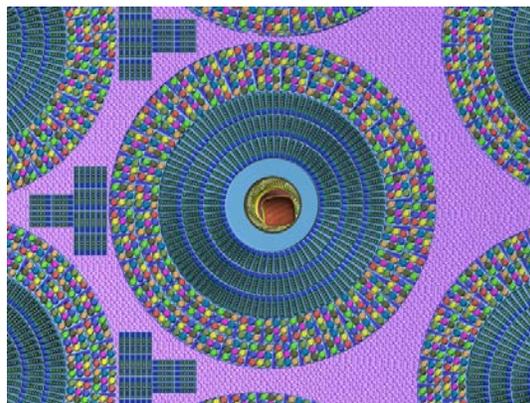
<sup>830</sup> Freitas RA Jr. *Nanomedicine Vol. IIA: Biocompatibility*. Landes Bioscience, Georgetown, TX, 2003, Section 15.4.2.3; <http://www.nanomedicine.com/NMI/15.4.2.3.htm>.

<sup>831</sup> Freitas RA Jr. *Nanomedicine Vol. IIA: Biocompatibility*. Landes Bioscience, Georgetown, TX, 2003, Section 15.4.2; <http://www.nanomedicine.com/NMI/15.4.2.htm>.

<sup>832</sup> Freitas RA Jr. Microbivores: Artificial mechanical phagocytes using digest and discharge protocol. *J Evol Technol* 2005;14:1-52; <http://www.jetpress.org/volume14/freitas.html>.

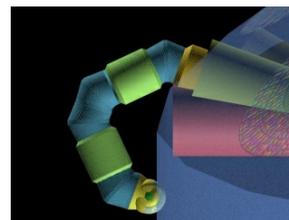
trapped microbes at a maximum throughput of 2 micron<sup>3</sup> of organic material per 30-second cycle. This “digest and discharge” protocol<sup>833</sup> is conceptually similar to the internalization and digestion process practiced by natural phagocytes, except that the artificial process should be a hundred-fold faster and also cleaner. For example, it is well-known that macrophages release biologically active compounds during bacteriophagy,<sup>834</sup> whereas well-designed microbivores need only release biologically inactive effluent.

The first task for the bloodborne microbivore is to reliably acquire a pathogen to be digested. If the correct bacterium bumps into the nanorobot surface, reversible species-specific binding sites on the microbivore hull can recognize and weakly bind to the bacterium. A set of 9 distinct antigenic markers should be specific enough,<sup>835</sup> since all 9 must register a positive binding event to confirm that a targeted microbe has been caught. There are 20,000 copies of these 9-marker receptor sets, distributed in 275 disk-shaped regions across the microbivore surface (image, right). Inside each receptor ring are more rotors to absorb ambient glucose and oxygen from the bloodstream to provide nanorobot power.



At the center of each 150-nm diameter receptor disk is a grapple silo (image, left). Once a bacterium has been captured by the reversible receptors, telescoping robotic grapples<sup>836</sup> rise up out of the microbivore surface and attach to the trapped bacterium, establishing secure anchorage to the microbe's cell wall, capsid, or plasma membrane. The microbivore grapple arms are about 100 nanometers long and have various rotating and telescoping joints that allow them to change their

position, angle, and length. After rising out of its silo, a grapple arm could execute complex twisting motions (image, right), and adjacent grapple arms can physically reach each other, allowing them to hand off bound objects as small as a virus particle. Grapple handoff



<sup>833</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 10.4.2.4.2; <http://www.nanomedicine.com/NMI/10.4.2.4.2.htm>.

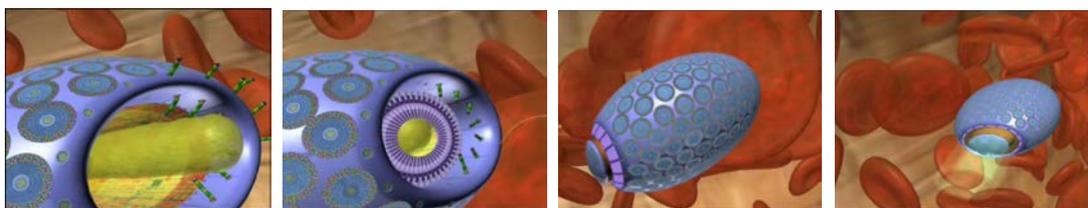
<sup>834</sup> Fincher EF 4th, Johannsen L, Kapás L, Takahashi S, Krueger JM. Microglia digest *Staphylococcus aureus* into low molecular weight biologically active compounds. Am J Physiol. 1996 Jul;271(1 Pt 2):R149-56; <http://www.ncbi.nlm.nih.gov/pubmed/8760216>.

<sup>835</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 8.5.2.2; <http://www.nanomedicine.com/NMI/8.5.2.2.htm>.

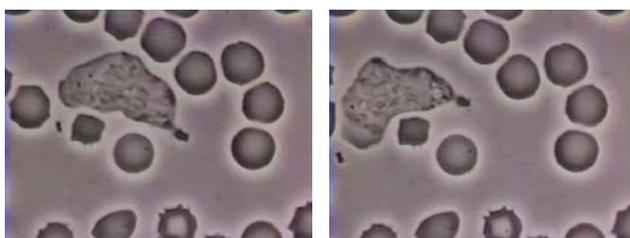
<sup>836</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 9.3.1.4; <http://www.nanomedicine.com/NMI/9.3.1.4.htm>.

motions could transport a large rod-shaped bacterium from its original capture site forward to the ingestion port at the front of the device. The captive organism would be rotated into the proper orientation as it approaches the open microbivore mouth. There the pathogen is internalized into a 2 micron<sup>3</sup> morcellation chamber under continuous control of mouth grapples and an internal mooring mechanism.

There are two concentric cylinders inside the microbivore. The bacterium will be minced into nanoscale pieces in the morcellation chamber (the smaller inner cylinder),<sup>837</sup> then the remains are pistoned into a separate 2 micron<sup>3</sup> digestion chamber (the larger outer cylinder). In a preprogrammed sequence, ~40 different engineered digestive enzymes will be successively injected and extracted six times during a single digestion cycle, progressively reducing the morcellate to monoresidue amino acids, mononucleotides, glycerol, free fatty acids and simple sugars, using an appropriate array of molecular sorting rotors. These basic molecules are then harmlessly discharged back into the bloodstream through the exhaust port at the rear of the device, completing the 30-second digestion cycle (images, below; artwork by Forrest Bishop). When treatment is finished, the doctor may transmit an ultrasound signal to tell the circulating microbivores that their work is done. The nanorobots may then exit the body through the kidneys and be excreted with the urine in due course.



A human neutrophil, the most common type of leukocyte or white cell, can capture and engulf a microbe in a minute or less (images, below), but complete digestion and excretion of the organism's remains can take an hour or longer. Our natural white cells – even when aided by antibiotics – can sometimes take weeks or months to completely clear bacteria from the bloodstream. By comparison, a single terabot (10<sup>12</sup>-nanorobot) dose of microbivores should be able to fully eliminate bloodborne pathogens in just minutes, or hours in the case of locally dense



infections. This is accomplished without increasing the risk of sepsis or septic shock because all bacterial components (including all cell-wall lipopolysaccharide) will be internalized and fully digested into harmless non-antigenic molecules prior to discharge from the microbivore device.

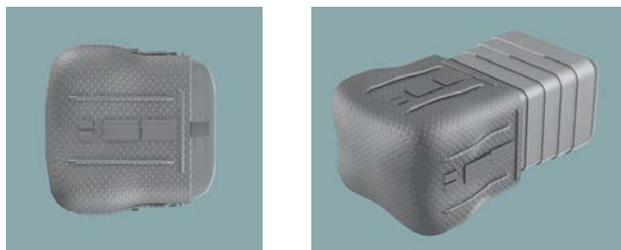
And no matter that a bacterium has acquired multiple drug resistance to antibiotics or to any other traditional treatment – the microbivore will eat it anyway. Microbivores would be up to ~1000 times faster-acting than antibiotic-based cures which often need weeks or months to work. The nanorobots would digest ~100 times more microbial material than an equal volume of natural

<sup>837</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 9.3.5.1; <http://www.nanomedicine.com/NMI/9.3.5.1.htm>.

white cells could digest in any given time period, and would have far greater maximum lifetime capacity for phagocytosis than natural white blood cells.

### 4.2.3 Chromalocyte-Class Nanorobots

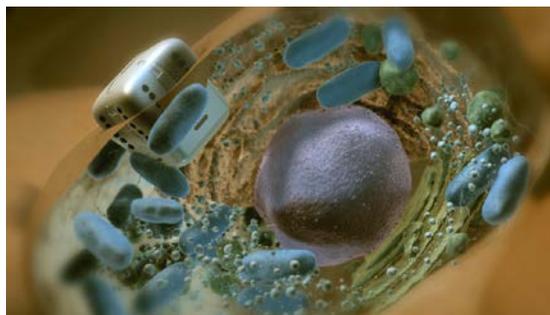
The chromalocyte<sup>838</sup> is a hypothetical mobile cell-repair nanorobot whose primary purpose is to perform chromosome replacement therapy (CRT). In CRT, the entire chromatin content of the nucleus in a living cell is extracted and promptly replaced with a new set of prefabricated chromosomes that have been artificially manufactured as defect-free copies of the originals.



The chromalocyte (images, left; artwork by Stimulacra) will be capable of limited vascular surface travel into the capillary bed of the targeted tissue or organ, followed by diapedesis (exiting a blood vessel into the tissues),<sup>839</sup> histonotation (locomotion through tissues),<sup>840</sup> cytopenetration (entry into the cell

interior; see images, below, by E-spaces),<sup>841</sup> and complete chromatin replacement in the nucleus of the target cell. The CRT mission ends with a return to the vasculature and subsequent extraction of the nanodevices from the body at the original infusion site.

This ~3 hour chromosome replacement process is expected to involve a 26-step sequence of distinct semi-autonomous sensor-driven activities which are described at length in a comprehensive published technical paper on the subject by Freitas<sup>842</sup> and in summary below, including: (1) injection, (2) extravasation, (3) ECM immigration, (4) cytopenetration (image, right), (5) inhibition of



<sup>838</sup> Freitas RA Jr. The ideal gene delivery vector: Chromalocytes, cell repair nanorobots for chromosome replacement therapy. *J Evol Technol* 2007;16:1-97; <http://jetpress.org/v16/freitas.pdf>.

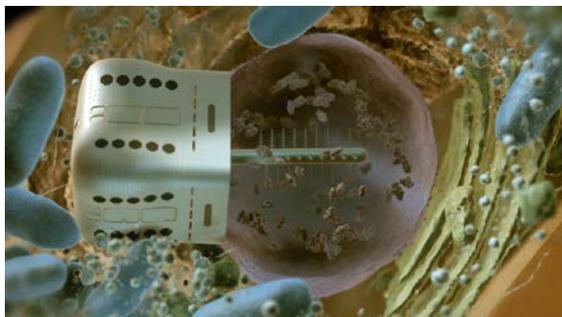
<sup>839</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 9.4.4.1; <http://www.nanomedicine.com/NMI/9.4.4.1.htm>.

<sup>840</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 9.4.4; <http://www.nanomedicine.com/NMI/9.4.4.htm>.

<sup>841</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 9.4.5; <http://www.nanomedicine.com/NMI/9.4.5.htm>.

<sup>842</sup> Freitas RA Jr. The ideal gene delivery vector: Chromalocytes, cell repair nanorobots for chromosome replacement therapy. *J Evol Technol* 2007;16:1-97; <http://jetpress.org/v16/freitas.pdf>.

mechanotransduction (to avoid nanorobot mechanical actions triggering unwanted cell responses), (6) nuclear localization, (7) nucleopenetration, (8) blockade of apoptosis (to prevent misinterpretation of CRT processes as damage demanding cell suicide), (9) arrest of DNA repair (to prevent misinterpretation of CRT processes as damage demanding repair), (10) blockade of inflammatory signals, (11) deactivation of transcription, (12) detachment of chromatin from inner



nuclear wall lamins (cortex proteins), (13) extension of the “Proboscis” into the cell nucleus (image, left), (14) rotation of the Proboscis, (15) deployment of the chromosomal collection funnel, (16) digestion of stray chromatin, (17) dispensation of new chromatin, (18) decondensation of the new chromatin, (19) re-anchoring of the dispensed chromatin to inner nuclear wall lamins, (20) reactivation of transcription, (21) reactivation of DNA repair and other DNA-related maintenance and usage

processes, (22) nuclear emigration, (23) cellular emigration, (24) ECM emigration, (25) return to original point of entry into the body, and (26) removal from the body. Treatment of an entire large human organ such as a liver, involving simultaneous CRT on all 250 billion hepatic tissue cells, might require the localized infusion of a ~1 terabot ( $10^{12}$  devices) or  $\sim 69 \text{ cm}^3$  chromalloy dose in a 1-liter (7% v/v nanorobots) saline suspension during a ~7 hour course of therapy. This nanodevice population draws 100-200 watts which lies within estimated nanorobot thermogenic limits consistent with maintenance of constant human body temperature.<sup>843</sup>

Replacement chromosome sets would be manufactured<sup>844</sup> in a desktop *ex vivo* chromosome sequencing and manufacturing facility (image, right),<sup>845</sup> then loaded into the nanorobots for delivery to specific targeted cells during CRT. The new DNA is manufactured to incorporate proper methylation for the target cell type and other post-translational modifications constituting the “histone code” used by the cell to encode various chromatin conformations and gene expression states.<sup>846</sup>



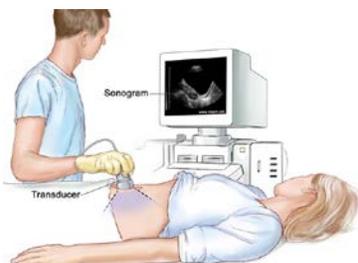
<sup>843</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 6.5.2; <http://www.nanomedicine.com/NMI/6.5.2.htm>. Freitas RA Jr. The ideal gene delivery vector: Chromalloyes, cell repair nanorobots for chromosome replacement therapy. J Evol Technol 2007;16:1-97, Section 3.6; <http://jetpress.org/v16/freitas.pdf>.

<sup>844</sup> Strickland E. DNA manufacturing enters the age of mass production. IEEE Spectrum, 23 Dec 2015; <http://spectrum.ieee.org/biomedical/devices/dna-manufacturing-enters-the-age-of-mass-production>.

<sup>845</sup> Freitas RA Jr. The ideal gene delivery vector: Chromalloyes, cell repair nanorobots for chromosome replacement therapy. J Evol Technol 2007;16:1-97, Section 4; <http://jetpress.org/v16/freitas.pdf>.

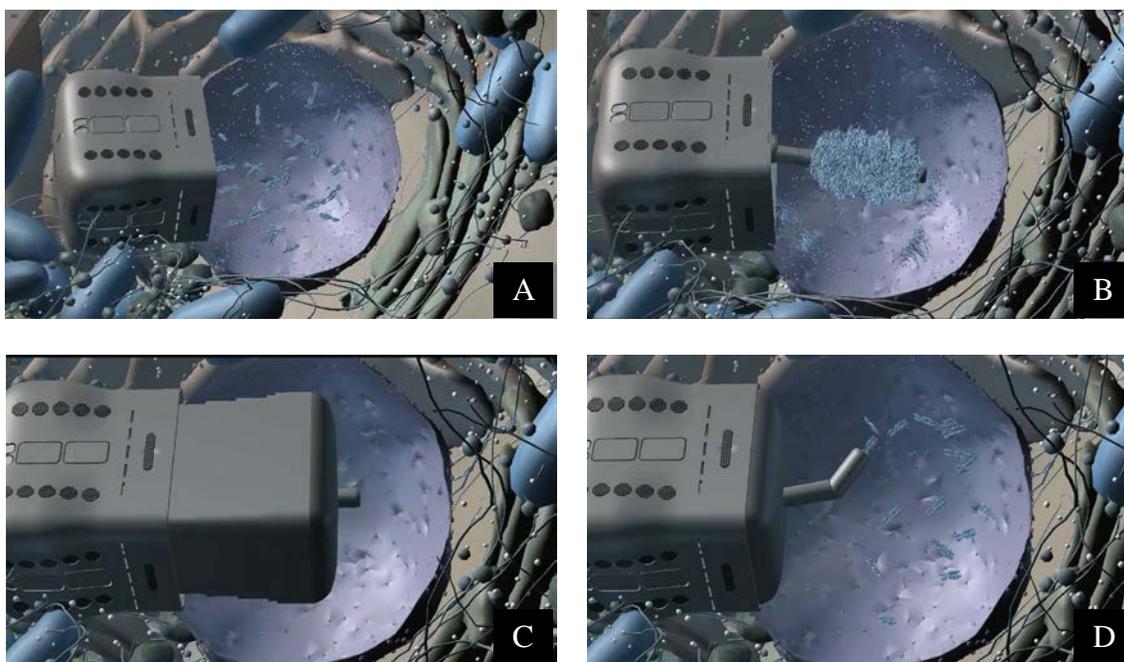
<sup>846</sup> Villar-Garea A, Imhof A. The analysis of histone modifications. Biochim Biophys Acta. 2006 Dec;1764(12):1932-9; <http://www.ncbi.nlm.nih.gov/pubmed/17015046>.

A single fully-loaded lozenge-shaped  $69 \text{ micron}^3$  chromalloyce can measure 4.18 microns and 3.28 microns along cross-sectional diameters and 5.05 microns in length, typically consuming 50-200 pW of power in normal operation and a maximum of 1000 pW in bursts during outmessaging, the most energy-intensive task. Onboard power can be provided acoustically from



the outside in an operating-table scenario (image, left) in which the patient is well-coupled to a medically-safe  $1000 \text{ W/m}^2$  0.5 MHz ultrasound transverse-plane-wave transmitter throughout the procedure. The American Institute of Ultrasound in Medicine (AIUM) deems 10,000-sec exposures to  $1000 \text{ W/m}^2$  ultrasound to be safe.<sup>847</sup>

The chromalloyce design includes an extensible primary manipulator 4 microns long and 0.55 microns in diameter called the Proboscis that is used to spool up chromatin strands via slow rotation when inserted into the cell nucleus (image sequence, below; images by E-spaces). After spooling (images A & B), a segmented funnel assembly is extended around the spooled bolus of DNA, fully enclosing and sequestering the old genetic material (image C). The new genetic material can then be discharged into the nucleus through the center of the Proboscis by pistoning from internal storage vaults (image D), while the old chromatin that is sequestered inside the sealed leakproof funnel assembly is forced into the storage vaults as space is vacated by the new chromatin that is simultaneously being pumped out.



<sup>847</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 6.4.1; <http://www.nanomedicine.com/NMI/6.4.1.htm>.

The chromalloyocyte can employ a mobility system similar to the microbivore grapple system, possibly including a solvation wave drive<sup>848</sup> to help ensure smooth its passage through cell plasma membrane and nuclear membrane.

---

<sup>848</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 9.4.5.3; <http://www.nanomedicine.com/NMI/9.4.5.3.htm>.

#### 4.2.4 Medical Nanorobot Biocompatibility

The safety, effectiveness, and utility of medical nanorobotic devices will critically depend upon their biocompatibility with human organs, tissues, cells, and biochemical systems. An entire technical book published by Freitas in 2003<sup>849</sup> describes the many biocompatibility issues surrounding the use of diamond-based nanorobots inside the human body, and broadens the definition of nanomedical biocompatibility to include all of the mechanical, physiological, immunological, cytological, and biochemical responses of the human body to the introduction of artificial medical nanodevices (**Table 1**).

Table 1. Issues in nanorobot biocompatibility	
Classical Nanorobot Biocompatibility Issues	New Nanorobot Biocompatibility Issues
<ul style="list-style-type: none"> <li>● Adhesive Interactions with Nanorobot Surfaces</li> <li>● Nanorobot Immunoreactivity</li> <li>● Complement Activation</li> <li>● Immunosuppression, Tolerization, and Camouflage</li> <li>● Immune Privilege and Immune Evasion</li> <li>● General and Nonspecific Inflammation</li> <li>● Coagulation and Thrombogenicity</li> <li>● Allergic and Other Sensitivity Reactions</li> <li>● Sternutogenesis, Nauseogenesis and Emetogenesis</li> <li>● Nanoid Shock</li> <li>● Nanopyrexia</li> <li>● Nanorobot Mutagenicity and Carcinogenicity</li> <li>● Protein Adsorption on Diamondoid Surfaces</li> <li>● Cell Response to Diamondoid Surfaces</li> <li>● Chemical Stability and Corrosion Degradation Effects</li> <li>● Nanorobot Hemolysis, Thrombocytolysis, and Leukocytolysis</li> </ul>	<ul style="list-style-type: none"> <li>● Geometrical Trapping of Bloodborne Medical Nanorobots</li> <li>● Phagocytosis of Bloodborne Microparticles</li> <li>● Particle Clearance from Tissues or Lymphatics</li> <li>● Phagocyte Avoidance and Escape</li> <li>● Nanorobotic Thermocompatibility and Electrocompatibility</li> <li>● Biofouling of Medical Nanorobots</li> <li>● Biocompatibility of Nanorobot Effluents and Leachates</li> <li>● Biocompatibility of Nanorobot Fragments <i>in vivo</i></li> <li>● Nanorobot Mechanocompatibility</li> <li>● Mechanical Peristaltogenesis and Mucosacompatibility</li> <li>● Nanorobotic Mechanical Vasculopathies</li> <li>● Mechanocompatibility with Extracellular Matrix and Tissue Cells</li> <li>● Mechanocompatibility with Nontissue Cells</li> <li>● Cytomembrane and Intracellular Mechanocompatibility</li> <li>● Disruption of Molecular Motors and Vesicular Transport</li> <li>● Mechanical Disruption of Intracellular Microzones</li> <li>● Mechanically-Induced Proteolysis, Apoptosis, or Prionosis</li> </ul>

<sup>849</sup> Freitas RA Jr. Nanomedicine Vol. IIA: Biocompatibility. Landes Bioscience, Georgetown, TX, 2003; <http://www.nanomedicine.com/NMIIA.htm>.

A large part of the 2003 work is an examination of the classical biocompatibility challenges including issues such as immune system reactions, complement activation, inflammation, thrombogenesis, and carcinogenesis that might be caused by medical nanorobots. This study of classical challenges suggested a number of new biocompatibility issues that must also be addressed in medical nanorobotics including, most importantly, the areas of mechanocompatibility, particle biodynamics and distribution, and phagocyte avoidance protocols. We will touch upon a few of these items only briefly here. Readers interested in biocompatibility issues that are not covered below can find a more comprehensive list of topics and associated discussions in the book *Nanomedicine, Vol. IIA*.<sup>850</sup>

---

<sup>850</sup> Freitas RA Jr. *Nanomedicine Vol. IIA: Biocompatibility*. Landes Bioscience, Georgetown, TX, 2003; <http://www.nanomedicine.com/NMIIA.htm>.

#### 4.2.4.1 Immune System Reactions

Whether the human immune system can recognize medical nanorobots may depend largely upon the composition of the nanorobot exterior surfaces. Pure diamond is generally considered non-immunogenic – e.g., chemical vapor deposition (CVD) diamond coatings for artificial joints are considered to have “low immunoreactivity”, and as of 2016 there were no reports in the literature of antibodies having been raised to pure crystalline diamond, though related searches continue.<sup>851</sup> But concerted experimental searches for antibodies to diamondoid materials have yet to be undertaken, and experimental failures rarely find their way into the literature.

It is conceivable that different antibodies may recognize distinct faces of a crystal (possibly including diamond or sapphire crystal faces exposed at the surfaces of medical nanorobots) in an interaction similar to that of antibodies for repetitive epitopes present on protein surfaces. For instance, one monoclonal antibody (mAb) to 1,4-dinitrobenzene crystals was shown to specifically interact with the molecularly flat, aromatic, and polar (101) face of these crystals, but not with other faces of the same crystal.<sup>852</sup>

Another concern is that antibodies may be raised against binding sites that are positioned on the nanorobot exterior, e.g., sorting rotor pockets<sup>853</sup> which may be similar to traditional bioreceptors, and that these antibodies could then act as antagonists<sup>854</sup> for such sites, since mAbs specific to biological binding sites are well known.

---

<sup>851</sup> Coyle BL, Rolandi M, Baneyx F. Carbon-binding designer proteins that discriminate between sp<sup>2</sup>- and sp<sup>3</sup>-hybridized carbon surfaces. *Langmuir*. 2013 Apr 16;29(15):4839-46; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3658162/>. Gabryelczyk B, Szilvay GR, Linder MB. The structural basis for function in diamond-like carbon binding peptides. *Langmuir*. 2014 Jul 29;30(29):8798-802; [https://www.researchgate.net/profile/Bartosz\\_Gabryelczyk/publication/263778978\\_The\\_Structural\\_Basis\\_of\\_Function\\_in\\_Diamond-like\\_Carbon\\_Binding\\_Peptides/links/5408141f0cf2c48563b8988e.pdf](https://www.researchgate.net/profile/Bartosz_Gabryelczyk/publication/263778978_The_Structural_Basis_of_Function_in_Diamond-like_Carbon_Binding_Peptides/links/5408141f0cf2c48563b8988e.pdf).

<sup>852</sup> Kessler N, Perl-Treves D, Addadi L, Eisenstein M. Structural and chemical complementarity between antibodies and the crystal surfaces they recognize. *Proteins*. 1999 Feb 15;34(3):383-94; [https://www.researchgate.net/profile/Miriam\\_Eisenstein/publication/13265535\\_Structural\\_and\\_chemical\\_complementarity\\_between\\_antibodies\\_and\\_the\\_crystal\\_surfaces\\_they\\_recognize/links/0deec51a74783aa686000000.pdf](https://www.researchgate.net/profile/Miriam_Eisenstein/publication/13265535_Structural_and_chemical_complementarity_between_antibodies_and_the_crystal_surfaces_they_recognize/links/0deec51a74783aa686000000.pdf).

<sup>853</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 3.4.2; <http://www.nanomedicine.com/NMI/3.4.2.htm>.

<sup>854</sup> A **receptor antagonist** blocks the action of a receptor by occupying the active site, effectively inactivating that site. Fauque J, Borgna JL, Rochefort H. A monoclonal antibody to the estrogen receptor inhibits *in vitro* criteria of receptor activation by an estrogen and an anti-estrogen. *J Biol Chem* 1985 Dec 15;260(29):15547-15553; <http://www.jbc.org/content/260/29/15547.full.pdf>. Wright LM, Brzozowski AM, Hubbard RE, Pike AC, Roberts SM, Skovgaard RN, Svendsen I, Vissing H, Bywater RP. Structure of Fab hGR-2 F6, a competitive antagonist of the glucagon receptor. *Acta Crystallogr D Biol Crystallogr*. 2000 May;56(Pt 5):573-80; <http://www.ncbi.nlm.nih.gov/pubmed/10771426>.

If antibodies to nanorobot exteriors are found to exist in the natural human antibody specificity repertoire, then to avoid immune recognition many techniques of immune evasion<sup>855</sup> may be borrowed from biology, for example:

(1) **Camouflage.** Coat the nanorobot with a layer of “self” proteins and carbohydrate moieties resembling fibroblast, platelet, or even RBC (red blood cell) plasma membrane.

(2) **Chemical Inhibition.** Nanorobots may slowly secrete chemical substances into the perirobotic environment to make it difficult for Ig molecules to adhere to an otherwise immunogenic nanorobot surface.

(3) **Decoys.** Release a cloud of soluble nanorobot-epitope antigens in the vicinity of the nanorobot (though this method has limited utility because sending out decoys may only expand the number of attacking elements to overwhelm the decoys).

(4) **Active Neutralization.** Equip the nanorobot with molecular sorting rotors designed with binding sites similar or identical to the nanorobot epitopes that raised the target antibodies.

(5) **Tolerization.** Using only traditional methods, nanorobots introduced into a newborn may train the neonatal immune system to regard these foreign materials as “native,” thus eliminating nanorobot-active antibodies via natural clonal deletion. And it now appears possible to tolerize an adult to any antigen by regenerating the adult’s thymus (the source of the newborn effect) and placing the antigen into the thymus where self-reactive clones are then deleted or anergized.<sup>856</sup>

(6) **Clonal Deletion.** Once the paratopes of antibodies that bind nanorobots are known, immunotoxin molecules can be engineered that display those paratopes, and upon injection into the patient, these targeted immunotoxins would bind to all T cell receptors that display this paratope, killing the nanorobot-sensitive T cells.

---

<sup>855</sup> Freitas RA Jr. Nanomedicine Vol. IIA: Biocompatibility. Landes Bioscience, Georgetown, TX, 2003, Section 15.2.3.6; <http://www.nanomedicine.com/NMI/15.2.3.6.htm>.

<sup>856</sup> Fahy GM. Apparent induction of partial thymic regeneration in a normal human subject: a case report. *J Anti Aging Med* 2003;6(3):219-227; <http://www.ncbi.nlm.nih.gov/pubmed/14987435>. Fahy GM. Method for the prevention of transplant rejection, U.S. Patent No. 7,166,569, 2007. Aspinall R. Chapter 7. Maintenance and Restoration of Immune System Function, in: Fahy GM, *et al.* (eds), *The Future of Aging: Pathways to Human Life Extension*. Springer, New York, 2010. Fahy GM. Chapter 4. Precedents for the Prevention and Reversal of Aging Processes, in: Fahy GM, *et al.* (eds), *The Future of Aging: Pathways to Human Life Extension*. Springer, New York, 2010.

#### 4.2.4.2 Inflammation

Could medical nanorobots trigger general inflammation in the human body? One early experiment<sup>857</sup> to determine the inflammatory effects of various implant substances emplaced subdermally into rat paws found that an injection of 2-10 mg/cm<sup>3</sup> (10- to 20-micron particles at 10<sup>5</sup>-10<sup>6</sup> particles/cm<sup>3</sup>) of natural diamond powder suspension caused a slight increase in volume of the treated paw relative to the control paw. However, the edematous effect subsided after 30-60 minutes at both concentrations of injected diamond powder that were tried, so this swelling could have been wholly caused by mechanical trauma of the injection and not the diamond powder. Another experiment<sup>858</sup> at the same laboratory found that intraarticular injection of diamond powder was not phlogistic (i.e., no erythematous or edematous changes) in rabbit bone joints and produced no inflammation. Diamond particles are traditionally regarded as biologically inert and noninflammatory for neutrophils<sup>859</sup> and are typically used as experimental null controls.<sup>860</sup>

Since the general inflammatory reaction is chemically mediated, it should also be possible to employ nanorobot surface-deployed molecular sorting rotors to selectively absorb kinins or other soluble inflammation activation factors such as HMGB1 (High Mobility Group Box Protein 1),<sup>861</sup>

---

<sup>857</sup> Royer RJ, Delongas JL, Netter P, Faure G, Mur JM, Burnel D, Gaucher A. Inflammatory effect of aluminium phosphate on rat paws. *Pathol Biol (Paris)*. 1982 Apr;30(4):211-5; <http://www.ncbi.nlm.nih.gov/pubmed/6283459>.

<sup>858</sup> Delongas JL, Netter P, Boz P, Faure G, Royer RJ, Gaucher A. Experimental synovitis induced by aluminium phosphate in rabbits. Comparison of the changes produced in synovial tissue and in articular cartilage by aluminium phosphate, carrageenin, calcium hydrogen phosphate dihydrate, and natural diamond powder. *Biomed Pharmacother*. 1984;38(1):44-8; <http://www.ncbi.nlm.nih.gov/pubmed/6087947>.

<sup>859</sup> Tse RL, Phelps P. Polymorphonuclear leukocyte motility *in vitro*. V. Release of chemotactic activity following phagocytosis of calcium pyrophosphate crystals, diamond dust, and urate crystals. *J Lab Clin Med* 1970 Sep;76(3):403-415; <http://www.translationalres.com/article/0022-2143%2870%2990049-1/abstract>. Higson FK, Jones OT. Oxygen radical production by horse and pig neutrophils induced by a range of crystals. *J Rheumatol*. 1984 Dec;11(6):735-40; <http://www.ncbi.nlm.nih.gov/pubmed/6097690>. Hedenborg M, Klockars M. Quartz-dust-induced production of reactive oxygen metabolites by human granulocytes. *Lung*. 1989;167(1):23-32; <http://www.ncbi.nlm.nih.gov/pubmed/2537915>. Aspenberg P, Anttila A, Kontinen YT, Lappalainen R, Goodman SB, Nordsletten L, Santavirta S. Benign response to particles of diamond and SiC: bone chamber studies of new joint replacement coating materials in rabbits. *Biomaterials* 1996 Apr;17(8):807-812; <http://www.ncbi.nlm.nih.gov/pubmed/8730965>.

<sup>860</sup> Delongas JL, Netter P, Boz P, Faure G, Royer RJ, Gaucher A. Experimental synovitis induced by aluminium phosphate in rabbits. Comparison of the changes produced in synovial tissue and in articular cartilage by aluminium phosphate, carrageenin, calcium hydrogen phosphate dihydrate, and natural diamond powder. *Biomed Pharmacother*. 1984;38(1):44-8; <http://www.ncbi.nlm.nih.gov/pubmed/6087947>.

<sup>861</sup> Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature* 2002 Jul 11;418(6894):191-5; [https://www.researchgate.net/profile/Marco\\_Bianchi2/publication/11267443\\_Scaffidi\\_P\\_Misteli\\_T\\_Bianchi\\_ME/Release\\_of\\_chromatin\\_protein\\_HMGB1\\_by\\_necrotic\\_cells\\_triggers\\_inflammation/Nature/418191-195/links/53ebf1c70cf202d087d00891.pdf](https://www.researchgate.net/profile/Marco_Bianchi2/publication/11267443_Scaffidi_P_Misteli_T_Bianchi_ME/Release_of_chromatin_protein_HMGB1_by_necrotic_cells_triggers_inflammation/Nature/418191-195/links/53ebf1c70cf202d087d00891.pdf).

thus short-circuiting the inflammatory process. Active semaphores<sup>862</sup> consisting of bound proteases such as gelatinase A could be deployed at the nanorobot surface to cleave and degrade monocyte chemoattractant molecules<sup>863</sup> or other chemokines, suppressing the cellular inflammatory response.

Conversely, key inflammatory inhibitors could be locally released by nanorobots. For instance, Hageman factor contact activation inhibitors such as the 22.5-kD endothelial cell-secreted protein HMG-I,<sup>864</sup> surface-immobilized unfractionated heparin,<sup>865</sup> and C1 inhibitor<sup>866</sup> would probably require lower release dosages than for aspirin or steroids, and therapeutic blockade of factor XII activation has been demonstrated.<sup>867</sup> As yet another example, platelet activating factor (PAF) is a cytokine mediator of immediate hypersensitivity which produces inflammation. PAF is produced by many different kinds of stimulated cells such as basophils, endothelial cells, macrophages, monocytes, and neutrophils. It is 100-10,000 times more vasoactive than histamine and aggregates platelets at concentrations as low as 0.01 pmol/cm<sup>3</sup>.<sup>868</sup> Various PAF antagonists and inhibitors are known.<sup>869</sup> These or related inhibitory molecules, if released or surface-displayed by medical nanorobots, may be useful in circumventing a general inflammatory response.

---

<sup>862</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 5.3.6; <http://www.nanomedicine.com/NMI/5.3.6.htm>.

<sup>863</sup> McQuibban GA, Gong JH, Tam EM, McCulloch CA, Clark-Lewis I, Overall CM. Inflammation dampened by gelatinase A cleavage of monocyte chemoattractant protein-3. *Science*. 2000 Aug 18;289(5482):1202-6; <http://science.sciencemag.org/content/sci/289/5482/1202.full.pdf>.

<sup>864</sup> Donaldson VH, Wagner CJ, Mitchell BH, Mandle R. An HMG-I protein from human endothelial cells apparently is secreted and impairs activation of Hageman factor (factor XII). *Proc Assoc Am Physicians*. 1998 Mar-Apr;110(2):140-9; <http://www.ncbi.nlm.nih.gov/pubmed/9542770>.

<sup>865</sup> Elgue G, Sanchez J, Egberg N, Olsson P, Riesenfeld J. Effect of surface-immobilized heparin on the activation of adsorbed factor XII. *Artif Organs* 1993 Aug;17(8):721-726; <http://www.ncbi.nlm.nih.gov/pubmed/8215954>.

<sup>866</sup> Cameron CL, Fisslthaler B, Sherman A, Reddigari S, Silverberg M. Studies on contact activation: effects of surface and inhibitors. *Med Prog Technol* 1989;15(1-2):53-62; <http://www.ncbi.nlm.nih.gov/pubmed/2530427>.

<sup>867</sup> Fuhrer G, Gallimore MJ, Heller W, Hoffmeister HE. FXII. *Blut* 1990 Nov;61(5):258-266; <http://www.ncbi.nlm.nih.gov/pubmed/2271772>.

<sup>868</sup> Mayes PA. Metabolism of acylglycerols and sphingolipids. In: Murray RK, Granner DK, Mayes PA, Rodwell VW (eds) *Harper's Biochemistry*, 23rd Edition. Appleton & Lange, Norwalk CT, 1993, pp. 241-249.

<sup>869</sup> Freitas RA Jr. *Nanomedicine Vol. IIA: Biocompatibility*. Landes Bioscience, Georgetown, TX, 2003, Section 15.2.4; <http://www.nanomedicine.com/NMI/15.2.4.htm>.

### 4.2.4.3 Phagocytosis

Invading microbes that readily attract phagocytes and are easily ingested and killed are generally unsuccessful as parasites. In contrast, most bacteria that are successful as parasites interfere to some extent with the activities of phagocytes or find some way to avoid their attention. Bacterial pathogens have devised numerous diverse strategies to avoid phagocytic engulfment and killing. These strategies are mostly aimed at blocking one or more of the steps in phagocytosis, thereby halting the process.<sup>870</sup>

Similarly, phagocytic cells presented with any significant concentration of medical nanorobots may attempt to internalize these nanorobots. Virtually every medical nanorobot placed inside the human body will physically encounter phagocytic cells many times during its mission. Thus all nanorobots that are of a size capable of ingestion by phagocytic cells must incorporate physical mechanisms and operational protocols for avoiding and escaping from phagocytes.<sup>871</sup> Engulfment may require from many seconds to many minutes to go to completion,<sup>872</sup> depending upon the size of the particle to be internalized, so medical nanorobots should have plenty of time to detect, and to actively prevent, this process. Detection by a medical nanorobot that it is being engulfed by a phagocyte may be accomplished using (1) hull-mounted chemotactic sensor pads equipped with artificial binding sites that are specific to phagocyte coat molecules, (2) continuous monitoring of the flow rates of nanorobot nutrient ingestion or waste ejection mechanisms (e.g., blocked glucose or O<sub>2</sub> import), (3) acoustic techniques,<sup>873</sup> (4) direct measurement of mechanical forces on the hull, or (5) various other means.

The basic anti-phagocyte strategy is first to avoid phagocytic contact,<sup>874</sup> recognition,<sup>875</sup> or binding and activation,<sup>876</sup> and secondly, if this fails, to inhibit phagocytic engulfment<sup>877</sup> or enclosure and

---

<sup>870</sup> Todar K. Evasion of host phagocytic defenses. University of Wisconsin-Madison website, 2000; <http://web.archive.org/web/20020223143807/http://www.bact.wisc.edu/microtextbook/disease/evadephago.html>.

<sup>871</sup> Freitas RA Jr. Nanomedicine Vol. IIA: Biocompatibility. Landes Bioscience, Georgetown, TX, 2003, Section 15.4.3.6; <http://www.nanomedicine.com/NMI/15.4.3.6.htm>.

<sup>872</sup> Freitas RA Jr. Nanomedicine Vol. IIA: Biocompatibility. Landes Bioscience, Georgetown, TX, 2003, Section 15.4.3.1; <http://www.nanomedicine.com/NMI/15.4.3.1.htm>.

<sup>873</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 4.8.2; <http://www.nanomedicine.com/NMI/4.8.2.htm>.

<sup>874</sup> Freitas RA Jr. Nanomedicine Vol. IIA: Biocompatibility. Landes Bioscience, Georgetown, TX, 2003, Section 15.4.3.6.1; <http://www.nanomedicine.com/NMI/15.4.3.6.1.htm>.

<sup>875</sup> Freitas RA Jr. Nanomedicine Vol. IIA: Biocompatibility. Landes Bioscience, Georgetown, TX, 2003, Section 15.4.3.6.2; <http://www.nanomedicine.com/NMI/15.4.3.6.2.htm>.

<sup>876</sup> Freitas RA Jr. Nanomedicine Vol. IIA: Biocompatibility. Landes Bioscience, Georgetown, TX, 2003, Section 15.4.3.6.3; <http://www.nanomedicine.com/NMI/15.4.3.6.3.htm>.

scission<sup>878</sup> of the phagosome. If trapped, the medical nanorobot can induce exocytosis of the phagosomal vacuole in which it is lodged<sup>879</sup> or inhibit both phagolysosomal fusion<sup>880</sup> and phagosome metabolism.<sup>881</sup> In rare circumstances, it may be necessary to kill the phagocyte<sup>882</sup> or to blockade the entire phagocytic system.<sup>883</sup>

Of course, the most direct approach for a fully-functional medical nanorobot is to employ its motility mechanisms to locomote out of, or away from, the phagocytic cell that is attempting to engulf it. This may involve reverse cytopenetration,<sup>884</sup> which must be done cautiously (e.g., the rapid exit of non-enveloped viruses from cells can be cytotoxic<sup>885</sup>). It is also possible that frustrated phagocytosis may induce a localized compensatory granulomatous reaction. Medical nanorobots therefore may need to employ simple but active defensive strategies to forestall granuloma formation.<sup>886</sup>

---

<sup>877</sup> Freitas RA Jr. Nanomedicine Vol. IIA: Biocompatibility. Landes Bioscience, Georgetown, TX, 2003, Section 15.4.3.6.4; <http://www.nanomedicine.com/NMI/15.4.3.6.4.htm>.

<sup>878</sup> Freitas RA Jr. Nanomedicine Vol. IIA: Biocompatibility. Landes Bioscience, Georgetown, TX, 2003, Section 15.4.3.6.5; <http://www.nanomedicine.com/NMI/15.4.3.6.5.htm>.

<sup>879</sup> Freitas RA Jr. Nanomedicine Vol. IIA: Biocompatibility. Landes Bioscience, Georgetown, TX, 2003, Section 15.4.3.6.6; <http://www.nanomedicine.com/NMI/15.4.3.6.6.htm>.

<sup>880</sup> Freitas RA Jr. Nanomedicine Vol. IIA: Biocompatibility. Landes Bioscience, Georgetown, TX, 2003, Section 15.4.3.6.7; <http://www.nanomedicine.com/NMI/15.4.3.6.7.htm>.

<sup>881</sup> Freitas RA Jr. Nanomedicine Vol. IIA: Biocompatibility. Landes Bioscience, Georgetown, TX, 2003, Section 15.4.3.6.8; <http://www.nanomedicine.com/NMI/15.4.3.6.8.htm>.

<sup>882</sup> Freitas RA Jr. Nanomedicine Vol. IIA: Biocompatibility. Landes Bioscience, Georgetown, TX, 2003, Section 15.4.3.6.9; <http://www.nanomedicine.com/NMI/15.4.3.6.9.htm>.

<sup>883</sup> Freitas RA Jr. Nanomedicine Vol. IIA: Biocompatibility. Landes Bioscience, Georgetown, TX, 2003, Section 15.4.3.6.10; <http://www.nanomedicine.com/NMI/15.4.3.6.10.htm>.

<sup>884</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 9.4.5; <http://www.nanomedicine.com/NMI/9.4.5.htm>.

<sup>885</sup> Oh JO. Immunology of viral infections. *Int Ophthalmol Clin*. 1985 Summer 25(2):107-16; <http://www.ncbi.nlm.nih.gov/pubmed/3874186>.

<sup>886</sup> Freitas RA Jr. Nanomedicine Vol. IIA: Biocompatibility. Landes Bioscience, Georgetown, TX, 2003, Section 15.4.3.5; <http://www.nanomedicine.com/NMI/15.4.3.5.htm>.

## 4.2.5 Advantages of Medical Nanorobots

Although biotechnology makes possible a greatly increased range and efficacy of treatment options compared to traditional approaches, with medical nanorobotics the range, efficacy, comfort and speed of possible medical treatments further expands enormously. Medical nanorobotics will be essential whenever the damage to the human body is extremely subtle, highly selective (e.g., specific organ, tissue, or cell type), or time-critical (as in head traumas, burns, or fast-spreading diseases), or when the damage is very massive, overwhelming the body's natural defenses and repair mechanisms – pathological conditions from which it is often difficult or impossible to recover at all using current or easily foreseeable biotechnological techniques.

While it is true that many classes of medical problems may be at least partially resolved using existing treatment alternatives, it is also true that as the chosen medical technology becomes more precise, active, and controllable, the range of options broadens and the quality of the options improves. Thus the question is not whether medical nanorobotics is absolutely required to accomplish a given medical objective. In some cases, it is not – though of course there are some things that only biotechnology and nanotechnology can do, and some other things that only nanotechnology can do. Rather, the important question is which approach offers a superior outcome for a given medical problem, using any reasonable metric of treatment efficacy. For virtually every class of medical challenge, a mature medical nanorobotics offers a wider and more effective range of treatment options than any other solution. A few of the most important advantages of medical nanorobotics over present-day and anticipated future biotechnology-based medical and surgical approaches include:<sup>887</sup>

1. **Speed of Treatment.** Doctors may be surprised by the incredible quickness of nanorobotic action when compared to methods relying on self-repair. We expect that mechanical nanorobotic therapeutic systems can reach their targets up to ~1,000 times faster, all else equal, and treatments which require  $\sim 10^5$  sec (~days) for biological systems to complete may require only  $\sim 10^2$  sec (~minutes) using nanorobotic systems.<sup>888</sup>

2. **Control of Treatment.** Present-day biotechnological entities are not very programmable and cannot easily be switched on and off conditionally (while following complex multidecision trees) during task execution. Even assuming that a digital biocomputer<sup>889</sup> could be

---

<sup>887</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 1.3.3; <http://www.nanomedicine.com/NMI/1.3.3.htm>.

<sup>888</sup> Freitas RA Jr. Microbivores: Artificial mechanical phagocytes using digest and discharge protocol. *J Evol Technol* 2005;14:1-52; <http://www.jetpress.org/volume14/freitas.html>.

<sup>889</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 10.2.3; <http://www.nanomedicine.com/NMI/10.2.3.htm>. Guet CC, Elowitz MB, Hsing W. Combinatorial synthesis of genetic networks. *Science*. 2002 May 24;296(5572):1466-70; <http://science.sciencemag.org/content/sci/296/5572/1466.full.pdf>. Yokobayashi Y, Weiss R, Arnold FH. Directed evolution of a genetic circuit. *Proc Natl Acad Sci U S A*. 2002 Dec 24;99(26):16587-91; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC139187/>. Basu S, Mehreja R, Thiberge S, Chen MT, Weiss

installed in, for example, a fibroblast, and that appropriate effector mechanisms could be attached, such a biorobotic system would necessarily have slower clock cycles,<sup>890</sup> less capacious memory per unit volume, and longer data access times, implying less diversity of action, poorer control, and less complex executable programs than would be available in diamondoid nanocomputer-controlled nanorobotic systems. The mechanical or electronic nanocomputer approach<sup>891</sup> emphasizes precise control of action,<sup>892</sup> including control of physical placement, timing, strength, structure, and interactions with other (especially biological) entities.

**3. Verification of Treatment.** Nanorobotic-enabled endoscopic nanosurgery<sup>893</sup> will include comprehensive sensory feedback enabling full VR telepresence permitting real-time surgery into cellular and subcellular tissue volumes. Using a variety of communication modalities,<sup>894</sup> nanorobots will be able to report back to the attending physician, with digital precision and ~MHz bandwidth,<sup>895</sup> a summary of diagnostically- or therapeutically-relevant data describing exactly what was found prior to treatment, what was done during treatment, and what problems were encountered after treatment, in every cell or tissue that was visited and treated by the nanorobot. A comparable biological-based approach relying primarily upon chemical messaging must necessarily be slow with only limited signaling capacity and bandwidth.

**4. Minimal Side Effects.** Almost all drugs have significant side effects, such as conventional cancer chemotherapy which typically causes hair loss and vomiting, although computer-designed drugs can have higher specificity and fewer side effects than earlier drugs.

R. Spatiotemporal control of gene expression with pulse-generating networks. *Proc Natl Acad Sci U S A*. 2004 Apr 27;101(17):6355-60; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC404049/>. Basu S, Gerchman Y, Collins CH, Arnold FH, Weiss R. A synthetic multicellular system for programmed pattern formation. *Nature*. 2005 Apr 28;434(7037):1130-4; [http://macpost.caltech.edu/groups/fha/publications/Basu\\_Nature2005.pdf](http://macpost.caltech.edu/groups/fha/publications/Basu_Nature2005.pdf).

<sup>890</sup> Basu S, Mehreja R, Thiberge S, Chen MT, Weiss R. Spatiotemporal control of gene expression with pulse-generating networks. *Proc Natl Acad Sci U S A*. 2004 Apr 27;101(17):6355-60; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC404049/>.

<sup>891</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 10.2; <http://www.nanomedicine.com/NMI/10.2.htm>.

<sup>892</sup> Freitas RA Jr. Computational tasks in medical nanorobotics. In: Eshaghian-Wilner MM (ed) *Bio-inspired and Nano-scale Integrated Computing*. Wiley, New York, 2009; <http://www.nanomedicine.com/Papers/NanorobotControl2009.pdf>.

<sup>893</sup> Freitas RA Jr. "Chapter 23. Comprehensive Nanorobotic Control of Human Morbidity and Aging," in Gregory M. Fahy, Michael D. West, L. Stephen Coles, and Steven B. Harris, eds, *The Future of Aging: Pathways to Human Life Extension*, Springer, New York, 2010, Section 6.3.5.3; <http://www.nanomedicine.com/Papers/Aging.pdf>.

<sup>894</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Chapter 7; <http://www.nanomedicine.com/NMI/7.1.htm>.

<sup>895</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 7.3; <http://www.nanomedicine.com/NMI/7.3.htm>.

Carefully tailored cancer vaccines under development starting in the late 1990s were expected unavoidably to affect some healthy cells. Even well-targeted drugs are distributed to unintended tissues and organs in low concentrations,<sup>896</sup> although some bacteria can target a few organs fairly reliably without being able to distinguish individual cells. By contrast, mechanical nanorobots may be targeted with virtually 100% accuracy to specific organs, tissues, or even individual cellular addresses within the human body.<sup>897</sup> Such nanorobots should have few if any side effects, and will remain safe even in large dosages because their actions can be digitally self-regulated using rigorous control protocols<sup>898</sup> that affirmatively prohibit device activation unless all necessary preconditions have been met, and remain continuously satisfied. More than a decade ago, Fahy<sup>899</sup> observed that these possibilities could transform “drugs” into “programmable machines with a range of sensory, decision-making, and effector capabilities [that] might avoid side effects and allergic reactions...attaining almost complete specificity of action...Designed smart pharmaceuticals might activate themselves only when, where, and if needed.” Additionally, nanorobots may be programmed to harmlessly remove themselves from the site of action, or conveniently excrete themselves from the body, after a treatment is completed. By contrast, spent biorobotic elements containing ingested foreign materials may have more limited post-treatment mobility, thus lingering at the worksite causing inflammation when naturally degraded *in situ* or removed. (It might be possible to design artificial eukaryotic biorobots having an apoptotic pathway<sup>900</sup> that could be activated to permit clean and natural self-destruction, but any indigestible foreign material that had been endocytosed by the biorobot could still cause inflammation in surrounding tissues when released).

**5. Faster and More Precise Diagnosis.** The analytic function of medical diagnosis requires rapid communication between the injected devices and the attending physician. If limited to chemical messaging, biotechnology-based devices such as biorobots will require minutes or hours to complete each diagnostic loop. Nanomachines, with their more diverse set of input-output mechanisms, will be able to outmessage complete results (both aggregated and

---

<sup>896</sup> Davis S. Chapter 16. Biomedical applications of particle engineering. In: Coombs RRH, Robinson DW (eds) *Nanotechnology in Medicine and the Biosciences*. Gordon & Breach Publishers, Netherlands, 1996, pp. 243-262.

<sup>897</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Chapter 8; <http://www.nanomedicine.com/NMI/8.1.htm>. Freitas RA Jr. Phagocytes: an ideal vehicle for targeted drug delivery. *J Nanosci Nanotechnol* 2006 Sep-Oct;6(9-10):2769-2775; <http://www.nanomedicine.com/Papers/JNNPharm06.pdf>.

<sup>898</sup> Freitas RA Jr. Computational tasks in medical nanorobotics. In: Eshaghian-Wilner MM (ed) *Bio-inspired and Nano-scale Integrated Computing*. Wiley, New York, 2009; <http://www.nanomedicine.com/Papers/NanorobotControl2009.pdf>.

<sup>899</sup> Fahy GM. Molecular nanotechnology and its possible pharmaceutical implications. In: Bezold C, Halperin JA, Eng JL (eds) *2020 Visions: Health Care Information Standards and Technologies*. U.S. Pharmacopeial Convention Inc., Rockville MD, 1993, pp. 152-159.

<sup>900</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 10.4.1.1; <http://www.nanomedicine.com/NMI/10.4.1.1.htm>.

individual outliers) of *in vivo* reconnaissance or testing to the physician, literally in seconds.<sup>901</sup> Such nanomachines could also run more complex tests of greater variety in far less time. Nanomechanical nanoinstrumentation will make comprehensive rapid cell mapping and cell interaction analysis possible. For example, new instances of novel bacterial resistance could be assayed at the molecular level in real time, allowing new treatment agents to be quickly composed using an FDA-approved formulary, then manufactured and immediately deployed on the spot.

**6. More Sensitive Response Threshold for High-Speed Action.** Unlike natural systems, an entire population of nanorobotic devices could be triggered globally by just a single local detection of the target antigen or pathogen. The natural immune system takes  $>10^5$  sec to become fully engaged after exposure to a systemic pathogen or other antigen-presenting intruder. A biotechnologically enhanced immune system that could employ the fastest natural unit replication time ( $\sim 10^3$  sec for some bacteria) would thus require at least  $\sim 10^4$  sec for full deployment post-exposure. By contrast, an artificial nanorobotic immune system<sup>902</sup> could probably be fully engaged (though not finished) in at most two blood circulation times, or  $\sim 10^2$  sec.

**7. More Reliable Operation.** Individual engineered macrophages would almost certainly operate less reliably than individual mechanical nanorobots. For example, many pathogens, such as *Listeria monocytogenes* and *Trypanosoma cruzi*, are known to be able to escape from phagocytic vacuoles into the cytoplasm.<sup>903</sup> While biotech drugs or cell manufactured proteins could be developed to prevent this (e.g., cold therapy drugs are entry-point blockers), nanorobotic trapping mechanisms could be more secure.<sup>904</sup> Proteins assembled by natural ribosomes typically incorporate one error per  $\sim 10^4$  amino acids placed; current gene and protein synthesizing machines utilizing biotechnological processes have similar error rates. A molecular nanotechnology approach should decrease these error rates by at least a millionfold.<sup>905</sup> Nanomechanical systems will also incorporate onboard sensors to determine if and when a particular task needs to be done, or when a task has been completed. Finally, and perhaps most

---

<sup>901</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Chapter 7; <http://www.nanomedicine.com/NMI/7.1.htm>.

<sup>902</sup> Freitas RA Jr. Microbivores: Artificial mechanical phagocytes using digest and discharge protocol. J Evol Technol 2005;14:1-52; <http://www.jetpress.org/volume14/freitas.html>.

<sup>903</sup> Stenger S, Hanson DA, Teitelbaum R, Dewan P, Niazi KR, Froelich CJ, Ganz T, Thoma-Uszynski S, Melián A, Bogdan C, Porcelli SA, Bloom BR, Krensky AM, Modlin RL. An antimicrobial activity of cytolytic T cells mediated by granulysin. Science. 1998 Oct 2;282(5386):121-5; <http://science.sciencemag.org/content/sci/282/5386/121.full.pdf>.

<sup>904</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 10.4.2; <http://www.nanomedicine.com/NMI/10.4.2.htm>. Freitas RA Jr. Microbivores: Artificial mechanical phagocytes using digest and discharge protocol. J Evol Technol 2005;14:1-52; <http://www.jetpress.org/volume14/freitas.html>.

<sup>905</sup> Drexler KE. Nanosystems: Molecular Machinery, Manufacturing, and Computation. John Wiley & Sons, New York, 1992, Section 8.3.4.

importantly, it is highly unlikely that natural microorganisms will be able to infiltrate rigid watertight diamondoid nanorobots or to co-opt their functions. By contrast, a biotech-based biorobot more readily could be diverted or defeated by microbes that would piggyback on its metabolism, interfere with its normal workings, or even incorporate the device wholesale into their own structures, causing the engineered biomachine to perform some new or different – and possibly pathological – function that was not originally intended. There are many examples of such co-option in natural biological systems, including the protozoan mixotrichs found in the termite gut that have assimilated bacteria into their bodies for use as motive engines,<sup>906</sup> and the nudibranch mollusks (marine snails without shells) that steal nematocysts (stinging cells) away from coelenterates such as jellyfish (i.e. a Portuguese man-of-war) and incorporate the stingers as defensive armaments in their own skins<sup>907</sup> – a process which Vogel<sup>908</sup> calls “stealing loaded guns from the army.”

**8. Nonbiodegradable Treatment Agents.** Diagnostic and therapeutic agents constructed of biomaterials generally are biodegradable *in vivo*, although there is a major branch of pharmacology devoted to designing drugs that are moderately non-biodegradable – e.g., anti-sense DNA analogs with unusual backbone linkages and peptide nucleic acids (PNAs) are difficult to break down. An engineered fibroblast may not stimulate an immune response when transplanted into a foreign host, but its biomolecules are subject to chemical attack *in vivo* by free radicals, acids, and enzymes. Even “mirror” biomolecules or “Doppelganger proteins” comprised exclusively of unnatural D-amino acids have a lifetime of only ~5 days inside the human body.<sup>909</sup> In contrast, suitably designed nanorobotic agents constructed of nonbiological materials need not be biodegradable. Nonbiological diamondoid materials are highly resistant to chemical breakdown or leukocytic degradation *in vivo*, and pathogenic biological entities cannot easily evolve useful attack strategies against these materials.<sup>910</sup> This means that medical nanorobots could be recovered intact from the patient and recycled, possibly reducing life-cycle energy consumption and treatment costs.

---

<sup>906</sup> Cleveland LR, Grimstone AV. The fine structure of the flagellate *Mixotrich paradoxa* and its associated microorganisms. Proc Roy Soc London B 1964;159:668-686; <http://rspb.royalsocietypublishing.org/content/royprsb/159/977/668.full.pdf>. Tamm SL. Flagellated ectosymbiotic bacteria propel a eukaryotic cell. J Cell Biol 1982 Sep;94(3):697-709; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2112208/>.

<sup>907</sup> Thompson TE, Bennett I. *Physalia* nematocysts utilized by mollusks for defense. Science 1969 Dec 19;166(3912):1532-3; <http://www.ncbi.nlm.nih.gov/pubmed/17742854>.

<sup>908</sup> Vogel S. Cats' Paws and Catapults: Mechanical Worlds of Nature and People. W.W. Norton and Company, New York, 1998.

<sup>909</sup> Robson B. Doppelganger proteins as drug leads. Nature Biotechnol 1996 Jul;14(7):892-3; <http://www.ncbi.nlm.nih.gov/pubmed/9631018>. Robson B, Pseudoproteins: Non-protein protein-like machines. 6th Foresight Conference on Molecular Nanotechnology, Nov 1998; <http://foresight.org/Conference/MNT6/Abstracts/Robson/index.html>.

<sup>910</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 9.3.5.3.6; <http://www.nanomedicine.com/NMI/9.3.5.3.6.htm>.

9. **Superior Materials.** Typical biological materials have tensile failure strengths in the  $10^6$ - $10^7$  N/m<sup>2</sup> range, with the strongest biological materials such as wet compact bone having a failure strength of  $\sim 10^8$  N/m<sup>2</sup>, all of which compare poorly to  $\sim 10^9$  N/m<sup>2</sup> for good steel,  $\sim 10^{10}$  N/m<sup>2</sup> for sapphire, and  $\sim 10^{11}$  N/m<sup>2</sup> for diamond and carbon fullerenes,<sup>911</sup> again showing a  $10^3$ - $10^5$  fold strength advantage for mechanical systems that use nonbiological, and especially diamondoid, materials. Nonbiological materials can be much stiffer, permitting the application of higher forces with greater precision of movement, and they also tend to remain more stable over a larger range of relevant conditions including temperature, pressure, salinity and pH. Proteins are heat sensitive in part because much of the functionality of their structure derives from the noncovalent bonds involved in folding, which are broken more easily at higher temperatures. In diamond, sapphire, and many other rigid materials, structural shape is covalently fixed, hence is far more temperature-stable. Most proteins also tend to become dysfunctional at cryogenic temperatures, unlike diamond-based mechanical structures,<sup>912</sup> so diamondoid nanorobots could more easily be used to repair frozen cells and tissues. Biomaterials are not ruled out for all nanomechanical systems, but they represent only a small subset of the full range of materials that can be employed in nanorobots. Nanorobotic systems may take advantage of a wider variety of atom types and molecular structures in their design and construction, making possible novel functional forms that might be difficult to implement in a purely biological-based system (e.g., steam engines<sup>913</sup> or nuclear power<sup>914</sup>). As another example, an application requiring the most effective bulk thermal conduction possible should use diamond, the best conductor available, not some biomaterial having inferior thermal performance.

---

<sup>911</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Table 9.3; <http://www.nanomedicine.com/NMI/Tables/9.3.jpg>.

<sup>912</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 10.5; <http://www.nanomedicine.com/NMI/10.5.htm>.

<sup>913</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 6.3.1; <http://www.nanomedicine.com/NMI/6.3.1.htm>.

<sup>914</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 6.3.7; <http://www.nanomedicine.com/NMI/6.3.7.htm>.

## 4.2.6 Nanorobotic Cell Mills

In our vision of future nanorobotic medicine, a desktop-style apparatus accepts as input the patient's DNA sequence, then manufactures human tissue cells or blood cells of any type in a convergent assembly type process,<sup>915</sup> as described in the following conceptual design of a cell mill with three modules. Output should approach ~1 kg/hr, roughly similar to a mature nanofactory.

The **first module** of the cell mill would synthesize copies of the patient's own autologous proteins and other relevant biomolecules, working from the patient's genome. As a proof of concept, functional copies of the human red cell **band 3 anion exchanger 1** (AE1, aka. Band 3, solute carrier family 4 member 1 (SLC4A1), or Band 3 anion transport protein; image, right), a proteinaceous transmembrane pump, have been self-assembled from sets of three, four or five complementary fragment "nanoparts" that were separately cloned in *Xenopus* oocytes.<sup>916</sup> The first module would also include the capacity to manufacture many duplicate copies of the patient's own DNA, suitably methylated to match the expression pattern (e.g., the "methylome," "transcriptome," etc.) and activation state of the particular cell type that was being constructed (Section 4.2.3). Custom protein factories have been created using cell-free microfabricated bioreactors (which eliminates the maintenance of living systems inside the device) and are already being used to facilitate the on-demand production of therapeutic proteins for medicines and biopharmaceuticals.<sup>917</sup>




---

<sup>915</sup> Freitas RA Jr, Merkle RC. Kinematic Self-Replicating Machines. Landes Bioscience, Georgetown, TX, 2004, Section 5.9.4, "Performance of Convergent Assembly Nanofactory Systems"; <http://www.molecularassembler.com/KSRM/5.9.4.htm>.

<sup>916</sup> Groves JD, Wang L, Tanner MJ. Functional reassembly of the anion transport domain of human red cell band 3 (AE1) from multiple and non-complementary fragments. FEBS Lett. 1998 Aug 21;433(3):223-7; <http://www.sciencedirect.com/science/article/pii/S0014579398009090/pdf?md5=5a6fd338036616de263d878735192af6&pid=1-s2.0-S0014579398009090-main.pdf>.

<sup>917</sup> Ron Walli, "ORNL cell-free protein synthesis is potential lifesaver," Oak Ridge National Laboratory, 29 Dec 2015; <https://www.ornl.gov/news/ornl-cell-free-protein-synthesis-potential-lifesaver>.

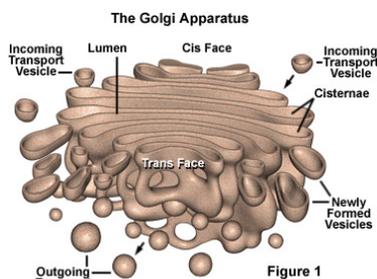
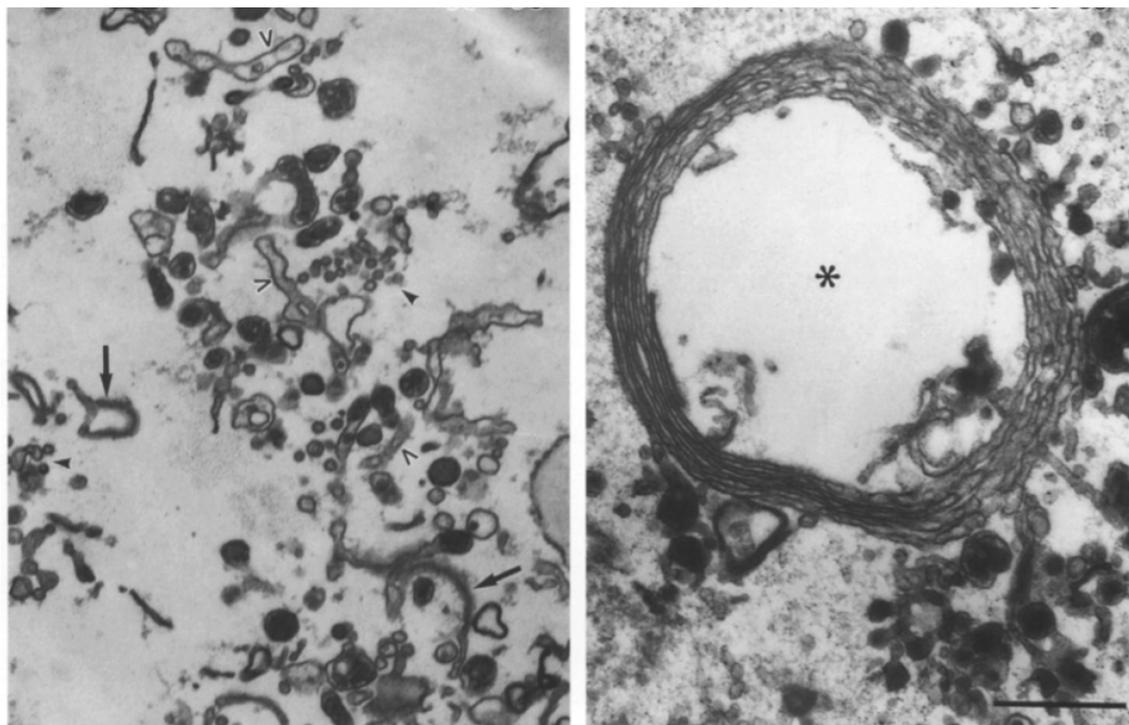


Figure 1

These fabricated molecular components would then be fed to the **second module** of the cell mill that assembles them into bulk quantities of artificially fabricated organelles, membranes, vesicles, granules, and other key intracellular structures.<sup>918</sup> Many such structures will self-assemble robustly.<sup>919</sup> As an experimental example of this, **Golgi stacks** (an important intracellular organelle; idealized schematic image at left) have been reassembled from isolated Golgi components (including random assortments of vesicles “▲”, tubules “>”, and cisternal remnants “→”; image, below left) to create a large reassembled Golgi stack (image, below right; scale bar = 0.5 μm).<sup>920</sup>

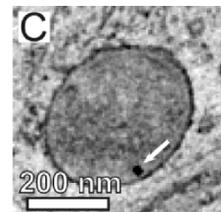


<sup>918</sup> Olson KJ, Ahmadzadeh H, Arriaga EA. Within the cell: analytical techniques for subcellular analysis. *Anal Bioanal Chem.* 2005 Jun;382(4):906-17; <http://www.ncbi.nlm.nih.gov/pubmed/15928950>. Satori CP, Kostal V, Arriaga EA. Review on recent advances in the analysis of isolated organelles. *Anal Chim Acta.* 2012 Nov 13;753:8-18; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3484375/>.

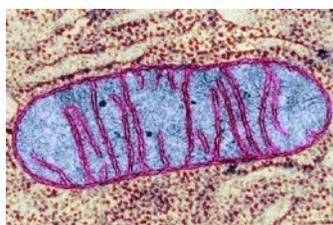
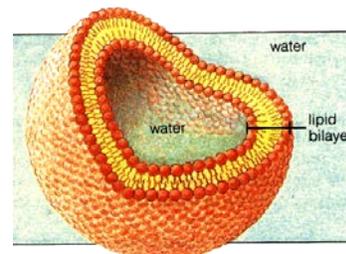
<sup>919</sup> Marrink SJ, Lindahl E, Edholm O, Mark AE. Simulation of the spontaneous aggregation of phospholipids into bilayers. *J Am Chem Soc.* 2001 Sep 5;123(35):8638-9; <http://www.rug.nl/research/portal/files/3618453/2001JAmChemSocMarrink1.pdf>.

<sup>920</sup> Rabouille C, Misteli T, Watson R, Warren G. Reassembly of Golgi stacks from mitotic Golgi fragments in a cell-free system. *J Cell Biol.* 1995 May;129(3):605-18; <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.273.3233&rep=rep1&type=pdf>.

**Endosomes** (membrane-bound intracellular compartments originating from the Golgi trans face containing molecules or ligands internalized from the plasma membrane that may be targeted to lysosomes for degradation; image, right) have been reconstituted *in vitro* from purified components.<sup>921</sup>



The automated assembly of uniform liposomes with lipid bilayers, the basic building block of **cell membranes**, has been demonstrated experimentally in a controlled, robust, efficient and simple way using chip-based microfluidics.<sup>922</sup>



Organelle biogenesis in biological systems can occur by *de novo* synthesis from (1) a pre-existing membrane source, (2) fission,<sup>923</sup> (3) fusion,<sup>924</sup> and (4) decay, such as through partitioning during cell division or autophagy.<sup>925</sup> For example, biogenesis of **mitochondria** (image, left) occurs by growth and division of pre-existing organelles,<sup>926</sup> a process that could presumably be artificially accelerated using a bioreactor setup or by other means.<sup>927</sup> The inventory of known proteins comprising all mammalian mitochondria (the full mitochondrial “parts list”) is almost complete.<sup>928</sup>

<sup>921</sup> Wollert T. Reconstituting multivesicular body biogenesis with purified components. *Methods Cell Biol.* 2012;108:73-92; <http://www.ncbi.nlm.nih.gov/pubmed/22325598>.

<sup>922</sup> Deshpande S, Caspi Y, Meijering AE, Dekker C. Octanol-assisted liposome assembly on chip. *Nat Commun.* 2016 Jan 22;7:10447; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4735860/>.

<sup>923</sup> Lowe M, Barr FA. Inheritance and biogenesis of organelles in the secretory pathway. *Nat Rev Mol Cell Biol.* 2007 Jun;8(6):429-39; <https://www.ncbi.nlm.nih.gov/pubmed/17505521/>.

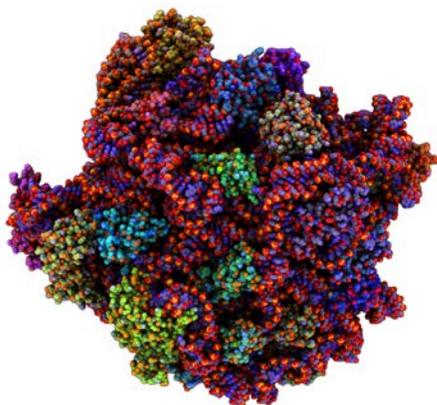
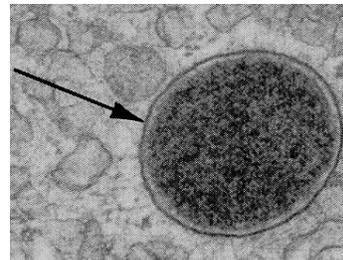
<sup>924</sup> Denesvre C, Malhotra V. Membrane fusion in organelle biogenesis. *Curr Opin Cell Biol.* 1996 Aug;8(4):519-23; <https://www.ncbi.nlm.nih.gov/pubmed/8791453/>.

<sup>925</sup> van der Vaart A, Mari M, Reggiori F. A picky eater: exploring the mechanisms of selective autophagy in human pathologies. *Traffic.* 2008 Mar;9(3):281-9; [https://www.researchgate.net/profile/Fulvio\\_Reggiori/publication/5859063\\_A\\_picky\\_eater\\_exploring\\_the\\_mechanisms\\_of\\_selective\\_autophagy\\_in\\_human\\_pathologies/links/542408dc0cf238c6ea6e896e.pdf](https://www.researchgate.net/profile/Fulvio_Reggiori/publication/5859063_A_picky_eater_exploring_the_mechanisms_of_selective_autophagy_in_human_pathologies/links/542408dc0cf238c6ea6e896e.pdf).

<sup>926</sup> Valero T. Mitochondrial biogenesis: pharmacological approaches. *Curr Pharm Des.* 2014;20(35):5507-9; <https://www.ncbi.nlm.nih.gov/pubmed/24606795>.

<sup>927</sup> Komen JC, Thorburn DR. Turn up the power - pharmacological activation of mitochondrial biogenesis in mouse models. *Br J Pharmacol.* 2014 Apr;171(8):1818-36; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3976607/>. Thevis M, Schänzer W. Emerging drugs affecting skeletal muscle function and mitochondrial biogenesis - Potential implications for sports drug

One of the first artificial **lysosome** (image, right) “models” was reported in 1969.<sup>929</sup> Lysosomal ion channel molecules have been successfully inserted into artificial membranes.<sup>930</sup>



**Ribosomes** (image, left) use positionally-controlled solution-phase mechanochemistry to fabricate proteins from amino acid building blocks,<sup>931</sup> a process that should be readily automated in a nanomechanical cell mill. A small number of autologous proteins fabricated in this manner can then self-assemble into complete ribosomes, a simple way to make lots of copies of this organelle. Steps toward the first artificial ribosome have also been reported.<sup>932</sup>

**Cytoskeleton**<sup>933</sup> and **chromatin**<sup>934</sup> molecules have been directly manipulated inside cells using optical and magnetic tweezers.

testing programs. *Rapid Commun Mass Spectrom.* 2016 Mar 15;30(5):635-51; <http://www.ncbi.nlm.nih.gov/pubmed/26842585>.

<sup>928</sup> Calvo SE, Clauser KR, Mootha VK. MitoCarta2.0: an updated inventory of mammalian mitochondrial proteins. *Nucleic Acids Res.* 2016 Jan 4;44(D1):D1251-7; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4702768/>.

<sup>929</sup> Sessa G, Weissman GJ. Formation of artificial lysosome *in vitro*. *J Clin Invest.* 1969 Jan 1;48(6):A76-A77. Sessa G, Weissmann G. Incorporation of lysozyme into liposomes. A model for structure-linked latency. *J Biol Chem.* 1970 Jul 10;245(13):3295-301; <http://www.jbc.org/content/245/13/3295.long>

<sup>930</sup> Venturi E, Sitsapesan R. Reconstitution of lysosomal ion channels into artificial membranes. *Methods Cell Biol.* 2015;126:217-36; <http://www.ncbi.nlm.nih.gov/pubmed/25665448>.

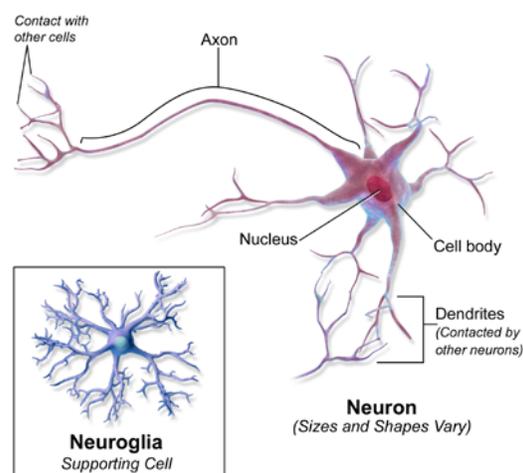
<sup>931</sup> Freitas RA Jr., Merkle RC. Kinematic Self-Replicating Machines. Landes Bioscience, Georgetown, TX, 2004, Section 4.2, “Ribosomes: Molecular Positional Assembly for Self-Replication,” pp. 96-101; <http://www.MolecularAssembler.com/KSRM/4.2.htm>

<sup>932</sup> Orelle C, Carlson ED, Szal T, Florin T, Jewett MC, Mankin AS. Protein synthesis by ribosomes with tethered subunits. *Nature.* 2015 Aug 6;524(7563):119-24; <http://www.ncbi.nlm.nih.gov/pubmed/26222032>.

<sup>933</sup> Felgner H, Frank R, Schliwa M. Flexural rigidity of microtubules measured with the use of optical tweezers. *J Cell Sci.* 1996 Feb;109 ( Pt 2):509-16; <http://jcs.biologists.org/content/joces/109/2/509.full.pdf>. de Vries AH, Krenn BE, van Driel R, Kanger JS. Micro magnetic tweezers for nanomanipulation inside live cells. *Biophys J.* 2005 Mar;88(3):2137-44; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1305265/>.

These mass-produced autologous organelles and other intracellular structures then serve as feedstock to the **third module**, wherein the pre-manufactured subcellular structures and materials are assembled into complete neural cells of the requisite types,<sup>935</sup> along with any extracellular matrix materials that might be required.

This could be done using cytomufacturing systems crudely analogous to 3D printing.<sup>936</sup> As long ago as 1970, an *Amoeba proteus* single-cell organism was physically reassembled from its major subcellular components – nucleus, cytoplasm, and cell membrane – taken from three different cells,<sup>937</sup> demonstrating the physical possibility of manually assembling living cells from more primitive parts. Others<sup>938</sup> later reported that “cell fractions from four different animals can be injected into the eviscerated ghost of a fifth amoeba, and a living functioning organism results.” Mammalian cells have also been assembled from separate nuclear and cytoplasmic parts,<sup>939</sup> and intracellular organelles have been individually manipulated both directly<sup>940</sup> and nanosurgically,<sup>941</sup> a key capability that will be needed to assemble whole cells.



**Neural Tissue**

<sup>934</sup> Kanger JS, Subramaniam V, van Driel R. Intracellular manipulation of chromatin using magnetic nanoparticles. *Chromosome Res.* 2008;16(3):511-22; [https://www.researchgate.net/profile/Vinod\\_Subramaniam/publication/5392507\\_Intracellular\\_manipulation\\_of\\_chromatin\\_using\\_magnetic\\_nanoparticles/links/00b7d52e03fa9b3e22000000.pdf](https://www.researchgate.net/profile/Vinod_Subramaniam/publication/5392507_Intracellular_manipulation_of_chromatin_using_magnetic_nanoparticles/links/00b7d52e03fa9b3e22000000.pdf). Hong J, Purwar P, Cha M, Lee J. Spatial control of chromosomal location in a live cell with functionalized magnetic particles. *Nanoscale.* 2015 Dec 7;7(45):19110-7; <http://www.ncbi.nlm.nih.gov/pubmed/26524004>.

<sup>935</sup> There are several hundred neuron types and subtypes in the entire human nervous system, as described at the NeuroMorpho database (<http://neuromorpho.org/>) and the “Neuroscience Lexicon” at <http://neurolex.org/wiki/Category:Neuron>. Some but not all of these may be relevant to AD patients.

<sup>936</sup> Mandrycky C, Wang Z, Kim K, Kim DH. 3D bioprinting for engineering complex tissues. *Biotechnol Adv.* 2015 Dec 23. pii: S0734-9750(15)30066-5; [https://www.researchgate.net/profile/Zongjie\\_Wang/publication/288040709\\_3D\\_Bioprinting\\_for\\_Engineering\\_Complex\\_Tissues/links/5684bb4b08aebccc4e0fecal.pdf](https://www.researchgate.net/profile/Zongjie_Wang/publication/288040709_3D_Bioprinting_for_Engineering_Complex_Tissues/links/5684bb4b08aebccc4e0fecal.pdf).

<sup>937</sup> Jeon KW, Lorch IJ, Danielli JF. Reassembly of living cells from dissociated components. *Science.* 1970 Mar 20;167(3925):1626-7; <http://science.sciencemag.org/content/sci/167/3925/1626.full.pdf>.

<sup>938</sup> Morowitz HJ. Manufacturing a living organism. *Hospital Practice* 1974; 9:210-215.

<sup>939</sup> Veomett G, Prescott DM, Shay J, Porter KR. Reconstruction of mammalian cells from nuclear and cytoplasmic components separated by treatment with cytochalasin B. *Proc Natl Acad Sci U S A.* 1974 May;71(5):1999-2002; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC388372/pdf/pnas00058-0421.pdf>. Veomett G, Prescott DM. Reconstruction of cultured mammalian cells from nuclear and cytoplasmic parts. *Methods Cell Biol* 1976;13:7-14; <http://www.ncbi.nlm.nih.gov/pubmed/1263853>.

Early cell mills might also make limited use of more traditional biotechnologies such as cloning, stem cells, tissue engineering, animal cell reactors,<sup>942</sup> cell-like bioreactors,<sup>943</sup> transdifferentiation<sup>944</sup> and nuclear reprogramming.<sup>945</sup> Lipid vesicles have been prepared containing polymerase enzymes that can synthesize RNA from externally added substrates, and the entire translation apparatus, including ribosomes, has now been captured in vesicles.<sup>946</sup>

Completed manufactured whole cells can be inspected, sorted,<sup>947</sup> and transported by optical tweezers,<sup>948</sup> microgrippers,<sup>949</sup> or other conventional means<sup>950</sup> to a collection area for export and prompt therapeutic use.

<sup>940</sup> Weber G, Greulich KO. Manipulation of cells, organelles, and genomes by laser microbeam and optical trap. *Int Rev Cytol.* 1992;133:1-41; <http://www.ncbi.nlm.nih.gov/pubmed/1577585>. Felgner H, Grolig F, Müller O, Schliwa M. *In vivo* manipulation of internal cell organelles. *Methods Cell Biol.* 1998;55:195-203; <http://www.ncbi.nlm.nih.gov/pubmed/9352518>. Bayoudh S, Mehta M, Rubinsztein-Dunlop H, Heckenberg NR, Critchley C. Micromanipulation of chloroplasts using optical tweezers. *J Microsc.* 2001 Aug;203(Pt 2):214-22; <http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2818.2001.00843.x/full>. Sacconi L, Tolić-Nørrelykke IM, Stringari C, Antolini R, Pavone FS. Optical micromanipulations inside yeast cells. *Appl Opt.* 2005 Apr 10;44(11):2001-7; [http://www.researchgate.net/profile/Francesco\\_Pavone/publication/7902546\\_Optical\\_micromanipulations\\_inside\\_yeast\\_cells/links/0c960520f4d61165f0000000.pdf](http://www.researchgate.net/profile/Francesco_Pavone/publication/7902546_Optical_micromanipulations_inside_yeast_cells/links/0c960520f4d61165f0000000.pdf). Maghelli N, Tolić-Nørrelykke IM. Optical trapping and laser ablation of microtubules in fission yeast. *Methods Cell Biol.* 2010;97:173-83; [https://publications.mpi-cbg.de/Maghelli\\_2010\\_5020.pdf](https://publications.mpi-cbg.de/Maghelli_2010_5020.pdf).

<sup>941</sup> Freitas RA Jr. "Chapter 23. Comprehensive Nanorobotic Control of Human Morbidity and Aging," in Gregory M. Fahy, Michael D. West, L. Stephen Coles, and Steven B. Harris, eds, *The Future of Aging: Pathways to Human Life Extension*, Springer, New York, 2010, pp. 685-805; Section 6.4.2 "Cell nanosurgery"; <http://www.nanomedicine.com/Papers/Aging.pdf>.

<sup>942</sup> Bliem R, Konopitzky K, Katinger H. Industrial animal cell reactor systems: aspects of selection and evaluation. *Adv Biochem Eng Biotechnol* 1991; 44:1-26; <http://www.ncbi.nlm.nih.gov/pubmed/1781316>. Nelson KL, Geyer S. Bioreactor and process design for large-scale mammalian cell culture manufacturing. *Bioprocess Technol* 1991; 13:112-143; <http://www.ncbi.nlm.nih.gov/pubmed/1367130>.

<sup>943</sup> Noireaux V, Libchaber A. A vesicle bioreactor as a step toward an artificial cell assembly. *Proc Natl Acad Sci U S A.* 2004 Dec 21;101(51):17669-74; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC539773/>.

<sup>944</sup> Collas P, Håkelién AM. Teaching cells new tricks. *Trends Biotechnol* 2003 Aug; 21:354-361; <http://www.ncbi.nlm.nih.gov/pubmed/12902172>.

<sup>945</sup> Tada T. Nuclear reprogramming: an overview. *Methods Mol Biol* 2006; 348:227-236; <http://www.ncbi.nlm.nih.gov/pubmed/16988383>.

<sup>946</sup> Deamer D. A giant step towards artificial life? *Trends Biotechnol.* 2005 Jul;23(7):336-8; <http://www.pai.utexas.edu/faculty/isaxena/BIO320/A%20giant%20step%20towards%20artificial%20life%20-%202005.pdf>.

<sup>947</sup> Grover SC, Skirtach AG, Gauthier RC, Grover CP. Automated single-cell sorting system based on optical trapping. *J Biomed Opt.* 2001 Jan;6(1):14-22; <http://www.ncbi.nlm.nih.gov/pubmed/11178576>.

### 4.3 Medical Nanorobots: Ingress to, and Egress from, the Brain



A crucial capability for medical nanorobots intended for neural repair is the ability of the devices to harmlessly enter and exit the live brain tissue of the patient.

Entry to brain tissue by large molecules and particulate matter is restricted by the presence of three distinct interfaces through which medical nanorobots seeking access must pass. These interfaces are called, respectively, the blood-brain barrier or BBB that separates the blood from direct contact with brain tissue (Section 4.3.1), the blood-cerebrospinal fluid barrier or BCB that separates the blood from direct contact with cerebrospinal fluid in the brain ventricles and spinal column (Section 4.3.2), and finally the CSF-brain interface or ependymal interface that separates the CSF from the brain tissue or neuropil (Section 4.3.3). This Section describes more than a dozen possible methods by which medical nanorobots can penetrate these barriers or interfaces to ingress into, and egress from, living human brain tissue.

---

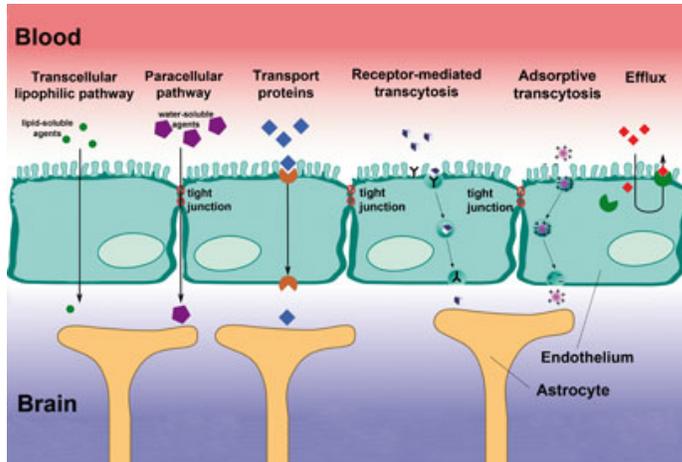
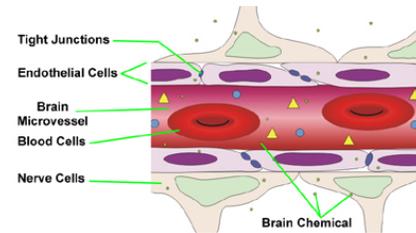
<sup>948</sup> Zhang H, Liu KK. Optical tweezers for single cells. *J R Soc Interface*. 2008 Jul 6;5(24):671-90; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2408388/>.

<sup>949</sup> Kim K, Liu X, Zhang Y, Sun Y. Nanonewton force-controlled manipulation of biological cells using a monolithic MEMS microgripper with two-axis force feedback. *J Micromech Microeng* 2008 Apr 1;18(5):055013; <http://amnl.mie.utoronto.ca/data/J35.pdf>. Leong TG, Randall CL, Benson BR, Bassik N, Stern GM, Gracias DH. Tetherless thermobiochemically actuated microgrippers. *Proc Natl Acad Sci U S A*. 2009 Jan 20;106(3):703-8; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2630075/>.

<sup>950</sup> Gach PC, Wang Y, Phillips C, Sims CE, Allbritton NL. Isolation and manipulation of living adherent cells by micromolded magnetic rafts. *Biomicrofluidics*. 2011 Sep;5(3):32002-3200212; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3194786/>.

### 4.3.1 Blood-Brain Barrier (BBB) Penetration

The blood-brain barrier (BBB) is a highly selective permeability barrier that separates the circulating blood from the neuropil of the brain in which the neurons reside. The BBB is formed by brain endothelial cells, which are connected by tight junctions along all capillaries in the brain (images, right and below). (Most capillaries in the



body lack tight junctions.) These endothelial cells largely prevent the diffusion of microscopic objects (e.g., bacteria) and large or hydrophilic molecules into the cerebrospinal fluid (CSF), while allowing the diffusion of small hydrophobic molecules ( $O_2$ ,  $CO_2$ , hormones) and also allowing active transport of metabolic chemicals such as glucose through the endothelial cell membrane and into the brain. This barrier also includes a thick basement membrane and

astrocytic endfeet, a combination sometimes called the “neurovascular unit” (image, below).



To effect a cure for Alzheimer’s disease, medical nanorobots may need to penetrate the blood-brain barrier, pass into the neuropil, and even enter neurons to conduct repairs, then exit the brain

by the same or another route. Here are 10 methods that have been proposed for doing this, all of which might be applicable to micron-size medical nanorobots:

(1) **Localized Osmotic Disruption.** The BBB can be temporarily and reversibly opened to allow small-particle passage by osmotic disruption<sup>951</sup> via intracarotid infusion of hypertonic saccharide solution, e.g., mannitol,<sup>952</sup> arabinose<sup>953</sup> or alkyl-glycerol,<sup>954</sup> which results in transient shrinkage of cerebrovascular endothelial cells with widening of the tight junctions to at least 20 nm and subsequent increased permeability of the BBB. This method has allowed the passage of ~40 nm hydrodynamic diameter<sup>955</sup> magnetite-dextran nanoparticles,<sup>956</sup> 70-90 nm diameter replication-defective adenovirus particles,<sup>957</sup> and even 150-200 nm diameter herpes simplex virus

---

<sup>951</sup> Rapoport SI, Hori M, Klatzo I. Testing of a hypothesis for osmotic opening of the blood-brain barrier. *Am J Physiol.* 1972 Aug;223(2):323-31; <http://www.ncbi.nlm.nih.gov/pubmed/5046750>. Bellavance MA, Blanchette M, Fortin D. Recent advances in blood-brain barrier disruption as a CNS delivery strategy. *AAPS J.* 2008;10(1):166-77; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2751463/>.

<sup>952</sup> Abbott NJ, Revest PA. Control of brain endothelial permeability. *Cerebrovasc Brain Metab Rev.* 1991 Spring;3(1):39-72; <http://www.ncbi.nlm.nih.gov/pubmed/2036300>.

<sup>953</sup> Rapoport SI. Osmotic opening of the blood-brain barrier: principles, mechanism, and therapeutic applications. *Cell Mol Neurobiol.* 2000 Apr;20(2):217-30; <http://www.ncbi.nlm.nih.gov/pubmed/10696511>.

<sup>954</sup> Lee HJ, Zhang Y, Pardridge WM. Blood-brain barrier disruption following the internal carotid arterial perfusion of alkyl glycerols. *J Drug Target.* 2002 Sep;10(6):463-7; <http://www.ncbi.nlm.nih.gov/pubmed/12575736>.

<sup>955</sup> Shen T, Weissleder R, Papisov M, Bogdanov A Jr, Brady TJ. Monocrystalline iron oxide nanocompounds (MION): physicochemical properties. *Magn Reson Med.* 1993 May;29(5):599-604; <http://www.ncbi.nlm.nih.gov/pubmed/8505895>.

<sup>956</sup> Muldoon LL, Nilaver G, Kroll RA, Pagel MA, Breakefield XO, Chiocca EA, Davidson BL, Weissleder R, Neuwelt EA. Comparison of intracerebral inoculation and osmotic blood-brain barrier disruption for delivery of adenovirus, herpesvirus, and iron oxide particles to normal rat brain. *Am J Pathol.* 1995 Dec;147(6):1840-51; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1869962/pdf/amjpathol00048-0324.pdf>. Rousseau V, Denizot B, Pouliquen D, Jallet P, Le Jeune JJ. Investigation of blood-brain barrier permeability to magnetite-dextran nanoparticles (MD3) after osmotic disruption in rats. *MAGMA.* 1997 Sep;5(3):213-22; <http://www.ncbi.nlm.nih.gov/pubmed/9351025>.

<sup>957</sup> Doran SE, Ren XD, Betz AL, Pagel MA, Neuwelt EA, Roessler BJ, Davidson BL. Gene expression from recombinant viral vectors in the central nervous system after blood-brain barrier disruption. *Neurosurgery.* 1995 May;36(5):965-70; <http://www.ncbi.nlm.nih.gov/pubmed/7791989>. Nilaver G, Muldoon LL, Kroll RA, Pagel MA, Breakefield XO, Davidson BL, Neuwelt EA. Delivery of herpesvirus and adenovirus to nude rat intracerebral tumors after osmotic blood-brain barrier disruption. *Proc Natl Acad Sci U S A.* 1995 Oct 10;92(21):9829-33; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC40896/>. Muldoon LL, Nilaver G, Kroll RA, Pagel MA, Breakefield XO, Chiocca EA, Davidson BL, Weissleder R, Neuwelt EA. Comparison of intracerebral inoculation and osmotic blood-brain barrier disruption for delivery of adenovirus, herpesvirus, and iron oxide particles to normal rat brain. *Am J Pathol.* 1995 Dec;147(6):1840-51; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1869962/pdf/amjpathol00048-0324.pdf>.

(HSV) particles<sup>958</sup> through the BBB endothelium and into the neuropil. Other chemicals such as sodium caprate<sup>959</sup> and sodium dodecyl sulfate surfactant<sup>960</sup> have also induced reversible BBB permeability. Fenart *et al*<sup>961</sup> notes that the customary drawback to methods that involve a systemic increase in BBB permeability is that there is poor specificity, with circulating blood compounds such as albumin gaining indiscriminant and pathological access to the brain. However, in the case of medical nanorobots these methods could be applied on a highly localized basis, followed by rapid entry in convoy formation.<sup>962</sup>

(2) **Acoustic and Thermal Disruption.** High intensity focused ultrasound (HIFU) or pulsed ultrasound employs acoustic energy to vibrate intravenously administered lipid-encased perfluorocarbon gas microbubbles near the target endothelium, whereupon the oscillating microbubbles create a mechanical stress on the endothelial tight junctions, causing them to open and admit therapeutic agents into the brain.<sup>963</sup> Similarly, localized heating,<sup>964</sup> vibrational

<sup>958</sup> Neuwelt EA, Pagel MA, Dix RD. Delivery of ultraviolet-inactivated 35S-herpesvirus across an osmotically modified blood-brain barrier. *J Neurosurg.* 1991 Mar;74(3):475-9; <http://www.ncbi.nlm.nih.gov/pubmed/1899695>. Muldoon LL, Nilaver G, Kroll RA, Pagel MA, Breakefield XO, Chiocca EA, Davidson BL, Weissleder R, Neuwelt EA. Comparison of intracerebral inoculation and osmotic blood-brain barrier disruption for delivery of adenovirus, herpesvirus, and iron oxide particles to normal rat brain. *Am J Pathol.* 1995 Dec;147(6):1840-51; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1869962/pdf/amjpathol00048-0324.pdf>. Nilaver G, Muldoon LL, Kroll RA, Pagel MA, Breakefield XO, Davidson BL, Neuwelt EA. Delivery of herpesvirus and adenovirus to nude rat intracerebral tumors after osmotic blood-brain barrier disruption. *Proc Natl Acad Sci U S A.* 1995 Oct 10;92(21):9829-33; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC40896/>.

<sup>959</sup> Preston E, Slinn J, Vinokourov I, Stanimirovic D. Graded reversible opening of the rat blood-brain barrier by intracarotid infusion of sodium caprate. *J Neurosci Methods.* 2008 Mar 15;168(2):443-9; <http://www.ncbi.nlm.nih.gov/pubmed/18155299>.

<sup>960</sup> Saija A, Princi P, Trombetta D, Lanza M, De Pasquale A. Changes in the permeability of the blood-brain barrier following sodium dodecyl sulphate administration in the rat. *Exp Brain Res.* 1997 Jul;115(3):546-51; <http://www.ncbi.nlm.nih.gov/pubmed/9262210>.

<sup>961</sup> Fenart L, Casanova A, Dehouck B, Duhem C, Slupek S, Cecchelli R, Betbeder D. Evaluation of effect of charge and lipid coating on ability of 60-nm nanoparticles to cross an *in vitro* model of the blood-brain barrier. *J Pharmacol Exp Ther.* 1999 Dec;291(3):1017-22; <http://jpet.aspetjournals.org/content/291/3/1017.long>.

<sup>962</sup> Freitas RA Jr. *Nanomedicine, Volume IIA: Biocompatibility.* Landes Bioscience, Georgetown, TX, 2003, Section 15.5.2.3 "Nanorobot Convoy Formation"; <http://www.nanomedicine.com/NMIIA/15.5.2.3.htm>.

<sup>963</sup> Hynynen K, McDannold N, Vykhodtseva N, Jolesz FA. Noninvasive MR imaging-guided focal opening of the blood-brain barrier in rabbits. *Radiology* 2001;220(3):640-646; <http://pubs.rsna.org/doi/full/10.1148/radiol.2202001804>. Sheikov N, McDannold N, Sharma S, Hynynen K. Effect of focused ultrasound applied with an ultrasound contrast agent on the tight junctional integrity of the brain microvascular endothelium. *Ultrasound Med Biol.* 2008;34(7):1093-1104; <http://dx.doi.org/10.1016/j.ultrasmedbio.2007.12.015>. Konofagou EE, Tung YS, Choi J, Deffieux T, Baseri B, Vlachos F. Ultrasound-induced blood-brain barrier opening. *Curr Pharm Biotechnol.* 2012

agitation of magnetic particles,<sup>965</sup> and more recent techniques such as laser interstitial thermotherapy, nonthermal irreversible electroporation,<sup>966</sup> and pulsed electric fields<sup>967</sup> can also temporarily open the BBB and allow passage of small particles. These methods could be applied locally by medical nanorobots with minimal non-local effects.

(3) **Tight Junction Signaling Pathways.** The BBB is disrupted during diseases such as experimental allergic encephalomyelitis,<sup>968</sup> HIV encephalitis,<sup>969</sup> Lyme disease,<sup>970</sup> and multiple

Jun;13(7):1332-45; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4038976/>. Leinenga G, Götz J. Scanning ultrasound removes amyloid- $\beta$  and restores memory in an Alzheimer's disease mouse model. *Sci Transl Med.* 2015 Mar 11;7(278):278ra33; <http://www.ncbi.nlm.nih.gov/pubmed/25761889>. Carpentier A, Canney M, Vignot A, Reina V, Beccaria K, Horodyckid C, Karachi C, Leclercq D, Lafon C, Chapelon JY, Capelle L, Cornu P, Sanson M, Hoang-Xuan K, Delattre JY, Idbah A. Clinical trial of blood-brain barrier disruption by pulsed ultrasound. *Sci Transl Med.* 2016 Jun 15;8(343):343re2; <http://stm.sciencemag.org/content/8/343/343re2.full>. See also: "Blood-brain barrier opened non-invasively for the first time in humans, using focused ultrasound," 10 Nov 2015; <http://www.kurzweilai.net/blood-brain-barrier-opened-non-invasively-for-the-first-time-in-humans-using-focused-ultrasound>.

<sup>964</sup> Dokladny K, Moseley PL, Ma TY. Physiologically relevant increase in temperature causes an increase in intestinal epithelial tight junction permeability. *Am J Physiol Gastrointest Liver Physiol.* 2006;290(2):G204-G212; <http://ajpgi.physiology.org/content/ajpgi/290/2/G204.full.pdf>. Kiyatkin EA, Sharma HS. Permeability of the blood-brain barrier depends on brain temperature. *Neuroscience* 2009;161(3):926-939; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2694729/>.

<sup>965</sup> Tabatabaei SN, Girouard H, Carret AS, Martel S. Remote control of the permeability of the blood-brain barrier by magnetic heating of nanoparticles: A proof of concept for brain drug delivery. *J Control Release.* 2015 May 28;206:49-57; <http://www.ncbi.nlm.nih.gov/pubmed/25724273>.

<sup>966</sup> Rodriguez A, Tatter SB, Debinski W. Neurosurgical techniques for disruption of the blood-brain barrier for glioblastoma treatment. *Pharmaceutics.* 2015 Aug 3;7(3):175-87; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4588193/>.

<sup>967</sup> Arena CB, Garcia PA, Sano MB, Olson JD, Rogers-Cotrone T, Rossmeisl JH Jr, Davalos RV. Focal blood-brain-barrier disruption with high-frequency pulsed electric fields. *Technology* 2014;02:206; <http://www.worldscientific.com/doi/abs/10.1142/S2339547814500186>. See also: <http://www.kurzweilai.net/blood-brain-barrier-disruption-with-high-frequency-pulsed-electric-fields>.

<sup>968</sup> Merodio M, Irache JM, Eclancher F, Mirshahi M, Villarroya H. Distribution of albumin nanoparticles in animals induced with the experimental allergic encephalomyelitis. *J Drug Target.* 2000;8(5):289-303; <http://www.ncbi.nlm.nih.gov/pubmed/11328657>. Calvo P, Gouritin B, Villarroya H, Eclancher F, Giannavola C, Klein C, Andreux JP, Couvreur P. Quantification and localization of PEGylated polycyanoacrylate nanoparticles in brain and spinal cord during experimental allergic encephalomyelitis in the rat. *Eur J Neurosci.* 2002 Apr;15(8):1317-26; <http://www.ncbi.nlm.nih.gov/pubmed/11994126>.

<sup>969</sup> Persidsky Y, Stins M, Way D, Witte MH, Weinand M, Kim KS, Bock P, Gendelman HE, Fiala M. A model for monocyte migration through the blood-brain barrier during HIV-1 encephalitis. *J Immunol.* 1997 Apr 1;158(7):3499-510; <http://www.ncbi.nlm.nih.gov/pubmed/9120312>.

sclerosis in which >1000-nm diameter T cells and macrophages invade neural tissue through BBB tight junctions.<sup>971</sup> During bacterial meningitis, bloodborne bacteria can induce tight junction disruption, allowing these 200-300 nm pathogens to cross the BBB and enter the brain.<sup>972</sup> Monocytes<sup>973</sup> and neutrophils<sup>974</sup> also enter the brain without destroying the tight junctional integrity of the BBB.

Similarly, nanorobots could purposely and locally manipulate the signaling pathways involved in BBB tight junction regulation,<sup>975</sup> possibly commanding junctional gaps to open or close at need. For example, ICAM-1-mediated signaling in brain endothelial cells is known to be a crucial regulatory step in the process of 6-12 micron lymphocyte recruitment and migration through the BBB.<sup>976</sup> Tight junction structure is apparently altered dynamically during BBB diapedesis by 8-

---

<sup>970</sup> Grab DJ, Perides G, Dumler JS, Kim KJ, Park J, Kim YV, Nikolskaia O, Choi KS, Stins MF, Kim KS. *Borrelia burgdorferi*, host-derived proteases, and the blood-brain barrier. *Infect Immun*. 2005 Feb;73(2):1014-22; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC546937/>.

<sup>971</sup> Markovic-Plese S, McFarland HF. Immunopathogenesis of the multiple sclerosis lesion. *Curr Neurol Neurosci Rep*. 2001 May;1(3):257-62; <http://www.ncbi.nlm.nih.gov/pubmed/11898527>.

<sup>972</sup> Quagliarello VJ, Ma A, Stukenbrok H, Palade GE. Ultrastructural localization of albumin transport across the cerebral microvasculature during experimental meningitis in the rat. *J Exp Med*. 1991 Sep 1;174(3):657-72; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2118932/>. Kim BJ, Hancock BM, Bermudez A, Del Cid N, Reyes E, van Sorge NM, Lauth X, Smurthwaite CA, Hilton BJ, Stotland A, Banerjee A, Buchanan J, Wolkowicz R, Traver D, Doran KS. Bacterial induction of Snail1 contributes to blood-brain barrier disruption. *J Clin Invest*. 2015 Jun;125(6):2473-83; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4497739/>.

<sup>973</sup> Giri R, Shen Y, Stins M, Du Yan S, Schmidt AM, Stern D, Kim KS, Zlokovic B, Kalra VK. beta-amyloid-induced migration of monocytes across human brain endothelial cells involves RAGE and PECAM-1. *Am J Physiol Cell Physiol*. 2000 Dec;279(6):C1772-81; <http://ajpcell.physiology.org/content/279/6/C1772.long>.

<sup>974</sup> Burns AR, Bowden RA, MacDonell SD, Walker DC, Odebunmi TO, Donnachie EM, Simon SI, Entman ML, Smith CW. Analysis of tight junctions during neutrophil transendothelial migration. *J Cell Sci*. 2000 Jan;113 ( Pt 1):45-57; <http://jcs.biologists.org/content/113/1/45.long>.

<sup>975</sup> Wolburg H, Lippoldt A. Tight junctions of the blood-brain barrier: development, composition and regulation. *Vascul Pharmacol*. 2002 Jun;38(6):323-37; <http://loyce2008.free.fr/MENINGO/Tight%20Junctions/Wolburg,%20Tight%20junctions%20of%20the%20BBB%20devlpmt%20compo%20regul,%20Vascular%20Pharmaco%202002.pdf>.

<sup>976</sup> Greenwood J, Etienne-Manneville S, Adamson P, Couraud PO. Lymphocyte migration into the central nervous system: implication of ICAM-1 signalling at the blood-brain barrier. *Vascul Pharmacol*. 2002 Jun;38(6):315-22; <http://www.ncbi.nlm.nih.gov/pubmed/12529926>.

10 micron leukocytes,<sup>977</sup> and the signaling molecules that regulate the BBB are now beginning to be understood,<sup>978</sup> including claudin-5<sup>979</sup> and occludin.<sup>980</sup>

A great variety of pathological conditions and agents are known to cause disruption of the BBB, including neurological conditions<sup>981</sup> that we aim to treat such as ALS. Some bacteria gain access to the brain by releasing neurotoxins such as pneumolysin<sup>982</sup> which have a direct toxic effect on brain endothelial cells and their tight junctions.<sup>983</sup> Many of these disruptive mechanisms can be safely adapted for use and applied locally by nanorobots that require passage through the BBB.

**(4) Exploit Microscopic Gaps in BBB.** The BBB is not a structurally perfect barrier. Gaps and imperfections of various sizes are naturally present. Nanorobots seeking entry to the neuropil from the bloodstream can search out and exploit these randomly-placed natural junctional gaps. BBB ultrastructure has been studied for decades<sup>984</sup> and 0.5-micron perijunctional gaps have been observed,<sup>985</sup> but no precise estimate of the number density or distribution of

---

<sup>977</sup> Winger RC, Koblinski JE, Kanda T, Ransohoff RM, Muller WA. Rapid remodeling of tight junctions during paracellular diapedesis in a human model of the blood-brain barrier. *J Immunol.* 2014 Sep 1;193(5):2427-37; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4138548/>.

<sup>978</sup> Bauer HC, Krizbai IA, Bauer H, Traweger A. “You Shall Not Pass”-tight junctions of the blood brain barrier. *Front Neurosci.* 2014 Dec 3;8:392; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4253952/>.

<sup>979</sup> Campbell M, Hanrahan F, Gobbo OL, Kelly ME, Kiang AS, Humphries MM, Nguyen AT, Ozaki E, Keaney J, Blau CW, Kerskens CM, Cahalan SD, Callanan JJ, Wallace E, Grant GA, Doherty CP, Humphries P. Targeted suppression of claudin-5 decreases cerebral oedema and improves cognitive outcome following traumatic brain injury. *Nat Commun.* 2012 May 22;3:849; <http://www.ncbi.nlm.nih.gov/pubmed/22617289>

<sup>980</sup> Tai LM, Holloway KA, Male DK, Loughlin AJ, Romero IA. Amyloid-beta-induced occludin down-regulation and increased permeability in human brain endothelial cells is mediated by MAPK activation. *J Cell Mol Med.* 2010 May;14(5):1101-12; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3822747/>.

<sup>981</sup> Rosenberg GA. Neurological diseases in relation to the blood-brain barrier. *J Cereb Blood Flow Metab.* 2012 Jul;32(7):1139-51; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3390801/>.

<sup>982</sup> Zysk G, Schneider-Wald BK, Hwang JH, Bejo L, Kim KS, Mitchell TJ, Hakenbeck R, Heinz HP. Pneumolysin is the main inducer of cytotoxicity to brain microvascular endothelial cells caused by *Streptococcus pneumoniae*. *Infect Immun.* 2001 Feb;69(2):845-52; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC97961/>.

<sup>983</sup> van Sorge NM, Doran KS. Defense at the border: the blood-brain barrier versus bacterial foreigners. *Future Microbiol.* 2012 Mar;7(3):383-94; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3589978/>.

<sup>984</sup> Stewart PA, Magliocco M, Hayakawa K, Farrell CL, Del Maestro RF, Girvin J, Kaufmann JC, Vinters HV, Gilbert J. A quantitative analysis of blood-brain barrier ultrastructure in the aging human. *Microvasc Res.* 1987 Mar;33(2):270-82; <http://www.ncbi.nlm.nih.gov/pubmed/3587079>.

<sup>985</sup> Fraser PA, Dallas AD. Permeability of disrupted cerebral microvessels in the frog. *J Physiol.* 1993 Feb;461:619-32; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1175276/>.

micron-size gaps throughout the entire BBB network of the human brain is yet available in the literature. One rat experiment<sup>986</sup> found that in control animals 0.4%-0.6% of circulating albumin appeared in the subendothelial space and in the basement membrane of control animals prior to osmotic disruption (rising to 56%, 30 minutes after osmotic disruption), so some number of gaps of some size clearly exist. Another study<sup>987</sup> reports 0.5%-2.4% BBB penetration by various peptide molecules prior to BBB disruption. Hypertension can produce measurably leaky venules<sup>988</sup> and other leaks in the BBB,<sup>989</sup> and the foreign body response due to a small implant placed in the brain causes the BBB near the implant to become leaky enough to allow spherical nanoparticles up to 500 nm in diameter to pass.<sup>990</sup> The BBB is disrupted during aging, dementia, stroke, and multiple sclerosis, and the capillaries in brain tumors exhibit a more leaky BBB.<sup>991</sup> A key product of Alzheimer's disease,  $\beta$ -amyloid, is also thought to have damaging effects on the vasculature resulting in microbleeds and symptomatic intracerebral hemorrhage.<sup>992</sup>

If a population of  $N_{\text{bot}}$  nanorobots of (assumed cubical) volume  $V_{\text{bot}}$  transit in convoy at velocity  $v_{\text{bot}}$  through randomly-placed  $>V_{\text{bot}}^{2/3}$ -area holes in the BBB (i.e., large enough to admit one nanorobot at a time) of collective hole area  $A_{\text{total}}$  with the objective of infusing the entire nanorobot population into the neuropil in  $t_{\text{infusion}}$  seconds, then  $v_{\text{bot}} = N_{\text{bot}} V_{\text{bot}} / A_{\text{total}} t_{\text{infusion}}$ . Taking  $N_{\text{bot}} = 10^9$  nanorobots,  $V_{\text{bot}} = 1 \mu\text{m}^3$ , and  $t_{\text{infusion}} = 1000 \text{ sec}$  ( $\sim 0.3 \text{ hr}$ ), then assuming a reasonable transit speed of  $v_{\text{bot}} = 10 \mu\text{m}/\text{sec}$ , the total area of all  $\sim$ micron-size holes need only be

---

<sup>986</sup> Vorbrodt AW, Dobrogowska DH, Tarnawski M, Lossinsky AS. A quantitative immunocytochemical study of the osmotic opening of the blood-brain barrier to endogenous albumin. *J Neurocytol.* 1994 Dec;23(12):792-800; <http://www.ncbi.nlm.nih.gov/pubmed/7897443>.

<sup>987</sup> Ermisch A, Rühle HJ, Landgraf R, Hess J. Blood-brain barrier and peptides. *J Cereb Blood Flow Metab.* 1985 Sep;5(3):350-7; <http://www.nature.com/jcbfm/journal/v5/n3/pdf/jcbfm198549a.pdf>.

<sup>988</sup> Mayhan WG, Faraci FM, Heistad DD. Disruption of the blood-brain barrier in cerebrum and brain stem during acute hypertension. *Am J Physiol.* 1986 Dec;251(6 Pt 2):H1171-5; <http://www.ncbi.nlm.nih.gov/pubmed/3098113>.

<sup>989</sup> Kemper TL, Blatt GJ, Killiany RJ, Moss MB. Neuropathology of progressive cognitive decline in chronically hypertensive rhesus monkeys. *Acta Neuropathol.* 2001 Feb;101(2):145-53; <http://www.ncbi.nlm.nih.gov/pubmed/11271369>.

<sup>990</sup> Sawyer AJ, Kyriakides TR. Nanoparticle-based evaluation of blood-brain barrier leakage during the foreign body response. *J Neural Eng.* 2013 Feb;10(1):016013; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3602133/>.

<sup>991</sup> Heye AK, Culling RD, Valdés Hernández Mdel C, Thrippleton MJ, Wardlaw JM. Assessment of blood-brain barrier disruption using dynamic contrast-enhanced MRI. A systematic review. *Neuroimage Clin.* 2014 Sep 10;6:262-74; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4215461/>.

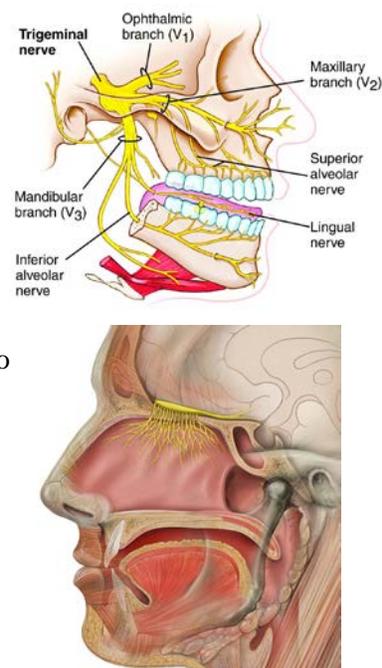
<sup>992</sup> Samarasekera N, Smith C, Al-Shahi Salman R. The association between cerebral amyloid angiopathy and intracerebral haemorrhage: systematic review and meta-analysis. *J Neurol Neurosurg Psychiatry.* 2012 Mar;83(3):275-81; <http://www.ncbi.nlm.nih.gov/pubmed/22056966>.

$A_{\text{total}} = 10^{-7} \text{ m}^2 (= 10^5 \text{ } \mu\text{m}^2)$  or just  $\sim 0.000001\%$  of the total BBB surface area of  $\sim 9.3 \text{ m}^2$ .<sup>993</sup> If the actual total area of micron-size holes  $A_{\text{total}}$  is less than  $10^5 \text{ } \mu\text{m}^2$ , the transit velocity  $v_{\text{bot}}$  or the infusion time  $t_{\text{infusion}}$  must be increased as required. Single white cell diapedesis through a blood vessel wall typically requires  $>200 \text{ sec}$  (implies  $<0.01 \text{ } \mu\text{m}/\text{sec}$ ), but if the opening already exists the nanorobots should require only milliseconds (implies  $>100 \text{ } \mu\text{m}/\text{sec}$ ) to pass in convoy.<sup>994</sup>

**(5) Exploit Unbarriered Pathways.** A small number of regions in the brain do not have a blood-brain barrier, or have a vascular endothelium that lacks tight junctions.

Most notably, there are nerve cells (i.e., the maxillary branch of the trigeminal nerve; image, right) that initiate in the brain and terminate in the nasal cavity at the olfactory neuroepithelium or respiratory epithelium (image, below right). These are the only externally exposed portions of the central nervous system and therefore represent the most direct method of noninvasive entry into the brain that bypasses the BBB.<sup>995</sup> A recent experiment demonstrated the intranasal transfer of 114 nm diameter chitosan-coated nanoparticles into mouse brain.<sup>996</sup>

The intranasal delivery method can even be used to transport large mesenchymal stem cells, 8-20 microns in size, into the brain with transit times ranging from a few hours to a few days,<sup>997</sup> entirely



<sup>993</sup> Freitas RA Jr. Nanomedicine, Volume IIA: Biocompatibility. Landes Bioscience, Georgetown, TX, 2003, Section 15.3.6.5 "Biocompatibility with Neural Cells"; <http://www.nanomedicine.com/NMIIA/15.3.6.5.htm>.

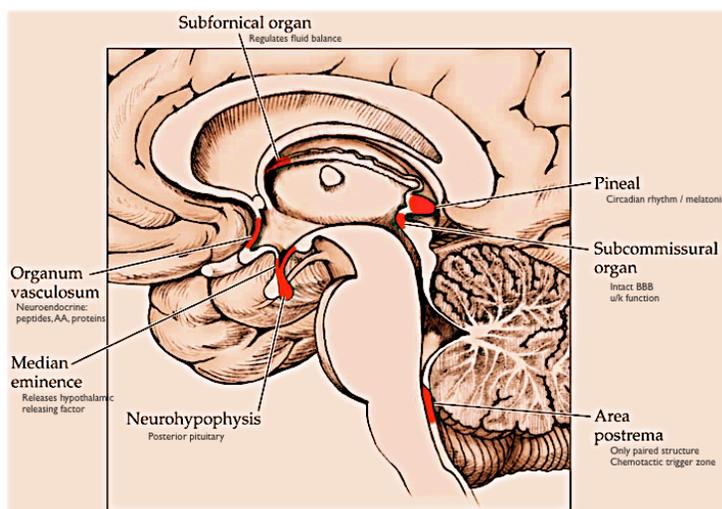
<sup>994</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 9.4.4.1 "Nanorobot Diapedesis"; <http://www.nanomedicine.com/NMI/9.4.4.1.htm>.

<sup>995</sup> Mittal D, Ali A, Md S, Baboota S, Sahni JK, Ali J. Insights into direct nose to brain delivery: current status and future perspective. Drug Deliv. 2014 Mar;21(2):75-86; <http://www.ncbi.nlm.nih.gov/pubmed/24102636>.

<sup>996</sup> Gartzandia O, Herran E, Pedraz JL, Carro E, Igartua M, Hernandez RM. Chitosan coated nanostructured lipid carriers for brain delivery of proteins by intranasal administration. Colloids Surf B Biointerfaces. 2015 Oct 1;134:304-13; <http://www.ncbi.nlm.nih.gov/pubmed/26209963>.

<sup>997</sup> Danielyan L, Schäfer R, von Ameln-Mayerhofer A, Buadze M, Geisler J, Klopfer T, Burkhardt U, Proksch B, Verleysdonk S, Ayturan M, Buniatian GH, Gleiter CH, Frey WH 2nd. Intranasal delivery of cells to the brain. Eur J Cell Biol. 2009 Jun;88(6):315-24; <http://www.ncbi.nlm.nih.gov/pubmed/19324456/>. Danielyan L, Schäfer R, von Ameln-Mayerhofer A, Bernhard F, Verleysdonk S, Buadze M, Lourhmati A, Klopfer T, Schaumann F, Schmid B, Koehle C, Proksch B, Weissert R, Reichardt HM, van den Brandt J, Buniatian GH, Schwab M, Gleiter CH, Frey WH 2nd. Therapeutic efficacy of intranasally delivered mesenchymal stem cells in a rat model of Parkinson disease. Rejuvenation Res. 2011 Feb;14(1):3-16; <http://www.ncbi.nlm.nih.gov/pubmed/21291297/>. Reitz M, Demestre M, Sedlacik J, Meissner H, Fiehler J, Kim SU, Westphal M, Schmidt NO. Intranasal delivery

bypassing the BBB. This method appears sufficient to accommodate the passage of a medical nanorobot measuring several microns in size that is attempting to enter the brain. Drug administration via nasal mucosal grafts has also been investigated.<sup>998</sup>



Also bypassing the BBB are the **circumventricular organs** (CVOs) which are small structures bordering the 3rd and 4th ventricles<sup>999</sup> of the brain. CVOs have an extensive vasculature with fenestrated capillaries but their vascular endothelium lacks tight junctions, hence are “leaky” compared to BBB. Nanorobots should have relatively easy access to CVOs from the bloodstream, as well as from the cerebrospinal fluid or CSF (image, left; and see Section 4.3.2).

CVOs can be classified into sensory and secretory organs. Sensory CVOs include the area postrema, the subfornical organ, and the vascular organ of the lamina terminalis. These have the ability to sense plasma molecules and then pass that information into other regions of the brain, thus providing direct information to the autonomic nervous system from the systemic circulation. Secretory CVOs include the subcommissural organ, the posterior pituitary or neurohypophysis, the pineal gland, and the median eminence. These organs secrete hormones and glycoproteins into the peripheral vascular system using feedback from both the brain environment and external stimuli.

---

of neural stem/progenitor cells: a noninvasive passage to target intracerebral glioma. *Stem Cells Transl Med.* 2012 Dec;1(12):866-73; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3659670/>. Balyasnikova IV, Prasol MS, Ferguson SD, Han Y, Ahmed AU, Gutova M, Tobias AL, Mustafi D, Rincón E, Zhang L, Aboody KS, Lesniak MS. Intranasal delivery of mesenchymal stem cells significantly extends survival of irradiated mice with experimental brain tumors. *Mol Ther.* 2014 Jan;22(1):140-8; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3978787/>.

<sup>998</sup> Bleier BS, Kohman RE, Feldman RE, Ramanlal S, Han X. Permeabilization of the blood-brain barrier via mucosal engrafting: implications for drug delivery to the brain. *PLoS One.* 2013 Apr 24;8(4):e61694; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3634848/>. Bleier BS, Kohman RE, Guerra K, Nocera AL, Ramanlal S, Kocharyan AH, Curry WT, Han X. Heterotopic Mucosal Grafting Enables the Delivery of Therapeutic Neuropeptides Across the Blood Brain Barrier. *Neurosurgery.* 2015 Sep 8; <http://www.ncbi.nlm.nih.gov/pubmed/26352099>. See also: <http://www.kurzweilai.net/a-drug-delivery-technique-to-bypass-the-blood-brain-barrier>.

<sup>999</sup> The ventricular system is a set of four interconnected cavities (ventricles) in the brain where cerebrospinal fluid (CSF) is produced (see Section 4.3.2).

(6) **Diapedesis with Cytocariage.** The commandeering of natural motile cells by medical nanorobots, known as cytocariage,<sup>1000</sup> offers another alternative mode of *in vivo* transport. During cytocariage, one or more medical nanorobots may enter a motile cell, ride or steer the cell to a desired destination inside the human body, then vacate the cell upon arrival. In particular, a variety of mesenchymal stem cells<sup>1001</sup> and leukocytes<sup>1002</sup> including monocytes<sup>1003</sup> and neutrophils<sup>1004</sup> can engage in paracellular diapedesis through the blood-brain barrier without destroying the tight junctional integrity of the BBB. Leukocytes typically have cell volume on the order of  $\sim 1000 \mu\text{m}^3$ ,<sup>1005</sup> so the internal presence of a few nanorobots of volume  $1\text{-}10 \mu\text{m}^3$  will not be unduly intrusive<sup>1006</sup> and should not impair the diapedesic movement of the cell. Once through the BBB, the nanorobots can exit the transiting cell and proceed to their programmed destinations.

(7) **Direct Cytopenetration.** Even in the complete absence of all of the aforementioned methods, properly mission-designed active motile nanorobots can employ a combination of cytopenetration,<sup>1007</sup> *in cyto* locomotion,<sup>1008</sup> and hystonation<sup>1009</sup> through the vascular endothelium of the BBB to achieve ready access to the neuropil.

---

<sup>1000</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 9.4.7 “Cytocariage”; <http://www.nanomedicine.com/NMI/9.4.7.htm>.

<sup>1001</sup> Lin MN, Shang DS, Sun W, Li B, Xu X, Fang WG, Zhao WD, Cao L, Chen YH. Involvement of PI3K and ROCK signaling pathways in migration of bone marrow-derived mesenchymal stem cells through human brain microvascular endothelial cell monolayers. *Brain Res.* 2013 Jun 4;1513:1-8; <http://www.ncbi.nlm.nih.gov/pubmed/23548598>.

<sup>1002</sup> Winger RC, Koblinski JE, Kanda T, Ransohoff RM, Muller WA. Rapid remodeling of tight junctions during paracellular diapedesis in a human model of the blood-brain barrier. *J Immunol.* 2014 Sep 1;193(5):2427-37; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4138548/>.

<sup>1003</sup> Giri R, Shen Y, Stins M, Du Yan S, Schmidt AM, Stern D, Kim KS, Zlokovic B, Kalra VK. beta-amyloid-induced migration of monocytes across human brain endothelial cells involves RAGE and PECAM-1. *Am J Physiol Cell Physiol.* 2000 Dec;279(6):C1772-81; <http://ajpcell.physiology.org/content/279/6/C1772.long>.

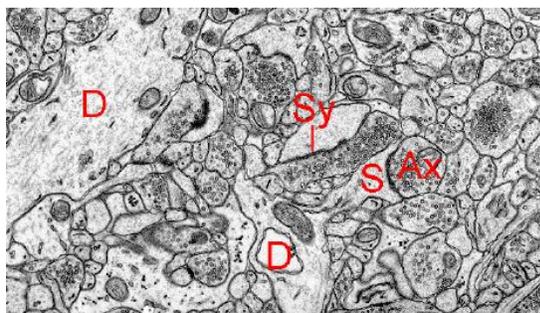
<sup>1004</sup> Burns AR, Bowden RA, MacDonell SD, Walker DC, Odebunmi TO, Donnachie EM, Simon SI, Entman ML, Smith CW. Analysis of tight junctions during neutrophil transendothelial migration. *J Cell Sci.* 2000 Jan;113 ( Pt 1):45-57; <http://jcs.biologists.org/content/113/1/45.long>.

<sup>1005</sup> Freitas RA Jr. Nanomedicine, Volume IIA: Biocompatibility. Landes Bioscience, Georgetown, TX, 2003, Section 15.4.3.1 “Phagocytes, Phagocytosis, and the RES”; <http://www.nanomedicine.com/NMIIA/15.4.3.1.htm>.

<sup>1006</sup> Freitas RA Jr. Nanomedicine, Volume IIA: Biocompatibility. Landes Bioscience, Georgetown, TX, 2003, Section 15.6.3.6 “Intracellular Nanorobot Intrusiveness”; <http://www.nanomedicine.com/NMIIA/15.6.3.6.htm>.

<sup>1007</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 9.4.5 “Cytopenetration”; <http://www.nanomedicine.com/NMI/9.4.5.htm>.

This may likely be necessary in any case because the neuropil is so tightly packed with cells (image, right).<sup>1010</sup>



(8) **Microscale Docking Modules.** This method entails implanting in the BBB wall a docking module which remains closed to all normal biochemical traffic, but which can be operated (e.g., triggered to open) by a nanorobot that requires egress. This module could be:

- (a) a purely biologic injectable, analogous to a bioengineered superporin molecule<sup>1011</sup> or the complement C9 monomers that self-assemble to form the membrane attack complex (MAC),<sup>1012</sup> only much larger and possessing an internal gating structure of some kind;
- (b) a genetic modification to the human genome in which the genes needed to naturally manufacture the relevant protein components (that self-assemble to make the docking module) described in (a) are permanently added to the brain endothelial cell genome of the patient; or
- (c) a basic nanomechanical spring-loaded gate system made of diamondoid materials, whose biocompatibility may be comparable to the closely related resident transmembrane penetrators.<sup>1013</sup> The system could operate like a simple one-way mechanical valve that the nanorobot pushes open and passes through, and which then springs shut or is pushed closed by the nanorobot after passage. The patient would have to have these installed beforehand, but once installed, they could be useful for many subsequent procedures involving all sorts of nanorobots.

Embedding  $\sim 10^6$  such docking modules – each of area  $\sim 10 \mu\text{m}^2$ , total area  $\sim 10 \text{mm}^2$  (i.e., a negligible fraction of the  $9,300,000 \text{mm}^2$  area of the BBB) in the BBB surface would allow 30

<sup>1008</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 9.4.6 “In Cyto Locomotion”; <http://www.nanomedicine.com/NMI/9.4.6.htm>.

<sup>1009</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 9.4.4 “Histone acetylation”; <http://www.nanomedicine.com/NMI/9.4.4.htm>.

<sup>1010</sup> <http://www.bio.davidson.edu/courses/genomics/method/brainparts.html>

<sup>1011</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999; <http://www.nanomedicine.com/NMI/10.4.1.4.htm#p8>.

<sup>1012</sup> Freitas RA Jr. Nanomedicine Vol. IIA: Biocompatibility. Landes Bioscience, Georgetown, TX, 2003, Section 15.2.3.2; <http://www.nanomedicine.com/NMIIA/15.2.3.2.htm>

<sup>1013</sup> Freitas RA Jr. Nanomedicine Vol. IIA: Biocompatibility. Landes Bioscience, Georgetown, TX, 2003, Section 15.5.7.2.3; <http://www.nanomedicine.com/NMIIA/15.5.7.2.3.htm>.

billion nanorobots to pass in 1 hour, assuming 10 nanorobots per second per module. Increasing the docking module area allocation by 1-2 orders or magnitude should be achievable if necessary.

**(9) Dedicated Transit Port.** More efficient would be to install a permanent port, as a permanent diamondoid implant, with a large enough opening to pass nanorobots *en masse*, quickly and efficiently, through the BBB. Consider a transmembrane penetrator with a  $3 \text{ mm}^2$  opening and a  $1 \text{ cm}^3$  collection depot on the bloodstream side of the membrane, geometrically arranged for convenience of mass transfer. Nanorobots arrive at the depot and are admitted until the depot is full. When full, the depot dilates its transmembrane aperture and squirts the depot contents through the BBB. The aperture closes and the depot resumes accumulating new arrivals. At a 20% packing density of  $1 \text{ }\mu\text{m}^3$  nanorobots inside the depot ( $\sim 0.2 \times 10^{12}$  nanorobots when full), this gives a viscosity of the particle suspension of about two times normal plasma for rigid spheres,<sup>1014</sup> or  $\sim 2 \times 10^{-3} \text{ kg/m-sec}$ . Using Poiseuille's Law<sup>1015</sup> and assuming a depot discharge flow velocity of 1 cm/sec and a transfer distance of 1 mm (1000  $\mu\text{m}$ ) through an egress aperture hole of radius  $\sim 1 \text{ mm}$ ,  $\sim 1 \text{ cm}^3$  of fluid may be transferred for a negligible pumping energy cost of  $\sim 5000 \text{ pW}$  in a pumping time of  $\sim 0.1 \text{ sec}$ , providing a gross egress rate of  $10^{12}$  nanorobots/sec during the discharge cycle.

The net egress rate is much slower, limited by the time required for a depot-full of nanorobots to make their way to the depot site, and then to enter it. If the  $4.84 \text{ cm}^2$  surface of the depot (radius 0.62 cm, assumed spherical) is on average 1% covered with arriving nanorobots at any time during the egress (giving a steady total of  $\sim 4$  million nanorobots on the depot surface and a mean center-to-center distance between them of 11  $\mu\text{m}$  on the depot surface) and they are transferred through the depot wall into the depot interior space within  $\sim 1$  second after arrival, then  $14 \times 10^9$  nanorobots can be loaded into the depot interior every hour. A 0.2 terabot dosage would then require  $\sim 14$  hours to transit from the bloodstream. Increasing the collection surface area improves the depot-loading times. If the arriving nanorobots can traverse the outer surface of the depot, seeking entrance to it, at a speed of 1 cm/sec, then a 1 second time budget allows each nanorobot to travel 1000  $\mu\text{m}$ , or  $\sim 100$  times their mean separation distance of 11  $\mu\text{m}$ , before obtaining entry, which seems sufficient. Note also that a mean separation distance of 11  $\mu\text{m}$  would equate to a mean number density of  $\sim 10^9$  nanorobots/ $\text{cm}^3$ , which equates to a  $\sim 4$  terabot dose in  $5400 \text{ cm}^3$  of blood, so the above 1% coverage figure is of a plausible order of magnitude, at least at the start of the transit process. After that, the nanorobots can concentrate themselves preferentially in the BBB due to their mobility – in principle, all the nanorobots present in the bloodstream could “precipitate out” during a single pass through the BBB, e.g., in a circulation time of just  $\sim 60$  seconds.

**(10) Direct Injection into the Brain.** As a last resort, a sharp needle or microcatheter can be passed directly through the skull or entirely through soft tissues (to avoid skull

---

<sup>1014</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999; <http://www.nanomedicine.com/NMI/Figures/9.13.jpg>.

<sup>1015</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999; <http://www.nanomedicine.com/NMI/9.2.5.htm>.

penetration), terminating precisely inside the neuropil or specialized tissue masses and allowing medical nanorobots to ingress or egress via that route. In one experiment, 90 nm spherical nanoparticles injected directly into mouse hippocampus bypassed the BBB and found their way into dentate granule cells, microglia, and neurons and were found in both intracellular and extracellular locations.<sup>1016</sup> Intrahippocampal infusion is a fairly popular technique in studies of anxiety,<sup>1017</sup> epilepsy,<sup>1018</sup> and Alzheimer's disease.<sup>1019</sup> Direct injection of bioactive substances<sup>1020</sup> into a variety of target sites in animal brains including, e.g., hypothalamus,<sup>1021</sup> striatum,<sup>1022</sup> sensorimotor cortex,<sup>1023</sup> lateral septum,<sup>1024</sup> and posterior insular cortex<sup>1025</sup> has also been reported.

---

<sup>1016</sup> Härtig W, Paulke BR, Varga C, Seeger J, Harkany T, Kacza J. Electron microscopic analysis of nanoparticles delivering thioflavin-T after intrahippocampal injection in mouse: implications for targeting beta-amyloid in Alzheimer's disease. *Neurosci Lett.* 2003 Feb 27;338(2):174-6; [http://www.researchgate.net/profile/Bernd-Reiner-Paulke/publication/10918856\\_Electron\\_microscopic\\_analysis\\_of\\_nanoparticles\\_delivering\\_thioflavin-T\\_after\\_intrahippocampal\\_injection\\_in\\_mouse\\_implications\\_for\\_targeting\\_beta-amyloid\\_in\\_Alzheimer's\\_disease/links/545c8a7a0cf2f1dbc4b84.pdf](http://www.researchgate.net/profile/Bernd-Reiner-Paulke/publication/10918856_Electron_microscopic_analysis_of_nanoparticles_delivering_thioflavin-T_after_intrahippocampal_injection_in_mouse_implications_for_targeting_beta-amyloid_in_Alzheimer's_disease/links/545c8a7a0cf2f1dbc4b84.pdf)

<sup>1017</sup> Engin E, Treit D. The role of hippocampus in anxiety: intracerebral infusion studies. *Behav Pharmacol.* 2007 Sep;18(5-6):365-74; <http://www.ncbi.nlm.nih.gov/pubmed/17762507>.

<sup>1018</sup> Bozzi Y, Costantin L, Antonucci F, Caleo M. Action of botulinum neurotoxins in the central nervous system: antiepileptic effects. *Neurotox Res.* 2006 Apr;9(2-3):197-203; <http://www.ncbi.nlm.nih.gov/pubmed/16785118>.

<sup>1019</sup> McLarnon JG, Ryu JK. Relevance of abeta1-42 intrahippocampal injection as an animal model of inflamed Alzheimer's disease brain. *Curr Alzheimer Res.* 2008 Oct;5(5):475-80; <http://www.ncbi.nlm.nih.gov/pubmed/18855589>. McLarnon JG. Correlated inflammatory responses and neurodegeneration in peptide-injected animal models of Alzheimer's disease. *Biomed Res Int.* 2014;2014:923670; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4005142/>.

<sup>1020</sup> Berges BK, Wolfe JH, Fraser NW. Transduction of brain by herpes simplex virus vectors. *Mol Ther.* 2007 Jan;15(1):20-9; <http://www.ncbi.nlm.nih.gov/pubmed/17164771/>.

<sup>1021</sup> Gonzalo-Ruiz A. Changes in neurotransmission systems after the injection of beta-amyloid protein beta (12-28) in the hypothalamus and anterior thalamus of the rat. *Rev Neurol.* 1999 May 16-31;28(10):931-41; <http://www.ncbi.nlm.nih.gov/pubmed/10416226>.

<sup>1022</sup> Liu W, Griffin G, Clarke T, Parente MK, Valentino RJ, Wolfe JH, Fraser NW. Bilateral single-site intracerebral injection of a nonpathogenic herpes simplex virus-1 vector decreases anxiogenic behavior in MPS VII mice. *Mol Ther Methods Clin Dev.* 2015 Jan 28;2:14059; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4448997/>. Paumier KL, Luk KC, Manfredsson FP, Kanaan NM, Lipton JW, Collier TJ, Steece-Collier K, Kemp CJ, Celano S, Schulz E, Sandoval IM, Fleming S, Dirr E, Polinski NK, Trojanowski JQ, Lee VM, Sortwell CE. Intra-striatal injection of pre-formed mouse  $\alpha$ -synuclein fibrils into rats triggers  $\alpha$ -synuclein pathology and bilateral nigrostriatal degeneration. *Neurobiol Dis.* 2015 Jun 17;82:185-199; <http://www.ncbi.nlm.nih.gov/pubmed/26093169>.

<sup>1023</sup> Yang P, Qin Y, Zhang W, Bian Z, Wang R. Sensorimotor Cortex Injection of Adeno-Associated Viral Vector Mediates Knockout of PTEN in Neurons of the Brain and Spinal Cord of Mice. *J Mol Neurosci.* 2015 Jul 11; <http://www.ncbi.nlm.nih.gov/pubmed/26160695>.

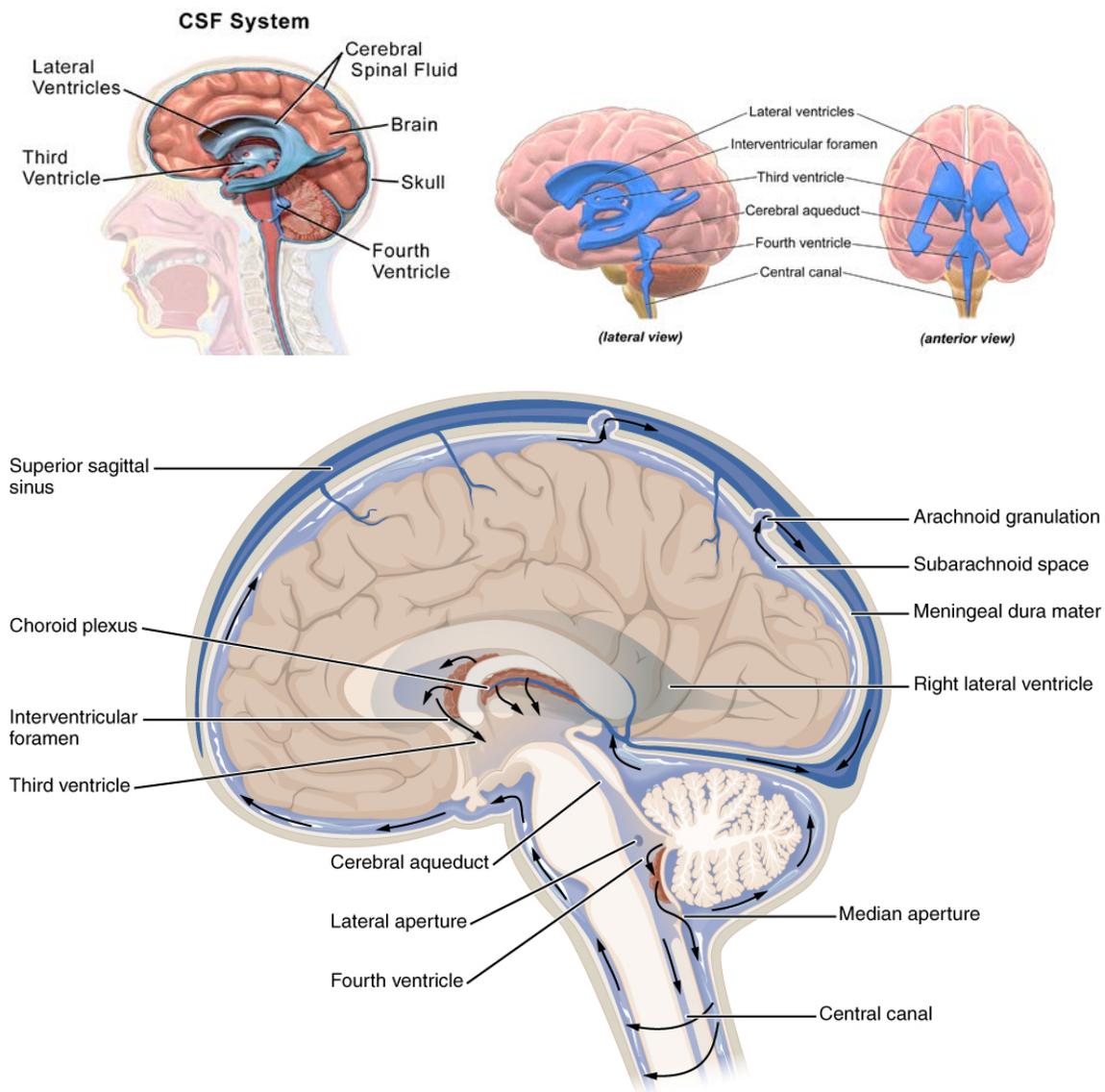
---

<sup>1024</sup> Castaneda MT, Lopez ED, Touhami A, Tovar R, Ortega MR, Rodriguez JM. Neuroprotection of medial septal cholinergic neurons by memantine after intralateral septal injection of A $\beta$ 1-40. *Neuroreport*. 2015 May 27;26(8):450-4; <http://www.ncbi.nlm.nih.gov/pubmed/25919992>.

<sup>1025</sup> Ranjbar A, Hatam M, Nasimi A. Cardiovascular and single-unit responses to l-glutamate injection into the posterior insular cortex in rat. *Neuroscience*. 2015 Oct 15;306:63-73; <http://www.ncbi.nlm.nih.gov/pubmed/26297894>.

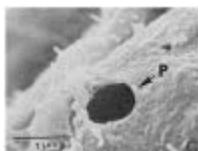
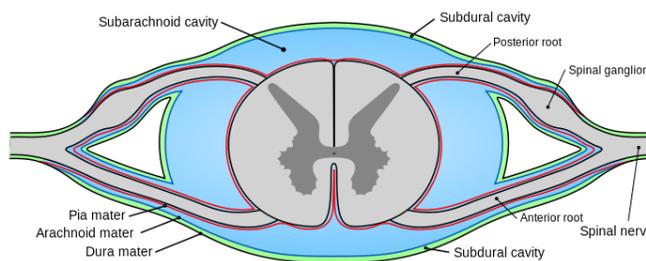
### 4.3.2 Blood-CSF Barrier (BCB) Penetration

Cerebrospinal fluid (CSF) is a clear, colorless body fluid found in the brain and spine. Roughly 500 ml/day of CSF is produced in the choroid plexuses of the ventricles of the brain, but the fluid is constantly reabsorbed so that only 100-160 ml is present at any one time. There are four choroid plexuses in the brain, one in each of the four ventricles. CSF acts as a cushion or buffer for the brain's cortex, providing basic mechanical and immunological protection to the brain inside the skull, and also serves a vital function in cerebral autoregulation of cerebral blood flow.



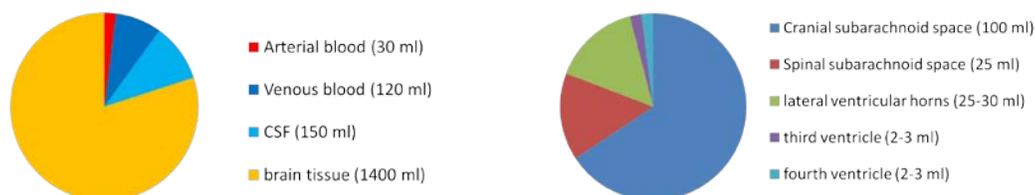
CSF slowly circulates through the ventricular system of the brain. The ventricles are an interconnected series of cavities filled with CSF, inside the brain. The majority of CSF is produced from within the two lateral ventricles. From there, the CSF passes through the interventricular foramina to the third ventricle, then the cerebral aqueduct to the fourth ventricle.

The fourth ventricle is an outpouching on the posterior part of the brainstem. From the fourth ventricle, the fluid passes through three openings to enter the subarachnoid space where most of the fluid volume resides (**Figure 14**). Fluid flowing through one of these openings, called the “arachnoid granulation,” must pass through the



“leptomeningeal pores” (image, left), which studies using radiolabeled size-calibrated microspheres show are at least 1  $\mu\text{m}$  in diameter,<sup>1026</sup> wide enough to admit nanorobots. The subarachnoid space covers the brain (image, previous page) and spinal cord (image, above). The CSF moves in a pulsatile manner throughout the CSF system with a nearly zero net flow.<sup>1027</sup>

Figure 14. Intracranial volumetric distribution of cerebrospinal fluid, blood, and brain parenchyma: all fluids (left), CSF only (right).<sup>1028</sup>



Clearly the CSF system offers an alternate route into the brain for bloodborne medical nanorobots. To enter the CSF system from the bloodstream, the nanorobots must pass through the blood-CSF barrier (BCB) – a pair of barriers that separates peripheral and cerebral blood flow from the cerebrospinal fluid.<sup>1029</sup> The BCB is composed of epithelial cells of the choroid plexus at the peripheral blood-CSF boundary and the arachnoid membrane at the cerebral blood-CSF boundary. The BCB serves the same purpose as the blood-brain barrier, but facilitates the

<sup>1026</sup> Papisov MI, Belov VV, Gannon KS. Physiology of the intrathecal bolus: the leptomeningeal route for macromolecule and particle delivery to CNS. *Mol Pharm*. 2013 May 6;10(5):1522-32; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3646927/>.

<sup>1027</sup> “Cerebrospinal fluid”; [https://en.wikipedia.org/wiki/Cerebrospinal\\_fluid](https://en.wikipedia.org/wiki/Cerebrospinal_fluid). “Ventricular system”; [https://en.wikipedia.org/wiki/Ventricular\\_system](https://en.wikipedia.org/wiki/Ventricular_system).

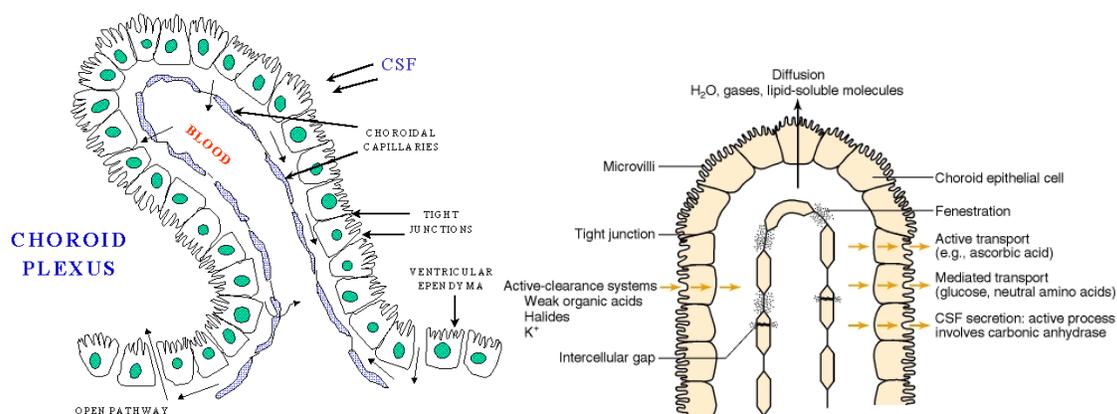
<sup>1028</sup> [https://en.wikipedia.org/wiki/Cerebrospinal\\_fluid#/media/File:Dist\\_csf.png](https://en.wikipedia.org/wiki/Cerebrospinal_fluid#/media/File:Dist_csf.png) and [https://en.wikipedia.org/wiki/Cerebrospinal\\_fluid#/media/File:Dist\\_vent.png](https://en.wikipedia.org/wiki/Cerebrospinal_fluid#/media/File:Dist_vent.png).

<sup>1029</sup> Lathera J, Keep R, Betz LA, Goldstein GW. “Blood–Cerebrospinal Fluid Barrier”. *Basic Neurochemistry: Molecular, Cellular and Medical Aspects*. (6th ed.). Philadelphia: Lippincott-Raven, 1999; <http://www.ncbi.nlm.nih.gov/books/NBK27998/>.

transport of different substances into the brain due to the dissimilar structural characteristics of the two barrier systems.

In the BBB the barrier is localized at the level of the endothelial cells, but in the BCB the blood-CSF barrier is established by choroid plexus epithelial cells.<sup>1030</sup> The choroid plexus is connected by tight junctions and consists of capillary networks enclosed by a single layer of epithelium cells.<sup>1031</sup> Unlike the capillaries that form the blood-brain barrier, choroid plexus capillaries are fenestrated and have no tight junctions so the capillary endothelium presents no barrier to the movement of small molecules (**Figure 15**). Instead, the barrier function of the BCB is performed by the epithelial cells and the tight junctions that link them. The other part of the blood-CSF barrier is the arachnoid membrane (aka. the “blood-arachnoid barrier”), which envelops the brain. The cells of this membrane also are linked by tight junctions. Microvilli are present on the CSF-facing surface, greatly increasing the surface area of the apical membrane and possibly aiding in fluid secretion. Diffusion, facilitated diffusion and active transport into CSF, as well as active transport of metabolites from CSF to blood, have been demonstrated in the choroid plexus.<sup>1032</sup>

Figure 15. Structure of the blood-CSF barrier



Methods that may allow medical nanorobots to penetrate the blood-cerebrospinal fluid barrier (BCB) and move from the bloodstream into the CSF may include:

<sup>1030</sup> Engelhardt B, Sorokin L. The blood-brain and the blood-cerebrospinal fluid barriers: function and dysfunction. *Semin Immunopathol.* 2009 Nov;31(4):497-511; <http://www.ncbi.nlm.nih.gov/pubmed/19779720>.

<sup>1031</sup> Johanson CE, Stopa EG, McMillan PN. The blood-cerebrospinal fluid barrier: structure and functional significance. *Methods Mol Biol.* 2011;686:101-31; <http://www.ncbi.nlm.nih.gov/pubmed/21082368>.

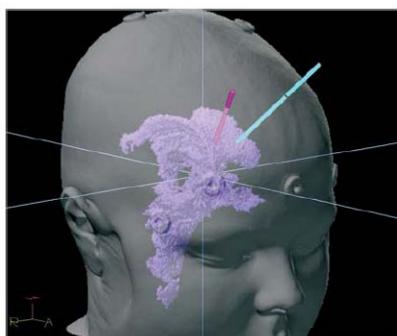
<sup>1032</sup> Lathera J, Keep R, Betz LA, Goldstein GW. “Blood–Cerebrospinal Fluid Barrier”. *Basic Neurochemistry: Molecular, Cellular and Medical Aspects.* (6th ed.). Philadelphia: Lippincott-Raven, 1999; <http://www.ncbi.nlm.nih.gov/books/NBK27998/>.

(1) **BBB Penetration Methods.** Any of the methods previously identified for possible use by medical nanorobots to penetrate the blood-brain barrier could also be applied to the tight-junction barrier that exists between the choroid plexus epithelial cells comprising the BCB.

(2) **Lumbar Injection.** Lumbar puncture (aka. spinal tap) is carried out under local anesthesia sterile conditions by inserting a needle into the subarachnoid space, usually between the third and fourth lumbar vertebrae.<sup>1033</sup> CSF is then extracted through the needle and tested. Medical nanorobots could also be inserted into, or extracted from, the CSF through a similar needle, as has already analogously been done in animals using drug-impregnated microspheres.<sup>1034</sup> Leptomeningeal (intrathecal) drug administration,<sup>1035</sup> aka. spinal drug delivery, is already a well-established practice.<sup>1036</sup>



(3) **Intraventricular Injection.** Medical nanorobots can be inserted into the CSF, not just via intrathecal injection, but also by direct injection into the four cerebral ventricles. The intraventricular administration of chemotherapeutic agents (most



typically, methotrexate) is sometimes used to treat patients with malignant CNS tumors, neuroleukemia, CNS lymphomas, and other disorders – distributing chemotherapeutic agents through the cerebrospinal fluid more uniformly and maintaining their concentrations for a longer period (images from a stereotactic positioning system are shown above).<sup>1037</sup>



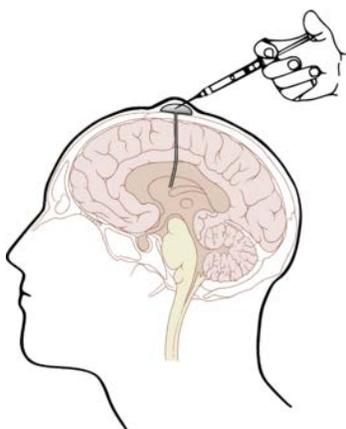
<sup>1033</sup> “Lumbar puncture”; [https://en.wikipedia.org/wiki/Lumbar\\_puncture](https://en.wikipedia.org/wiki/Lumbar_puncture)

<sup>1034</sup> Lagarce F, Faisant N, Desfontis JC, Marescaux L, Gautier F, Richard J, Menei P, Benoit JP. Baclofen-loaded microspheres in gel suspensions for intrathecal drug delivery: *in vitro* and *in vivo* evaluation. *Eur J Pharm Biopharm.* 2005 Oct;61(3):171-80; [http://www.researchgate.net/profile/Frederic\\_Lagarce/publication/7775091\\_Baclofen-loaded\\_microspheres\\_in\\_gel\\_suspensions\\_for\\_intrathecal\\_drug\\_delivery\\_in\\_vitro\\_and\\_in\\_vivo\\_evaluation/links/55312ad60cf2f2a588ad158a.pdf](http://www.researchgate.net/profile/Frederic_Lagarce/publication/7775091_Baclofen-loaded_microspheres_in_gel_suspensions_for_intrathecal_drug_delivery_in_vitro_and_in_vivo_evaluation/links/55312ad60cf2f2a588ad158a.pdf)

<sup>1035</sup> Papisov MI, Belov VV, Gannon KS. Physiology of the intrathecal bolus: the leptomeningeal route for macromolecule and particle delivery to CNS. *Mol Pharm.* 2013 May 6;10(5):1522-32; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3646927/>.

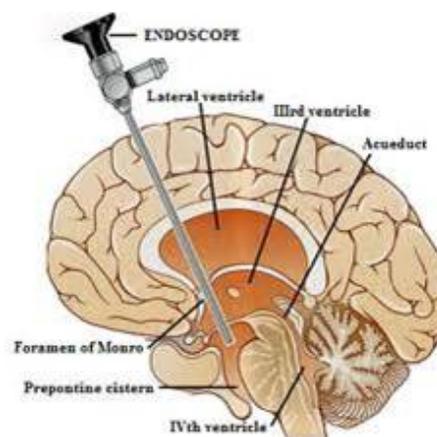
<sup>1036</sup> “Intrathecal administration”; [https://en.wikipedia.org/wiki/Intrathecal\\_administration](https://en.wikipedia.org/wiki/Intrathecal_administration).

<sup>1037</sup> Ozerov SS, Mel’nikov AV, Ibragimova DI, Tereshchenko GV, Rachkov VE. Placement of the Ommaya reservoir in narrow and slit-like ventricles using a neuronavigation system. *Zh Vopr Neurokhir Im*



A **ventricular catheter system** (also known as the Ommaya reservoir; image, left)<sup>1038</sup> is the most commonly used method for repeated introduction of chemotherapeutic agents into the cerebral ventricles. An Ommaya reservoir is an intraventricular catheter system, often with ultrasound-guided placement,<sup>1039</sup> that can be used for the aspiration of cerebrospinal fluid or for the delivery of drugs (e.g., chemotherapy) into the cerebrospinal fluid. It consists of a catheter in one lateral ventricle attached to a reservoir implanted under the scalp. It is used to treat brain tumors,<sup>1040</sup> leukemia/lymphoma or leptomeningeal disease by intrathecal drug administration. In the palliative care of terminal cancer, an Ommaya reservoir can be inserted for intracerebroventricular injection of morphine. This sort of system could also be used to inject medical nanorobots into CSF in the ventricles.

A related intraventricular procedure is **endoscopic third ventriculostomy** – a surgical procedure for treatment of hydrocephalus (a medical condition involving an abnormal accumulation of cerebrospinal fluid in the brain) in which an opening is created in the floor of the third ventricle using an endoscope placed within the ventricular system through a burr hole (image, right).<sup>1041</sup> Nanorobots could be introduced into the CSF by this means.



These BCB penetration methods would also enable nanorobot access to the circumventricular organs or CVOs (Section 4.3.1) in the brain.

N N Burdenko. 2014;78(3):38-43;

[http://www.mediasphera.ru/uppic/Journal\\_of\\_Neurosurgery\\_named\\_after\\_NNBurdenko/2014/3/5/Neirohirurgija\\_2014\\_03\\_034.pdf](http://www.mediasphera.ru/uppic/Journal_of_Neurosurgery_named_after_NNBurdenko/2014/3/5/Neirohirurgija_2014_03_034.pdf).

<sup>1038</sup> “Ommaya reservoir”; [https://en.wikipedia.org/wiki/Ommaya\\_reservoir](https://en.wikipedia.org/wiki/Ommaya_reservoir).

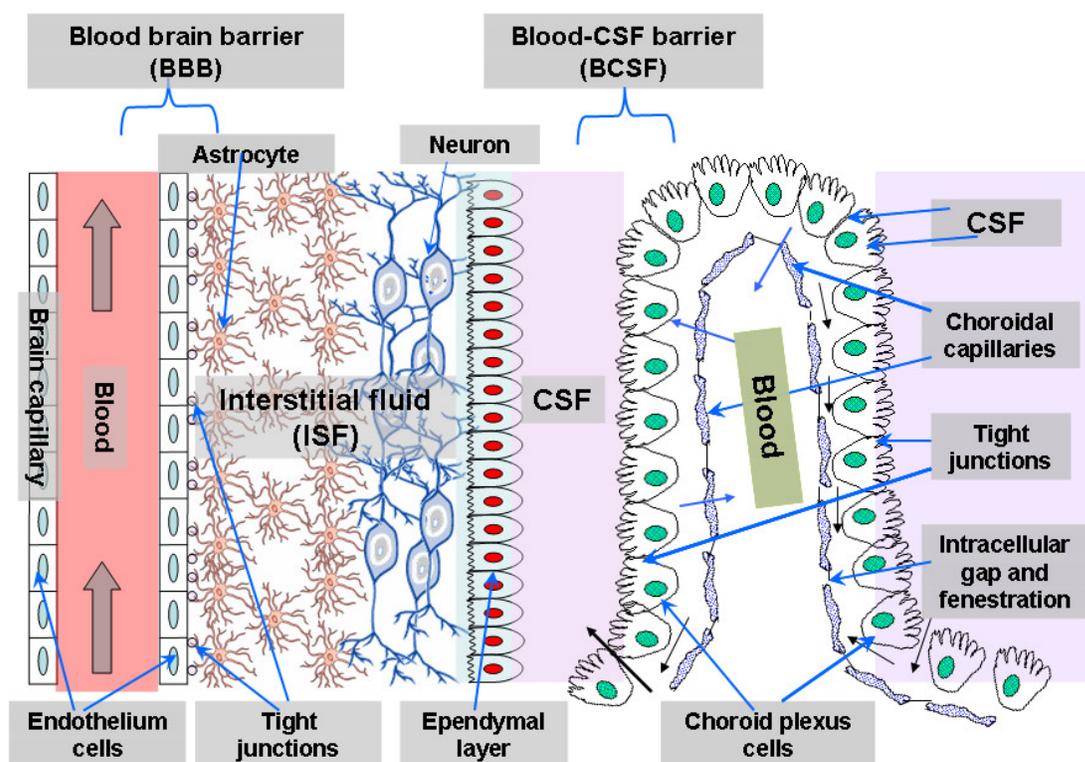
<sup>1039</sup> Jakola AS, Reinertsen I, Selbekk T, Solheim O, Lindseth F, Gulati S, Unsgård G. Three-dimensional ultrasound-guided placement of ventricular catheters. *World Neurosurg.* 2014 Sep-Oct;82(3-4):536; <http://www.ncbi.nlm.nih.gov/pubmed/23973451>.

<sup>1040</sup> Takahashi M, Yamada R, Tabei Y, Nakamura O, Shinoura N. Navigation-guided Ommaya reservoir placement: implications for the treatment of leptomeningeal metastases. *Minim Invasive Neurosurg.* 2007 Dec;50(6):340-5; <http://www.ncbi.nlm.nih.gov/pubmed/18210356>.

<sup>1041</sup> “Endoscopic third ventriculostomy”; [https://en.wikipedia.org/wiki/Endoscopic\\_third\\_ventriculostomy](https://en.wikipedia.org/wiki/Endoscopic_third_ventriculostomy).

### 4.3.3 CSF-Brain Interface Penetration

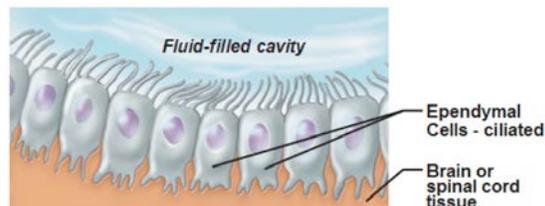
Although there is a blood-brain barrier (BBB) (Section 4.3.1) and a blood-CSF barrier (BCB) (Section 4.3.2), there is no CSF-brain barrier because CSF in the ventricles is separated from the brain by ependymal cells – and the ependyma is highly permeable, with no tight junctions. Thus a drug molecule or anesthetic agent injected into the CSF, e.g., by lumbar puncture, can readily reach the brain and spinal cord. The fact that large molecules can penetrate into the brain parenchyma from the cerebrospinal fluid (CSF) was established more than four decades ago.<sup>1042</sup> Large proteins the size of ferritin (MW ~ 445,000) readily diffuse through the paracellular space to cross the ependyma and thus easily move between CSF and brain.<sup>1043</sup> Accordingly, the ependymal interface between ventricular fluid and underlying brain is not regarded as a CSF-brain “barrier”.



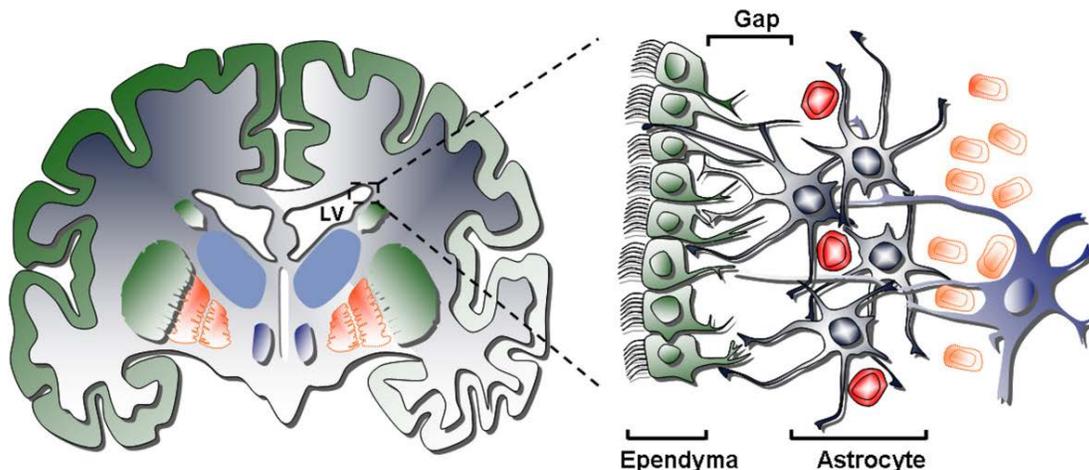
<sup>1042</sup> Wagner HJ, Pilgrim C, Brandl J. Penetration and removal of horseradish peroxidase injected into the cerebrospinal fluid: role of cerebral perivascular spaces, endothelium and microglia. *Acta Neuropathol.* 1974 Apr 30;27(4):299-315; <http://www.ncbi.nlm.nih.gov/pubmed/4366438/>.

<sup>1043</sup> Johanson C, Stopa E, McMillan P, Roth D, Funk J, Krinke G. The distributional nexus of choroid plexus to cerebrospinal fluid, ependyma and brain: toxicologic/pathologic phenomena, periventricular destabilization, and lesion spread. *Toxicol Pathol.* 2011 Jan;39(1):186-212; <http://tpx.sagepub.com/content/39/1/186.long>.

More than being a structural boundary between ventricular CSF and brain, the ependyma is a dynamic interface mediating the movement of cilia, CSF, and migrating neuroblasts. On the ependymal apical surface is a network of cilia (image, right) that beat in coordination to facilitate CSF circulation. Each cilium is a subcellular organelle emanating from the cell's interior.



Ependymal cells line the ventricles of the brain and the central canal of the spinal cord. They're arranged in a single-layered columnar epithelium, and have many of the histological characteristics of simple epithelium but are usually cuboidal and  $\sim 8 \mu\text{m}$  in size.<sup>1044</sup> The ependyma forming the ventricular lining do not connect to a basal lamina, but rest directly upon underlying nervous tissue. The subventricular zone is illustrated in the image below.<sup>1045</sup> Four layers are typically observed throughout the lateral ventricular (LV) wall: (1) a monolayer of ependymal cells, (2) a hypocellular gap, (3) a ribbon of cells composed of astrocytes (a type of glial cell, providing infrastructure support for neurons), and (4) a transitional zone into the brain parenchyma (where neurons reside).<sup>1046</sup>



<sup>1044</sup> Bjugn R, Bøe R, Haugland HK. A stereological study of the ependyma of the mouse spinal cord. With a comparative note on the choroid plexus ependyma. *J Anat.* 1989 Oct;166:171-8; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1256750/>.

<sup>1045</sup> "Subventricular zone"; [https://en.wikipedia.org/wiki/Subventricular\\_zone](https://en.wikipedia.org/wiki/Subventricular_zone).

<sup>1046</sup> Quiñones-Hinojosa A, Sanai N, Soriano-Navarro M, Gonzalez-Perez O, Mirzadeh Z, Gil-Perotin S, Romero-Rodriguez R, Berger MS, Garcia-Verdugo JM, Alvarez-Buylla A. Cellular composition and cytoarchitecture of the adult human subventricular zone: a niche of neural stem cells. *J Comp Neurol.* 2006 Jan 20;494(3):415-34; <http://www.ncbi.nlm.nih.gov/pubmed/16320258>.

Medical nanorobots possessing modest motility should be able to easily penetrate the ependymal interface and directly enter the brain parenchyma or neuropil, using one or more of the methods previously described above. The evidence for this comes from two sources.

First, motile biological cells readily migrate through the ependymal layer. For example, micron-size T-cells injected intracerebroventricularly move quickly from the lateral ventricles into the brain parenchyma in mice.<sup>1047</sup> Adenovirus viral 20-nm nanoparticles, assisted by mannitol infusion, are delivered into the brain following intracerebral ventricular injection.<sup>1048</sup>

Second, nanorobot-sized microparticles can migrate through the ependymal layer, readily entering the brain even though they lack the additional motility mechanisms that medical nanorobots could employ to assist in this process. One study<sup>1049</sup> used rats injected intraventricularly with MPIOs (iron oxide microparticles) or “Bangs particles” – 1.63  $\mu\text{m}$  diameter polystyrene microparticles encapsulating a 0.9  $\mu\text{m}$  iron oxide core. The uptake of these MPIOs into the subventricular zone is  $\sim 30\%$  in adult rodents,<sup>1050</sup> likely usually carried by migrating neuroblasts in a process analogous to cytocarriage and not via simple diffusion. Another study showed cell-independent migration of MPIOs through the subventricular region,<sup>1051</sup> and yet another study found that 150-nm dextran-coated super-paramagnetic iron oxide nanoparticles (SPIO) injected into a rat ventricle passed the subventricular zone via transcytosis<sup>1052</sup> through ependymal cells,<sup>1053</sup> though paracellular transport<sup>1054</sup> could not be ruled

<sup>1047</sup> Fisher Y, Strominger I, Biton S, Nemirovsky A, Baron R, Monsonego A. Th1 polarization of T cells injected into the cerebrospinal fluid induces brain immunosurveillance. *J Immunol.* 2014 Jan 1;192(1):92-102; <http://www.jimmunol.org/content/192/1/92.long>.

<sup>1048</sup> Janson CG, Romanova LG, Leone P, Nan Z, Belur L, McIvor RS, Low W. Comparison of Endovascular and Intraventricular Gene Therapy With Adeno-Associated Virus- $\alpha$ -L-Iduronidase for Hurler Disease. *Neurosurgery.* 2014 Jan;74(1):99-111; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4116107/>.

<sup>1049</sup> Yang J, Liu J, Niu G, Chan KC, Wang R, Liu Y, Wu EX. *In vivo* MRI of endogenous stem/progenitor cell migration from subventricular zone in normal and injured developing brains. *Neuroimage.* 2009 Nov 1;48(2):319-28; <http://www.ncbi.nlm.nih.gov/pubmed/19591946>.

<sup>1050</sup> Sumner JP, Shapiro EM, Maric D, Conroy R, Koretsky AP. *In vivo* labeling of adult neural progenitors for MRI with micron sized particles of iron oxide: quantification of labeled cell phenotype. *Neuroimage.* 2009 Feb 1;44(3):671-8; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2967480/>.

<sup>1051</sup> Granot D, Shapiro EM. Accumulation of micron sized iron oxide particles in endothelin-1 induced focal cortical ischemia in rats is independent of cell migration. *Magn Reson Med.* 2014 Apr;71(4):1568-74; <http://www.ncbi.nlm.nih.gov/pubmed/23661604>.

<sup>1052</sup> **Transcytosis** is the process by which macromolecules or particles are transported through the interior of a cell. The particles are captured in vesicles on one side of the cell, drawn across the cell, and ejected on the other side; <http://en.wikipedia.org/wiki/Transcytosis>.

<sup>1053</sup> Panizzo RA, Kyratatos PG, Price AN, Gadian DG, Ferretti P, Lythgoe MF. *In vivo* magnetic resonance imaging of endogenous neuroblasts labelled with a ferumoxide-polycation complex. *Neuroimage.* 2009 Feb 15;44(4):1239-46; [http://www.ucl.ac.uk/cabi/PDF/2009\\_Panizzo\\_et\\_al\\_NeuroImage.pdf](http://www.ucl.ac.uk/cabi/PDF/2009_Panizzo_et_al_NeuroImage.pdf).

out in this experiment. The oft-cited 2006 study by Shapiro *et al.* used micron-sized Bangs particles injected into rats:<sup>1055</sup> “Five weeks after injection, electron microscopy detected intracellular MPIOs in astrocytes, microglia, ependymal, and migratory cells. Of all the cellular types that were labeled with MPIOs, astrocytes and microglia showed greater amounts of iron particles in electron micrographs, a fact that is probably related to their phagocytosing capacity.”

This evidence suggests that injection into the CSF should provide medical nanorobots with ready access to neurons in the brain.

---

<sup>1054</sup> **Paracellular transport** is the transfer of particles across an epithelium by passing through the intercellular space between the cells, as distinguished from transcellular transport or transcytosis in which the materials travel through the cell, passing through both the apical membrane and basolateral membrane; [http://en.wikipedia.org/wiki/Paracellular\\_transport](http://en.wikipedia.org/wiki/Paracellular_transport).

<sup>1055</sup> Shapiro EM, Gonzalez-Perez O, Manuel García-Verdugo J, Alvarez-Buylla A, Koretsky AP. Magnetic resonance imaging of the migration of neuronal precursors generated in the adult rodent brain. *Neuroimage*. 2006 Sep;32(3):1150-7; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4035244/>.

### 4.3.4 Nanorobotic Transit Through Extracellular Spaces

Brain tissue typically contains only ~5% extracellular space and consists for the most part of densely-packed axons and dendrites with virtually no gaps between them, so neuron-targeted motile nanorobots may often transit plasma membranes between neighboring cells rather than intercellular spaces. Because cell bodies containing the nucleus may be relatively far apart, such specialized nanorobots should be engineered to migrate either (1) inside the larger-diameter axons without ruining neural function or (2) external to the axons without disturbing the local ionic environment. This may require active nanorobotic monitoring and localized remediation of the ECM chemical environment<sup>1056</sup> during nanorobot locomotion, given that the minimal extracellular space in the brain controls the concentrations of extracellular ions that cross and re-enter the cell membrane during and after action potentials.

Another approach is to osmotically expand the extracellular space on a local basis to allow relatively large nanorobotic devices to migrate wherever they need to go. Considerable expansion may be tolerable: A.U. Smith's classic hamster freezing experiments<sup>1057</sup> showed that >60% of the water in the brain can be converted into extracellular ice without apparent brain damage, a distortion far in excess of what would be needed to enable nanorobot traffic. Unpublished observations by G. Fahy<sup>1058</sup> at 21st Century Medicine show that when ice forms in the brain even at low temperatures in the presence of cryoprotectants, neurons and nerve processes are neatly packaged and are not torn apart, supporting the idea that the extracellular space can be significantly locally expanded without lasting harm.

The migration of newly-generated neurons through the brain provides additional evidence that the organ can tolerate significant local distortion of the extracellular space. For example, neurogenesis (formation of new neurons) in the hippocampus is followed by neurons or their precursors migrating out of the hippocampus over large distances to other parts of the brain,<sup>1059</sup> a

---

<sup>1056</sup> Freitas RA Jr. "Chapter 23. Comprehensive Nanorobotic Control of Human Morbidity and Aging," in Gregory M. Fahy, Michael D. West, L. Stephen Coles, and Steven B. Harris, eds, *The Future of Aging: Pathways to Human Life Extension*, Springer, New York, 2010, pp. 685-805; Section 6.4.8 "Intracellular Environmental Maintenance"; <http://www.nanomedicine.com/Papers/Aging.pdf>.

<sup>1057</sup> Smith AU, Lovelock JE, Parkes AS. Resuscitation of hamsters after supercooling or partial crystallization at body temperature below 0 degrees C. *Nature*. 1954 Jun 12;173(4415):1136-7; <http://www.ncbi.nlm.nih.gov/pubmed/13165726>. Smith AU, Lovelock JE. Studies on golden hamsters during cooling to and rewarming from body temperatures below 0 degrees C. III. Biophysical aspects and general discussion. *Proc R Soc Lond B Biol Sci*. 1956 Jul 24;145(920):427-42; <http://www.ncbi.nlm.nih.gov/pubmed/13359396>. Smith AU. Problems in freezing organs and their component cells and tissues. *Fed Proc*. 1965 Mar-Apr;24:S196-203; <http://www.ncbi.nlm.nih.gov/pubmed/14314566>.

<sup>1058</sup> Fahy G. Personal communication to R. Freitas, 2008.

<sup>1059</sup> Ehninger D, Kempermann G. Neurogenesis in the adult hippocampus. *Cell Tissue Res*. 2008 Jan;331(1):243-50; <http://www.ncbi.nlm.nih.gov/pubmed/17938969>.

mechanical process that is normal and apparently well-tolerated. Microglial cells, the immune system phagocytes in the brain, have been observed (via two-photon imaging of mammalian neocortex) to have extremely motile processes and protrusions, with their cell bodies sometimes moving 0.02-0.03  $\mu\text{m}/\text{min}$  but their filopodia-like processes extending and retracting at 1-4  $\mu\text{m}/\text{min}$ .<sup>1060</sup>

---

<sup>1060</sup> Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. *Science*. 2005 May 27;308(5726):1314-8; <http://www.sciencemag.org/content/308/5726/1314.long>.

### 4.3.5 Nanocatheter Transport into the Brain

As the era of surgical nanorobotics arrives,<sup>1061</sup> today's smallest millimeter-diameter flexible catheters will shrink to 1-10 micron diameter bundles that could be steered<sup>1062</sup> through the tiniest blood vessels (including capillaries) or could even be inserted directly through the skin<sup>1063</sup> into organs without pain or discomfort. Tactile,<sup>1064</sup> haptic,<sup>1065</sup> and other sensory feedback will also allow surgical practitioners to steer the nanocatheter into a patient to either emplace or remove materials from inside the brain, then to withdraw bloodlessly from the body. The nanosurgeon may control the procedure via hand-guided interfaces similar to various medical exoskeletal appliances,<sup>1066</sup> instrumented gloves<sup>1067</sup> and hand-held surgical robots<sup>1068</sup> that have been under

---

<sup>1061</sup> Freitas RA Jr. "Chapter 23. Comprehensive Nanorobotic Control of Human Morbidity and Aging," in Gregory M. Fahy, Michael D. West, L. Stephen Coles, and Steven B. Harris, eds, *The Future of Aging: Pathways to Human Life Extension*, Springer, New York, 2010, pp. 685-805; Section 6.3.5.3 "Endoscopic Nanosurgery and Surgical Nanorobots"; <http://www.nanomedicine.com/Papers/Aging.pdf>.

<sup>1062</sup> Glozman D, Shoham M. Flexible needle steering for percutaneous therapies. *Comput Aided Surg* 2006 Jul; 11(4):194-201; <http://www.ncbi.nlm.nih.gov/pubmed/17060077>.

<sup>1063</sup> Wang PM, Cornwell M, Prausnitz MR. Minimally invasive extraction of dermal interstitial fluid for glucose monitoring using microneedles. *Diabetes Technol Ther* 2005 Feb; 7(1):131-141; <http://www.ncbi.nlm.nih.gov/pubmed/15738711>.

<sup>1064</sup> Ku J, Mraz R, Baker N, Zakzanis KK, Lee JH, Kim IY, Kim SI, Graham SJ. A data glove with tactile feedback for FMRI of virtual reality experiments. *Cyberpsychol Behav*. 2003 Oct;6(5):497-508; <http://www.ncbi.nlm.nih.gov/pubmed/14583125>. Winter SH, Bouzit M. Use of magnetorheological fluid in a force feedback glove. *IEEE Trans Neural Syst Rehabil Eng* 2007 Mar;15(1):2-8; <http://www.ncbi.nlm.nih.gov/pubmed/17436869>.

<sup>1065</sup> McColl R, Brown I, Seligman C, Lim F, Alsaraira A. Haptic rendering for VR laparoscopic surgery simulation. *Australas Phys Eng Sci Med* 2006;29:73-78; <http://dspace2.flinders.edu.au/xmlui/bitstream/handle/2328/9552/2006017101.pdf?sequence=1>. De Rossi D, Carpi F, Carbonaro N, Tognetti A, Scilingo EP. Electroactive polymer patches for wearable haptic interfaces. *Conf Proc IEEE Eng Med Biol Soc*. 2011;2011:8369-72; <http://www.ncbi.nlm.nih.gov/pubmed/22256288>.

<sup>1066</sup> Fleischer C, Wege A, Kondak K, Hommel G. Application of EMG signals for controlling exoskeleton robots. *Biomed Tech (Berl)* 2006 Dec;51(5-6):314-319; <http://pdv.cs.tu-berlin.de/ExoSkeleton/Paper/bmte.51.5-6.314.pdf>. Cavallaro EE, Rosen J, Perry JC, Burns S. Real-time myoprocessors for a neural controlled powered exoskeleton arm. *IEEE Trans Biomed Eng* 2006 Nov;53(11):2387-2396; [http://bionics.seas.ucla.edu/publications/JP\\_12.pdf](http://bionics.seas.ucla.edu/publications/JP_12.pdf). Gordon KE, Ferris DP. Learning to walk with a robotic ankle exoskeleton. *J Biomech* 2007;40(12):2636-2644; <http://www.ncbi.nlm.nih.gov/pubmed/17275829>. Ben-Tzvi P, Ma Z. Sensing and Force-Feedback Exoskeleton (SAFE) Robotic Glove. *IEEE Trans Neural Syst Rehabil Eng*. 2015 Nov;23(6):992-1002; <http://www.ncbi.nlm.nih.gov/pubmed/25494512>.

<sup>1067</sup> Castro MC, Cliquet A. A low-cost instrumented glove for monitoring forces during object manipulation. *IEEE Trans Rehabil Eng* 1997 Jun;5(2):140-147; <http://www.ncbi.nlm.nih.gov/pubmed/9184900>. Yun MH, Cannon D, Freivalds A, Thomas G. An

development for several decades, and may include multisensory interfaces with individual cells.<sup>1069</sup>

A nanocatheter is a specialized syringe-like nanomechanical probe instrument with nanorobotic mechanisms embedded in its external surfaces to assist in actively propelling the telescoping apparatus gently through the tissues.<sup>1070</sup> The probe samples the chemical environment (e.g., concentrations of oxygen, glucose, hormones, cytokines) along the way<sup>1071</sup> and provides a torrent of mechanical and optical sensory feedback together with precision positional metrology to allow the surgeon to know exactly where his tools are at all times, and also where his “virtual presence” is in relation to his targets. Internal hollow spaces inside the nanocatheter can be used to transport tools, sensors, fluids, drugs, nanorobots, replacement cells, or debridement detritus between patient and physician. The tip of the nanocatheter may include a working head with thousands of independent manipulators and sensors branching outward from the central trunk on retractile stalks, from which data can be encoded in real time and passed to external computers along an optical data bus located inside each nanocatheter. Surgeons will gain the ability to easily control many more than one active surgical instrument or surgical task at a time<sup>1072</sup> – the endoscopic nanosurgeon’s ability to multitask ultimately may extend to many thousands of nanocatheters and millions of simultaneously occurring mechanical and chemical processes during a single surgical procedure.

instrumented glove for grasp specification in virtual-reality-based point-and-direct telerobotics. *IEEE Trans Syst Man Cybern B Cybern* 1997 Oct;27(5):835-846; <http://groklab.org/files/2007/02/An-instrumented-glove-for-grasp-specification-in-virtual-reality-based-point-and-direct-telerobotics.pdf>. Skinner A, Lathan C. Assessment of laparoscopic surgical skill acquisition and retention. *Stud Health Technol Inform*. 2012;173:478-82; <http://www.ncbi.nlm.nih.gov/pubmed/22357040>. Saggio G, Lazzaro A, Sberini L, Carrano FM, Passi D, Corona A, Panetta V, Gaspari AL, Di Lorenzo N. Objective Surgical Skill Assessment: An Initial Experience by Means of a Sensory Glove Paving the Way to Open Surgery Simulation? *J Surg Educ*. 2015 Sep-Oct;72(5):910-7; <http://www.ncbi.nlm.nih.gov/pubmed/26089159>.

<sup>1068</sup> Tonet O, Focacci F, Piccigallo M, Cavallo F, Uematsu M, Megali G, Dario P. Comparison of control modes of a hand-held robot for laparoscopic surgery. *Med Image Comput Comput Assist Interv*. 2006;9(Pt 1):429-36; [https://www.researchgate.net/profile/Oliver\\_Tonet/publication/6452106\\_Comparison\\_of\\_control\\_modes\\_of\\_a\\_hand-held\\_robot\\_for\\_laparoscopic\\_surgery/links/09e4150e5adb7137c1000000.pdf](https://www.researchgate.net/profile/Oliver_Tonet/publication/6452106_Comparison_of_control_modes_of_a_hand-held_robot_for_laparoscopic_surgery/links/09e4150e5adb7137c1000000.pdf).

<sup>1069</sup> Koerner M, Wait E, Winter M, Bjornsson C, Kokovay E, Wang Y, Goderie SK, Temple S, Cohen AR. Multisensory interface for 5D stem cell image volumes. *Conf Proc IEEE Eng Med Biol Soc*. 2014;2014:1178-81; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4321857/>

<sup>1070</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 9.4.4 “Histonatation”; <http://www.nanomedicine.com/NMI.htm>.

<sup>1071</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Chapter 4 “Nanosensors and nanoscale Sensing”; <http://www.nanomedicine.com/NMI.htm>.

<sup>1072</sup> Zhijiang D, Zhiheng J, Minxiu K. Virtual reality-based telesurgery via teleprogramming scheme combined with semi-autonomous control. *Conf Proc IEEE Eng Med Biol Soc* 2005;2:2153-2156; <http://www.ncbi.nlm.nih.gov/pubmed/17282656>.

Also, and as an extension of today's surgical microrobotics (a young but thriving field of experimental research),<sup>1073</sup> populations of individual surgical nanorobots could be introduced into the body from the ends of nanocatheters into various vessels and other cavities within the body, and later retrieved when their job is done. A future surgical nanorobot, programmed or guided by a human surgeon, would act as a semi-autonomous on-site surgeon inside the human body, assisting activities occurring near the tip of the nanocatheter and coordinated by an on-board computer while maintaining contact with the supervising surgeon via coded ultrasound signals.

Insertion of the nanocatheter through brain tissue should not create any significant damage if done slowly and carefully enough. Active nanocatheter tips festooned with sensors and manipulators will permit the nanosurgeon to steer the nanocatheter exclusively through tissue while avoiding bone and vascular puncture events. For example, cryobiologists have found that neurons and nerve processes are not torn apart when ice forms in the brain even at low temperatures in the presence of cryoprotectants.<sup>1074</sup> This supports the idea that the extracellular space can be significantly locally expanded without lasting harm. The migration of newly-generated neurons through the brain provides additional evidence that the organ can tolerate significant local distortion of the extracellular space. Migration of neurons or their precursors out of the hippocampus over large distances to other parts of the brain during neurogenesis<sup>1075</sup> is a

---

<sup>1073</sup> Ishiyama K, Sendoh M, Arai KI. Magnetic micromachines for medical applications. *J Magnetism Magnetic Mater* 2002; 242-245:1163-1165; [http://emma.inrim.it/twiki/pub/MagMicMach/TutorialMmm/6\\_Magnetic\\_micromachinesformedicalapplication.pdf](http://emma.inrim.it/twiki/pub/MagMicMach/TutorialMmm/6_Magnetic_micromachinesformedicalapplication.pdf). Mathieu JB, Martel S, Yahia LH, Soulez G, Beaudoin G. MRI systems as a mean of propulsion for a microdevice in blood vessels. *Biomed Mater Eng* 2005; 15:367; <http://wiki.polymtl.ca/nano/images/4/48/C-2003-MRSUB-EMBS-JBMathieu.pdf>. Yesin KB, Exner P, Vollmers K, Nelson BJ. Design and control of in-vivo magnetic microrobots. *Med Image Comput Comput Assist Interv.* 2005;8(Pt 1):819-26; <http://www.msrl.ethz.ch/content/dam/ethz/special-interest/mavt/robotics-n-intelligent-systems/multiscaleroboticlabb-dam/documents/microrobotics/Yesin2005.pdf>. Martel S, Mathieu JB, Felfoul O, Chanu A, Aboussouan E, Tamaz S, Pouponneau P, Yahia LH, Beaudoin G, Soulez G, Mankiewicz M. Automatic navigation of an untethered device in the artery of a living animal using a conventional clinical magnetic resonance imaging system. *Appl Phys Lett* 2007;90:114105; <http://wiki.polymtl.ca/nano/fr/images/1/14/J-2007-MRSUB-APL-Sylvain2.pdf>. Behkam B, Sitti M. Bacterial flagella-based propulsion and on/off motion control of microscale objects. *Appl Phys Lett* 2007; 90:023902; <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.206.2159&rep=rep1&type=pdf>. Cole E. Fantastic Voyage: Departure 2009. *Wired Magazine.* 2007 Jan 18; <http://www.wired.com/medtech/health/news/2007/01/72448>. Nelson BJ, Kaliakatsos IK, Abbott JJ. Microrobots for minimally invasive medicine. *Annu Rev Biomed Eng.* 2010 Aug 15;12:55-85; [http://www.msrlpublications.ch/btw/files/Nelson\\_ARBE.pdf](http://www.msrlpublications.ch/btw/files/Nelson_ARBE.pdf). Olamaei N, Cheriet F, Beaudoin G, Martel S. MRI visualization of a single 15  $\mu\text{m}$  navigable imaging agent and future microrobot. *Conf Proc IEEE Eng Med Biol Soc.* 2010;2010:4355-8; <http://www.ncbi.nlm.nih.gov/pubmed/21096004>. Ullrich F, Bergeles C, Pokki J, Ergeneman O, Erni S, Chatzipirpiridis G, Pané S, Framme C, Nelson BJ. Mobility experiments with microrobots for minimally invasive intraocular surgery. *Invest Ophthalmol Vis Sci.* 2013 Apr 23;54(4):2853-63; [http://www.msrlpublications.ch/btw/files/Ullrich\\_IOVS\\_2013.pdf](http://www.msrlpublications.ch/btw/files/Ullrich_IOVS_2013.pdf).

<sup>1074</sup> Fahy G. Personal communication to R. Freitas, 2008.

<sup>1075</sup> Ehninger D, Kempermann G. Neurogenesis in the adult hippocampus. *Cell Tissue Res.* 2008 Jan;331(1):243-50; <http://www.ncbi.nlm.nih.gov/pubmed/17938969>.

mechanical process that is normal and apparently well tolerated. The passage of a sensor-guided tip-mobile nanocatheter should likewise produce minimal tissue damage during insertion and retraction of the device.

The nanocatheter could rapidly and painlessly import macroscale quantities of new cells into the brain. For example, a 1 cm<sup>3</sup> volume of 125 million 20-micron tissue cells, arranged in planar 10-cell slabs moving perpendicular to the slab plane through a tube, could be imported at ~1 m/sec through a single 10-cm long nanocatheter<sup>1076</sup> with a 100 micron inside diameter (possibly internally coated with mechanical cilia to facilitate efficient transport) to virtually anywhere inside the human body in ~250 sec (~4 min). A small array of 100 safe and painless nanocatheters (analogous to microneedles<sup>1077</sup>) having a total ~1 mm<sup>2</sup> penetration cross-section for the entire array could transport ~124 cm<sup>3</sup> of neural cells – or ~10% of the entire cellular volume of the human brain, the expected normal cell volume loss by age 90 solely due to ageing – into the body during a ~300 sec (~5 min) transfer. A second nanocatheter array can export a matching volume of body fluid or comminuted pathological tissue to precisely maintain conservation of volume/mass, if necessary. The slow traversal of conventional vein-infused self-targeting neural stem cells<sup>1078</sup> to their designated destinations would take many orders of magnitude longer and would not be 100% reliable and complete, as compared to using nanocatheters to quickly transport the same cells.

The nanocatheter can also be employed to remove unwanted materials from inside the brain.<sup>1079</sup> Carefully poking a needle-like 100-micron diameter nanosensor-tipped self-steering nanocatheter quickly (~1000 µm/sec) through all intervening soft tissues to the immediate vicinity of a target block of unwanted material should cause minimal permanent damage,<sup>1080</sup> much like bloodless

<sup>1076</sup> Freitas RA Jr. “Chapter 23. Comprehensive Nanorobotic Control of Human Morbidity and Aging,” in Gregory M. Fahy, Michael D. West, L. Stephen Coles, and Steven B. Harris, eds, *The Future of Aging: Pathways to Human Life Extension*, Springer, New York, 2010, pp. 685-805; Section 6.3.5.4 “Nanosyringoscopy”; <http://www.nanomedicine.com/Papers/Aging.pdf>.

<sup>1077</sup> Roxhed N, Samel B, Nordquist L, Griss P, Stemme G. Painless drug delivery through microneedle-based transdermal patches featuring active infusion. *IEEE Trans Biomed Eng.* 2008 Mar;55(3):1063-71; <http://www.ncbi.nlm.nih.gov/pubmed/18334398>. Chua JY, Pendharkar AV, Wang N, Choi R, Andres RH, Gaeta X, Zhang J, Moseley ME, Guzman R. Intra-arterial injection of neural stem cells using a microneedle technique does not cause microembolic strokes. *J Cereb Blood Flow Metab.* 2011 May;31(5):1263-71; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3099630/>.

<sup>1078</sup> Kim SU, de Vellis J. Stem cell-based cell therapy in neurological diseases: a review. *J Neurosci Res.* 2009 Aug 1;87(10):2183-200; <http://www.ncbi.nlm.nih.gov/pubmed/19301431>.

<sup>1079</sup> Feichtinger M, Zemann W, Kärcher H. Removal of a pellet from the left orbital cavity by image-guided endoscopic navigation. *Int J Oral Maxillofac Surg* 2007 Apr;36(4):358-361; <http://www.ncbi.nlm.nih.gov/pubmed/17229549>.

<sup>1080</sup> Song S, Song S, Cao C, Lin X, Li K, Sava V, Sanchez-Ramos J. Hippocampal neurogenesis and the brain repair response to brief stereotaxic insertion of a microneedle. *Stem Cells Int.* 2013;2013:205878; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3608357/>.

painless microneedles.<sup>1081</sup> After penetration,  $\sim 10^{10}$   $\text{micron}^3/\text{sec}$  of nanorobots flowing at 1 m/sec through the tube (typical syringe fluid velocity) could surround a cubic  $1 \text{ cm}^3$  target object (e.g., a solid tumor mass) to a coating thickness of 100 microns in  $\sim 10$  seconds. The coating nanorobots could then dig out 1 micron wide channels at a volumetric excavation rate of 1% nanorobot volume per second to partition the  $1 \text{ cm}^3$  target object into  $10^6$  100-micron microcubes in  $\sim 300$  seconds, after which the target object microcubes are transported out of the patient in single file at 1 m/sec through the nanocatheter in  $\sim 100$  seconds, followed by the exiting nanorobots taking  $\sim 10$  seconds, completing a  $\sim 7$  minute  $\sim 1 \text{ cm}^3$  object-removal nanocatheter procedure through a  $\sim 100$ -micron diameter hole.

Finally, mobile surgical nanorobots imported into the brain through the nanocatheter can also physically manipulate cells<sup>1082</sup> arriving through the nanocatheter, and reposition those cells as desired within tissue voids or other spaces previously cleared or debrided (Section 5.3.2.2) inside the brain.

For those concerned about the possible discomfort or damage to human tissue being simultaneously penetrated by many hundreds or thousands of nanocatheters, it should again be noted that microneedle penetration can be painless<sup>1083</sup> and non-damaging.<sup>1084</sup>

Microneedle density ranges from very low-density arrays such as the MEMS polymer array (**15 microneedles/cm<sup>2</sup>**)<sup>1085</sup> and the dissolving microneedles (**36 microneedles/cm<sup>2</sup>**; image, below, at

<sup>1081</sup> Oh J, Liu K, Medina T, Kralick F, Noh HM. A novel microneedle array for the treatment of hydrocephalus. *Microsyst Technol.* 2014 Jun 1;20(6):1169-1179; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4084597/>.

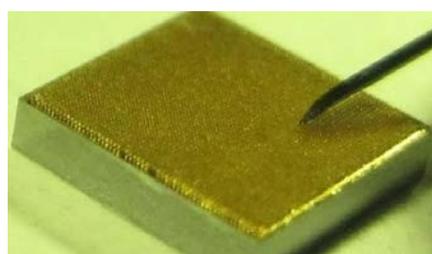
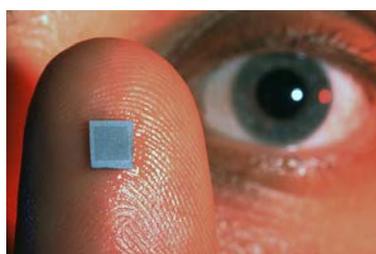
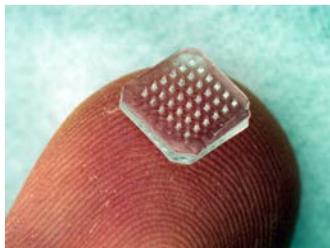
<sup>1082</sup> Leong TG, Randall CL, Benson BR, Bassik N, Stern GM, Gracias DH. Tetherless thermobiochemically actuated microgrippers. *Proc Natl Acad Sci U S A.* 2009 Jan 20;106(3):703-8; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2630075/>. Gach PC, Wang Y, Phillips C, Sims CE, Allbritton NL. Isolation and manipulation of living adherent cells by micromolded magnetic rafts. *Biomicrofluidics.* 2011 Sep;5(3):32002-3200212; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3194786/>.

<sup>1083</sup> Roxhed N, Samel B, Nordquist L, Griss P, Stemme G. Painless drug delivery through microneedle-based transdermal patches featuring active infusion. *IEEE Trans Biomed Eng.* 2008 Mar;55(3):1063-71; <http://www.ncbi.nlm.nih.gov/pubmed/18334398>.

<sup>1084</sup> Chua JY, Pendharkar AV, Wang N, Choi R, Andres RH, Gaeta X, Zhang J, Moseley ME, Guzman R. Intra-arterial injection of neural stem cells using a microneedle technique does not cause microembolic strokes. *J Cereb Blood Flow Metab.* 2011 May;31(5):1263-71; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3099630/>.

<sup>1085</sup> Wang PC, Wester BA, Rajaraman S, Paik SJ, Kim SH, Allen MG. Hollow Polymer Microneedle Array Fabricated by Photolithography Process Combined with Micromolding Technique. 31st Annual International Conference of the IEEE EMBS, Minneapolis, Minnesota, USA, 2-6 Sep 2009; <http://mems.seas.upenn.edu/publications/2009/Hollow%20Polymer%20Microneedle%20Array%20Fabricated%20by%20Photolithography%20Process%20Combined%20with%20Micromolding%20Technique.pdf>.

top),<sup>1086</sup> to very high-density arrays such as the drug-delivery patch (2500 microneedles/cm<sup>2</sup>; image, below, middle left)<sup>1087</sup> and the microprojection arrays (18,750 microneedles/cm<sup>2</sup>; image, below, middle right).<sup>1088</sup> Intermediate in areal number density are microneedle arrays attached to a syringe to permit easy injection of fluids, such as the AdminPen 600 microneedle liquid injection system manufactured by NanoBioSciences LLC (187 microneedles/cm<sup>2</sup>; image, below, at bottom).<sup>1089</sup>



<sup>1086</sup> Shana Leonard, “Dissolving microneedles could eliminate use of sharps, spreading of disease,” QMed MedTech Pulse Blog, 23 Jul 2010; <http://www.qmed.com/mpmn/medtechpulse/dissolving-microneedles-could-eliminate-use-sharps-spreading-disease>.

<sup>1087</sup> “Taking the ‘ouch’ out of needles: Arrays of micron-scale ‘microneedles’ offer new technique for drug delivery,” 22 Jun 1998; <http://gtresearchnews.gatech.edu/newsrelease/NEEDLES.html#copyright>.

<sup>1088</sup> Robinson KJ, Coffey JW, Muller DA, Young PR, Kendall MA, Thurecht KJ, Grøndahl L, Corrie SR. Comparison between polyethylene glycol and zwitterionic polymers as antifouling coatings on wearable devices for selective antigen capture from biological tissue. *Biointerphases*. 2015 Dec 7;10(4):04A305; <http://www.ncbi.nlm.nih.gov/pubmed/26446192>. See also: <http://www.smh.com.au/technology/sci-tech/uq-team-develops-needlefree-disease-detection-through-nanotechnology-patch-20150917-gjoyod.html>.

<sup>1089</sup> “AdminPen 600 microneedle liquid injection system,” NanoBioSciences LLC, AdminMed, 2015; <http://www.adminmed.com/adminpen600>.

Of course, nanocatheters must be inserted deep into the brain and thus will have much greater lengths than commercial microneedles which are typically less than a millimeter long. Might a long skinny nanocatheter buckle when pushed through the brain? If the force required to coaxially push a nanocatheter cylinder of length  $L_{\text{cath}}$  and radius  $R_{\text{cath}}$  at a velocity  $v_{\text{cath}}$  through brain tissue of viscosity  $\eta_{\text{brain}}$  is  $F_{\text{push}} = (4\pi \eta_{\text{brain}} L_{\text{cath}} v_{\text{cath}}) / (1 + \ln(L_{\text{cath}}^2/R_{\text{cath}}^2))$  and if the Euler buckling force  $F_{\text{buckle}} = \pi^3 E_{\text{cath}} (R_{\text{cath}}^4 - r_{\text{cath}}^4) / (4 L_{\text{cath}}^2)$  for a hollow nanocatheter of inside radius  $r_{\text{cath}}$  composed of material with Young's modulus  $E_{\text{cath}}$ ,<sup>1090</sup> and ignoring bony skull penetration (which may be accomplished by conventional surgery), then the **maximum velocity at which a nanocatheter can be pushed through brain tissue** without buckling occurs when the pushing force  $F_{\text{push}}$  equals the buckling force  $F_{\text{buckle}}$ , or  $v_{\text{cathMAX}} = 2300 \mu\text{m/sec}$ , taking  $R_{\text{cath}} = 50 \mu\text{m}$ ,  $r_{\text{cath}} = 47.5 \mu\text{m}$  (i.e.,  $5 \mu\text{m}$  nanocatheter wall thickness),  $L_{\text{cath}} \sim V_{\text{brain}}^{1/3} = 11.5 \text{ cm}$  where adult male human brain volume  $V_{\text{brain}} = 1510 \text{ cm}^3$ ,<sup>1091</sup>  $E_{\text{cath}} = 10^{12} \text{ N/m}^2$  for diamond, and a shear viscosity of human brain tissue at 25-50 Hz of  $\eta_{\text{brain}} \sim 3.4 \text{ Pa-sec}$ .<sup>1092</sup>

---

<sup>1090</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999; Section 9.4.2.4, <http://www.nanomedicine.com/NMI/9.4.2.4.htm>, and Section 9.3.1.2, <http://www.nanomedicine.com/NMI/9.3.1.2.htm>.

<sup>1091</sup> Lüders E, Steinmetz H, Jäncke L. Brain size and grey matter volume in the healthy human brain. Neuroreport. 2002 Dec 3;13(17):2371-4; <http://www.genling.nw.ru/Staff/Psycholinguistics/Brain%20Size.pdf>.

<sup>1092</sup> Sack I, Beierbach B, Hamhaber U, Klatt D, Braun J. Non-invasive measurement of brain viscoelasticity using magnetic resonance elastography. NMR Biomed. 2008 Mar;21(3):265-71; <http://www.ncbi.nlm.nih.gov/pubmed/17614101>.

## Chapter 5. Nanorobotic Treatment: The Alzheimer Protocols

The proposed nanorobotic treatment for Alzheimer's disease, hereinafter called the Alzheimer Protocols, can be conceptually organized as a series of three specific protocols which are aimed at three distinct clinical objectives. These objectives are: **Genetic Derisking** (Section 5.1), **Tissue Rejuvenation** (Section 5.2), and **Neural Reconstruction** (Section 5.3). Note that these are conceptual categories, a convenient way of organizing what needs to be done to treat and to cure AD.

In an actual clinical treatment process, the requirements and modalities of all three protocols will be applied to a patient to the extent needed. Specific activities that might be called for in multiple protocols and which require the same nanorobot instrumentalities can be combined for efficiency into a single coherent treatment plan to cure the particular patient. For example, all AD patients should receive the genotyping (Section 5.1) and brain mapping (Section 5.3.1) procedures, both to ensure a correct AD diagnosis and to acquire complete information prior to designing a comprehensive patient-specific treatment that will be undertaken in the clinic. Chromosome replacement therapies required for both the genetic derisking and anti-aging protocols might be combined into a single application of chromalloy nanorobots, and so forth.

Having applied the proposed Alzheimer Protocols to a particular patient (as described at length in the rest of this Chapter), what sorts of outcomes might we reasonably expect?

For **preclinical patients**, the treatments associated with the first two Alzheimer Protocols should largely suffice to hold Alzheimer's disease at bay indefinitely, though likely requiring periodic checkups and partial retreatments, perhaps decadal. Eliminating AD permanently without the need for periodic checkups and retreatment would probably require (1) comprehensive re-engineering of the human genome, (2) incorporation of permanent nanorobotic implants into the human body, or (3) replacement of the biological human body with an engineered synthetic body – all of which are beyond the scope of the present discussion.

For diagnosed AD patients (Section 2.3), Alzheimer Protocol #3 will be required for maximum recovery, with the following anticipated results:

**Stage 1 pre-dementia patients** with “mild cognitive impairment” should expect a complete recovery to preclinical levels of function, probably with memories fully intact.

**Stage 2 early AD patients** should recover all physical abilities and most memories, and a complete recovery is possible.

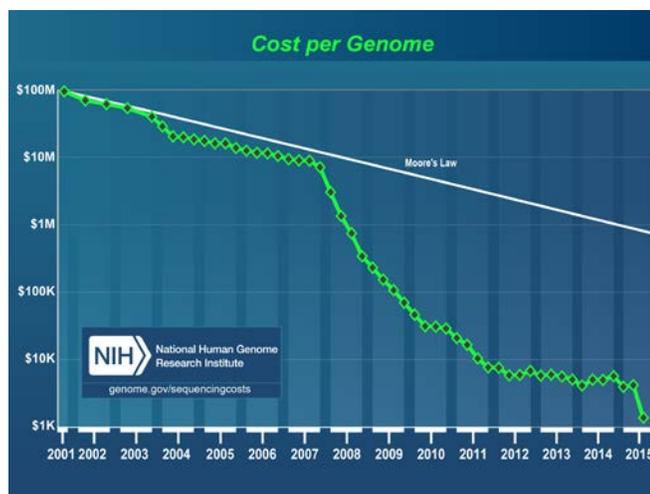
**Stage 3 moderate AD patients** have a good chance of recovering all physical abilities and their original behavioral persona. Many memories can be recovered or reconstructed, but some memories will remain lost.

**Stage 4 advanced AD patients** have a good chance of recovering all physical abilities and much of their former behavioral persona. Some memories can be recovered or reconstructed, but many memories will remain lost. Offsetting this loss will be a regained ability to make and retain new memories during the retraining process (Section 5.3.3), allowing the patient to retain their core identity and to resume normal relationships with family, friends, and associates.

## 5.1 First Alzheimer Protocol: Genetic Derisking

To begin the First Alzheimer Protocol, each patient is fully genotyped using increasingly inexpensive gene sequencing technologies (image, right).<sup>1093</sup>

Even without the advent of nanotechnology-based approaches, the first \$1000 genome was already available by the end of 2015.<sup>1094</sup> By the time medical nanorobotics is fully available, it is highly likely that the cost of sequencing may have fallen as low as ~\$1/genome, and software tools for organizing and analyzing this data should be commonplace.



Upon full genotyping and analysis, it may be discovered (in a modest number of cases) that the patient has inherited a small subset of genes that increase the person's risk of developing Alzheimer's disease – possibly at an early age. Some of these patients might already be displaying clinical signs of AD. All such genetically at-risk patients should first be treated with a gene editing procedure<sup>1095</sup> to correct these inherited genetic defects that produce increased susceptibility to AD. Required genetic corrections may include replacement of familial Alzheimer's disease gene mutations (Section 5.1.1), replacement of apolipoprotein ApoE4 genes (Section 5.1.2) and other high-risk genes (Section 5.1.3), and elimination of AD-related pathological genetic mosaicism (Section 5.1.4). Once identified, all required genetic corrections can be applied in a single procedure directed to all relevant target cells.

<sup>1093</sup> Freitas RA Jr. The ideal gene delivery vector: Chromalloyocytes, cell repair nanorobots for chromosome replacement therapy. *J Evol Technol* 2007;16:1-97, Section 4.2; <http://jetpress.org/v16/freitas.pdf>.

<sup>1094</sup> "Veritas Genetics Breaks \$1,000 Whole Genome Barrier," 29 Sep 2015; <http://www.prnewswire.com/news-releases/veritas-genetics-breaks-1000-whole-genome-barrier-300150585.html>.

<sup>1095</sup> Conventional biotech techniques for *in vivo* gene editing such as CRISPR are being tried and may work on a gene-by-gene basis. Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. Genome engineering using the CRISPR-Cas9 system. *Nat Protoc*. 2013 Nov;8(11):2281-308; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3969860/>. But these methods are still immature and can produce incomplete gene alterations along with off-target effects on other genes. Liang P, Xu Y, Zhang X, Ding C, Huang R, Zhang Z, Lv J, Xie X, Chen Y, Li Y, Sun Y, Bai Y, Songyang Z, Ma W, Zhou C, Huang J. CRISPR/Cas9-mediated gene editing in human triploid zygotes. *Protein Cell*. 2015 May;6(5):363-72; <http://link.springer.com/content/pdf/10.1007%2Fs13238-015-0153-5.pdf>. See also Section 3.2.12.

For the genetic derisking protocol, the patient's genotype will be analyzed and edited,<sup>1096</sup> resulting in a corrected (derisked) genotype sequence. Working from this corrected data file, replacement chromosome sets will be fabricated in an *ex vivo* desktop chromosome manufacturing facility,<sup>1097</sup> then loaded into billions of nanorobots for delivery to every one of the patient's affected cells (Section 4.2.3). These replacement sets will contain chromosomes from which the defective or excessive genes that lead to AD have been deleted and replaced with nondefective genes that do not lead to AD.

For example, correcting high-risk genes that are present in all of the patient's neurons would require an injected dose of  $N_{\text{bot}} = 86$  billion chromalloyte nanorobots (total  $\sim 6 \text{ cm}^3$  volume, assuming  $69 \mu\text{m}^3$  device volume per nanorobot; Section 4.2.3) that can individually target each of the 86 billion neurons in the human brain,<sup>1098</sup> with each nanorobot delivering one complete replacement chromosome set into the nucleus of every neuron.

The time requirement for this procedure has been estimated as approximately 3 hours for essential pre- and post-operative work, to which must be added another 4 hours for every  $10^{12}$ -nanorobot dose of treatment chromalloytes that are infused into the patient (with each nanorobot targeting a single nucleus in a cell). This assumes a conservative nanorobot infusion rate of  $\sim 1 \text{ cm}^3/\text{min}$  and restricts the maximum whole-body generation of waste heat to maximum acceptable levels  $< 100$  watts.<sup>1099</sup> By this means, **all 86 billion neurons in a human brain could be cleared of pro-Alzheimer's genes in  $\sim 7$  hours of chromalloyte treatment time.**

It may be deemed necessary in most if not all cases to clear high-risk pro-AD genes not just from all brain cells, but from all  $\sim 10$  trillion cell nuclei in the human body. This could be accomplished using a series of 10 dosage cycles of chromalloytes, using a  $\sim 1$  terabot dose per cycle. **Whole-body pro-AD gene clearance would require  $\sim 48$  hours of continuous treatment**, which includes an extra 5 hours for special-case requirements, with a maximum of  $10^{12}$  chromalloytes ( $\sim 69 \text{ cm}^3$  volume) present in the human body ( $\sim 60,000 \text{ cm}^3$  volume) at any one time.

---

<sup>1096</sup> Freitas RA Jr. The ideal gene delivery vector: Chromalloytes, cell repair nanorobots for chromosome replacement therapy. J Evol Technol 2007;16:1-97, Section 4.1; <http://jetpress.org/v16/freitas.pdf>.

<sup>1097</sup> Freitas RA Jr. The ideal gene delivery vector: Chromalloytes, cell repair nanorobots for chromosome replacement therapy. J Evol Technol 2007;16:1-97, Section 4.3; <http://jetpress.org/v16/freitas.pdf>.

<sup>1098</sup> Azevedo FA, Carvalho LR, Grinberg LT, Farfel JM, Ferretti RE, Leite RE, Jacob Filho W, Lent R, Herculano-Houzel S. Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. J Comp Neurol. 2009 Apr 10;513(5):532-41; <http://www.sakkyndig.com/psykologi/artvit/frederico2009.pdf>.

<sup>1099</sup> Freitas RA Jr. The ideal gene delivery vector: Chromalloytes, cell repair nanorobots for chromosome replacement therapy. J Evol Technol 2007;16:1-97, Section 3.6; <http://jetpress.org/v16/freitas.pdf>.

### 5.1.1 Replace Familial Alzheimer's Gene Mutations

Familial Alzheimer's disease (FAD), aka. early onset familial Alzheimer's disease (EOFAD), accounts for 1%-5% of all Alzheimer's cases.<sup>1100</sup> It usually strikes between 40-65 years of age, though occasionally earlier. It is inherited in an autosomal dominant fashion,<sup>1101</sup> mostly attributable to mutations in one of three genes: the presenilin 1 gene (PSEN1 located on Chromosome 14),<sup>1102</sup> the presenilin 2 gene (PSEN2 located on Chromosome 1),<sup>1103</sup> and the amyloid precursor protein gene (APP located on Chromosome 21).<sup>1104</sup> Hundreds of different mutations of these three genes have been identified so far. EOFAD may be caused by overproduction of A $\beta$  due to mutations in the aforementioned three genes, which are essential components of the  $\gamma$ -secretase complexes responsible for cleavage and release of A $\beta$ .<sup>1105</sup>

Patients expressing any one of these mutations can be genetically derisked by having their defective gene(s) on Chromosomes 1, 14, and 21 replaced with normal gene variants that do not cause AD, using a single treatment with chromalloy nanorobots.

It may also be possible to replace any of the AD-susceptibility genes with an alternative gene variant that induces protection against Alzheimer's, rather than susceptibility to it, without creating new problems elsewhere. The best-known example is a coding mutation (A673T) in the APP gene that confers protection against Alzheimer's disease and protection against cognitive decline in the elderly without Alzheimer's disease.<sup>1106</sup> This is a rare natural APP variant that

<sup>1100</sup> Reitz C, Brayne C, Mayeux R. Epidemiology of Alzheimer disease. *Nat Rev Neurol*. 2011 Mar;7(3):137-52; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3339565/>.

<sup>1101</sup> **Autosomes** are pairs of chromosomes other than the sex-linked X and Y chromosomes; autosomal dominant inheritance occurs via the non-sex-linked chromosomes.

<sup>1102</sup> Taddei K, Fisher C, Laws SM, Martins G, Paton A, Clarnette RM, Chung C, Brooks WS, Hallmayer J, Miklossy J, Relkin N, St George-Hyslop PH, Gandy SE, Martins RN. Association between presenilin-1 Glu318Gly mutation and familial Alzheimer's disease in the Australian population. *Mol Psychiatry*. 2002;7(7):776-81; <http://www.nature.com/mp/journal/v7/n7/full/4001072a.html>.

<sup>1103</sup> Cai Y, An SS, Kim S. Mutations in presenilin 2 and its implications in Alzheimer's disease and other dementia-associated disorders. *Clin Interv Aging*. 2015 Jul 14;10:1163-72; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4507455/>.

<sup>1104</sup> Rossor MN, Newman S, Frackowiak RS, Lantos P, Kennedy AM. Alzheimer's disease families with amyloid precursor protein mutations. *Ann N Y Acad Sci*. 1993 Sep 24;695:198-202; <http://www.ncbi.nlm.nih.gov/pubmed/8239283>.

<sup>1105</sup> Blennow K, de Leon MJ, Zetterberg H. Alzheimer's disease. *Lancet*. 2006 Jul 29;368(9533):387-403; <https://www.ncbi.nlm.nih.gov/pubmed/16876668>.

<sup>1106</sup> Jonsson T, *et al*. A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature*. 2012 Aug 2;488(7409):96-9; <http://www.nature.com/nature/journal/vaop/ncurrent/full/nature11283.html>.

encodes an alanine-to-threonine substitution at residue 673. The substitution is adjacent to the aspartyl protease  $\beta$ -site in APP and reduces the formation of amyloidogenic peptides *in vitro* by ~40%, while also mildly decreasing A $\beta$  aggregation.<sup>1107</sup> The strong protective effect of the A673T substitution against Alzheimer's disease provides proof of principle that reducing the  $\beta$ -cleavage of APP may protect against the disease.

Chromalloytes could perform this and similar replacements as well.

---

<sup>1107</sup> Maloney JA, Bainbridge T, Gustafson A, Zhang S, Kyauk R, Steiner P, van der Brug M, Liu Y, Ernst JA, Watts RJ, Atwal JK. Molecular mechanisms of Alzheimer disease protection by the A673T allele of amyloid precursor protein. *J Biol Chem*. 2014 Nov 7;289(45):30990-1000; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4223305/>. Zheng X, Liu D, Roychaudhuri R, Teplow DB, Bowers MT. Amyloid  $\beta$ -Protein Assembly: Differential Effects of the Protective A2T Mutation and Recessive A2V Familial Alzheimer's Disease Mutation. *ACS Chem Neurosci*. 2015 Oct 21;6(10):1732-40; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4618051/>.

## 5.1.2 Replace Apolipoprotein ApoE4 Gene

Apolipoprotein E (aka. APOE; Section 2.4.5) is a class of apolipoprotein found in the chylomicron and intermediate-density lipoprotein that is essential for the normal catabolism of triglyceride-rich lipoprotein constituents. APOE is primarily produced by the liver and macrophages in peripheral tissues and by astrocytes in the central nervous system, and transports cholesterol to neurons via APOE receptors which are members of the low density lipoprotein receptor gene family.<sup>1108</sup>

The three polymorphic alleles (ApoE2, ApoE3, ApoE4) of the human APOE gene on Chromosome 19 have a worldwide frequency of 8.4%, 77.9% and 13.7%, respectively, with the frequency of the ApoE4 allele dramatically increased to ~40% in patients with AD.<sup>1109</sup> The lifetime risk estimate of developing AD for individuals with two copies of the ApoE4 allele (~2% of the population) is ~60% by the age of 85, and for those with one copy of the ApoE4 allele (~25% of the population) ~30%. In comparison, the lifetime risk of AD for those with two copies of the ApoE3 allele is ~10% by the age of 85. Between 40% and 80% of people with AD possess at least one ApoE4 allele.<sup>1110</sup> Thus, ApoE4 is considered the foremost late-onset AD gene.<sup>1111</sup>

ApoE4 is clearly undesirable.<sup>1112</sup> Amyloid A $\beta$  deposition in the form of senile plaques is more abundant in ApoE4 carriers compared with noncarriers, with 40.7% of ApoE4 carriers having senile plaques compared with 8.2% of noncarriers among individuals aged 50-59 years.<sup>1113</sup>

---

<sup>1108</sup> Liu CC, Kanekiyo T, Xu H, Bu G. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nat Rev Neurol*. 2013 Feb;9(2):106-18; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3726719/>.

<sup>1109</sup> Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, Pericak-Vance MA, Risch N, van Duijn CM. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA*. 1997 Oct 22-29;278(16):1349-56; <https://www.ncbi.nlm.nih.gov/pubmed/9343467>.

<sup>1110</sup> Mahley RW, Weisgraber KH, Huang Y. Apolipoprotein E4: a causative factor and therapeutic target in neuropathology, including Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America*. 2006;103(15):5644-51; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1414631/>.

<sup>1111</sup> Genin E, Hannequin D, Wallon D, Sleegers K, Hiltunen M, Combarros O, Bullido MJ, Engelborghs S, De Deyn P, Berr C, *et al*. APOE and Alzheimer disease: A major gene with semi-dominant inheritance. *Mol Psychiatry*. 2011;16:903-907; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3162068/>.

<sup>1112</sup> Genin E, *et al*. APOE and Alzheimer disease: a major gene with semi-dominant inheritance. *Mol Psychiatry*. 2011 Sep;16(9):903-7; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3162068/>.

<sup>1113</sup> Kok E, Haikonen S, Luoto T, Huhtala H, Goebeler S, Haapasalo H, Karhunen PJ. Apolipoprotein E-dependent accumulation of Alzheimer disease-related lesions begins in middle age. *Ann Neurol*. 2009 Jun;65(6):650-7; <https://www.ncbi.nlm.nih.gov/pubmed/19557866>.

Independently of A $\beta$ , ApoE4 triggers inflammatory cascades that cause neurovascular dysfunction, including BBB breakdown, leakage of blood-derived toxic proteins into the brain, and reduction in the length of small vessels.<sup>1114</sup>

ApoE3 is considered the “neutral” or natural variant, largely avoiding the pro-AD effects of the ApoE4 allele, although the ApoE2 variant appears slightly less pro-AD than ApoE3. There is evidence that the ApoE2 allele might serve a protective role in AD<sup>1115</sup> – patients with two copies of ApoE4 have 25 times higher risk of AD as patients with two copies of ApoE2 (who have the lowest risk of AD).<sup>1116</sup> Initial concerns<sup>1117</sup> that ApoE2 might be linked to Parkinson’s disease have been largely discounted following a larger population association study,<sup>1118</sup> and there is some evidence that ApoE2 may be protective against myocardial infarction.<sup>1119</sup> On the cautionary side, the ApoE2 allele has been linked to increased risk for Type-III hyperlipoproteinemia,<sup>1120</sup> though the presence of ApoE2 appears essential but not sufficient to induce this rare condition.<sup>1121</sup> ApoE2 patients may also be at a higher risk of intracerebral hemorrhage.<sup>1122</sup>

<sup>1114</sup> Bell RD, Winkler EA, Singh I, Sagare AP, Deane R, Wu Z, Holtzman DM, Betsholtz C, Armulik A, Sallstrom J, Berk BC, Zlokovic BV. Apolipoprotein E controls cerebrovascular integrity via cyclophilin A. *Nature*. 2012 May 16;485(7399):512-6; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4047116/>.

<sup>1115</sup> Corder EH, Saunders AM, Risch NJ, Strittmatter WJ, Schmechel DE, Gaskell PC Jr, Rimmler JB, Locke PA, Conneally PM, Schmechel KE, *et al*. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. *Nat Genet*. 1994 Jun;7(2):180-4; <http://www.ncbi.nlm.nih.gov/pubmed/7920638>.

<sup>1116</sup> Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, Pericak-Vance MA, Risch N, van Duijn CM. Effects of age, sex, and ethnicity on the association between apolipoprotein genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA*. 1997 Oct 22-29;278(16):1349-56; <http://www.ncbi.nlm.nih.gov/pubmed/9343467>.

<sup>1117</sup> Huang X, Chen PC, Poole C. APOE-[epsilon]2 allele associated with higher prevalence of sporadic Parkinson disease. *Neurology*. 2004 Jun 22;62(12):2198-202; <https://www.ncbi.nlm.nih.gov/pubmed/15210882>

<sup>1118</sup> Federoff M, Jimenez-Rolando B, Nalls MA, Singleton AB. A large study reveals no association between APOE and Parkinson’s disease. *Neurobiol Dis*. 2012 May;46(2):389-92; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3323723/>.

<sup>1119</sup> Wang YL, Sun LM, Zhang L, Xu HT, Dong Z, Wang LQ, Wang ML. Association between Apolipoprotein E polymorphism and myocardial infarction risk: A systematic review and meta-analysis. *FEBS Open Bio*. 2015 Oct 23;5:852-8; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4637359/>.

<sup>1120</sup> Breslow JL, Zannis VI, SanGiacomo TR, Third JL, Tracy T, Glueck CJ. Studies of familial type III hyperlipoproteinemia using as a genetic marker the apoE phenotype E2/2. *J Lipid Res*. 1982 Nov;23(8):1224-35; <http://www.jlr.org/content/23/8/1224.long>.

<sup>1121</sup> Phillips MC. Apolipoprotein E isoforms and lipoprotein metabolism. *IUBMB Life*. 2014 Sep;66(9):616-23; <http://onlinelibrary.wiley.com/doi/10.1002/iub.1314/full>

<sup>1122</sup> Viswanathan A, Greenberg SM. Cerebral amyloid angiopathy in the elderly. *Ann Neurol*. 2011 Dec;70(6):871-80; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4004372/>.

While a final choice between ApoE2 and ApoE3 may be premature at this time, a strong recommendation to use chromalloy nanorobots to replace the ApoE4 isoform with either ApoE3 or ApoE2 variants seems warranted as part of the genetic derisking procedure in the First Alzheimer Protocol.

### 5.1.3 Replace Other High-Risk Genes

In addition to the APOE locus encoding apolipoprotein E (Section 5.1.2), genome-wide Alzheimer's association studies in individuals of European ancestry during 2009-2013 identified 11 other genomic regions associated with late-onset AD as "susceptibility loci," including:

(1) CLU (aka. APOJ) on Chromosome 8,<sup>1123</sup> encoding clusterin or apolipoprotein J; CLU has a supporting role in the clearance of beta amyloid peptide.

(2) CR1 on Chromosome 1,<sup>1124</sup> encoding the complement component (3b/4b) receptor 1; CR1 has a supporting role in the clearance of beta amyloid peptide. A variant in CR1 is also associated with increased risk of cerebral amyloid angiopathy-related intracerebral hemorrhage.<sup>1125</sup>

(3) PICALM (phosphatidylinositol-binding clathrin assembly protein) on Chromosome 11,<sup>1126</sup> involved in clathrin-mediated endocytosis which directs the trafficking of proteins that play a prominent role in the fusion of synaptic vesicles to the presynaptic membrane in neurotransmitter release, a process crucial to neuronal function.

(4) BIN1 (bridging integrator 1) on Chromosome 2,<sup>1127</sup> one of two amphiphysins expressed most abundantly in the brain and muscle. Amphiphysins promote caspase-independent apoptosis and also play a critical role in neuronal membrane organization and clathrin-mediated synaptic vesicle formation, a process disrupted by A $\beta$ .

---

<sup>1123</sup> Harold D, *et al.* Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat Genet.* 2009 Oct;41(10):1088-93; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2845877/>. Lambert JC, *et al.* Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nat Genet.* 2009 Oct;41(10):1094-9; <http://www.ncbi.nlm.nih.gov/pubmed/19734903>.

<sup>1124</sup> Lambert JC, *et al.* Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nat Genet.* 2009 Oct;41(10):1094-9; <http://www.ncbi.nlm.nih.gov/pubmed/19734903>.

<sup>1125</sup> Biffi A, *et al.* Genetic variation at CR1 increases risk of cerebral amyloid angiopathy. *Neurology.* 2012 Jan 31;78(5):334-41; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3280047/>.

<sup>1126</sup> Harold D, *et al.* Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat Genet.* 2009 Oct;41(10):1088-93; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2845877/>.

<sup>1127</sup> Seshadri S, *et al.* Genome-wide analysis of genetic loci associated with Alzheimer disease. *JAMA.* 2010 May 12;303(18):1832-40; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2989531/>.

(5) The 19q13.3 locus (rs597668) near EXOC3L2/BLOC1S3/MARK4 on Chromosome 19,<sup>1128</sup> that seems independent of APOE but is currently of unknown function. Two genes adjacent to this locus are part of pathways linked to Alzheimer pathology.

(6) ABCA7 on Chromosome 19,<sup>1129</sup> aka. ATP-binding cassette transporter, sub-family A, member 7. ABCA7 is highly expressed in brain, especially hippocampal CA1 neurons and in microglia.

(7) The membrane spanning MS4A gene cluster on Chromosome 11,<sup>1130</sup> a family of cell surface proteins.

(8) CD2AP (a scaffold/adaptor protein) on Chromosome 6.<sup>1131</sup>

(9) CD33 (cluster of differentiation 33, an endocytic receptor) on Chromosome 19.<sup>1132</sup>

(10) EPHA1 (ephrin receptor A1) on Chromosome 7, with roles in cell and axon guidance and in synaptic development and plasticity.<sup>1133</sup>

<sup>1128</sup> Seshadri S, *et al.* Genome-wide analysis of genetic loci associated with Alzheimer disease. JAMA. 2010 May 12;303(18):1832-40; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2989531/>.

<sup>1129</sup> Hollingworth P, *et al.* Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet. 2011 May;43(5):429-35; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3084173/>.

<sup>1130</sup> Hollingworth P, *et al.* Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet. 2011 May;43(5):429-35; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3084173/>. Naj AC, *et al.* Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. Nat Genet. 2011 May;43(5):436-41; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3090745/>.

<sup>1131</sup> Hollingworth P, *et al.* Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet. 2011 May;43(5):429-35; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3084173/>. Naj AC, *et al.* Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. Nat Genet. 2011 May;43(5):436-41; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3090745/>.

<sup>1132</sup> Hollingworth P, *et al.* Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet. 2011 May;43(5):429-35; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3084173/>. Naj AC, *et al.* Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. Nat Genet. 2011 May;43(5):436-41; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3090745/>.

<sup>1133</sup> Hollingworth P, *et al.* Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet. 2011 May;43(5):429-35; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3084173/>. Naj AC, *et al.* Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. Nat Genet. 2011 May;43(5):436-41; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3090745/>.

(11) Heterozygous rare variants in TREM2 (triggering receptor expressed on myeloid cells 2) on Chromosome 6.<sup>1134</sup>

An even more extensive genome-wide Alzheimer's association study completed in 2014 found yet another 11 new genetic susceptibility loci,<sup>1135</sup> bringing the number of currently-known genetic loci associated with late-onset AD up to 22, including:

(12) CASS4 on Chromosome 20, in the APP pathway, tau pathology, and in cytoskeletal function and axonal transport;

(13) CELF1 on Chromosome 11, in cytoskeletal function and axonal transport;

(14) FERMT2 on Chromosome 14, in tau pathology;

(15,16) HLA-DRB5 and HLA-DRB1 on Chromosome 6, in immune response and inflammation;

(17) INPP5D on Chromosome 2, in regulation of gene expression and post-translational modification of proteins, microglial and myeloid cell function, and in immune response and inflammation;

(18) MEF2C on Chromosome 5, in hippocampal synaptic function and in immune response and inflammation;

(19) NME8 on Chromosome 7, in cytoskeletal function and axonal transport;

(20) PTK2B on Chromosome 8, in cell migration and hippocampal synaptic function;

(21) SORL1 (sortilin-related receptor) on Chromosome 11, in the APP pathway and in lipid transport and endocytosis; and

(22) the ZCWPW1 gene on Chromosome 7, encoding zinc finger and modulating epigenetic regulation.

These 22 new risk alleles<sup>1136</sup> have a much smaller effect on late-onset AD susceptibility than does ApoE4 (Section 5.1.2), with estimates for the “population-attributable fractions” for these new

---

<sup>1134</sup> Jonsson T, *et al.* Variant of TREM2 associated with the risk of Alzheimer's disease. *N Engl J Med.* 2013 Jan 10;368(2):107-16; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3677583/>. Guerreiro R, *et al.* TREM2 variants in Alzheimer's disease. *N Engl J Med.* 2013 Jan 10;368(2):117-27; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3631573/>.

<sup>1135</sup> Lambert JC, *et al.* Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet.* 2013 Dec;45(12):1452-8; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3896259/>.

loci in the range of 1%-8%.<sup>1137</sup> Nevertheless, 1%-8% of all people is still a significant patient population, and an appropriate treatment by chromalloy nanorobots should suffice to replace all AD-susceptible gene variants with safer variants that do not confer susceptibility to Alzheimer's disease.

What about mitochondrial DNA mutations? While inherited stable mtDNA variations (mitochondrial haplogroups such as H5)<sup>1138</sup> have been associated with the development of neurodegenerative disease,<sup>1139</sup> so far it appears that pathogenic inherited mtDNA mutations do not constitute a major etiological factor in sporadic AD.<sup>1140</sup> A few mitochondrial gene variants have been proposed to increase susceptibility to AD but researchers remain skeptical – e.g., one polymorphic poly-T variant (rs10524523) in the translocase of the outer mitochondrial membrane 40 homolog (TOMM40) gene was implicated in one study<sup>1141</sup> but could not be replicated.<sup>1142</sup>

<sup>1136</sup> There may be a few other risk alleles for late-onset AD. For example, the rare N660Y variant of the APP gene on Chromosome 21 that was identified in one case from a late-onset AD family, and a few rare variants of PSEN1 exist with similar effect. Cruchaga C, *et al.* Rare variants in APP, PSEN1 and PSEN2 increase risk for AD in late-onset Alzheimer's disease families. PLoS One. 2012;7(2):e31039; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3270040/>.

<sup>1137</sup> Zou Z, Liu C, Che C, Huang H. Clinical genetics of Alzheimer's disease. Biomed Res Int. 2014;2014:291862; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4052685/>.

<sup>1138</sup> Santoro A, *et al.* Evidence for sub-haplogroup h5 of mitochondrial DNA as a risk factor for late onset Alzheimer's disease. PLoS One. 2010 Aug 6;5(8):e12037; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2917370/>.

<sup>1139</sup> Keogh MJ, Chinnery PF. Mitochondrial DNA mutations in neurodegeneration. Biochim Biophys Acta. 2015 Nov;1847(11):1401-11; <http://www.ncbi.nlm.nih.gov/pubmed/26014345>.

<sup>1140</sup> Elson JL, Herrstadt C, Preston G, Thal L, Morris CM, Edwardson JA, Beal MF, Turnbull DM, Howell N. Does the mitochondrial genome play a role in the etiology of Alzheimer's disease? Hum Genet. 2006 Apr;119(3):241-54; <http://www.ncbi.nlm.nih.gov/pubmed/16408223>.

<sup>1141</sup> Roses AD, Lutz MW, Amrine-Madsen H, Saunders AM, Crenshaw DG, Sundseth SS, Huentelman MJ, Welsh-Bohmer KA, Reiman EM. A TOMM40 variable-length polymorphism predicts the age of late-onset Alzheimer's disease. Pharmacogenomics J. 2010 Oct;10(5):375-84; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2946560/>.

<sup>1142</sup> Cruchaga C, Nowotny P, Kauwe JS, Ridge PG, Mayo K, Bertelsen S, Hinrichs A, Fagan AM, Holtzman DM, Morris JC, Goate AM; Alzheimer's Disease Neuroimaging Initiative. Association and expression analyses with single-nucleotide polymorphisms in TOMM40 in Alzheimer disease. Arch Neurol. 2011 Aug;68(8):1013-9; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3204798/>.

### 5.1.4 Eliminate Pathological Genetic Mosaicism

It has long been known that having an extra copy of the APP gene on Chromosome 21 can cause rare “familial” Alzheimer’s disease (<5% of AD cases), and this APP duplication can be passed on genetically and thus be present in all the cells of a patient’s body.

Early studies of the far more common “sporadic” Alzheimer’s disease (>95% of AD cases) showed no difference in the number of APP genes found in tissue samples, including whole brain. But it appears these studies may have been conducted without adequate appreciation of the possibility of brain “mosaicism” (i.e., when cells with varied DNA are present in the same person), and thus individual neurons were not investigated.

A more recent study<sup>1143</sup> used five different types of experiments to examine the DNA content of single neurons for possible mosaicism and found a possible additional genetic source of excess beta amyloid (A $\beta$ ) generation. The researchers discovered that individual neurons from people with Alzheimer’s disease contained more DNA – on average, hundreds of millions of DNA base pairs more – and also more copies of the APP gene, with some neurons containing up to 12 copies. Cortical nuclei of Alzheimer’s brains displayed increased average copy numbers (~3.8-4.0 copies) significantly higher than for control samples (~1.7-2.2 copies), with increased frequencies of high copy number nuclei (6 or more copies) primarily occurring in prefrontal cortex samples of AD brains. All these extra copies of the APP gene could cause overexpression of APP in all affected cells in the brain, leading, in turn, to overproduction of beta amyloid. The mosaicism itself could be caused by a variety of environmental factors known to produce genetic damage, or by physical trauma, or even by cellular aging.

A single-pass treatment by chromalloyte nanorobots should suffice to remove all excess (>2) copies of the APP gene, via direct replacement of the entire Chromosome 21.

---

<sup>1143</sup> Bushman DM, Kaeser GE, Siddoway B, Westra JW, Rivera RR, Rehen SK, Yung YC, Chun J. Genomic mosaicism with increased amyloid precursor protein (APP) gene copy number in single neurons from sporadic Alzheimer’s disease brains. *Elife*. 2015 Feb 4;4; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4337608/>.

## 5.2 Second Alzheimer Protocol: Tissue Rejuvenation

Although Alzheimer's disease has not traditionally been regarded as a normal part of growing older, almost everyone agrees that the greatest risk factor for AD is increasing age, with the risk doubling every five years after age 65.<sup>1144</sup> The role of aging in AD is increasingly coming under closer scrutiny.<sup>1145</sup> Biogerontologist Aubrey de Grey goes further, asserting that AD is a disease that everyone would get eventually, if only they lived long enough.<sup>1146</sup> Our synoptic view of the genesis of Alzheimer's disease (Section 2.4.8) suggests that the natural progress of aging gradually reduces the effectiveness of the biochemical pathways that control the normal processing and degradation of proteins, leading to a failure of proteostasis and subsequently to the accumulation of excess A $\beta$ , tau protein, and a cascade of pathological biochemical events, manifesting eventually in the clinical symptoms of AD.

If this view is mostly correct, then it should be possible to indefinitely postpone the arrival of AD by indefinitely postponing aging, using the full suite of anti-aging treatments<sup>1147</sup> made possible by medical nanorobots (Section 4.2) manufactured in nanofactories (Section 4.1). Some forms of aging-related damage are measurable as early as 30 years of age,<sup>1148</sup> including declining cognitive function,<sup>1149</sup> increasing difficulty with balance and motor skill tests,<sup>1150</sup> dementia-associated

---

<sup>1144</sup> "Risk Factors," Alzheimer's Association, 2015; [http://www.alz.org/alzheimers\\_disease\\_causes\\_risk\\_factors.asp](http://www.alz.org/alzheimers_disease_causes_risk_factors.asp).

<sup>1145</sup> Kerchner GA, Wyss-Coray T. The role of aging in Alzheimer's disease. In Sierra F, Kohanski R, eds., *Advances in Geroscience*, Springer, 2016, pp. 197-227; [http://link.springer.com/chapter/10.1007/978-3-319-23246-1\\_7](http://link.springer.com/chapter/10.1007/978-3-319-23246-1_7).

<sup>1146</sup> "All of the major diseases of old age are things that we would all get if we didn't die of something else first. They result from the gradual lifelong accumulation of precursors of those diseases. Those precursors continue to accumulate as inevitable side effects of normal metabolism, so inevitably we're going to get them, once we live long enough, as long as we don't die of something else first." See: Big Think interview with Aubrey de Grey; <http://bigthink.com/videos/big-think-interview-with-aubrey-de-grey>. See also: <http://bigthink.com/articles/will-everyone-get-alzheimers-if-they-live-long-enough>.

<sup>1147</sup> Freitas RA Jr. "Chapter 23. Comprehensive Nanorobotic Control of Human Morbidity and Aging," in Gregory M. Fahy, Michael D. West, L. Stephen Coles, and Steven B. Harris, eds, *The Future of Aging: Pathways to Human Life Extension*, Springer, New York, 2010, pp. 685-805; <http://www.nanomedicine.com/Papers/Aging.pdf>.

<sup>1148</sup> Belsky DW, Caspi A, Houts R, Cohen HJ, Corcoran DL, Danese A, Harrington H, Israel S, Levine ME, Schaefer JD, Sugden K, Williams B, Yashin AI, Poulton R, Moffitt TE. Quantification of biological aging in young adults. *Proc Natl Acad Sci U S A*. 2015 Jul 28;112(30):E4104-10; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4522793/>.

<sup>1149</sup> Salthouse, TA. What and when of cognitive aging. *Current directions in psychological science*, 13(4), 2004, 140-144. <http://faculty.virginia.edu/cogage/publications2/What%20and%20When%20of%20Cognitive%20Aging.pdf>

microvascular changes,<sup>1151</sup> and the peaking of different mental abilities<sup>1152</sup> at ages ranging from the late teens to the late 40s. But except for a few patients who are genetically at risk for “familial” or “early-onset” Alzheimer’s disease (Section 5.1.1), people under 30-50 years of age generally do not display clinical signs of AD. As a result, arresting brain aging in pre-clinical Alzheimer’s individuals should halt the accumulation of further damage and should suffice to indefinitely postpone the development of clinically observable Alzheimer’s disease.

How do we arrest aging? According to a foundational paper in the field of anti-aging research:<sup>1153</sup>

Aging is a three-stage process: metabolism, damage, and pathology. The biochemical processes that sustain life generate toxins as an intrinsic side effect. These toxins cause damage, of which a small proportion cannot be removed by any endogenous repair process and thus accumulates. This accumulating damage ultimately drives age-related degeneration. Interventions can be designed at all three stages. However, intervention in metabolism can only modestly postpone pathology, because production of toxins is so intrinsic a property of metabolic processes that greatly reducing that production would entail fundamental redesign of those processes. Similarly, intervention in pathology is a losing battle if the damage that drives it is accumulating unabated. By contrast, **intervention to remove the accumulating damage** would sever the link between metabolism and pathology, and so **has the potential to postpone aging indefinitely**. The term “negligible senescence” was coined<sup>1154</sup> to denote the absence of a statistically detectable increase with organismal age in a species’ mortality rate.

The use of medical nanorobots to arrest aging throughout the human body has been described at length in a previous work.<sup>1155</sup> Seven major categories of accumulative age-related damage have

<sup>1150</sup> Belsky DW, Caspi A, Houts R, Cohen HJ, Corcoran DL, Danese A, Harrington H, Israel S, Levine ME, Schaefer JD, Sugden K, Williams B, Yashin AI, Poulton R, Moffitt TE. Quantification of biological aging in young adults. *Proc Natl Acad Sci U S A*. 2015 Jul 28;112(30):E4104-10; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4522793/>.

<sup>1151</sup> Heringa SM, Bouvy WH, van den Berg E, Moll AC, Kappelle LJ, Biessels GJ. Associations between retinal microvascular changes and dementia, cognitive functioning, and brain imaging abnormalities: a systematic review. *J Cereb Blood Flow Metab*. 2013 Jul;33(7):983-95; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3705441/>.

<sup>1152</sup> Hartshorne JK, Germine LT. When does cognitive functioning peak? The asynchronous rise and fall of different cognitive abilities across the life span. *Psychol Sci*. 2015 Apr;26(4):433-43; <http://www.ncbi.nlm.nih.gov/pubmed/25770099/>.

<sup>1153</sup> de Grey AD, Ames BN, Andersen JK, Bartke A, Campisi J, Heward CB, McCarter RJ, Stock G. Time to talk SENS: critiquing the immutability of human aging. *Ann N Y Acad Sci*. 2002 Apr;959:452-62; discussion 463-5; <http://www.ncbi.nlm.nih.gov/pubmed/11976218>.

<sup>1154</sup> Finch CE. *Longevity, Senescence and the Genome*. University of Chicago Press, Chicago, IL, 1990.

<sup>1155</sup> Freitas RA Jr. “Chapter 23. Comprehensive Nanorobotic Control of Human Morbidity and Aging,” in Gregory M. Fahy, Michael D. West, L. Stephen Coles, and Steven B. Harris, eds, *The Future of Aging: Pathways to Human Life Extension*, Springer, New York, 2010, pp. 685-805; <http://www.nanomedicine.com/Papers/Aging.pdf>.

been recommended for periodic correction<sup>1156</sup> and are briefly described below: removing extracellular aggregates (Section 5.2.1), removing intracellular aggregates (Section 5.2.2), correcting cancer, nuclear mutations and epimutations (Section 5.2.3), replacing mutant mitochondria (Section 5.2.4), eliminating dysfunctional or senescent cells (Section 5.2.5), restoring essential lost or atrophied cells (Section 5.2.6), and removing extracellular crosslinks (Section 5.2.7). In the present work, each of these categories is discussed largely in the context of the aging brain. The previous work should be consulted for a more complete discussion of anti-aging methods applied more broadly to the entire human body, not just the brain.

The methods described here would repair existing cellular damage, though new damage would slowly re-accumulate because the basic biological processes have not been altered. But the continuing arrest of brain degradation, neuron mass loss, and aging in pre-clinical Alzheimer's patients, using medical nanorobots with periodic re-treatment perhaps every decade or so, should constitute a complete preventative treatment against the development of clinically observable Alzheimer's disease in most individuals.

---

<sup>1156</sup> de Grey A, Rae M. Ending Aging: The Rejuvenation Breakthroughs that Could Reverse Human Aging in Our Lifetime. St. Martin's Griffin, NY, 2007; <https://www.amazon.com/Ending-Aging-Rejuvenation-Breakthroughs-Lifetime/dp/0312367074>.

## **5.2.1 Remove Extracellular Aggregates**

Extracellular aggregates are biomaterials that have accumulated and/or aggregated into deposits outside of the cell. These biomaterials are biochemical byproducts with no further useful physiological or structural function that have proven resistant to natural biological degradation and disposal.

The discussion here focuses on the removal of amyloid (Section 5.2.1.1) aggregates and excess glutamate (Section 5.2.1.2), which are of greatest concern in the AD brain.

### 5.2.1.1 Amyloid Plaques

The most notorious insoluble extracellular aggregates in the AD brain are the toxic amyloid plaques (Section 2.4.1), comprising  $M_{A\beta} \sim 10$  mg of deposited  $A\beta_{42}$  (mostly in the gray matter of the cortex) in a human AD brain of assumed mass 1150.<sup>1157</sup> Certain brain regions appear more prone than others to produce plaques: concentrations are seen in the amygdala, the hippocampus, and certain regions of the cortex, but not in basal ganglia and the cerebellum.<sup>1158</sup>

Amyloid plaques, however, are not pure  $A\beta$ . One comprehensive proteomic analysis<sup>1159</sup> of senile plaques from AD brain detected at least 26 proteins that were co-located with amyloid plaques and quantitatively enriched at least two-fold (typically 2-8 fold) in comparison with surrounding non-plaque tissues, and another 462 proteins that were also co-located but not significantly enriched. In this study, samples of 2000 amyloid plaques extracted from tissue slices taken from two human AD brains contained 2  $\mu$ g of total protein ( $\sim 1$  ng/plaque or  $\sim 1000 \mu\text{m}^3$ /plaque). The protein included classic plaque components such as  $A\beta$  (enriched 80-fold),  $\alpha 1$ -antichymotrypsin, apolipoprotein E, collagen type XXV, cystatin C,  $\alpha$ -synuclein, proteoglycans, and clusterin. If we conservatively assume that the mass of the non- $A\beta$  protein exceeds the mass of all forms of  $A\beta$  in an amyloid plaque by 9-fold (e.g.,  $M_{\text{non-}A\beta} \sim 9M_{A\beta}$ ), then we would estimate total amyloid plaque mass as  $M_{\text{plaque}} = M_{A\beta} + M_{\text{non-}A\beta} \sim 100$  mg per 1150 gm human AD brain. This is roughly consistent with early reports of  $\sim 5 \times 10^5$  plaques recoverable per gram of “optimal grey matter material”,<sup>1160</sup> which would predict up to (500,000 plaques/gm) ( $\sim 1$  ng/plaque) (1150 gm/brain) (42% cortex grey matter)  $\sim 240$  mg of plaques per whole AD brain.

Selective molecular binding sites for  $A\beta$  are well-known, such as the nucleotide decamer consensus sequence “KGGRKTGGGG” in DNA which is a general  $A\beta$ -interacting domain,<sup>1161</sup> and the protein amphoterin which contains an amyloidogenic peptide fragment with high affinity

---

<sup>1157</sup> Karran E, Mercken M, De Strooper B. The amyloid cascade hypothesis for Alzheimer’s disease: an appraisal for the development of therapeutics. *Nature Reviews Drug Discovery* 2011 Sep;10:698-712; <http://www.nature.com/nrd/journal/v10/n9/full/nrd3505.html>.

<sup>1158</sup> Wenk GL. Neuropathologic changes in Alzheimer’s disease. *J Clin Psychiatry*. 2003;64 Suppl 9:7-10; <http://faculty.psy.ohio-state.edu/wenk/documents/JClinPsychiatry2003.pdf>.

<sup>1159</sup> Liao L, Cheng D, Wang J, Duong DM, Losik TG, Gearing M, Rees HD, Lah JJ, Levey AI, Peng J. Proteomic characterization of postmortem amyloid plaques isolated by laser capture microdissection. *J Biol Chem*. 2004 Aug 27;279(35):37061-8; <http://www.jbc.org/content/279/35/37061.full>.

<sup>1160</sup> Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K. Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc Natl Acad Sci U S A*. 1985 Jun;82(12):4245-9; <http://www.pnas.org/content/82/12/4245.full.pdf>.

<sup>1161</sup> Maloney B, Lahiri DK. The Alzheimer’s amyloid  $\beta$ -peptide ( $A\beta$ ) binds a specific DNA  $A\beta$ -interacting domain ( $A\beta$ ID) in the APP, BACE1, and APOE promoters in a sequence-specific manner: characterizing a new regulatory motif. *Gene*. 2011 Nov 15;488(1-2):1-12; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3381326/>.

for A $\beta$  as a free peptide or while part of the native protein.<sup>1162</sup> Several microglial pattern recognition receptors for A $\beta$ <sub>42</sub> plaque fibrils have been reported, including the class B scavenger receptor CD36,<sup>1163</sup> the TLR4-interacting molecule CD14,<sup>1164</sup> and the microglial TLR2 receptor,<sup>1165</sup> and blood-derived microglial cells are chemoattracted to amyloid plaques.<sup>1166</sup> The presence of other proteins in the plaque affords additional recognition, confirmation, and binding opportunities.

Plaque binding sites can be installed on the external recognition modules of tissue-mobile microbivore-class (Section 4.2.2) scavenging nanorobots, allowing them to quickly seek, bind, ingest, and fully digest existing amyloid plaques throughout the relevant tissues, in the manner of artificial mechanical macrophages. The only significant material effluent from these synthetic digestive processes would be harmless free amino acids.<sup>1167</sup> The microbivore-class devices would enter and exit the brain by any of several means described earlier in Section 4.3. Assuming an injected dose of  $N_{\text{bot}} = 86$  billion nanorobots ( $\sim 1 \text{ cm}^3$  dose, assuming  $12.1 \mu\text{m}^3$  device volume per nanorobot; Section 4.2.2) which allows one for each of the 86 billion neurons in the brain,<sup>1168</sup> with each nanorobot able to digest  $V_{\text{plaque}} = 2 \mu\text{m}^3$  per cycle (Section 4.2.2) of amyloid plaque with average density  $\rho_{\text{amyloid}} \sim \rho_{\text{A}\beta} = 1200 \text{ kg/m}^3$ ,<sup>1169</sup> operating on a leisurely  $\tau =$

<sup>1162</sup> Kallijärvi J, Haltia M, Baumann MH. Amphoterin includes a sequence motif which is homologous to the Alzheimer's beta-amyloid peptide (Abeta), forms amyloid fibrils *in vitro*, and binds avidly to Abeta. *Biochemistry*. 2001 Aug 28;40(34):10032-7; <http://www.ncbi.nlm.nih.gov/pubmed/11513581>.

<sup>1163</sup> El Khoury JB, Moore KJ, Means TK, Leung J, Terada K, Toft M, Freeman MW, Luster AD. CD36 mediates the innate host response to beta-amyloid. *J Exp Med*. 2003 Jun 16;197(12):1657-66; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2193948/>.

<sup>1164</sup> Liu Y, Walter S, Stagi M, Cherny D, Letiembre M, Schulz-Schaeffer W, Heine H, Penke B, Neumann H, Fassbender K. LPS receptor (CD14): a receptor for phagocytosis of Alzheimer's amyloid peptide. *Brain*. 2005 Aug;128(Pt 8):1778-89; <http://www.ncbi.nlm.nih.gov/pubmed/15857927/>.

<sup>1165</sup> Liu S, Liu Y, Hao W, Wolf L, Kiliaan AJ, Penke B, Rube CE, Walter J, Heneka MT, Hartmann T, Menger MD, Fassbender K. TLR2 is a primary receptor for Alzheimer's amyloid  $\beta$  peptide to trigger neuroinflammatory activation. *J Immunol*. 2012 Feb 1;188(3):1098-107; <http://www.jimmunol.org/content/188/3/1098.long>.

<sup>1166</sup> Simard AR, Soulet D, Gowing G, Julien JP, Rivest S. Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. *Neuron*. 2006 Feb 16;49(4):489-502; [http://www.cell.com/neuron/pdf/S0896-6273\(06\)00075-4.pdf](http://www.cell.com/neuron/pdf/S0896-6273(06)00075-4.pdf)

<sup>1167</sup> Freitas RA Jr. Microbivores: Artificial mechanical phagocytes using digest and discharge protocol. *J Evol Technol* 2005;14:1-52; <http://www.jetpress.org/volume14/freitas.html>.

<sup>1168</sup> Azevedo FA, Carvalho LR, Grinberg LT, Farfel JM, Ferretti RE, Leite RE, Jacob Filho W, Lent R, Herculano-Houzel S. Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. *J Comp Neurol*. 2009 Apr 10;513(5):532-41; <http://www.sakkyndig.com/psykologi/artvit/frederico2009.pdf>.

<sup>1169</sup> Benditt EP, Eriksen N. Amyloid protein SAA is associated with high density lipoprotein from human serum. *Proc Natl Acad Sci U S A*. 1977 Sep;74(9):4025-8; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC431828/>.

3600 sec (1 hr) search/digest cycle, then **the entire AD human brain can be cleared of all amyloid plaques in**  $t_{\text{clearance}} = \text{Max}[\tau, \tau M_{\text{plaque}} / (N_{\text{bot}} V_{\text{plaque}} \rho_{\text{plaque}})] = \text{Max}[3600, 1744] = \mathbf{3600}$  sec (1 hr).

Nanorobot activities at a particular plaque may need to be accompanied by the emission of small aliquots of an engineered enzyme designed to separate the plaque body from any neurites, synapses, neuronal processes, elements of brain extracellular matrix,<sup>1170</sup> or other vital extracellular structures around which the plaque body might be wrapped or weakly bonded. These enzymes can later be retrieved using sorting rotors equipped with binding sites having high affinity for the engineered enzyme. Alternatively, the nanorobot can incorporate mechanisms and mechanical procedures designed to avoid damage to key extracellular structures while the plaque is being extracted and digested.

Based on estimates of neuronal  $A\beta_{42}$  production, the amount of  $A\beta_{42}$  deposited in the AD brain as plaque is equivalent to approximately 6-7 years of total  $A\beta_{42}$  production.<sup>1171</sup> (Animal data suggests that once the first seeding of plaques occurs, available amyloid- $\beta$  is rapidly accumulated into them.) As a result, multi-annual treatments of the kind described above may suffice to keep the patient's brain essentially clear of amyloid plaques.

Free-floating toxic amyloid oligomers, e.g., ADDLs (Section 2.4.1), are a closely related form of extracellular aggregate that should also be removed. While they're removing plaques, the nanorobots described above can simultaneously extract and safely digest  $A\beta$  oligomers, possibly using binding sites derived from known molecular receptors that are specific to amyloid oligomers (such as PrP<sup>C</sup>),<sup>1172</sup> further reducing the rate of formation of new amyloid plaques. Nanorobots can also use available binding sites to extract free  $A\beta$ , tau (Section 5.2.2.1),  $\alpha$ -synuclein (Section 5.2.2.3), and other proteins associated with neurodegenerative disorders that are often released into the extracellular space, where they may spread to other cells and possibly seed abnormal protein aggregation – e.g., tau is toxic to neurons when present in the extracellular space.<sup>1173</sup>

<sup>1170</sup> Ruoslahti E. Brain extracellular matrix. *Glycobiology*. 1996 Jul;6(5):489-92; <http://glycob.oxfordjournals.org/content/6/5/489.long>. Soleman S, Filippov MA, Dityatev A, Fawcett JW. Targeting the neural extracellular matrix in neurological disorders. *Neuroscience*. 2013 Dec 3;253:194-213; <http://www.ncbi.nlm.nih.gov/pubmed/24012743/>. Morawski M, Filippov M, Tzinia A, Tsilibary E, Vargova L. ECM in brain aging and dementia. *Prog Brain Res*. 2014;214:207-27; <http://www.ncbi.nlm.nih.gov/pubmed/25410360>.

<sup>1171</sup> Karran E, Mercken M, De Strooper B. The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. *Nature Reviews Drug Discovery* 2011 Sep;10:698-712; <http://www.nature.com/nrd/journal/v10/n9/full/nrd3505.html>.

<sup>1172</sup> Gunther EC, Strittmatter SM. Beta-amyloid oligomers and cellular prion protein in Alzheimer's disease. *J Mol Med (Berl)*. 2010 Apr;88(4):331-8; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2846635/>.

<sup>1173</sup> Gómez-Ramos A, Díaz-Hernández M, Cuadros R, Hernández F, Avila J. Extracellular tau is toxic to neuronal cells. *FEBS Lett*. 2006;580:4842–50; <http://www.ncbi.nlm.nih.gov/pubmed/16914144>.

### 5.2.1.2 Glutamate

Glutamate is a useful excitatory neurotransmitter of the nervous system, although excessive extracellular amounts in the brain can lead to cell death through a process called excitotoxicity which consists of the overstimulation of glutamate receptors.<sup>1174</sup> Excitotoxicity occurs not only in Alzheimer's disease, but also in other neurological diseases such as Parkinson's disease and multiple sclerosis.

Inflammation around the senile amyloid plaques upregulates the production of prostaglandins and leads to the increase in the extracellular concentration of glutamate which contributes to the ultimate death of neurons.<sup>1175</sup>

While nanorobots are busy eliminating the amyloid plaques (Section 5.2.1.1), they can also employ molecular sorting rotors (**Appendix C**) equipped with glutamate binding sites (e.g., iGluR receptors,<sup>1176</sup> mGluR receptors,<sup>1177</sup> etc.) to pump excess glutamate in the extracellular space down to acceptably low concentrations, storing the chemical in nanorobot onboard tanks to be carried out of the brain when the devices exit the patient. Extracting pro-inflammatory cytokines may also help to downregulate the production of excess glutamate.

---

<sup>1174</sup> Kim SH, Steele JW, Lee SW, Clemenson GD, Carter TA, Treuner K, Gadiant R, Wedel P, Glabe C, Barlow C, Ehrlich ME, Gage FH, Gandy S. Proneurogenic Group II mGluR antagonist improves learning and reduces anxiety in Alzheimer A $\beta$  oligomer mouse. *Mol Psychiatry*. 2014 Nov;19(11):1235-42; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4217144/>.

<sup>1175</sup> Wenk GL. Neuropathologic changes in Alzheimer's disease. *J Clin Psychiatry*. 2003;64 Suppl 9:7-10; <http://faculty.psy.ohio-state.edu/wenk/documents/JClinPsychiatry2003.pdf>.

<sup>1176</sup> Green T, Nayeem N. The multifaceted subunit interfaces of ionotropic glutamate receptors. *J Physiol*. 2015 Jan 1;593(1):73-81; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4293055/>.

<sup>1177</sup> Vaidya A, Jain S, Jain AK, Agrawal A, Kashaw SK, Jain SK, Agrawal RK. Metabotropic glutamate receptors: a review on perspectives and therapeutic aspects. *Mini Rev Med Chem*. 2013 Nov;13(13):1967-81; <http://www.ncbi.nlm.nih.gov/pubmed/22530579>.

## 5.2.2 Remove Intracellular Aggregates

Intracellular aggregates are highly heterogeneous lipid and protein biomaterials that have accumulated and aggregated into clumps inside of the cell.<sup>1178</sup> These biomaterials are normal intracellular molecules that have become chemically modified so that they no longer work and are resistant to the normal processes of degradation. Intracellular aggregates often accumulate inside lysosomes, organelles that contain the most powerful degradation machinery in the cell. But if the lysosomes become congested and engorged, the cell will stop working properly – crudely analogous to a house whose toilets have all backed up. Intracellular aggregates that accumulate elsewhere than in the lysosome are also a contributing factor in several types of neurodegeneration.

Nanorobotic clearance of three categories of intracellular aggregates of greatest concern in the AD brain are discussed below: (1) neurofibrillary tangles (NFTs) or “tau tangles” composed mostly of hyperphosphorylated tau protein (Section 5.2.2.1), (2) lipofuscin granules (Section 5.2.2.2), and (3)  $\alpha$ -synuclein as oligomers and Lewy body aggregates (also a significant contributor to Parkinson’s disease) (Section 5.2.2.3).

---

<sup>1178</sup> SENS Research Foundation, “A Reimagined Research Strategy for Aging,” 2015; <http://www.sens.org/research/introduction-to-sens-research>.

### 5.2.2.1 Tau Protein and Neurofibrillary Tangles

Tau protein, which is normally most abundant in axons, becomes hyperphosphorylated and mislocalized to the neuronal soma and dendritic spines, forming intracellular inclusions called neurofibrillary tangles (NFT) that disrupt the cytoskeleton (Section 2.4.2). One early study of human cerebral cortex tissue in AD brains found 2.1 mg/100 gm tissue of tau (of which at most 20% was derived from neurofibrillary tangles) and 0.8 mg/100 gm tissue of hyperphosphorylated tau (of which at most 26% was derived from neurofibrillary tangles).<sup>1179</sup> This implies that human AD brain tissue contains at most  $(20\%)(2.1 \text{ mg}) + (26\%)(0.8 \text{ mg}) = 0.6 \text{ mg/100 gm tissue}$ , or  $M_{\text{tangles}} \sim 7.2 \text{ mg}$  of NFT tau tangles per AD human brain of assumed mass 1150 gm. Even if all of the remaining AD tau protein was pathologically present as hyperphosphorylated tau oligomers, then human AD brain tissue could contain at most  $(80\%)(2.1 \text{ mg}) + (74\%)(0.8 \text{ mg}) = 2.3 \text{ mg/100 gm tissue}$ , or  $M_{\text{oligomers}} \sim 26.1 \text{ mg}$  of tau oligomers per 1150 gm AD human brain.

Selective molecular binding activity to phosphorylated tau protein has been found in phosphorylated pSmad2 and pSmad3 proteins<sup>1180</sup> and curcumin,<sup>1181</sup> but numerous phosphorylation-dependent anti-tau antibodies (e.g., Alz50, AP422, AT100, MC1, TG-3) are also known to specifically recognize pathological tau species.<sup>1182</sup> For example, monoclonal antibody AD2<sup>1183</sup> and PHF-1<sup>1184</sup> recognize the phosphorylated serine 396 and serine 404 sites, whereas

<sup>1179</sup> Kopke E, Tung YC, Shaikh S, Alonso AC, Iqbal K, Grundke-Iqbal I. Microtubule-associated protein tau. Abnormal phosphorylation of a non-paired helical filament pool in Alzheimer disease. *J Biol Chem.* 1993;268:24374–24384; <http://www.ncbi.nlm.nih.gov/pubmed/8226987> and <http://www.jbc.org/content/268/32/24374.long>.

<sup>1180</sup> Chalmers KA, Love S. Phosphorylated Smad 2/3 colocalizes with phospho-tau inclusions in Pick disease, progressive supranuclear palsy, and corticobasal degeneration but not with alpha-synuclein inclusions in multiple system atrophy or dementia with Lewy bodies. *J Neuropathol Exp Neurol.* 2007 Nov;66(11):1019-26; <http://www.ncbi.nlm.nih.gov/pubmed/17984683>.

<sup>1181</sup> Mutsuga M, Chambers JK, Uchida K, Tei M, Makibuchi T, Mizorogi T, Takashima A, Nakayama H. Binding of curcumin to senile plaques and cerebral amyloid angiopathy in the aged brain of various animals and to neurofibrillary tangles in Alzheimer's brain. *J Vet Med Sci.* 2012 Jan;74(1):51-7; [https://www.jstage.jst.go.jp/article/jvms/74/1/74\\_11-0307/pdf](https://www.jstage.jst.go.jp/article/jvms/74/1/74_11-0307/pdf).

<sup>1182</sup> Schraen-Maschke S, Sergeant N, Dhaenens CM, Bombois S, Deramecourt V, Caillet-Boudin ML, Pasquier F, Maurage CA, Sablonnière B, Vanmechelen E, Buée L. Tau as a biomarker of neurodegenerative diseases. *Biomark Med.* 2008 Aug;2(4):363-84; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2993973/>. Loeffler DA, Klaver AC, Coffey MP. ELISA measurement of specific antibodies to phosphorylated tau in intravenous immunoglobulin products. *Int Immunopharmacol.* 2015 Oct;28(2):1108-12; <http://www.ncbi.nlm.nih.gov/pubmed/26330100>.

<sup>1183</sup> Torreilles F, Roquet F, Granier C, Pau B, Mourton-Gilles C. Binding specificity of monoclonal antibody AD2: influence of the phosphorylation state of tau. *Brain Res Mol Brain Res.* 2000 May 31;78(1-2):181-5; <http://www.ncbi.nlm.nih.gov/pubmed/10891598>.

<sup>1184</sup> Otvos L Jr, Feiner L, Lang E, Szendrei GI, Goedert M, Lee VM. Monoclonal antibody PHF-1 recognizes tau protein phosphorylated at serine residues 396 and 404. *J Neurosci Res.* 1994 Dec 15;39(6):669-73; <http://www.ncbi.nlm.nih.gov/pubmed/7534834>.

PHF-9 shows phosphorylation-dependent binding to purified PHF and recognizes phosphorylated serine 404 of tau independently of phosphorylated serine 396.<sup>1185</sup> AT100 recognizes phosphorylated threonines 212-217 and serine 214, while antibody TG3 recognizes phosphothreonine 231 which is found in pretangles, intracellular tangles and extracellular tangles.<sup>1186</sup> Monoclonal antibody AP422 binds to the phosphorylated serine 422 site.<sup>1187</sup> Other monoclonal antibodies target multiple neighboring hyperphosphorylation sites<sup>1188</sup> and individual tau phosphopeptides with high specificity.<sup>1189</sup>

Binding sites for tau oligomers might be fashioned from known tau oligomer-specific monoclonal antibodies.<sup>1190</sup> For example, one tau oligomer-specific monoclonal antibody (ADx215) stains specific low-order tau oligomers in diseased AD brain, while two others (ADx201, ADx210) additionally react to higher-order tau oligomers and presumed prefibrillar structures.<sup>1191</sup>

Binding to entire neurofibrillary tangles has been demonstrated using a BODIPY-based Zn(II) complex that binds preferentially to peptides presenting phosphorylated groups, only weakly to

<sup>1185</sup> Zemlan FP, Dean GE. Monoclonal antibody PHF-9 recognizes phosphorylated serine 404 of tau protein and labels paired helical filaments. *J Neurosci Res.* 1996 Oct 1;46(1):90-7; <http://www.ncbi.nlm.nih.gov/pubmed/8892109>.

<sup>1186</sup> Schraen-Maschke S, Sergeant N, Dhaenens CM, Bombois S, Deramecourt V, Caillet-Boudin ML, Pasquier F, Maurage CA, Sablonnière B, Vanmechelen E, Buée L. Tau as a biomarker of neurodegenerative diseases. *Biomark Med.* 2008 Aug;2(4):363-84; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2993973/>.

<sup>1187</sup> Hasegawa M, Jakes R, Crowther RA, Lee VM, Ihara Y, Goedert M. Characterization of mAb AP422, a novel phosphorylation-dependent monoclonal antibody against tau protein. *FEBS Lett.* 1996 Apr 8;384(1):25-30; <http://www.sciencedirect.com/science/article/pii/0014579396002712>.

<sup>1188</sup> Singer D, Lehmann J, Hanisch K, Härtig W, Hoffmann R. Neighbored phosphorylation sites as PHF-tau specific markers in Alzheimer's disease. *Biochem Biophys Res Commun.* 2006 Aug 4;346(3):819-28; <http://www.ncbi.nlm.nih.gov/pubmed/16781671>

<sup>1189</sup> Shih HH, Tu C, Cao W, Klein A, Ramsey R, Fennell BJ, Lambert M, Ní Shúilleabháin D, Autin B, Kouranova E, Laxmanan S, Braithwaite S, Wu L, Ait-Zahra M, Milici AJ, Dumin JA, LaVallie ER, Arai M, Corcoran C, Paulsen JE, Gill D, Cunningham O, Bard J, Mosyak L, Finlay WJ. An ultra-specific avian antibody to phosphorylated tau protein reveals a unique mechanism for phosphoepitope recognition. *J Biol Chem.* 2012 Dec 28;287(53):44425-34; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3531756/>.

<sup>1190</sup> Castillo-Carranza DL, Sengupta U, Guerrero-Muñoz MJ, Lasagna-Reeves CA, Gerson JE, Singh G, Estes DM, Barrett AD, Dineley KT, Jackson GR, Kaye R. Passive immunization with Tau oligomer monoclonal antibody reverses tauopathy phenotypes without affecting hyperphosphorylated neurofibrillary tangles. *J Neurosci.* 2014 Mar 19;34(12):4260-72; <http://www.jneurosci.org/content/34/12/4260.long>.

<sup>1191</sup> Rosseels J, Van den Brande J, Violet M, Jacobs D, Grognet P, Lopez J, Huvent I, Caldara M, Swinnen E, Papegaey A, Cailliez R, Buée-Scherrer V, Engelborghs S, Lippens G, Colin M, Buée L, Galas MC, Vanmechelen E, Winderickx J. Tau monoclonal antibody generation based on humanized yeast models: impact on Tau oligomerization and diagnostics. *J Biol Chem.* 2015 Feb 13;290(7):4059-74; <http://www.ncbi.nlm.nih.gov/pubmed/25540200>.

artificially prepared aggregates of the nonphosphorylated tau protein, and clearly discriminates between NFTs and the amyloid plaques.<sup>1192</sup> Casein kinase II (CK-II) antiserum immunoreacts with NFTs,<sup>1193</sup> and a PET tracer compound <sup>18</sup>F-THK5351 selectively binds to neurofibrillary tangles.<sup>1194</sup> Congo Red, Thiazine Red, and Thioflavin S are fluorescent dyes commonly used for staining neurofibrillary tangles and monitoring tau aggregation,<sup>1195</sup> and Gallyas silver is a traditional stain specific to NFTs.<sup>1196</sup>

Binding sites for phosphorylated tau protein, tau oligomer, or NFT can be installed on the external recognition modules of tissue-mobile microbivore-class (Section 4.2.2) scavenging nanorobots, allowing them to quickly seek, bind, ingest, and fully digest existing neurofibrillary tangles throughout the relevant tissues, in the manner of artificial mechanical macrophages. The only significant material effluent from these synthetic digestive processes would be harmless free amino acids.<sup>1197</sup> The microbivore-class devices would enter and exit the brain by any of several means described earlier in Section 4.3. Assuming an injected dose of  $N_{\text{bot}} = 86$  billion nanorobots ( $\sim 1 \text{ cm}^3$  dose, assuming  $12.1 \mu\text{m}^3$  device volume per nanorobot; Section 4.2.2) applying one for each of the 86 billion neurons in the brain,<sup>1198</sup> with each nanorobot able to digest  $V_{\text{tau}} = 2 \mu\text{m}^3$  per cycle (Section 4.2.2) of tau tangles with average density  $\rho_{\text{tangles}} = 1300 \text{ kg/m}^3$ ,<sup>1199</sup>

---

<sup>1192</sup> Ojida A, Sakamoto T, Inoue MA, Fujishima SH, Lippens G, Hamachi I. Fluorescent BODIPY-based Zn(II) complex as a molecular probe for selective detection of neurofibrillary tangles in the brains of Alzheimer's disease patients. *J Am Chem Soc.* 2009 May 13;131(18):6543-8; <http://www.ncbi.nlm.nih.gov/pubmed/19368380>.

<sup>1193</sup> Iimoto DS, Masliah E, DeTeresa R, Terry RD, Saitoh T. Aberrant casein kinase II in Alzheimer's disease. *Brain Res.* 1990 Jan 22;507(2):273-80; <http://www.ncbi.nlm.nih.gov/pubmed/2337767>.

<sup>1194</sup> Harada R, Okamura N, Furumoto S, Furukawa K, Ishiki A, Tomita N, Tago T, Hiraoka K, Watanuki S, Shidahara M, Miyake M, Ishikawa Y, Matsuda R, Inami A, Yoshikawa T, Funaki Y, Iwata R, Tashiro M, Yanai K, Arai H, Kudo Y. <sup>18</sup>F-THK5351: A Novel PET Radiotracer for Imaging Neurofibrillary Pathology in Alzheimer's Disease. *J Nucl Med.* 2015 Nov 5. pii; <http://www.ncbi.nlm.nih.gov/pubmed/26541774>.

<sup>1195</sup> Lira-De León KI, García-Gutiérrez P, Serratos IN, Palomera-Cárdenas M, Figueroa-Corona Mdel P, Campos-Peña V, Meraz-Ríos MA. Molecular mechanism of tau aggregation induced by anionic and cationic dyes. *J Alzheimers Dis.* 2013;35(2):319-34; <http://www.ncbi.nlm.nih.gov/pubmed/23435411>.

<sup>1196</sup> Braak H, Braak E, Ohm T, Bohl J. Silver impregnation of Alzheimer's neurofibrillary changes counterstained for basophilic material and lipofuscin pigment. *Stain Technol.* 1988 Jul;63(4):197-200; <http://www.ncbi.nlm.nih.gov/pubmed/2464205>.

<sup>1197</sup> Freitas RA Jr. Microbivores: Artificial mechanical phagocytes using digest and discharge protocol. *J Evol Technol* 2005;14:1-52; <http://www.jetpress.org/volume14/freitas.html>.

<sup>1198</sup> Azevedo FA, Carvalho LR, Grinberg LT, Farfel JM, Ferretti RE, Leite RE, Jacob Filho W, Lent R, Herculano-Houzel S. Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. *J Comp Neurol.* 2009 Apr 10;513(5):532-41; <http://www.sakkyndig.com/psykologi/artvit/frederico2009.pdf>.

<sup>1199</sup> Rosenberg KJ, Ross JL, Feinstein HE, Feinstein SC, Israelachvili J. Complementary dimerization of microtubule-associated tau protein: Implications for microtubule bundling and tau-mediated pathogenesis.

operating on a leisurely  $\tau = 3600$  sec (1 hr) search/digest cycle, then **the entire AD human brain can be cleared of all tau neurofibrillary tangles in**  $t_{\text{clearance}} = \text{Max}[\tau, \tau M_{\text{tau}} / (N_{\text{bot}} V_{\text{tau}} \rho_{\text{tangles}})]$   
 $= \text{Max}[3600, 536] = \mathbf{3600 \text{ sec}}$  (1 hr), taking  $M_{\text{tau}} = M_{\text{tangles}} + M_{\text{oligomers}} = 33.3$  mg as the maximum possible quantity of pathological tau per 1150 gm AD human brain. Nanorobot activities near a particular tangle may need to be accompanied by the emission of small aliquots of an engineered enzyme designed to separate the neurofibrillary material from any cytoskeletal elements or other vital intracellular structures around which it might be wrapped or weakly bonded. These enzymes can later be retrieved using sorting rotors equipped with binding sites having high affinity for the engineered enzyme. Alternatively, the nanorobot can incorporate mechanisms and procedures designed to avoid damage to key intracellular structures while the tangles are being extracted and digested. Free-floating toxic tau oligomers are also extracted and safely digested by the nanorobots, possibly using binding sites derived from known molecular receptors that are specific to phosphorylated tau oligomers as described above, further reducing the rate of formation of new neurofibrillary tangles.

### 5.2.2.2 Lipofuscin Granules

Lipofuscin consists of yellow-brown insoluble age-pigment lysosomal granules that collect inside many of our cells starting as early in life as 11 years old, are typically 1-3 microns in diameter, and may occupy up to 20% of brainstem neuron volume at age 20 and up to 50% of cell volume by age 90.<sup>1200</sup> While brain cell lipofuscin is not normally associated with mental<sup>1201</sup> or motor<sup>1202</sup> abnormalities or with other detrimental neuronal function,<sup>1203</sup> hereditary ceroid lipofuscinosis<sup>1204</sup> or neuronal ceroid-lipofuscinosis (NCL) diseases<sup>1205</sup> (Section 6.2.6) can lead to premature death. Interestingly, AD-brain neurons lacking neurofibrillary tangles (NFT) have about three times more lipofuscin than age-matched neurons with NFTs, suggesting that a breakdown in the capacity for making lipofuscin may reduce the neuronal inability to store toxic waste.<sup>1206</sup>

Lipofuscin granules are composed of lipid-containing residues of lysosomal digestion, usually arranged around the cell nucleus, surrounded by lysosomal, autophagosomal, or autolipophagosomal membrane. Lipofuscin is likely the product of the oxidation of unsaturated fatty acids (e.g., lipid peroxidation products derived from malondialdehyde and glycation products).<sup>1207</sup> Lipofuscin may be symptomatic of membrane damage or damage to mitochondria and lysosomes, and might be the result of incomplete lysosomal degradation of damaged

---

<sup>1200</sup> West CD. A quantitative study of lipofuscin accumulation with age in normals and individuals with Down's syndrome, phenylketonuria, progeria and transneuronal atrophy. *J Comp Neurol*. 1979 Jul 1;186(1):109-16; <http://www.ncbi.nlm.nih.gov/pubmed/156741>.

<sup>1201</sup> Drach LM, Bohl J, Goebel HH. The lipofuscin content of nerve cells of the inferior olivary nucleus in Alzheimer's disease. *Dementia*. 1994 Sep-Oct;5(5):234-9; <http://www.ncbi.nlm.nih.gov/pubmed/7951678>. West CD (1979), *ibid*.

<sup>1202</sup> McHolm GB, Aguilar MJ, Norris FH. Lipofuscin in amyotrophic lateral sclerosis. *Arch Neurol*. 1984 Nov;41(11):1187-8; <http://www.ncbi.nlm.nih.gov/pubmed/6487103>.

<sup>1203</sup> Davies I, Fotheringham A, Roberts C. The effect of lipofuscin on cellular function. *Mech Ageing Dev*. 1983 Nov-Dec;23(3-4):347-56; <http://www.ncbi.nlm.nih.gov/pubmed/6656318>.

<sup>1204</sup> Shotelersuk V, Gahl WA. Hermansky-Pudlak syndrome: models for intracellular vesicle formation. *Mol Genet Metab*. 1998 Oct;65(2):85-96; <http://www.ncbi.nlm.nih.gov/pubmed/9787100>.

<sup>1205</sup> Kida E, Golabek AA, Wisniewski KE. Cellular pathology and pathogenic aspects of neuronal ceroid lipofuscinoses. *Adv Genet*. 2001;45:35-68; <http://www.ncbi.nlm.nih.gov/pubmed/11332776>.

<sup>1206</sup> Stojanovic A, Roher AE, Ball MJ. Quantitative analysis of lipofuscin and neurofibrillary tangles in the hippocampal neurons of Alzheimer disease brains. *Dementia*. 1994 Sep-Oct;5(5):229-33; <http://www.ncbi.nlm.nih.gov/pubmed/7951677>.

<sup>1207</sup> Horie K, Miyata T, Yasuda T, Takeda A, Yasuda Y, Maeda K, Sobue G, Kurokawa K. Immunohistochemical localization of advanced glycation end products, pentosidine, and carboxymethyllysine in lipofuscin pigments of Alzheimer's disease and aged neurons. *Biochem Biophys Res Commun*. 1997 Jul 18;236(2):327-32; <http://www.ncbi.nlm.nih.gov/pubmed/9240434>.

mitochondria.<sup>1208</sup> Along with a large lipid content and crosslinked protein residues, lipofuscin is also known to contain sugars and metals, including mercury, aluminum, iron, copper and zinc.<sup>1209</sup>

**Table 2. Lipofuscin concentration in normal human brain as a function of age.<sup>1210</sup>**

Mean Age	16.8 yr	45.6 yr	66.8 yr	78.8 yr
Lipofuscin per brain	25.1 mg	50.7 mg	88.4 mg	72.0 mg

How much lipofuscin is present in human brain? One early study<sup>1211</sup> obtained several normal human brains of various ages, chopped them up and extracted all the lipofuscin, with the results (**Table 2**)

confirming that the lipofuscin content of normal brain generally rises with age, apparently increasing very roughly at the rate of ~1 mg/brain-yr. Another study<sup>1212</sup> of progeric, phenylketonuric, and Down's syndrome brains over a 14-92 year age range found no significant difference in the amount of lipofuscin accumulated with age compared to normal brains, so these values seem likely to be approximately correct for Alzheimer's brains as well. For our scaling estimate, we'll assume the maximum mass of lipofuscin to be cleared is  $M_{\text{lipofuscin}} \sim 100$  mg per aging human AD brain of assumed mass 1150 gm.

How to create binding sites for such a heterogeneous material consisting of lipids, proteins, carbohydrates and a small amount of metals? One approach is to find or create receptors for specific molecules found in lipofuscin membranes. For example, 186 different proteins<sup>1213</sup> were identified in the coatings of retinal epithelium lipofuscin granules, none of which were found in the purified lipofuscin when the coatings were chemically removed.<sup>1214</sup> A small array of receptors for a small number of these proteins should provide an unambiguous identification of a lipofuscin granule. Lipofuscin granules contain excessive amounts of the free fatty acids palmitic

<sup>1208</sup> Gray DA, Woulfe J. Lipofuscin and aging: a matter of toxic waste. *Sci Aging Knowledge Environ.* 2005 Feb 2;2005(5):re1; <http://www.ncbi.nlm.nih.gov/pubmed/15689603>.

<sup>1209</sup> Terman A, Brunk U (2004). Lipofuscin. *Int J Biochem Cell Biol* 36 (8): 1400–4; <http://www.ncbi.nlm.nih.gov/pubmed/15147719>.

<sup>1210</sup> Siakotos AN, Armstrong D, Koppang N, Muller J. Biochemical Significance of Age Pigment in Neurones. In K. Nandy, ed., *The Aging Brain and Senile Dementia*, Springer, 1977, pp. 99-118.

<sup>1211</sup> Siakotos AN, Armstrong D, Koppang N, Muller J. Biochemical Significance of Age Pigment in Neurones. In K. Nandy, ed., *The Aging Brain and Senile Dementia*, Springer, 1977, pp. 99-118.

<sup>1212</sup> West CD. A quantitative study of lipofuscin accumulation with age in normals and individuals with Down's syndrome, phenylketonuria, progeria and transneuronal atrophy. *J Comp Neurol.* 1979 Jul 1;186(1):109-16; <http://www.ncbi.nlm.nih.gov/pubmed/156741>.

<sup>1213</sup> [http://www.mcponline.org/content/suppl/2008/04/30/M700525-MCP200.DC1/Ng\\_et\\_al\\_LF\\_Proteomics\\_supplemental\\_data.pdf](http://www.mcponline.org/content/suppl/2008/04/30/M700525-MCP200.DC1/Ng_et_al_LF_Proteomics_supplemental_data.pdf).

<sup>1214</sup> Ng KP, Gugiu B, Renganathan K, Davies MW, Gu X, Crabb JS, Kim SR, Rózanowska MB, Bonilha VL, Rayborn ME, Salomon RG, Sparrow JR, Boulton ME, Hollyfield JG, Crabb JW. Retinal pigment epithelium lipofuscin proteomics. *Mol Cell Proteomics.* 2008 Jul;7(7):1397-405; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2493379/>.

acid, arachidonic acid, and oleic acid and a significant decrease in diacylglycerols.<sup>1215</sup> The granules are also immunoreactive to various monoclonal antibodies including mouse anti-nitrotyrosine mAb, mouse anti-2-( $\omega$ -carboxyethyl)pyrrole (CEP) mAb, and rabbit polyclonal anti-iso[4]levuglandin E2 (iso[4]LGE2),<sup>1216</sup> histochemical lipofuscin-staining antibodies (HPA025226, HPA041736, HPA045679),<sup>1217</sup> and antisera to lipoic acid,<sup>1218</sup> any of which could serve as the basis for a binding site. A large number of stains are employed that preferentially bind to lipofuscin granules as an aid to visualization. For example, Sudan Black B (SBB) is a lipophilic histochemical stain that identifies lipofuscin and is applicable for *in vitro* and *in situ* studies.<sup>1219</sup> Other traditional positive stains include Schmorl's reaction, Oil red O, carbol lipofuscin stain, Periodic acid-Schiff, Ziehl-Neelsen acid fast stain, and the lysosomal acid phosphatase and esterase stains,<sup>1220</sup> D-PAS and Fontana Masson,<sup>1221</sup> and crotonaldehyde fuchsin following performic acid oxidation.<sup>1222</sup> Finally, if naturally-occurring lipofuscinolytic bacteria

---

<sup>1215</sup> Bazan HE, Bazan NG, Feeney-Burns L, Berman ER. Lipids in human lipofuscin-enriched subcellular fractions of two age populations. Comparison with rod outer segments and neural retina. Invest Ophthalmol Vis Sci. 1990 Aug;31(8):1433-43; <http://www.ncbi.nlm.nih.gov/pubmed/2387677/>.

<sup>1216</sup> Ng KP, Gugiu B, Renganathan K, Davies MW, Gu X, Crabb JS, Kim SR, Rózanowska MB, Bonilha VL, Rayborn ME, Salomon RG, Sparrow JR, Boulton ME, Hollyfield JG, Crabb JW. Retinal pigment epithelium lipofuscin proteomics. Mol Cell Proteomics. 2008 Jul;7(7):1397-405; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2493379/>.

<sup>1217</sup> Sjöstedt E, Djureinovic D, Mulder J, Kampf C. Identification of proteins in lipofuscin using antibodies within the Human Protein Atlas; <https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma-Aldrich/Posters/1/identification-of-proteins-in-lipofuscin.pdf>.

<sup>1218</sup> Zhu X, Castellani RJ, Moreira PI, Aliev G, Shenk JC, Siedlak SL, Harris PL, Fujioka H, Sayre LM, Szweda PA, Szweda LI, Smith MA, Perry G. Hydroxynonenal-generated crosslinking fluorophore accumulation in Alzheimer disease reveals a dichotomy of protein turnover. Free Radic Biol Med. 2012 Feb 1;52(3):699-704; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3268699/>.

<sup>1219</sup> Gatenby JB, Moussa TA. The sudan black B technique in cytology. J R Microsc Soc. 1949 May;69(2):72-5; <http://www.ncbi.nlm.nih.gov/pubmed/18128449>. Jung T, Höhn A, Grune T. Lipofuscin: detection and quantification by microscopic techniques. Methods Mol Biol. 2010;594:173-93; <http://www.ncbi.nlm.nih.gov/pubmed/20072918>. Georgakopoulou EA, Tsimaratou K, Evangelou K, Fernandez Marcos PJ, Zoumpourlis V, Trougakos IP, Kletsas D, Bartek J, Serrano M, Gorgoulis VG. Specific lipofuscin staining as a novel biomarker to detect replicative and stress-induced senescence. A method applicable in cryo-preserved and archival tissues. Aging (Albany NY). 2013 Jan;5(1):37-50; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3616230/>.

<sup>1220</sup> <http://www.pathologyoutlines.com/topic/lymphnodeslipofuscin.html>.

<sup>1221</sup> Sjöstedt E, Djureinovic D, Mulder J, Kampf C. Identification of proteins in lipofuscin using antibodies within the Human Protein Atlas; <https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma-Aldrich/Posters/1/identification-of-proteins-in-lipofuscin.pdf>.

<sup>1222</sup> Braak H, Braak E, Ohm T, Bohl J. Silver impregnation of Alzheimer's neurofibrillary changes counterstained for basophilic material and lipofuscin pigment. Stain Technol. 1988 Jul;63(4):197-200; <http://www.ncbi.nlm.nih.gov/pubmed/2464205>.

exist,<sup>1223</sup> they may possess some means for chemically recognizing the pigment granules in order to digest them.

Binding sites for sensing lipofuscin materials or lipofuscin granule membrane can be installed on the external recognition modules of tissue-mobile microbivore-class (Section 4.2.2) scavenging nanorobots, allowing them to quickly seek, bind, ingest, and fully digest existing lipofuscin granules throughout the relevant tissues, in the manner of artificial mechanical macrophages. The effluent from these synthetic digestive processes would be mostly harmless free amino acids, fatty acids and carbohydrates,<sup>1224</sup> but any metal atoms present in the processed lipofuscin probably should be sequestered onboard the nanorobots. The microbivore-class devices would enter and exit the brain by any of several means described earlier in Section 4.3. Assuming an injected dose of  $N_{\text{bot}} = 86$  billion nanorobots ( $\sim 1 \text{ cm}^3$  dose, assuming  $12.1 \mu\text{m}^3$  device volume per nanorobot; Section 4.2.2) applying one for each of the 86 billion neurons in the brain,<sup>1225</sup> with each nanorobot able to digest  $V_{\text{lipofuscin}} = 2 \mu\text{m}^3$  per cycle (Section 4.2.2) of lipofuscin granules with average density  $\rho_{\text{lipofuscin}} \sim 1160 \text{ kg/m}^3$ ,<sup>1226</sup> operating on a leisurely  $\tau = 3600 \text{ sec}$  (1 hr) search/digest cycle, then **the entire AD human brain can be cleared of all lipofuscin deposits** in  $t_{\text{clearance}} = \text{Max}[\tau, \tau M_{\text{lipofuscin}} / (N_{\text{bot}} V_{\text{lipofuscin}} \rho_{\text{lipofuscin}})] = \text{Max}[3600, 1804] = \mathbf{3600 \text{ sec}}$  (1 hr).

It is not yet firmly established how quickly fresh lipofuscin would re-deposit in aging brain cells that have been completely cleared of the material. If the active deposition rate is similar to the  $\sim 1 \text{ mg/brain-yr}$  rate for age-dependent accumulation, then decadal cleanouts may result in brain cells carrying only  $\leq 10\%$  of their natural lipofuscin load, reducing pathological effects to minimal levels.

---

<sup>1223</sup> de Grey AD, Alvarez PJ, Brady RO, Cuervo AM, Jerome WG, McCarty PL, Nixon RA, Rittmann BE, Sparrow JR. Medical bioremediation: prospects for the application of microbial catabolic diversity to aging and several major age-related diseases. *Ageing Res Rev.* 2005 Aug;4(3):315-38; [https://www.researchgate.net/profile/W\\_Jerome/publication/7705795\\_Medical\\_bioremediation\\_prospects\\_for\\_the\\_application\\_of\\_microbial\\_catabolic\\_diversity\\_to\\_aging\\_and\\_several\\_major\\_age-related\\_diseases/links/004635268741f62fab000000.pdf](https://www.researchgate.net/profile/W_Jerome/publication/7705795_Medical_bioremediation_prospects_for_the_application_of_microbial_catabolic_diversity_to_aging_and_several_major_age-related_diseases/links/004635268741f62fab000000.pdf).

<sup>1224</sup> Freitas RA Jr. Microbivores: Artificial mechanical phagocytes using digest and discharge protocol. *J Evol Technol* 2005;14:1-52; <http://www.jetpress.org/volume14/freitas.html>.

<sup>1225</sup> Azevedo FA, Carvalho LR, Grinberg LT, Farfel JM, Ferretti RE, Leite RE, Jacob Filho W, Lent R, Herculano-Houzel S. Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. *J Comp Neurol.* 2009 Apr 10;513(5):532-41; <http://www.sakkyndig.com/psykologi/artvit/frederico2009.pdf>.

<sup>1226</sup> Kiefer CR, McKenney JB, Trainor JF, Lambrecht RW, Bonkovsky HL, Lifshitz LM, Valeri CR, Snyder LM. Porphyrin loading of lipofuscin granules in inflamed striated muscle. *Am J Pathol.* 1998 Sep;153(3):703-8; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1853029/>.

### 5.2.2.3 $\alpha$ -Synuclein and Lewy Bodies

$\alpha$ -synuclein is a 140-residue protein that is abundant in the human brain, primarily found in neural tissue and sometimes making up as much as 0.5% of all proteins in the cytosol of brain cells.<sup>1227</sup> It is predominantly expressed in the neocortex, hippocampus, substantia nigra, thalamus, and cerebellum, as well as in neuroglial cells, with subcellular localization studies<sup>1228</sup> indicating the heaviest concentrations in presynaptic terminals and nucleus,<sup>1229</sup> and relatively dense accumulations in some mitochondria. In rat brain,  $\alpha$ -synuclein is highly expressed in the mitochondria in the olfactory bulb, hippocampus, striatum, and thalamus, where the cytosolic  $\alpha$ -synuclein is also rich, whereas the cerebral cortex and cerebellum contains rich cytosolic  $\alpha$ -synuclein but very low or even undetectable levels of mitochondrial  $\alpha$ -synuclein.<sup>1230</sup>

In disorders known as  $\alpha$ -synucleinopathies,<sup>1231</sup>  $\alpha$ -synuclein aggregates to form insoluble fibrils characterized by intracellular Lewy bodies.  $\alpha$ -synuclein is the primary structural component of Lewy body fibrils, and while Lewy bodies sometimes contain tau protein,<sup>1232</sup>  $\alpha$ -synuclein and tau constitute two distinctive subsets of filaments in the same inclusion bodies known as Lewy bodies. Lewy bodies appear as spherical masses that displace other cell components. The two morphological types are classical (brain stem) Lewy bodies and cortical Lewy bodies. A classical Lewy body is an eosinophilic cytoplasmic inclusion consisting of a dense core ~10  $\mu$ m in

---

<sup>1227</sup> Iwai A, Masliah E, Yoshimoto M, Ge N, Flanagan L, de Silva HA, Kittel A, Saitoh T. The precursor protein of non-A beta component of Alzheimer's disease amyloid is a presynaptic protein of the central nervous system. *Neuron*. 1995 Feb;14(2):467-75; [http://www.cell.com/neuron/pdf/0896-6273\(95\)90302-X.pdf](http://www.cell.com/neuron/pdf/0896-6273(95)90302-X.pdf).

<sup>1228</sup> Zhang L, Zhang C, Zhu Y, Cai Q, Chan P, Uéda K, Yu S, Yang H. Semi-quantitative analysis of alpha-synuclein in subcellular pools of rat brain neurons: an immunogold electron microscopic study using a C-terminal specific monoclonal antibody. *Brain Res*. 2008 Dec 9;1244:40-52; <https://www.ncbi.nlm.nih.gov/pubmed/18817762>.

<sup>1229</sup> Yu S, Li X, Liu G, Han J, Zhang C, Li Y, Xu S, Liu C, Gao Y, Yang H, Uéda K, Chan P. Extensive nuclear localization of alpha-synuclein in normal rat brain neurons revealed by a novel monoclonal antibody. *Neuroscience*. 2007 Mar 16;145(2):539-55; <https://www.ncbi.nlm.nih.gov/pubmed/17275196>.

<sup>1230</sup> Liu G, Zhang C, Yin J, Li X, Cheng F, Li Y, Yang H, Uéda K, Chan P, Yu S. alpha-Synuclein is differentially expressed in mitochondria from different rat brain regions and dose-dependently down-regulates complex I activity. *Neurosci Lett*. 2009 May 1;454(3):187-92; <https://www.ncbi.nlm.nih.gov/pubmed/19429081>.

<sup>1231</sup> McCann H, Stevens CH, Cartwright H, Halliday GM.  $\alpha$ -Synucleinopathy phenotypes. *Parkinsonism Relat Disord*. 2014 Jan;20 Suppl 1:S62-7; <https://www.ncbi.nlm.nih.gov/pubmed/24262191>.

<sup>1232</sup> Arima K, Hirai S, Sunohara N, Aoto K, Izumiyama Y, Uéda K, Ikeda K, Kawai M. Cellular co-localization of phosphorylated tau- and NACP/alpha-synuclein-epitopes in Lewy bodies in sporadic Parkinson's disease and in dementia with Lewy bodies. *Brain Res*. 1999 Oct 2;843(1-2):53-61; <https://www.ncbi.nlm.nih.gov/pubmed/10528110>.

diameter<sup>1233</sup> surrounded by a halo of 10-nm-wide radiating fibrils, the primary structural component of which is  $\alpha$ -synuclein. Cortical Lewy bodies are also composed of  $\alpha$ -synuclein fibrils, but are less defined and lack halos. In brain regions where Lewy bodies appear, such as the substantia nigra and locus coeruleus, up to 1-5 Lewy bodies per neuron have been observed.<sup>1234</sup> Assuming the total mass of Lewy bodies does not exceed the total for tau-based neurofibrillary tangles (~33.3 mg/brain) and lipofuscin granules (~100 mg/brain) as previously discussed, a population of 86 billion microbivore-class nanorobots should be able to **clear the human brain of Lewy bodies in a treatment time of ~1 hour**. Binding sites can probably be designed from many sources, e.g., monoclonal antibodies such as the 3D5 monoclonal antibody that recognizes the C-terminal 115-121 amino acids of  $\alpha$ -synuclein<sup>1235</sup> and the 2E3 antibody that recognizes amino acids 134-138 of the protein;<sup>1236</sup> both 3D5 and 2E3 detect presynaptic  $\alpha$ -synuclein but only 3D5 detects nuclear  $\alpha$ -synuclein.

Alternatively, Lewy bodies could be chemically bonded onto probes, loaded into onboard storage tanks of chromalocyte-class nanorobots, and transported intact out of the patient's body for external disposal. Lewy bodies could also be removed via digestion to harmless effluents *in situ* using appropriate synthetic digestive enzymes that are temporarily injected into organelles, and then retrieved, by nanorobots.

Extracellular oligomeric  $\alpha$ -synuclein<sup>1237</sup> can be extracted similarly as for amyloid oligomers like ADDLs (Section 2.4.1), using binding sites with structures similar to oligomeric  $\alpha$ -synuclein-specific antibodies such FILA-1.<sup>1238</sup> Brain concentrations of total  $\alpha$ -synuclein are typically 0.05-

<sup>1233</sup> Duyckaerts C, Hauw JJ. Lewy bodies, a misleading marker for Parkinson's disease? Bull Acad Natl Med. 2003;187(2):277-92; discussion 292-3; <http://www.ncbi.nlm.nih.gov/pubmed/14556441>.

<sup>1234</sup> Turner RS, D'Amato CJ, Chervin RD, Blaivas M. The pathology of REM sleep behavior disorder with comorbid Lewy body dementia. Neurology. 2000 Dec 12;55(11):1730-2; [https://www.researchgate.net/profile/Raymond\\_Turner3/publication/12215385\\_The\\_pathology\\_of\\_REM\\_sleep\\_behavior\\_disorder\\_with\\_comorbid\\_Lewy\\_body\\_dementia/links/0d1c84f4d12f17fe40000000.pdf](https://www.researchgate.net/profile/Raymond_Turner3/publication/12215385_The_pathology_of_REM_sleep_behavior_disorder_with_comorbid_Lewy_body_dementia/links/0d1c84f4d12f17fe40000000.pdf)

<sup>1235</sup> Zhang L, Zhang C, Zhu Y, Cai Q, Chan P, Uéda K, Yu S, Yang H. Semi-quantitative analysis of alpha-synuclein in subcellular pools of rat brain neurons: an immunogold electron microscopic study using a C-terminal specific monoclonal antibody. Brain Res. 2008 Dec 9;1244:40-52; <https://www.ncbi.nlm.nih.gov/pubmed/18817762>.

<sup>1236</sup> Yu S, Li X, Liu G, Han J, Zhang C, Li Y, Xu S, Liu C, Gao Y, Yang H, Uéda K, Chan P. Extensive nuclear localization of alpha-synuclein in normal rat brain neurons revealed by a novel monoclonal antibody. Neuroscience. 2007 Mar 16;145(2):539-55; <https://www.ncbi.nlm.nih.gov/pubmed/17275196>.

<sup>1237</sup> Bliederhaeuser C, Grozdanov V, Speidel A, Zondler L, Ruf WP, Bayer H, Kiechle M, Feiler MS, Freischmidt A, Brenner D, Witting A, Hengerer B, Fändrich M, Ludolph AC, Weishaupt JH, Gillardon F, Danzer KM. Age-dependent defects of alpha-synuclein oligomer uptake in microglia and monocytes. Acta Neuropathol. 2015 Nov 17; <http://www.ncbi.nlm.nih.gov/pubmed/26576561>.

<sup>1238</sup> Paleologou KE, Kragh CL, Mann DM, Salem SA, Al-Shami R, Allsop D, Hassan AH, Jensen PH, El-Agnaf OM. Detection of elevated levels of soluble alpha-synuclein oligomers in post-mortem brain extracts from patients with dementia with Lewy bodies. Brain. 2009 Apr;132(Pt 4):1093-101; <http://brain.oxfordjournals.org/content/132/4/1093.long>.

0.25  $\mu\text{g/ml}$ ,<sup>1239</sup> or 0.07-0.35 mg per 1400  $\text{cm}^3$  human brain. Additionally,  $\alpha$ -synuclein (aka. non-A $\beta$  component protein or NABP) is one of many proteins associated with amyloid plaques in the AD brain. This material is likely to be swept out of the brain along with the amyloid during the nanorobotic procedure previously described in Section 5.2.1.1.

---

<sup>1239</sup> Paleologou KE, Kragh CL, Mann DM, Salem SA, Al-Shami R, Allsop D, Hassan AH, Jensen PH, El-Agnaf OM. Detection of elevated levels of soluble alpha-synuclein oligomers in post-mortem brain extracts from patients with dementia with Lewy bodies. *Brain*. 2009 Apr;132(Pt 4):1093-101; <http://brain.oxfordjournals.org/content/132/4/1093.long>.

### 5.2.3 Correct Cancer, Nuclear Mutations and Epimutations

Despite a sophisticated DNA self-repair system, chromosomes in the cell nucleus slowly acquire two types of irreversible age-related damage. First, there can be mutations, which are changes to the DNA sequence. Second, there can be epimutations or epigenetic modification,<sup>1240</sup> which are changes to the chemical decorations of the DNA molecule (e.g., mis-methylation<sup>1241</sup> and excess methylation<sup>1242</sup>) or to the histone modifications (e.g., excess acetylation),<sup>1243</sup> that control DNA's propensity to be decoded into proteins, collectively representing the "epigenetic state" or "epigenetic signature" of the cell. (In a given patient, different cell types have the same DNA sequences but have different epigenetic states.) When DNA damage of these types leads to uncontrolled rapid cell replication, the result is fast tumor growth, aka. cancer. Other loss of gene function unrelated to cancer can also occur. DNA damage and mutation may be a significant cause of cell toxicity and cell depletion because cells can either commit suicide or go into a senescent non-dividing state as a pre-emptive response to DNA damage that stops the cell from developing into cancer.<sup>1244</sup>

As part of the anti-aging protocol for pre-AD or AD patients, chromalocytes would be employed to replace the chromosome set in every brain cell with newly manufactured chromosomes in which all of the irreversible age-related DNA damage has been repaired. In this protocol, the

---

<sup>1240</sup> Marques S, Outeiro TF. Epigenetics in Parkinson's and Alzheimer's diseases. *Subcell Biochem.* 2013;61:507-25; <http://www.ncbi.nlm.nih.gov/pubmed/23150265>. Wang J, Yu JT, Tan MS, Jiang T, Tan L. Epigenetic mechanisms in Alzheimer's disease: implications for pathogenesis and therapy. *Ageing Res Rev.* 2013 Sep;12(4):1024-41; <http://www.ncbi.nlm.nih.gov/pubmed/23688931>. Millan MJ. The epigenetic dimension of Alzheimer's disease: causal, consequence, or curiosity? *Dialogues Clin Neurosci.* 2014 Sep;16(3):373-93; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4214179/>.

<sup>1241</sup> Yu L, Chibnik LB, Srivastava GP, Pochet N, Yang J, Xu J, Kozubek J, Obholzer N, Leurgans SE, Schneider JA, Meissner A, De Jager PL, Bennett DA. Association of Brain DNA methylation in SORL1, ABCA7, HLA-DRB5, SLC24A4, and BIN1 with pathological diagnosis of Alzheimer disease. *JAMA Neurol.* 2015 Jan;72(1):15-24; <http://www.ncbi.nlm.nih.gov/pubmed/25365775>. Chibnik LB, Yu L, Eaton ML, Srivastava G, Schneider JA, Kellis M, Bennett DA, De Jager PL. Alzheimer's loci: epigenetic associations and interaction with genetic factors. *Ann Clin Transl Neurol.* 2015 Jun;2(6):636-47; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4479524/>. Foraker J, Millard SP, Leong L, Thomson Z, Chen S, Keene CD, Bekris LM, Yu CE. The APOE Gene is Differentially Methylated in Alzheimer's Disease. *J Alzheimers Dis.* 2015 Oct 1;48(3):745-55; <http://www.ncbi.nlm.nih.gov/pubmed/26402071>.

<sup>1242</sup> Piaceri I, Raspanti B, Tedde A, Bagnoli S, Sorbi S, Nacmias B. Epigenetic modifications in Alzheimer's disease: cause or effect? *J Alzheimers Dis.* 2015;43(4):1169-73; <http://www.ncbi.nlm.nih.gov/pubmed/25159670>.

<sup>1243</sup> Narayan PJ, Lill C, Faull R, Curtis MA, Dragunow M. Increased acetyl and total histone levels in post-mortem Alzheimer's disease brain. *Neurobiol Dis.* 2015 Feb;74:281-94; <http://www.ncbi.nlm.nih.gov/pubmed/25484284>.

<sup>1244</sup> SENS Research Foundation, "A Reimagined Research Strategy for Aging," 2015; <http://www.sens.org/research/introduction-to-sens-research>.

replacement chromosomes constitute a consensus pre-damage nucleotide sequence such as might have existed in a youthful or natal cell prior to the occurrence of any damage.<sup>1245</sup> Replacement of all chromosomes in all 10 trillion cell nuclei in the human body would require a total continuous treatment time of ~48 hours (Section 5.1).

For genetically damaged brain cells, the optimal nanorobotic solution to nuclear mutation and epimutation is to employ chromalloytes (Section 4.2.3) to replace all of the randomly damaged chromosomes with newly-manufactured correctly-methylated mutation-free chromosome sets in all the cells of the body, including the brain. This should effectively **cure any pre-existing cancers**, which are proposed to be the principal negative impact of mutated nuclear DNA on health and aging.<sup>1246</sup> As another benefit, this chromosome replacement therapy (CRT) will automatically repair any somatic mutations in tumor-suppression genes, thus reinvigorating other components of the body's natural defenses against brain cancer – a repair that would likely be impractical using conventional biotechnology.

As yet another benefit, with pre-existing cancer cells already corrected, the installed new chromosome sets can be safely manufactured with their telomeres re-extended to full neonatal reserve length, essentially “rolling back the clock” on chromosome age and effectively implementing comprehensive cellular genetic rejuvenation.

Of course, the cumulative burden of random somatic nucleotide base mutations with age can be substantial. According to one recent analysis,<sup>1247</sup> each nonreplicating diploid cell (such as a neuron) could accumulate 400-4,000 mutations by the age of 60, while proliferative cells such as those in the intestinal epithelium and the epidermis could contain 4,000-40,000 mutations, with the result that the 60-year-old intestinal epithelium may harbor  $>10^9$  independent mutations such that “nearly every genomic site is likely to have acquired a mutation in at least one cell in this single organ.” But all of these mutations are readily corrected using chromalloytes, which replace all of the DNA with new pre-mutation consensus copies.

Because biology is highly complicated, the earliest implementations of nanorobotic CRT need not depend on knowing which DNA sequences and epigenetic states are “correct” (in the ideal functional sense), but merely on knowing which ones appear “normal” for a particular patient, with chromalloytes then reinstalling whatever is normal for each cell type. Normal can be measured by widespread sampling of DNA in the patient's native cells and statistically averaging out the observed random variations.<sup>1248</sup> In later implementations of CRT, we will know enough

---

<sup>1245</sup> Freitas RA Jr. The ideal gene delivery vector: Chromalloytes, cell repair nanorobots for chromosome replacement therapy. *J Evol Technol* 2007;16:1-97; <http://jetpress.org/v16/freitas.pdf>.

<sup>1246</sup> de Grey AD. Protagonistic pleiotropy: Why cancer may be the only pathogenic effect of accumulating nuclear mutations and epimutations in aging. *Mech Ageing Dev* 128, 2007;456-459; <http://www.sens.org/nucmutPP.pdf>

<sup>1247</sup> Lynch M. Rate, molecular spectrum, and consequences of human mutation. *Proc Natl Acad Sci U S A*. 2010 Jan 19;107(3):961-8; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2824313/>.

<sup>1248</sup> Freitas RA Jr. The ideal gene delivery vector: Chromalloytes, cell repair nanorobots for chromosome replacement therapy. *J Evol Technol* 2007;16:1-97, Section 4.1; <http://jetpress.org/v16/freitas.pdf>.

about the ideal epigenetic state of all cell types to be able to implement it just as precisely as we will be able to edit native DNA sequences or delete foreign sequences, using the same nanorobots.

It will usually be most efficient to combine the generic CRT therapy described here with the gene editing protocol of Section 5.1, in those few cases where the presence of correctable genetic defects dictates the simultaneous application of the First Alzheimer Protocol.

## 5.2.4 Replace Mutant Mitochondria

Besides nuclear DNA, cellular organelles called mitochondria possess their own DNA that is susceptible to mutation, causing the mutated mitochondrion to malfunction leading to respiration-driven (i.e., oxidative damage-mediated) aging.<sup>1249</sup> Outright mitochondrial DNA deletions of over 3% in the cortex and up to 12% in the putamen have been measured in the brains of normal individuals over the age of 80,<sup>1250</sup> and the pattern of deletion differs for normal vs. AD brains<sup>1251</sup> and for normal vs. bipolar-disorder brains.<sup>1252</sup> There also remains the possibility of mitochondrial epigenetic dysfunction in Alzheimer's disease etiology.<sup>1253</sup>

Mitochondria are the principal source of chemical energy in the cell, metabolizing oxygen and nutrients to carbon dioxide and water, producing energy-charged molecules of ATP that provide power for many important intracellular biochemical processes. Of the ~1000 proteins present in the mitochondrion, only 13 (totaling under 4000 amino acids) are encoded by its own DNA. All the rest are encoded in the cell's nuclear DNA and are manufactured in the cytosol, then transported through the mitochondrial membrane wall by a complicated apparatus called the TIM/TOM complex.<sup>1254</sup> It has been proposed to add the genes encoding the unique 13 mitochondrial proteins to the better-protected nuclear chromosome content,<sup>1255</sup> allowing these

---

<sup>1249</sup> Harman D. The biologic clock: the mitochondria? *J Am Geriatr Soc.* 1972 Apr;20(4):145-7; <http://onlinelibrary.wiley.com/doi/10.1111/j.1532-5415.1972.tb00787.x/abstract>. de Grey AD. The Mitochondrial Free Radical Theory of Aging. Landes Bioscience, Austin, TX, 1999. de Grey AD. Reactive oxygen species production in the mitochondrial matrix: implications for the mechanism of mitochondrial mutation accumulation. *Rejuvenation Res.* 2005 Spring;8(1):13-7; <http://www.sens.org/AKDH.pdf>.

<sup>1250</sup> Corral-Debrinski M, Horton T, Lott MT, Shoffner JM, Beal MF, Wallace DC. Mitochondrial DNA deletions in human brain: regional variability and increase with advanced age. *Nat Genet.* 1992 Dec;2(4):324-9; <http://www.ncbi.nlm.nih.gov/pubmed/1303288>.

<sup>1251</sup> Corral-Debrinski M, Horton T, Lott MT, Shoffner JM, McKee AC, Beal MF, Graham BH, Wallace DC. Marked changes in mitochondrial DNA deletion levels in Alzheimer brains. *Genomics.* 1994 Sep 15;23(2):471-6; <http://www.ncbi.nlm.nih.gov/pubmed/7835898>.

<sup>1252</sup> Kato T, Stine OC, McMahon FJ, Crowe RR. Increased levels of a mitochondrial DNA deletion in the brain of patients with bipolar disorder. *Biol Psychiatry.* 1997 Nov 15;42(10):871-5; <http://www.ncbi.nlm.nih.gov/pubmed/9359971>.

<sup>1253</sup> Devall M, Mill J, Lunnon K. The mitochondrial epigenome: a role in Alzheimer's disease? *Epigenomics.* 2014;6(6):665-75; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4329914/>.

<sup>1254</sup> Rehling P, Wiedemann N, Pfanner N, Truscott KN. The mitochondrial import machinery for preproteins. *Crit Rev Biochem Mol Biol.* 2001;36(3):291-336; <http://www.tandfonline.com/doi/pdf/10.1080/20014091074200>.

<sup>1255</sup> Zullo SJ, Parks WT, Chloupkova M, Wei B, Weiner H, Fenton WA, Eisenstadt JM, Merrill CR. Stable transformation of CHO Cells and human NARP cybrids confers oligomycin resistance (oli(r)) following transfer of a mitochondrial DNA-encoded oli(r) ATPase6 gene to the nuclear genome: a model system for

proteins to be produced when the mitochondria fail to do so and then to be imported through the organelle wall,<sup>1256</sup> thus maintaining adequate energy-producing function even in mutated organelles.

There are many possible nanorobotic strategies for dealing with mutant mitochondria:

First, chromalloytes could deliver into the nucleus of each cell in the human body a new set of manufactured chromosomes that incorporate genes encoding the 13 unique mitochondrial proteins, thus comprehensively effectuating the above proposal in a ~7 hour therapy for a single large organ such as the brain or up to ~48 hours for a continuously-performed whole-body CRT procedure (Section 5.1).

Second, chromalloytes could employ a revised CRT treatment in which mitochondrial DNA is removed from each intracellular organelle in each brain cell and replaced with corrected versions of mtDNA,<sup>1257</sup> a more time-consuming approach.

Third, replacement whole mitochondria containing non-mutated DNA could be manufactured in external clinical cell mills (Section 4.2.6),<sup>1258</sup> then delivered into the cytoplasmic compartment of target brain cells by chromalloyte-class nanorobots. Short-lifetime marker molecules<sup>1259</sup> would distinguish new mitochondria from old, facilitating subsequent deportation of the old from the cell using exiting (now-empty) nanorobots, leaving behind only the new and also ensuring the removal of any mitophages<sup>1260</sup> that might be present, effectuating an all-cell comprehensive mitochondrial transplant operation.

Fourth, replacement mitochondria re-engineered to contain no endogenous DNA could be installed in all brain cells by chromalloyte-class nanorobots, after other chromalloytes have

mtDNA gene therapy. Rejuvenation Res. 2005 Spring;8(1):18-28;  
<http://www.ncbi.nlm.nih.gov/pubmed/15798371>.

<sup>1256</sup> Gearing DP, Nagley P. Yeast mitochondrial ATPase subunit 8, normally a mitochondrial gene product, expressed *in vitro* and imported back into the organelle. EMBO J. 1986 Dec 20;5(13):3651-5;  
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1167406/>.

<sup>1257</sup> Freitas RA Jr. The ideal gene delivery vector: Chromalloytes, cell repair nanorobots for chromosome replacement therapy. J Evol Technol 2007;16:1-97, Section 6.6; <http://jetpress.org/v16/freitas.pdf>.

<sup>1258</sup> Freitas RA Jr. "Chapter 23. Comprehensive Nanorobotic Control of Human Morbidity and Aging," in Gregory M. Fahy, Michael D. West, L. Stephen Coles, and Steven B. Harris, eds, The Future of Aging: Pathways to Human Life Extension, Springer, New York, 2010, Section 6.3.5.2;  
<http://www.nanomedicine.com/Papers/Aging.pdf>.

<sup>1259</sup> Freitas RA Jr. The ideal gene delivery vector: Chromalloytes, cell repair nanorobots for chromosome replacement therapy. J Evol Technol 2007;16:1-97, Section 6.6; <http://jetpress.org/v16/freitas.pdf>.

<sup>1260</sup> Sasser D, Beninati T, Bandi C, Bouman EA, Sacchi L, Fabbi M, Lo N. 'Candidatus Midichloria mitochondrii', an endosymbiont of the tick *Ixodes ricinus* with a unique intramitochondrial lifestyle. Int J Syst Evol Microbiol. 2006 Nov;56(Pt 11):2535-40; <http://www.ncbi.nlm.nih.gov/pubmed/17082386>.

replaced nuclear DNA with new DNA containing the missing mitochondrial DNA, a treatment that would constitute a complete and permanent cure for intra-mitochondrion mutation. Nuclear mutations would continue to occur, and it has been claimed by some<sup>1261</sup> that the mutation rate of genes encoding mitochondrial proteins might be higher in the nucleus than in the mitochondria. In that case, the aforementioned strategy would be a way of greatly delaying but not permanently curing the problem of mitochondrial mutation.

---

<sup>1261</sup> Hayashi J, Ohta S, Kagawa Y, Kondo H, Kaneda H, Yonekawa H, Takai D, Miyabayashi S. Nuclear but not mitochondrial genome involvement in human age-related mitochondrial dysfunction. Functional integrity of mitochondrial DNA from aged subjects. *J Biol Chem.* 1994 Mar 4;269(9):6878-83; <http://www.jbc.org/content/269/9/6878.long>.

## 5.2.5 Repair or Eliminate Toxic, Dysfunctional, and Senescent Cells

One major source of age-related damage is the accumulation of unwanted death-resistant cells that secrete substances toxic to other cells throughout the body. These toxic cells are of several types: (1) fat cells (i.e., visceral adipocytes, which promote insulin resistance and lead to type 2 diabetes), (2) senescent cells (which often accumulate in joint cartilage, skin, white blood cells, and atherosclerotic plaques, cannot divide when they should, and secrete abnormal amounts of certain proteins), (3) memory cytotoxic T cells (which can become too numerous, crowding out other immune cells from the useful immunological space, and which frequently become dysfunctional), (4) immune cells hostile to endogenous antigens (autoimmune T and B cells, producing autoimmune disorders), and (5) other types of immune cells which seem to become dysfunctional during aging (e.g., inability to divide, or immunosenescence).<sup>1262</sup> Some of these toxic cells are found in the AD brain, and toxic cells in other parts of the body may have indirect effects on the brain or nervous system.

There are three types of cellular senescence<sup>1263</sup> – replicative senescence (due to telomere shortening), stress-induced premature senescence (due to DNA damage caused by oxidative stress, UV radiation, etc.), and oncogene-induced senescence (due to oncogene activation).<sup>1264</sup> Dysfunctional cells can secrete substances toxic to other cells, including factors that promote inflammation and cancer,<sup>1265</sup> as well as proteins that inflame the immune system and degrade the normal supporting tissue architecture.<sup>1266</sup> In the central nervous system, a senescence-like phenotype has been observed in postmitotic neurons *in vivo* – specifically, in Purkinje and cortical neurons in response to DNA damage or after exposure to oxidative and metabolic

---

<sup>1262</sup> de Grey ADNJ. Foreseeable pharmaceutical repair of age-related extracellular damage. *Curr Drug Targets*. 2006 Nov;7(11):1469-77; <http://sens.org/files/pdf/excellPP.pdf>. Aspinall R, Mitchell WA. Chapter 7. Maintenance and Restoration of Immune System Function. In: Fahy GM, *et al* (eds), *The Future of Aging: Pathways to Human Life Extension*. Springer, New York, 2010, pp. 489-520; [http://link.springer.com/chapter/10.1007/978-90-481-3999-6\\_15](http://link.springer.com/chapter/10.1007/978-90-481-3999-6_15). “ApoptoSENS: Removing dysfunctional cells,” SENS Research Foundation, 2016; <http://www.sens.org/research/introduction-to-sens-research/deathresistant-cells>.

<sup>1263</sup> The term **cellular senescence** originally described a state of growth arrest observed in aging cells, but now includes alterations in cellular metabolism, secreted cytokines, epigenetic regulation and protein expression.

<sup>1264</sup> Kuilman T, Michaloglou C, Mooi WJ, Peeper DS. The essence of senescence. *Genes Dev*. 2010 Nov 15;24(22):2463-79; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2975923/>.

<sup>1265</sup> Campisi J, Robert L. Cell senescence: role in aging and age-related diseases. *Interdiscip Top Gerontol*. 2014;39:45-61; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4211612/>.

<sup>1266</sup> “ApoptoSENS: Removing dysfunctional cells,” <http://www.sens.org/research/introduction-to-sens-research/deathresistant-cells>.

stress.<sup>1267</sup> Neural senescence has also been observed in response to neurotoxin exposure,<sup>1268</sup> and some evidence is emerging for cellular senescence in a wide variety of neural cell types including neurons, astrocytes, oligodendrocytes, and microglia.<sup>1269</sup>

Senescent cells can exacerbate aging throughout the body, though they prevent cancer from spreading by stopping cell division. In young people the immune system sweeps these cells out of the body on a regular basis before they can cause problems, but during aging the body can't get rid of the dead cells as quickly and they can build up, stopping new cells from regenerating. Senescent cells produce factors that damage adjacent cells and cause chronic inflammation, which is closely associated with frailty and age-related diseases. In one recent experiment,<sup>1270</sup> mice who received a special compound called AP20187 to trigger genes into ramping up their removal of senescent cells lived 35% longer than those allowed to age normally, and the test mice also stayed stronger and healthier for longer. Agents aimed at the removal of senescent cells are called senolytics,<sup>1271</sup> and at least one company is directly pursuing this approach.<sup>1272</sup>

To ensure that cellular dysfunction and senescence cannot contribute to the development of AD, these problems can be corrected in affected cells mostly via chromosome replacement therapy using medical nanorobots (Section 5.2.3), wherein dysfunctional cells are effectively de-senesced using chromalloyocytes to wholly replace their nuclear genome with newly manufactured

<sup>1267</sup> Jurk D, Wang C, Miwa S, Maddick M, Korolchuk V, Tzolou A, Gonos ES, Thrasivoulou C, Saffrey MJ, Cameron K, von Zglinicki T. Postmitotic neurons develop a p21-dependent senescence-like phenotype driven by a DNA damage response. *Aging Cell*. 2012 Dec;11(6):996-1004; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3533793/>.

<sup>1268</sup> Wan C, Liu J, Nie X, Zhao J, Zhou S, Duan Z, Tang C, Liang L, Xu G. 2, 3, 7, 8-Tetrachlorodibenzo-P-dioxin (TCDD) induces premature senescence in human and rodent neuronal cells via ROS-dependent mechanisms. *PLoS One*. 2014 Feb 24;9(2):e89811; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3933666/>.

<sup>1269</sup> Tan FCC, Hutchison ER, Eitan E, Mattson MP. Are there roles for brain cell senescence in aging and neurodegenerative disorders? *Biogerontology*. 2014 Dec;15(6):643-60; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4264619/>.

<sup>1270</sup> Baker DJ, Childs BG, Durik M, Wijers ME, Sieben CJ, Zhong J, A Saltness R, Jeganathan KB, Verzosa GC, Pezeshki A, Khazaie K, Miller JD, van Deursen JM. Naturally occurring p16Ink4a-positive cells shorten healthy lifespan. *Nature*. 2016 Feb 3. doi: 10.1038/nature16932; <http://www.nature.com/nature/journal/v530/n7589/full/nature16932.html>.

<sup>1271</sup> Kirkland JL, Tchkonja T. Clinical strategies and animal models for developing senolytic agents. *Exp Gerontol*. 2015 Aug;68:19-25; <http://www.ncbi.nlm.nih.gov/pubmed/25446976>. Zhu Y et al. The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. *Aging Cell*. 2015 Aug;14(4):644-58; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4531078/>. Zhu Y, Tchkonja T, Fuhrmann-Stroissnigg H, Dai HM, Ling YY, Stout MB, Pirtskhalava T, Giorgadze N, Johnson KO, Giles CB, Wren JD, Niedernhofer LJ, Robbins PD, Kirkland JL. Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of anti-apoptotic factors. *Aging Cell*. 2016 Jun;15(3):428-35; <http://onlinelibrary.wiley.com/doi/10.1111/acer.12445/epdf>.

<sup>1272</sup> Unity Biotechnology; <http://unitybiotechnology.com/>.

chromosomes (Section 4.2.3). Correction of cellular dysfunction might also involve removal of accumulated intracellular aggregates such as lipofuscin (Section 5.2.2.2) and import of healthy organelles (e.g., lysosomes) that are manufactured in an *ex vivo* facility (Section 4.2.6),<sup>1273</sup> loaded into onboard storage tanks of chromalloy-class nanorobots, and then transported intact into the patient's body on a cell-by-cell basis.

If the damage in a particular cell is too extensive, toxic senescent cells that are disrupting their neighbors can be removed – an approach that should be applied sparingly to neural cells in the human brain where cell removal might entail a loss of mental processing capacity or memory. Following the NENS anti-aging protocol,<sup>1274</sup> tissue-mobile microbivore-class nanorobots (Section 4.2.2) can quickly and completely remove the toxic or unwanted neurally-noncritical cells either by digesting them into harmless byproducts *in situ* or by sequestering their contents and transporting the compacted biomaterial out of the body for external disposal. Autoimmune T and B cells can be selectively deleted by medical nanorobots, somewhat analogously to clonal deletion,<sup>1275</sup> eliminating neural autoimmune disorders such as multiple sclerosis (Section 6.2.7).

---

<sup>1273</sup> Freitas RA Jr. “Chapter 23. Comprehensive Nanorobotic Control of Human Morbidity and Aging,” in Gregory M. Fahy, Michael D. West, L. Stephen Coles, and Steven B. Harris, eds, *The Future of Aging: Pathways to Human Life Extension*, Springer, New York, 2010, pp. 685-805; Section 6.3.5.2 “Tissue Printing, Cell Mills and Organ Mills”; <http://www.nanomedicine.com/Papers/Aging.pdf>.

<sup>1274</sup> Freitas RA Jr. “Chapter 23. Comprehensive Nanorobotic Control of Human Morbidity and Aging,” in Gregory M. Fahy, Michael D. West, L. Stephen Coles, and Steven B. Harris, eds, *The Future of Aging: Pathways to Human Life Extension*, Springer, New York, 2010, pp. 685-805; Section 7.1.3 “Eliminating Toxic Death-Resistant Cells”; <http://www.nanomedicine.com/Papers/Aging.pdf>.

<sup>1275</sup> **Clonal deletion** is the selective deactivation or destruction of B cells and T cells after they have expressed receptors for self-antigens. See: Weinberg AD, Bourdette DN, Sullivan TJ, Lemon M, Wallin JJ, Maziarz R, Davey M, Palida F, Godfrey W, Engleman E, Fulton RJ, Offner H, Vandembark AA. Selective depletion of myelin-reactive T cells with the anti-OX-40 antibody ameliorates **autoimmune encephalomyelitis**. *Nat Med*. 1996 Feb;2(2):183-9; <http://www.ncbi.nlm.nih.gov/pubmed/8574963>. Saavedra-Lozano J, McCoig C, Xu J, Cao Y, Keiser P, Ghetie V, Siliciano RF, Siliciano JD, Picker LJ, Ramilo O, Vitetta ES. An anti-CD45RO immunotoxin kills latently infected human immunodeficiency virus (HIV) CD4 T cells in the blood of HIV-positive persons. *J Infect Dis*. 2002 Feb 1;185(3):306-14; <http://jid.oxfordjournals.org/content/185/3/306.long>. Venanzi ES, Benoist C, Mathis D. Good riddance: Thymocyte clonal deletion prevents **autoimmunity**. *Curr Opin Immunol*. 2004 Apr;16(2):197-202; <http://cbdm.hms.harvard.edu/assets/Publications/2004pub/VenanziCurrOP.pdf>. Zhang GX, Liu TT, Ventura ES, Chen Y, Rostami A. Reversal of spontaneous progressive **autoimmune encephalomyelitis** by myelin basic protein-induced clonal deletion. *Autoimmunity*. 1999;31(4):219-27; <http://www.ncbi.nlm.nih.gov/pubmed/10789987>. Preda-Pais A, Stan AC, Casares S, Bona C, Brumeanu TD. Efficacy of clonal deletion vs. anergy of self-reactive CD4 T-cells for the prevention and reversal of **autoimmune diabetes**. *J Autoimmun*. 2005 Aug;25(1):21-32; <http://www.ncbi.nlm.nih.gov/pubmed/16005609>. Rose NR. Molecular mimicry and **clonal deletion**: A fresh look. *J Theor Biol*. 2015 Jun 21;375:71-6; <http://www.sciencedirect.com/science/article/pii/S0022519314004950>.

Nanorobots could also extract all DNA from each toxic cell and the genome-free cell could then be flagged for natural phagocytic removal following a “neuter and release” protocol.<sup>1276</sup>

Alternatively, nanocatheters (Section 4.3.5) can be employed to directly export any unwanted biological material from inside the brain.

---

<sup>1276</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 10.4.1.2, <http://www.nanomedicine.com/NMI/10.4.1.2.htm>, and Section 10.4.2.5.2, <http://www.nanomedicine.com/NMI/10.4.2.5.2.htm>.

## 5.2.6 Restore Essential Lost or Atrophied Cells

Cell depletion is another major source of age-related damage that involves cell loss without equivalent replacement, most commonly in the heart, the brain, and in muscles.<sup>1277</sup> Some damaged cells are repaired, while others are either destroyed or commit “cellular suicide” (apoptosis). Some of the lost cells are replaced by the pools of specialized, tissue-specific stem cells, but the degenerative aging process makes these stem cell pools less effective at repair over time. Over the course of decades, long-lived tissues like the brain begin to progressively lose cells and their function becomes increasingly compromised. The brain loses neurons, contributing to cognitive decline and dementia, as well as to loss of control over fine muscle movements (a process that ends in Parkinson’s disease).<sup>1278</sup> Missing cells leave gaps in tissues which may be filled by: (1) enlargement of adjacent similar cells (e.g., heart), (2) invasion by dissimilar cells or fibrous acellular material (e.g., heart, brain), or (3) general tissue shrinkage (e.g., muscle).

Replacing neurons in the AD brain is not as simple as replacing tissue cells elsewhere in the body, in part because neurons with their dendritic connections to other neurons embody unique information which may be difficult to recover if those neurons are completely missing. Methods for comprehensive neural reconstruction involving large numbers of lost or damaged neurons in the AD brain are described in Section 5.3. In this Section, we consider only the replacement of brain cells as required to offset the normal random loss of neurons due to aging, particularly for non-AD brains in persons who are as yet showing no signs of clinical dementia.

The human brain is composed of neurons (**Figure 16**), glial cells, and blood vessels. More quantitatively, the male adult human brain is composed of  $86.1 \pm 8.1$  billion neurons, of which 16.3 billion or ~19% of all brain neurons (82% of total brain mass) are located in the cerebral cortex (including subcortical white matter), 69.0 billion or ~80% of all brain neurons are in the cerebellum (~10% of brain mass), with  $\leq 1\%$  of all brain neurons located in the rest of the brain, comprising basal ganglia, diencephalon, and brainstem. The male adult human brain also contains  $84.6 \pm 9.8$  billion non-neuronal cells (mostly glia), including 60.8 billion cells or 72% of all non-neuronal cells in the cerebral cortex, 16.0 billion cells (19%) in the cerebellum, and 7.7 billion cells (9%) in the rest of the brain.<sup>1279</sup> The whole brain of a healthy young adult male has a mass of  $1508.9 \pm 299.1$  gm and  $170.7 \pm 13.9$  billion cells. The mean volume of the healthy young adult male human brain has been measured as  $1510 \text{ cm}^3$ , including  $820 \text{ cm}^3$  (54%) of grey

---

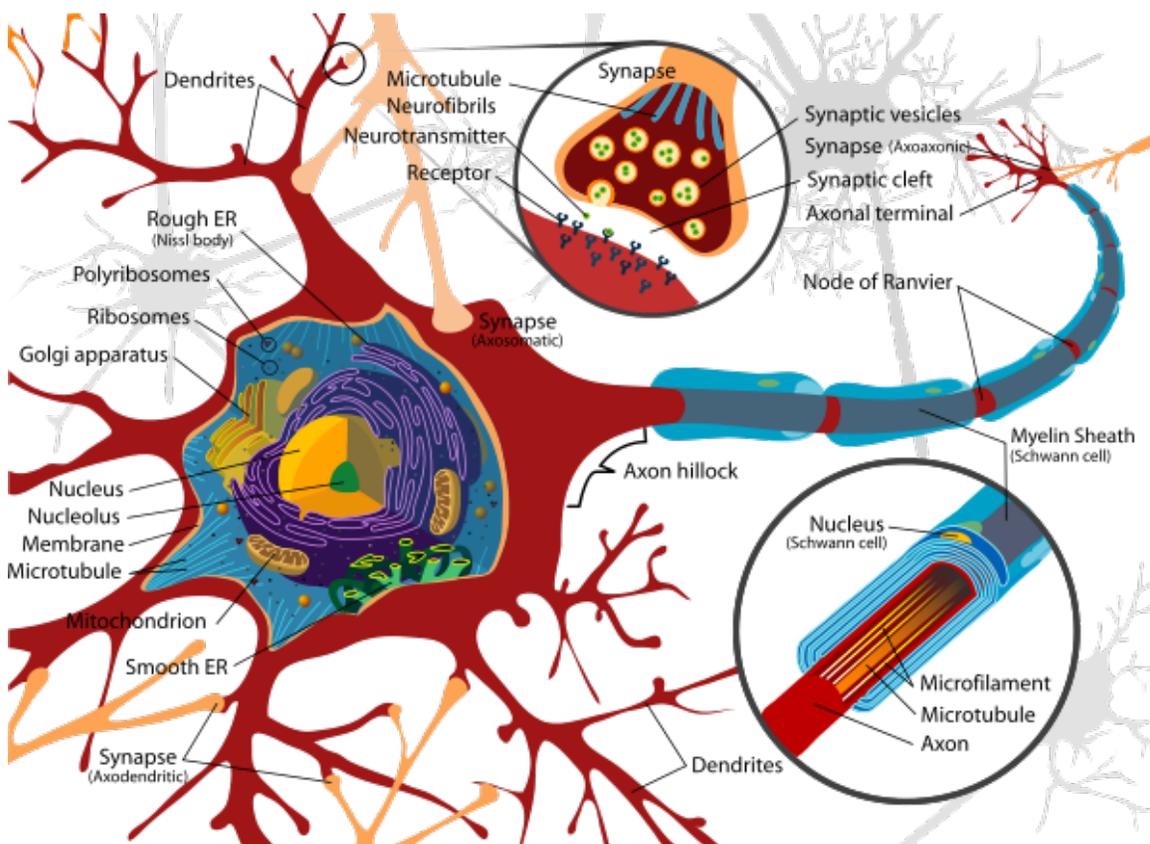
<sup>1277</sup> “RepleniSENS: Replacing lost cells,” <http://www.sens.org/research/introduction-to-sens-research/cell-loss-and-atrophy>.

<sup>1278</sup> “RepleniSENS: Replacing lost cells,” <http://www.sens.org/research/introduction-to-sens-research/cell-loss-and-atrophy>.

<sup>1279</sup> Azevedo FA, Carvalho LR, Grinberg LT, Farfel JM, Ferretti RE, Leite RE, Jacob Filho W, Lent R, Herculano-Houzel S. Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. *J Comp Neurol.* 2009 Apr 10;513(5):532-41; <http://www.sakkyndig.com/psykologi/artvit/frederico2009.pdf>.

matter,  $420 \text{ cm}^3$  (28%) of white matter, and  $270 \text{ cm}^3$  (18%) of cerebrospinal fluid.<sup>1280</sup> The average per-cell volume in the human brain is  $(1510 \text{ cm}^3 - 270 \text{ cm}^3 = 1240 \text{ cm}^3) / (170.7 \text{ billion cells}) = 7264 \mu\text{m}^3/\text{brain cell}$  (whether neuronal or non-neuronal).

Figure 16. Diagram of a common myelinated vertebrate motor neuron, typically found in the spinal cord.<sup>1281</sup>



Adult neurons generally do not reproduce and cannot replace themselves once destroyed. Early workers in the 1950s attempted the first assessment of the long-term rate of natural attrition of brain cells.<sup>1282</sup> Losses ranged from none at all to very many in various parts of the organ, but the

<sup>1280</sup> Lüders E, Steinmetz H, Jäncke L. Brain size and grey matter volume in the healthy human brain. *Neuroreport*. 2002 Dec 3;13(17):2371-4; <http://www.genling.nw.ru/Staff/Psycholinguistics/Brain%20Size.pdf>.

<sup>1281</sup> [https://en.wikipedia.org/wiki/Neuron#Anatomy\\_and\\_histology](https://en.wikipedia.org/wiki/Neuron#Anatomy_and_histology).

<sup>1282</sup> Brody H. Organization of the cerebral cortex. III. A study of aging in the human cerebral cortex. *J Comp Neurol*. 1955 Apr;102(2):511-6; <http://www.ncbi.nlm.nih.gov/pubmed/14381544>.

brainwide average loss was ~100,000 neurons per day (~1 neuron/sec), a rate consistent with loss of all brain cells (in some parts of the organ) over a period of about 250-350 years. More recent work found a normal loss of ~10% of all neocortical neurons in normal human brains over a 70-year period from 20 to 90 years of age in both genders,<sup>1283</sup> a 0.25%/yr long-term average loss rate of ~1 neuron/sec across the entire brain; another study found a 0.5%/yr decline in whole-brain volume and weight.<sup>1284</sup> A longitudinal MRI analysis of brain volume in patients aged 31-84 years found 0.32%/yr atrophy in the whole brain, 0.68%/yr in the temporal lobe, 0.82%/yr in the hippocampus, and ventricular enlargement of 650 mm<sup>3</sup>/yr.<sup>1285</sup> Yet another study confirmed a 0.3%/yr neuronal loss in the human vestibular nuclear complex from 40 to 90 years of age.<sup>1286</sup>

The NENS approach<sup>1287</sup> starts with the manufacture of any needed replacement whole living cells, either very quickly with ideal quality control using external clinical cell mills (Section 4.2.6) or several orders of magnitude slower with inferior quality control using some variant of conventional mammalian cell reactors.<sup>1288</sup> These replacement cells may include manufactured pluripotent neural stem cells.<sup>1289</sup> Nanocatheters (Section 4.3.5) can then be employed to transport the new cells directly into the brain. The slow traversal of conventional vein-infused self-targeting neural stem cells<sup>1290</sup> to their designated destinations would take many orders of magnitude longer and might not be 100% reliable and complete, as compared to using nanocatheters to transport the same cells.

---

<sup>1283</sup> Pakkenberg B, Gundersen HJ. Neocortical neuron number in humans: effect of sex and age. *J Comp Neurol.* 1997 Jul 28;384(2):312-20; <http://www.ncbi.nlm.nih.gov/pubmed/9215725>.

<sup>1284</sup> Svennerholm L, Boström K, Jungbjer B. Changes in weight and compositions of major membrane components of human brain during the span of adult human life of Swedes. *Acta Neuropathol.* 1997 Oct;94(4):345-52; <http://www.ncbi.nlm.nih.gov/pubmed/9341935/>.

<sup>1285</sup> Scahill RI, Frost C, Jenkins R, Whitwell JL, Rossor MN, Fox NC. A longitudinal study of brain volume changes in normal aging using serial registered magnetic resonance imaging. *Arch Neurol.* 2003 Jul;60(7):989-94; <http://archneur.jamanetwork.com/article.aspx?articleid=784396>.

<sup>1286</sup> Lopez I, Honrubia V, Baloh RW. Aging and the human vestibular nucleus. *J Vestib Res.* 1997 Jan-Feb;7(1):77-85; <http://www.ncbi.nlm.nih.gov/pubmed/9057161>.

<sup>1287</sup> Freitas RA Jr. "Chapter 23. Comprehensive Nanorobotic Control of Human Morbidity and Aging," in Gregory M. Fahy, Michael D. West, L. Stephen Coles, and Steven B. Harris, eds, *The Future of Aging: Pathways to Human Life Extension*, Springer, New York, 2010, pp. 685-805; Section 7.1.4 "Restoring Essential Lost or Atrophied Cells"; <http://www.nanomedicine.com/Papers/Aging.pdf>.

<sup>1288</sup> Nelson KL, Geyer S. Bioreactor and process design for large-scale mammalian cell culture manufacturing. *Bioprocess Technol.* 1991;13:112-43; <http://www.ncbi.nlm.nih.gov/pubmed/1367130>.

<sup>1289</sup> Suksuphew S, Noisa P. Neural stem cells could serve as a therapeutic material for age-related neurodegenerative diseases. *World J Stem Cells.* 2015 Mar 26;7(2):502-11; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4369507/>.

<sup>1290</sup> Kim SU, de Vellis J. Stem cell-based cell therapy in neurological diseases: a review. *J Neurosci Res.* 2009 Aug 1;87(10):2183-200; <http://www.ncbi.nlm.nih.gov/pubmed/19301431>.

If neuron losses due to aging are more or less randomly distributed and do not exceed the 10% figure mentioned above, then it is unlikely that existing connectivity networks will have become so thoroughly disrupted by aging alone as to produce serious information loss, assuming that estimates of from 10-fold to 1000-fold redundancy in human neocortical memory patterns<sup>1291</sup> are essentially correct. In this case, dysfunctional or missing neurons might be replaced by infusing neural stem cells (Section 3.2.14) as described above, then allowing normal memory-reinforcing cognitive processes to provide continuous network retraining (Section 5.3.3). *Ex vivo*-cultured neural stem cells have been induced to differentiate and replace lost neurons after injection into the brain.<sup>1292</sup> Irretrievably dead neurons can be replaced by introducing stem or precursor cells that differentiate appropriately.<sup>1293</sup> Brain cell losses can also be offset by inducing compensatory regeneration and reproduction of existing neurons as *in situ* replacements, i.e., by stimulating endogenous neurogenesis.<sup>1294</sup> Successful neuron re-growth in response to growth factors, with associated cognitive benefits, has been reported in rats,<sup>1295</sup> and self-assembling peptide nanofiber scaffolds can create a permissive environment for axons to regenerate through the site of an acute injury and also to knit the brain tissue together, as demonstrated by the experimental reversal of lost vision in one animal model.<sup>1296</sup>

Of course, patterned neuronal networks, once thoroughly disrupted to the point of serious information loss, cannot be restored by randomly infused stem cells. More aggressive means will be required to recover the lost information in these circumstances (Section 5.3).

---

<sup>1291</sup> Kurzweil R. How to Create a Mind. Viking Press, 2012, pp. 39-40, 48. See also: <https://www.quora.com/Is-there-any-redundancy-in-human-memory>.

<sup>1292</sup> Armstrong RJ, Svendsen CN. Neural stem cells: from cell biology to cell replacement. *Cell Transplant*. 2000 Mar-Apr;9(2):139-52; <http://www.ncbi.nlm.nih.gov/pubmed/10811389>.

<sup>1293</sup> Sugaya K, Brannen CL. Stem cell strategies for neuroreplacement therapy in Alzheimer's disease. *Med Hypotheses*. 2001 Dec;57(6):697-700; <http://www.ncbi.nlm.nih.gov/pubmed/11918428>.

<sup>1294</sup> Tatebayashi Y, Lee MH, Li L, Iqbal K, Grundke-Iqbal I. The dentate gyrus neurogenesis: a therapeutic target for Alzheimer's disease. *Acta Neuropathol*. 2003 Mar;105(3):225-32; <http://www.ncbi.nlm.nih.gov/pubmed/12557008>.

<sup>1295</sup> Chen KS, Masliah E, Mallory M, Gage FH. Synaptic loss in cognitively impaired aged rats is ameliorated by chronic human nerve growth factor infusion. *Neuroscience*. 1995 Sep;68(1):19-27; <http://www.ncbi.nlm.nih.gov/pubmed/7477924>.

<sup>1296</sup> Ellis-Behnke RG, Liang YX, Tay DK. Using nanotechnology to repair the body. Strategies for Engineered Negligible Senescence (SENS). In: Third Conference, Queens' College, Cambridge, England, Sep 2007, pp. 6-10.

## 5.2.7 Remove Extracellular Crosslinks

While intracellular proteins are regularly recycled to keep them in a generally undamaged state, many extracellular proteins are laid down early in life and are never, or only rarely, recycled. These long-lived proteins (mainly collagen and elastin) usually serve passive structural functions in the extracellular matrix (ECM) and give human body tissue its elasticity (e.g., artery wall), transparency (e.g., eye lens), or high tensile strength (e.g., ligaments). Note that the extracellular spaces in the human brain occupy 20% of total brain volume but only ~5% in the vicinity of most neurons, including a large number of very narrow (avg. 38-64 nm) spaces<sup>1297</sup> ranging from 0.3-10 nm around the cell bodies of oligodendrocytes to 4-154 nm width around the cell bodies of astrocytes.<sup>1298</sup>

Occasional chemical reactions with other molecules in the extracellular space may little affect these functions, but over time cumulative reactions can lead to random chemical bonding (crosslinks) between two nearby long-lived proteins that were previously unbonded and thus able to slide across or along each other.<sup>1299</sup> For example, in artery walls such crosslinking makes the vessels more rigid and contributes to high blood pressure, a pathology that may also apply to blood vessels in the brain.<sup>1300</sup> As a result, removing crosslinks is usually considered an important aspect of an anti-aging protocol.

Brain ECM has lattice-forming chondroitin sulfate proteoglycans that form into dense lattice-like structures, called perineuronal nets, which enwrap sub-populations of neurons and restrict plasticity.<sup>1301</sup> There are also abundant proteoglycan lecticans (e.g., versican, aggrecan, neurocan, brevican), with the heparin sulfate proteoglycan perlecan found only in amyloid plaques but with relatively low content of fibrous proteins (e.g., collagen, fibronectin, vitronectin) and basement

---

<sup>1297</sup> Thorne RG, Nicholson C. *In vivo* diffusion analysis with quantum dots and dextrans predicts the width of brain extracellular space. *Proc Natl Acad Sci U S A*. 2006 Apr 4;103(14):5567-72; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1459394/>.

<sup>1298</sup> Chvátal A, Anderová M, Syková E. Analysis of K<sup>+</sup> accumulation reveals privileged extracellular region in the vicinity of glial cells *in situ*. *J Neurosci Res*. 2004 Dec 1;78(5):668-82; <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.147.2808&rep=rep1&type=pdf>.

<sup>1299</sup> “GlycoSENS: Breaking extracellular crosslinks,” <http://www.sens.org/research/introduction-to-sens-research/extracellular-crosslinks>.

<sup>1300</sup> Roggendorf W, Opitz H, Schuppan D. Altered expression of collagen type VI in brain vessels of patients with chronic hypertension. A comparison with the distribution of collagen IV and procollagen III. *Acta Neuropathol*. 1988;77(1):55-60; <http://www.ncbi.nlm.nih.gov/pubmed/3239376>.

<sup>1301</sup> Soleman S, Filippov MA, Dityatev A, Fawcett JW. Targeting the neural extracellular matrix in neurological disorders. *Neuroscience*. 2013 Dec 3;253:194-213; <http://www.ncbi.nlm.nih.gov/pubmed/24012743/>.

membrane proteins (e.g., laminin).<sup>1302</sup> Collagen is present in the brain both intracellularly<sup>1303</sup> and extracellularly<sup>1304</sup> where it is normally crosslinked,<sup>1305</sup> often beneficially,<sup>1306</sup> and increased collagen expression may be neuroprotective against A $\beta$ <sub>42</sub> damage.<sup>1307</sup> Elastin is present in the meninges and the microvasculature of the normal human brain,<sup>1308</sup> and there are other ECM glycoproteins in the brain.<sup>1309</sup> The integrity of the pre-existing brain ECM scaffold can be severely damaged by amyloid deposition.<sup>1310</sup>

---

<sup>1302</sup> Ruoslahti E. Brain extracellular matrix. *Glycobiology*. 1996 Jul;6(5):489-92; <http://glycob.oxfordjournals.org/content/6/5/489.long>.

<sup>1303</sup> Seppänen A, Suuronen T, Hofmann SC, Majamaa K, Alafuzoff I. Distribution of collagen XVII in the human brain. *Brain Res*. 2007 Jul 16;1158:50-6; <http://www.ncbi.nlm.nih.gov/pubmed/17555727>.

<sup>1304</sup> Cheng IH, Lin YC, Hwang E, Huang HT, Chang WH, Liu YL, Chao CY. Collagen VI protects against neuronal apoptosis elicited by ultraviolet irradiation via an Akt/phosphatidylinositol 3-kinase signaling pathway. *Neuroscience*. 2011 Jun 2;183:178-88; <http://www.ncbi.nlm.nih.gov/pubmed/21459131>.

<sup>1305</sup> Bronson NW, Hamilton JS, Han M, Li PA, Hornstra I, Horowitz JM, Horwitz BA. LOXL null mice demonstrate selective dentate structural changes but maintain dentate granule cell and CA1 pyramidal cell potentiation in the hippocampus. *Neurosci Lett*. 2005 Dec 23;390(2):118-22; <http://www.ncbi.nlm.nih.gov/pubmed/16157454>. Laczko R, Szauter KM, Jansen MK, Hollosi P, Muranyi M, Molnar J, Fong KS, Hinek A, Csiszar K. Active lysyl oxidase (LOX) correlates with focal adhesion kinase (FAK)/paxillin activation and migration in invasive astrocytes. *Neuropathol Appl Neurobiol*. 2007 Dec;33(6):631-43; <http://www.ncbi.nlm.nih.gov/pubmed/17931358>.

<sup>1306</sup> Suttkus A, Holzer M, Morawski M, Arendt T. The neuronal extracellular matrix restricts distribution and internalization of aggregated Tau-protein. *Neuroscience*. 2015 Nov 24. pii: S0306-4522(15)01039-8; <http://www.ncbi.nlm.nih.gov/pubmed/26621125>

<sup>1307</sup> Cheng JS, Dubal DB, Kim DH, Legleiter J, Cheng IH, Yu GQ, Tesseur I, Wyss-Coray T, Bonaldo P, Mucke L. Collagen VI protects neurons against A $\beta$  toxicity. *Nat Neurosci*. 2009 Feb;12(2):119-21; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2812922/>. Bianchi FT, Camera P, Ala U, Imperiale D, Migheli A, Boda E, Tempia F, Berto G, Bosio Y, Oddo S, LaFerla FM, Taraglio S, Dotti CG, Di Cunto F. The collagen chaperone HSP47 is a new interactor of APP that affects the levels of extracellular beta-amyloid peptides. *PLoS One*. 2011;6(7):e22370; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3145648/>.

<sup>1308</sup> Jung S, Hinek A, Tsugu A, Hubbard SL, Ackerley C, Becker LE, Rutka JT. Astrocytoma cell interaction with elastin substrates: implications for astrocytoma invasive potential. *Glia*. 1999 Jan 15;25(2):179-89; <http://www.ncbi.nlm.nih.gov/pubmed/9890632>.

<sup>1309</sup> Barros CS, Franco SJ, Müller U. Extracellular matrix: functions in the nervous system. *Cold Spring Harb Perspect Biol*. 2011 Jan 1;3(1):a005108; <http://cshperspectives.cshlp.org/content/3/1/a005108.full>.

<sup>1310</sup> Syková E, Vorisek I, Antonova T, Mazel T, Meyer-Luehmann M, Jucker M, Hájek M, Ort M, Bures J. Changes in extracellular space size and geometry in APP23 transgenic mice: a model of Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2005 Jan 11;102(2):479-84; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC544312/>. Ajmo JM, Bailey LA, Howell MD, Cortez LK, Pennypacker KR, Mehta HN, Morgan D, Gordon MN, Gottschall PE. Abnormal post-translational and extracellular processing of brevican in plaque-bearing mice over-expressing APPsw. *J Neurochem*. 2010 May;113(3):784-95; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2855738/>.

The potential pathologies of both intracellular<sup>1311</sup> and extracellular<sup>1312</sup> crosslinking of structural proteins in the AD brain have been only lightly investigated to date. One suggestive early study found ~1 crosslinked residue per 10,000 residues in normal brain but 30-50 crosslinked residues per 10,000 residues in Alzheimer's cortex and cerebellum tissues.<sup>1313</sup> Beyond these few studies and excluding crosslinking within amyloid plaques<sup>1314</sup> (whose removal is already described in Section 5.2.1.1), no other specific therapeutic targets for correcting ECM crosslinking in the progression of AD have yet been identified.<sup>1315</sup> Should any be found in the future, there are multiple conventional strategies for targeting problematic ECM molecules, including chondroitinase ABC, ECM crosslinking antibodies, anti-integrin antibodies, and ECM-degrading enzymes such as matrix metalloproteinases,<sup>1316</sup> though care must be taken with these to avoid unwanted damage to desirable ECM components. There may be drugs or molecules that can selectively sever the crosslinks (which have very unusual chemical structures).<sup>1317</sup>

---

<sup>1311</sup> Cheng J, Wang F, Yu DF, Wu PF, Chen JG. The cytotoxic mechanism of malondialdehyde and protective effect of carnosine via protein cross-linking/mitochondrial dysfunction/reactive oxygen species/MAPK pathway in neurons. *Eur J Pharmacol.* 2011 Jan 10;650(1):184-94; <http://www.ncbi.nlm.nih.gov/pubmed/20868662>.

<sup>1312</sup> Wilhelmus MM, Grunberg SC, Bol JG, van Dam AM, Hoozemans JJ, Rozemuller AJ, Drukarch B. Transglutaminases and transglutaminase-catalyzed cross-links colocalize with the pathological lesions in Alzheimer's disease brain. *Brain Pathol.* 2009 Oct;19(4):612-22; <http://www.ncbi.nlm.nih.gov/pubmed/18673368>. de Jager M, Drukarch B, Hofstee M, Brevé J, Jongenelen CA, Bol JG, Wilhelmus MM. Tissue transglutaminase-catalysed cross-linking induces Apolipoprotein E multimers inhibiting Apolipoprotein E's protective effects towards amyloid-beta-induced toxicity. *J Neurochem.* 2015 Sep;134(6):1116-28; <http://www.ncbi.nlm.nih.gov/pubmed/26088696>.

<sup>1313</sup> Kim SY, Grant P, Lee JH, Pant HC, Steinert PM. Differential expression of multiple transglutaminases in human brain. Increased expression and cross-linking by transglutaminases 1 and 2 in Alzheimer's disease. *J Biol Chem.* 1999 Oct 22;274(43):30715-21; <http://www.jbc.org/content/274/43/30715.long>.

<sup>1314</sup> Sitkiewicz E, Olędzki J, Poznański J, Dadlez M. Di-tyrosine cross-link decreases the collisional cross-section of aβ peptide dimers and trimers in the gas phase: an ion mobility study. *PLoS One.* 2014 Jun 19;9(6):e100200; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4063900/>.

<sup>1315</sup> Morawski M, Filippov M, Tzinia A, Tsilibary E, Vargova L. ECM in brain aging and dementia. *Prog Brain Res.* 2014;214:207-27; <http://www.ncbi.nlm.nih.gov/pubmed/25410360>.

<sup>1316</sup> Berezin V, Walmod PS, Filippov M, Dityatev A. Targeting of ECM molecules and their metabolizing enzymes and receptors for the treatment of CNS diseases. *Prog Brain Res.* 2014;214:353-88; [http://www.researchgate.net/profile/Alexander\\_Dityatev/publication/268791743\\_Targeting\\_of\\_ECM\\_molecules\\_and\\_their\\_metabolizing\\_enzymes\\_and\\_receptors\\_for\\_the\\_treatment\\_of\\_CNS\\_diseases/links/548c58820cf225bf669f92ba.pdf](http://www.researchgate.net/profile/Alexander_Dityatev/publication/268791743_Targeting_of_ECM_molecules_and_their_metabolizing_enzymes_and_receptors_for_the_treatment_of_CNS_diseases/links/548c58820cf225bf669f92ba.pdf).

<sup>1317</sup> "GlycoSENS: Breaking extracellular crosslinks," <http://www.sens.org/research/introduction-to-sens-research/extracellular-crosslinks>.

Alternatively, given specific targets, nanorobots should also do an excellent job of crosslink removal.<sup>1318</sup>

---

<sup>1318</sup> Freitas RA Jr. "Chapter 23. Comprehensive Nanorobotic Control of Human Morbidity and Aging," in Gregory M. Fahy, Michael D. West, L. Stephen Coles, and Steven B. Harris, eds, *The Future of Aging: Pathways to Human Life Extension*, Springer, New York, 2010, pp. 685-805; Section 7.1.2 "Removing Extracellular Crosslinks"; <http://www.nanomedicine.com/Papers/Aging.pdf>.

### 5.3 Third Alzheimer Protocol: Neural Reconstruction

With genetic susceptibilities eliminated, accumulated damage repaired, and existing cells and tissues rejuvenated into a relatively youthful state on a recurring schedule, we can now begin the process of reconstructing any neural tissue that is either too damaged to repair or has been destroyed, leaving physical gaps in the AD patient's brain structure.

As noted by the EuroStemCell organization,<sup>1319</sup> the FDA,<sup>1320</sup> and others, the successful use of neural stem cells to treat Alzheimer's disease (the "biotechnology" approach) would minimally require the ability to:

- (1) provide a supply of healthy, working, autologous neural stem cells;
- (2) transplant those cells safely;
- (3) travel into the multiple areas of the brain where damage has occurred;
- (4) produce the many different types of neurons needed to replace the damaged or lost cells;
- (5) do this in a way that enables the new neurons to integrate effectively into the brain, making connections to replace the lost parts of a complex network;
- (6) ensure that intravenous injections of stem cells don't block capillaries, causing embolisms and damaging brain tissue;
- (7) ensure that stem cells don't give rise to tumors,<sup>1321</sup> or make the wrong kind of cells or connections in the brain, leading to side effects like chronic pain;
- (8) ensure that the brain retains the ability to integrate new neurons properly, once Alzheimer's has taken hold; and
- (9) ensure that the newly-arriving transplanted stem cells are not themselves damaged by the ongoing amyloid and tau protein tangle buildup in the brain, which would mean that a transplant could have only a temporary effect.<sup>1322</sup>

---

<sup>1319</sup> EuroStemCell, "Alzheimer's disease: how could stem cells help?" 22 Jan 2014; <http://www.eurostemcell.org/factsheet/alzheimer%E2%80%99s-disease-how-could-stem-cells-help>.

<sup>1320</sup> AlzForum, "Ready or Not: Stem Cell Therapies Poised to Enter Trials for Alzheimer's," 20 Nov 2014; <http://www.alzforum.org/news/conference-coverage/ready-or-not-stem-cell-therapies-poised-enter-trials-alzheimers>.

<sup>1321</sup> Borlongan CV. Recent preclinical evidence advancing cell therapy for Alzheimer's disease. *Exp Neurol.* 2012 Sep;237(1):142-6; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4082718/>.

<sup>1322</sup> Fan X, Sun D, Tang X, Cai Y, Yin ZQ, Xu H. Stem-cell challenges in the treatment of Alzheimer's disease: a long way from bench to bedside. *Med Res Rev.* 2014 Sep;34(5):957-78; [https://www.researchgate.net/profile/Haiwei\\_Xu/publication/260117547\\_Stem-Cell\\_Challenges\\_in\\_the\\_Treatment\\_of\\_Alzheimer's\\_Disease\\_A\\_Long\\_Way\\_from\\_Bench\\_to\\_Bedside/links/0046352f580f990cf4000000.pdf](https://www.researchgate.net/profile/Haiwei_Xu/publication/260117547_Stem-Cell_Challenges_in_the_Treatment_of_Alzheimer's_Disease_A_Long_Way_from_Bench_to_Bedside/links/0046352f580f990cf4000000.pdf).

Another researcher provides a similar list of requirements for the successful application of cell replacement therapy to AD:

For effective cell replacement strategies for AD, neural stem cells would first need to **migrate** to multiple areas of the brain and then **differentiate** and **mature** into multiple neuronal subtypes. These neurons would then also need to **re-innervate** appropriate targets and establish physiologically relevant afferent **connectivity**, in essence recapitulating much of the complex brain circuitry that develops *in utero*.<sup>1323</sup>

It will be difficult, if not impossible, to meet all of these requirements using treatments with stem cells alone. However, we can meet all of the requirements using nanorobots.

Our proposed Third Alzheimer Protocol begins with extensive brain mapping and the compilation of a neural repair plan (Section 5.3.1).

The next step is reconstruction of the missing neural tissue (Section 5.3.2). Reconstruction requires manufacturing replacement neural cells (Section 5.3.2.1), debridement of neural detritus in heavily damaged or envoided areas (Section 5.3.2.2), insertion and emplacement of replacement cells via a nanocatheter array (Section 5.3.2.3), and incorporation of the replacement cells into the existing neural tissues (Section 5.3.2.4).

Finally, full incorporation is elicited and guided by an intensive program of neural network retraining (Section 5.3.3), enabling significant restoration of function to the AD patient. Network retraining relies on the experimentally-proven beneficial effects of environmental enrichment (Section 5.3.3.1), which includes activities designed to elicit recovery of brain memory data (Section 5.3.3.2) and mental algorithms (Section 5.3.3.3).

Please note that while most of the tasks of the First Alzheimer Protocol might in principle be achievable using some very advanced form of genetic engineering (perhaps derived from CRISPR techniques), and while many of the numerous anti-aging tasks of the Second Alzheimer Protocol might conceivably be accomplished using some future form of advanced biotechnology,<sup>1324</sup> it seems unlikely that most of the tasks of the Third Alzheimer Protocol can be achieved without resorting to the very sophisticated mapping and cell-by-cell interventions that are uniquely enabled by medical nanorobots possessing advanced onboard communication, navigation, computation, and manipulation capabilities, as noted in Section 4.2.5.

Please also note that the proposed treatment methodology described here is conceptually fairly conservative and minimalist, relying as much as possible on natural biological processes to carry neural reconstruction to completion. More aggressive techniques – e.g., mapping via comprehensive whole-brain scans to nanometer resolution coupled with positionally-controlled

---

<sup>1323</sup> Chen WW, Blurton-Jones M. Concise review: Can stem cells be used to treat or model Alzheimer's disease? *Stem Cells*. 2012 Dec;30(12):2612-8; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3508338/>.

<sup>1324</sup> de Grey A, Rae M. *Ending Aging: The Rejuvenation Breakthroughs that Could Reverse Human Aging in Our Lifetime*. St. Martin's Griffin, NY, 2007; <https://www.amazon.com/Ending-Aging-Rejuvenation-Breakthroughs-Lifetime/dp/0312367074>.

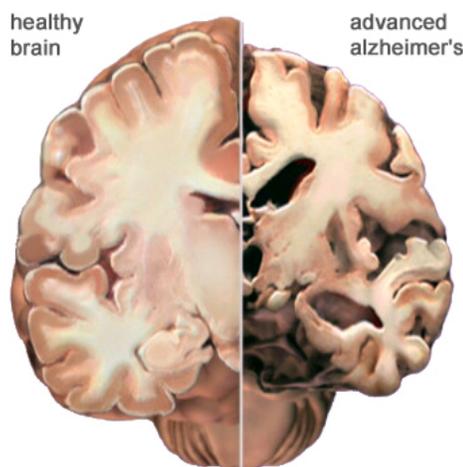
molecule-by-molecule *in situ* brain tissue printing, or brain retraining using continuous real-time *in vivo* action-potential monitoring<sup>1325</sup> during neural reconfiguration coupled with participatory conscious feedback from the patient – are theoretically possible and may produce superior results, but these are second-generation approaches that are beyond the scope of this initial presentation.

---

<sup>1325</sup> Martins NRB, Erlhagen W, Freitas RA Jr. Action Potential Monitoring Using Neuronanorobots: Neuroelectric Nanosensors. *Intl J Nanomater Nanostructures* 2015 Jun; 1:20-41; <http://materials.journalspub.info/index.php/IJNN/article/view/107>.

### 5.3.1 Brain Mapping and Neural Repair Plan

Where does damage occur in the brain of an Alzheimer's patient, as compared to a normally aging brain in a person who does not have Alzheimer's (image, right)? AD causes a larger loss in brain weight and volume than aging, and affects some brain regions and neuronal populations more than others. Although AD clearly causes loss of neurons in specific brain regions, much of the overall loss of brain volume appears to be due to the shrinkage and loss of neuronal processes. Studies using MRI and PET have documented reductions in the size of specific brain regions in people with AD as they progressed from mild cognitive impairment to Alzheimer's disease, and in comparison with similar images from healthy older adults (**Figure 17**).<sup>1326</sup>



The rate of whole-brain atrophy due to normal aging is 0.2%-0.5%/yr, roughly equivalent to ~1 neuron/sec (Section 5.2.6). The whole-brain atrophy rate in Alzheimer brains can be ten times faster. One MRI study<sup>1327</sup> reported a 1.9%/yr atrophy rate and rates of whole brain atrophy for AD brains are typically reported at 2%/yr,<sup>1328</sup> with a range of 1%-4%/yr brain volume loss among different populations.<sup>1329</sup>

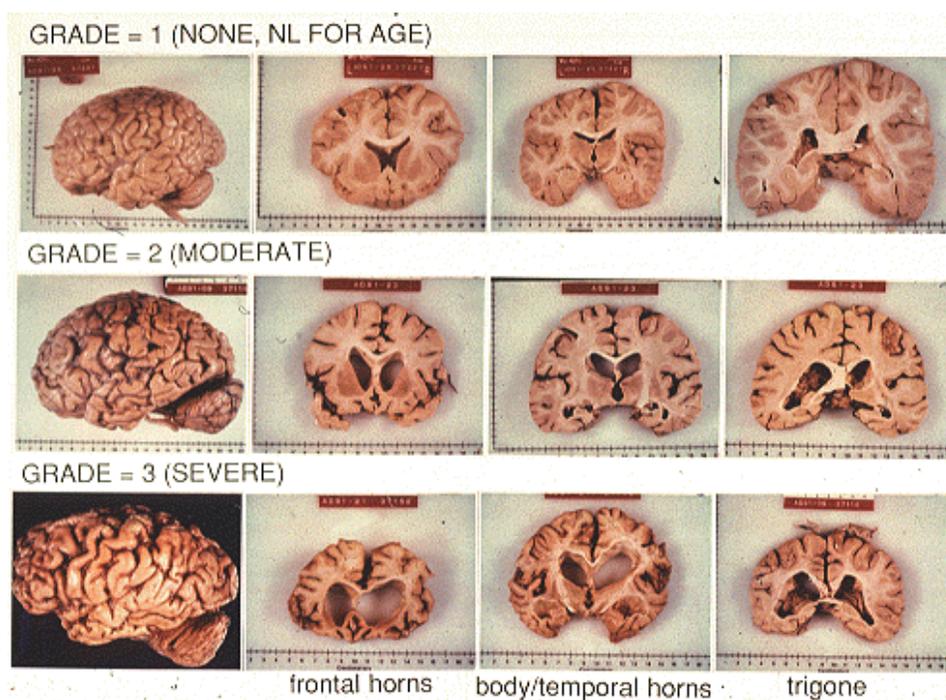
<sup>1326</sup> Desikan RS, Cabral HJ, Hess CP, Dillon WP, Glastonbury CM, Weiner MW, Schmansky NJ, Greve DN, Salat DH, Buckner RL, Fischl B. Automated MRI Measures Identify Individuals with Mild Cognitive Impairment and Alzheimer's Disease. *Brain*. Aug 2009;132(Pt 8):2048-57; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2714061/>. Moan R. MRI Software Accurately IDs Preclinical Alzheimer's Disease. *Diagnostic Imaging*. 20 Jul 2009; <http://www.diagnosticimaging.com/news/display/article/113619/1428344>.

<sup>1327</sup> Sluimer JD, Vrenken H, Blankenstein MA, Fox NC, Scheltens P, Barkhof F, van der Flier WM. Whole-brain atrophy rate in Alzheimer disease: identifying fast progressors. *Neurology*. 2008 May 6;70(19 Pt 2):1836-41; <http://dspace.uvu.vu.nl/bitstream/handle/1871/19214/dissertation.pdf?sequence=12#page=105>.

<sup>1328</sup> Schott JM, Price SL, Frost C, Whitwell JL, Rossor MN, Fox NC. Measuring atrophy in Alzheimer disease: a serial MRI study over 6 and 12 months. *Neurology*. 2005 Jul 12;65(1):119-24; <http://www.ncbi.nlm.nih.gov/pubmed/16009896>.

<sup>1329</sup> Jack CR Jr, Shiung MM, Gunter JL, O'Brien PC, Weigand SD, Knopman DS, Boeve BF, Ivnik RJ, Smith GE, Cha RH, Tangalos EG, Petersen RC. Comparison of different MRI brain atrophy rate measures with clinical disease progression in AD. *Neurology*. 2004 Feb 24;62(4):591-600; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2730165/>. Boyes RG, Rueckert D, Aljabar P, Whitwell J, Schott JM, Hill DL, Fox NC. Cerebral atrophy measurements using Jacobian integration: comparison with the boundary shift integral. *Neuroimage*. 2006 Aug 1;32(1):159-69; <http://www.doc.ic.ac.uk/~pa100/pubs/boyesNeuroImage2006.pdf>.

Figure 17. Visual standards for brain atrophy in several stages of AD.<sup>1330</sup>



Over a typical 5-10 year course of Alzheimer's disease, a 2%-4%/yr atrophy rate can amount to a 20%+ loss of whole-brain volume, over and above the expected 2% brain volume loss due to normal aging. Losses in specialized areas of the AD brain can be much higher. For example, one study<sup>1331</sup> found that at time of death, AD patients with the mildest clinically detectable dementia had 32% fewer entorhinal cortex<sup>1332</sup> neurons than controls (vs. 69% for severe AD brains), 40% fewer layer IV neurons (vs. 70% for severe AD) and 60% fewer layer II neurons (vs. 90% for severe AD). An analysis of postmortem brain samples demonstrated that the average number of cholinergic neurons in the basal forebrain was 79% lower in AD patients when compared with non-AD individuals.<sup>1333</sup>

<sup>1330</sup> <http://unionlosangeles.com/2012/09/21/pink-is-for-breast-cancer-purple-is-for-alzheimers-disease/>.

<sup>1331</sup> Gomez-Isla T, Price JL, McKeel DW, Jr, Morris JC, Growdon JH, Hyman BT. Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *J Neurosci*. 1996;16:4491-4500; <http://www.jneurosci.org/content/16/14/4491.long>.

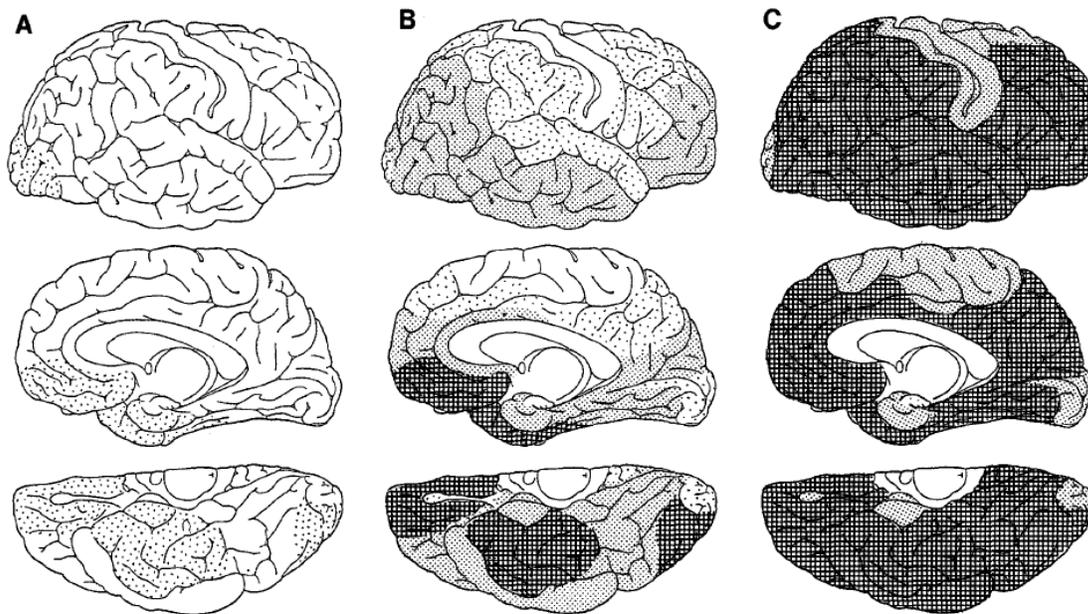
<sup>1332</sup> The **entorhinal cortex** is located in the medial temporal lobe and functions as a hub in a widespread network for memory and navigation, serving as the main interface between the hippocampus and neocortex and mediating declarative (autobiographical/episodic/semantic) and spatial memories.

<sup>1333</sup> Wenk GL. Neuropathologic changes in Alzheimer's disease: potential targets for treatment. *J Clin Psychiatry*. 2006;67 Suppl 3:3-7; quiz 23; <http://faculty.psy.ohio-state.edu/wenk/documents/JClinPsychiatry2006.pdf>.

The influential Braak work<sup>1334</sup> was among the first to map the physical location of amyloid plaques and neurofibrillary tangles in the AD brain to begin the study of damage localization and quantification of the disease.

For amyloid plaques (**Figure 18**), in early AD, small plaque deposits are found in basal portions of the frontal, temporal, and occipital lobes in the isocortex. In middle-stage AD, amyloid is present in virtually all isocortical association areas, the hippocampal formation is only mildly involved, and the external glial layer remains devoid of amyloid. By late-stage AD, deposits exist in all areas of the isocortex including sensory and motor core fields. The subthalamic and red nucleus show deposits, the molecular layer of the cerebellar cortex can exhibit patches of amyloid, but the substantia nigra (esp. pars compacta) remains virtually devoid of them.

*Figure 18. Distribution pattern of amyloid deposits in early (A), middle (B), and late (C) stage Alzheimer's disease, with darker shading indicating increasing numbers of amyloid deposits in several different cross-sections of the human AD brain (Braak, 1991).<sup>1335</sup>*

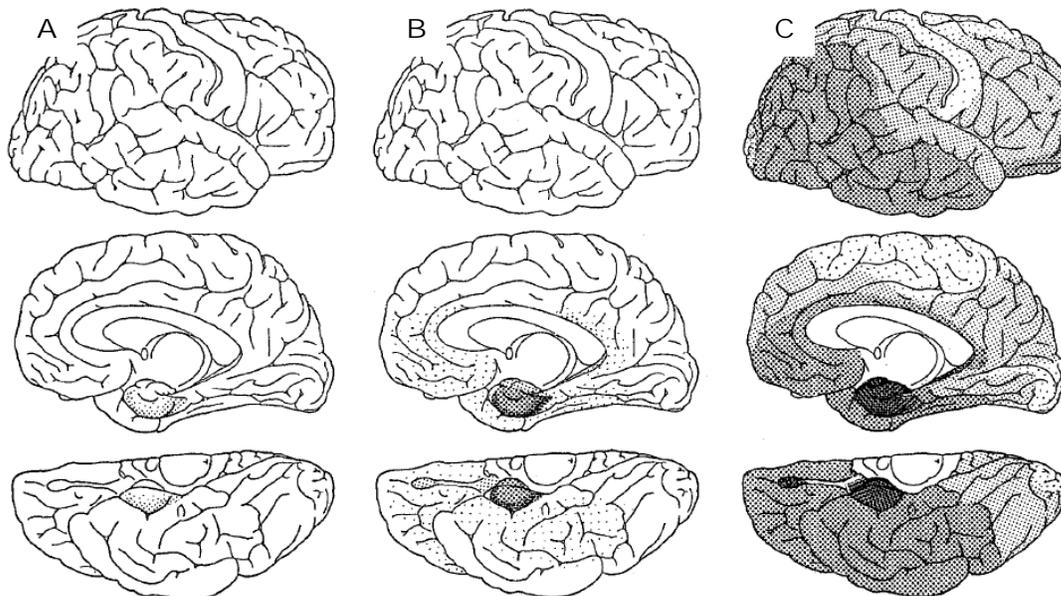


<sup>1334</sup> Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol.* 1991;82(4):239-59; [http://info-centre.jenage.de/assets/pdfs/library/braak\\_braak\\_ACTA\\_NEUROPATHOL\\_1991.pdf](http://info-centre.jenage.de/assets/pdfs/library/braak_braak_ACTA_NEUROPATHOL_1991.pdf).

<sup>1335</sup> Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol.* 1991;82(4):239-59; [http://info-centre.jenage.de/assets/pdfs/library/braak\\_braak\\_ACTA\\_NEUROPATHOL\\_1991.pdf](http://info-centre.jenage.de/assets/pdfs/library/braak_braak_ACTA_NEUROPATHOL_1991.pdf).

For neurofibrillary tangles (**Figure 19**), the earliest or “transentorhinal” stage (left) is characterized by either mild or severe alteration of the transentorhinal layer Entorhinal-Pre- $\alpha$  neurons. The middle or “limbic” stage (middle) is marked by a conspicuous affection of layer Pre- $\alpha$  in both transentorhinal region and proper entorhinal cortex, with mild involvement of the first Ammon’s horn sector. By the late or “isocortical” stage (right), we see the destruction of virtually all isocortical association areas, with considerable loss of nerve cells in layers Pre- $\alpha$  and Pri- $\alpha$  accompanied by large numbers of ghost tangles.

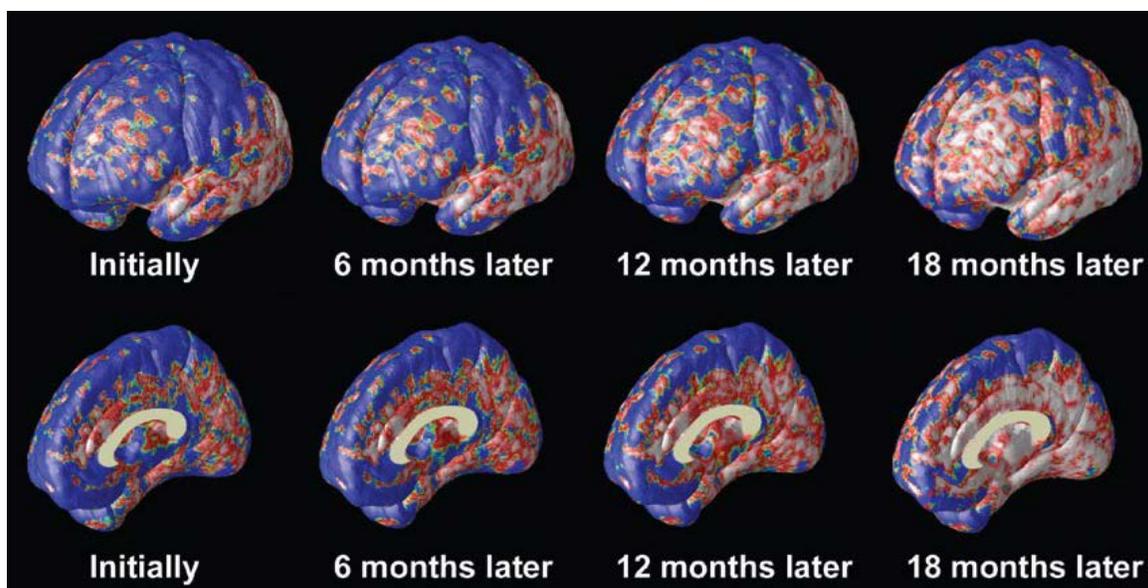
*Figure 19. Distribution pattern of neurofibrillary tangles in pre-clinical (A), early (B), and middle-late (C) stage Alzheimer’s disease, with darker shading indicating more numerous tangles in several different cross-sections of the human AD brain (Braak, 1991).<sup>1336</sup>*



<sup>1336</sup> Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol.* 1991;82(4):239-59; [http://info-centre.jenage.de/assets/pdfs/library/braak\\_braak\\_ACTA\\_NEUROPATHOL\\_1991.pdf](http://info-centre.jenage.de/assets/pdfs/library/braak_braak_ACTA_NEUROPATHOL_1991.pdf).

Since Braak's early work, research in neural imaging has rapidly advanced. In 2006, Paul Thompson of UCLA (now at the USC Neuroimaging and Informatics Institute)<sup>1337</sup> created remarkable 3D images of the spread of damage in actual human AD brains by overlaying data sets collected from various scanning techniques, including structural and functional MRI (**Figure 20**). Among the areas where significant cell death may first occur are in the hippocampus, the seahorse-shaped brain structures behind the ears in the temporal lobes that control memory processing. As AD progresses, this is followed by damage to other structures in the limbic system, affecting emotions, and to the frontal lobe areas that affect decisionmaking and self-control. The pattern of damage helps explain the sequence of thought and behavior problems that commonly arise as Alzheimer's disease becomes more severe.

*Figure 20. Spread of Alzheimer's disease in the brain over an 18-month period of observation. Exterior views (top row) and midline views (bottom row) through the brain show healthy brain activity as red and blue areas, and rapidly spreading areas of cell death as gray areas.<sup>1338</sup>*



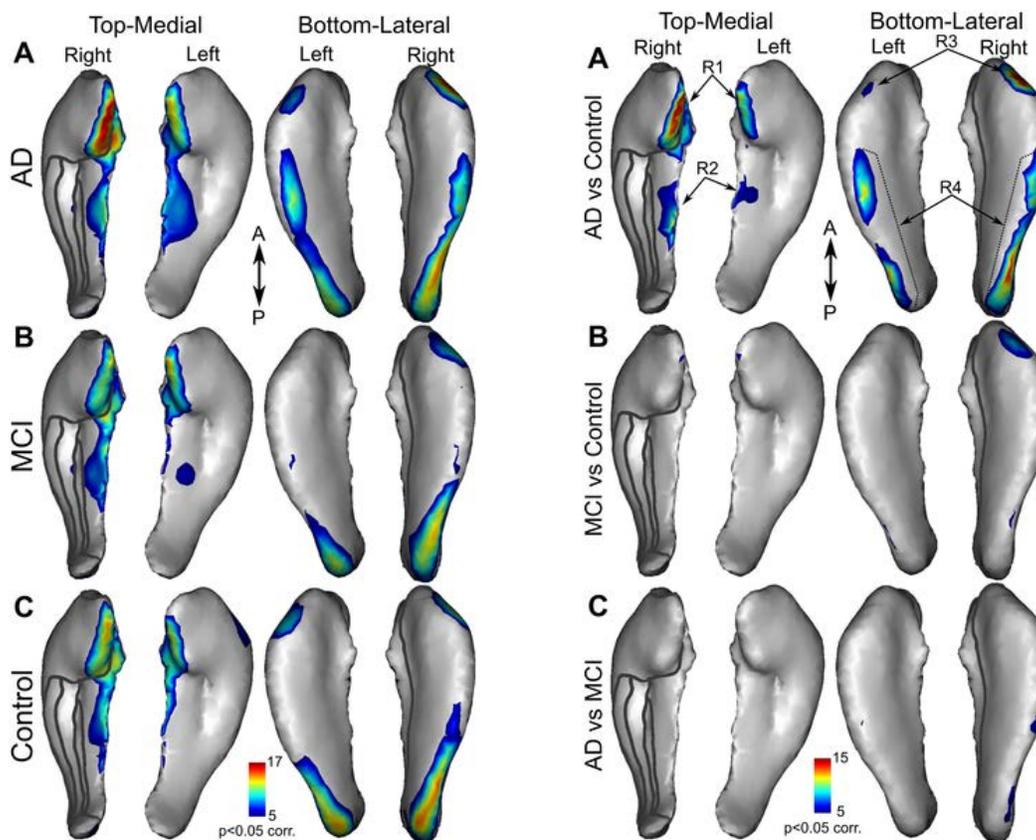
Once we have a comprehensive brain neuron map of the AD patient, the map can be computationally compared to the map of a normal elderly brain to obtain a “delta” map that indicates where the AD patient's brain may differ from normal aging. This tells us where damage has occurred and to what extent, thus the locations to which AD-specific repair activities should be directed. For example, a recent study of hippocampal damage rates in AD brains used this subtractive technique on data from MRI scans of human patients to computationally compare AD

<sup>1337</sup> <http://users.ioni.usc.edu/~thompson/thompson.html>.

<sup>1338</sup> Toby Bilanow, Preserving Your Memory, The Magazine of Health and Hope, Winter 2007; <https://www.alzinfo.org/articles/visualizing-alzheimers/>.

damage to MCI (mild cognitive impairment) elderly brains and normal elderly brains (**Figure 21**).<sup>1339</sup> In this study the method was applied on a statistical basis to provide general insight, but the same method could be employed to create an AD damage map (analogous to the “AD vs. Control” images at upper right in **Figure 21**) to help prepare a preliminary brain repair plan for a specific individual AD patient.

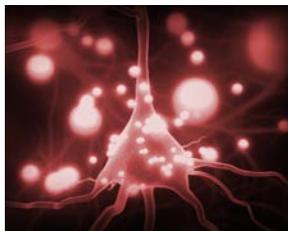
*Figure 21. Left images: Significant atrophy rates for the right and left hippocampi in patients with AD, MCI, and controls. Right images: Statistical “delta” maps showing location and severity of hippocampal damage between compared groups. (Frankó et al, 2013)*



Of course, present-day commercially-available MRI has only limited imaging resolution, typically, e.g., ~1000  $\mu\text{m}$  voxels, as in the Franko study. While not as commonly available, somewhat higher MRI spatial resolutions have been reported in the research literature using

<sup>1339</sup> Frankó E, Joly O; Alzheimer’s Disease Neuroimaging Initiative. Evaluating Alzheimer’s disease progression using rate of regional hippocampal atrophy. PLoS One. 2013 Aug 12;8(8):e71354; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3741167/>.

ultra-high field (7 Tesla) whole-body MRI and specialized techniques – e.g., **600  $\mu\text{m}$**  in schizophrenia patients,<sup>1340</sup> **500  $\mu\text{m}$** <sup>1341</sup> and **195-286  $\mu\text{m}$** <sup>1342</sup> in MS patients, and **400  $\mu\text{m}$** <sup>1343</sup> and **120  $\mu\text{m}$** <sup>1344</sup> spatial resolution (the best achieved so far) for normal live human subjects.



Micro-CT scanners allow non-destructive high-resolution tomography with spatial resolution of  $\sim 2 \mu\text{m}$ , and state-of-the-art commercially-available nano-CT scanners offer the highest spatial resolutions of **50-500 nm**. For instance, the Phoenix Nanotom M<sup>1345</sup> and the SkyScan-2211<sup>1346</sup> provide **200-400 nm** voxels for specimens the size of a human brain, and the Xradia nanoXCT<sup>1347</sup> claims **50-300 nm** resolution – but unlike MRI or medical nanorobots, none of these methods can be used for *in vivo* scanning of the brain of a living human AD patient. STED

<sup>1340</sup> Palaniyappan L, Mahmood J, Balain V, Mougou O, Gowland PA, Liddle PF. Structural correlates of formal thought disorder in schizophrenia: An ultra-high field multivariate morphometry study. *Schizophr Res*. 2015 Oct;168(1-2):305-12; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4604249/>.

<sup>1341</sup> Harrison DM, Roy S, Oh J, Izbudak I, Pham D, Courtney S, Caffo B, Jones CK, van Zijl P, Calabresi PA. Association of Cortical Lesion Burden on 7-T Magnetic Resonance Imaging With Cognition and Disability in Multiple Sclerosis. *JAMA Neurol*. 2015 Sep;72(9):1004-12; <http://www.ncbi.nlm.nih.gov/pubmed/26192316>.

<sup>1342</sup> Metcalf M, Xu D, Okuda DT, Carvajal L, Srinivasan R, Kelley DA, Mukherjee P, Nelson SJ, Vigneron DB, Pelletier D. High-resolution phased-array MRI of the human brain at 7 tesla: initial experience in multiple sclerosis patients. *J Neuroimaging*. 2010 Apr;20(2):141-7; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2874605/>.

<sup>1343</sup> Dinse J, Härtwich N, Waehnert MD, Tardif CL, Schäfer A, Geyer S, Preim B, Turner R, Bazin PL. A cytoarchitecture-driven myelin model reveals area-specific signatures in human primary and secondary areas using ultra-high resolution *in-vivo* brain MRI. *Neuroimage*. 2015 Jul 1;114:71-87; <http://www.sciencedirect.com/science/article/pii/S1053811915003134>.

<sup>1344</sup> Stucht D, Danishad KA, Schulze P, Godenschweger F, Zaitsev M, Speck O. Highest Resolution *In Vivo* Human Brain MRI Using Prospective Motion Correction. *PLoS One*. 2015 Jul 30;10(7):e0133921; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4520483/>.

<sup>1345</sup> “Phoenix Nanoatom M,” General Electric Measurement & Control, 2016; <http://www.ge-mcs.com/en/radiography-x-ray/ct-computed-tomography/nanotom-s.html>.

<sup>1346</sup> “X-ray Nanotomograph, Multiscale X-ray nano-CT System,” Bruker Corporation 2016; <https://www.bruker.com/products/microtomography/micro-ct-for-sample-scanning/skyscan-2211/overview.html>.

<sup>1347</sup> Tkachuk A, Duewer F, Hongtao C, Feser M, Wang S, Wenbing Y. X-ray computed tomography in Zernike phase contrast mode at 8 keV with 50 nm resolution using Cu rotation anode X-ray source. *Zeitschrift für Kristallographie*. 2007; 222(11): 650-655; [http://xrm.phys.northwestern.edu/research/pdf\\_papers/2007/tkachuk\\_zk\\_2007.pdf](http://xrm.phys.northwestern.edu/research/pdf_papers/2007/tkachuk_zk_2007.pdf).

fluorescence nanoscopy has produced 70-250 nm resolution scans of the cerebral cortex of a live gene-altered mouse whose neurons are tagged with fluorescent proteins,<sup>1348</sup> but this technique would also not be generally applicable to whole-brain human AD patients.

There are other high-resolution destructive techniques that cannot be used in living patients because the brain is destroyed in the process. For example, a structural 3D whole-brain map to **20  $\mu\text{m}$**  resolution has been completed, creating the first human whole-brain cytoarchitectural map using data obtained from physical brain slices.<sup>1349</sup> A  $\sim 1 \mu\text{m}$  spatial resolution brain model to capture details of single cell morphology (**Figure 22**) is planned for the future, and a few efforts are underway to destructively map the whole human brain at the synaptic level of resolution in the decades ahead.<sup>1350</sup> Ultramicrotome brain sections 30-100 nm thick can already be scanned by transmission electron microscopy, or by serial block-face scanning electron microscopy, or by optical microscopy, with automation of the collection of ultrathin serial sections for large volume transmission electron microscope reconstructions.<sup>1351</sup> FIB-SEM brain tissue scans to below **10 nm** resolution have been demonstrated,<sup>1352</sup> some with pixel size as small as **4 nm**,<sup>1353</sup> and similar

---

<sup>1348</sup> Berning S, Willig KI, Steffens H, Dibaj P, Hell SW. Nanoscopy in a living mouse brain. *Science*. 2012 Feb 3;335(6068):551; <http://classic.sciencemag.org/content/335/6068/551.long>.

<sup>1349</sup> Amunts K, Lepage C, Borgeat L, Mohlberg H, Dickscheid T, Rousseau M $\acute{E}$ , Bludau S, Bazin PL, Lewis LB, Oros-Peusquens AM, Shah NJ, Lippert T, Zilles K, Evans AC. BigBrain: an ultrahigh-resolution 3D human brain model. *Science*. 2013 Jun 21;340(6139):1472-5; <http://www.sciencemag.org/content/340/6139/1472.long>.

<sup>1350</sup> Collman F, Buchanan J, Phend KD, Micheva KD, Weinberg RJ, Smith SJ. Mapping synapses by conjugate light-electron array tomography. *J Neurosci*. 2015 Apr 8;35(14):5792-807; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4388933/>. Open Connectome Project; <http://www.openconnectomeproject.org>.

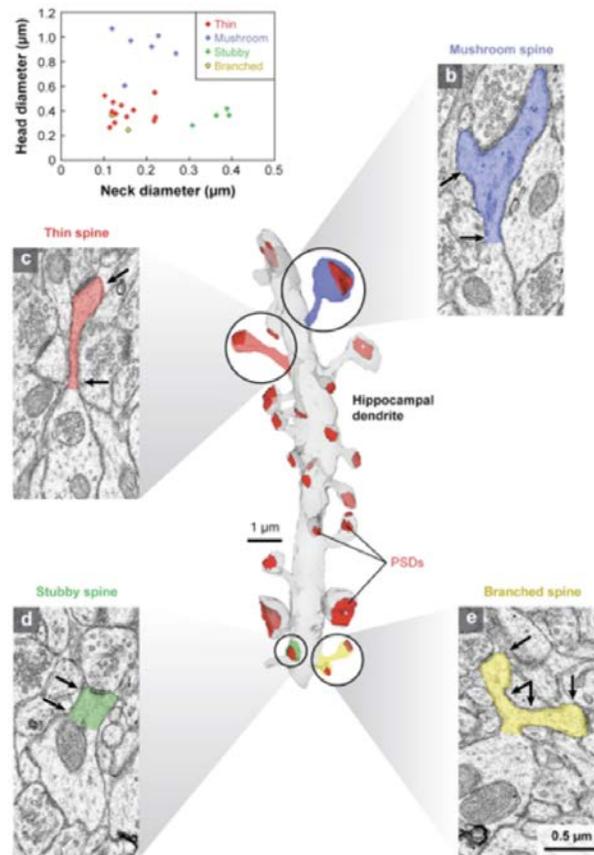
<sup>1351</sup> Hayworth KJ, Kasthuri N, Schalek R, Lichtman JW. Automating the collection of ultrathin serial sections for large volume TEM reconstructions. *Microsc Microanal*. 2006; 12 (Suppl 2): 86-7; <http://homes.mpimf-heidelberg.mpg.de/~mhelmsta/pdf/2006%20Hayworth%20Kasthuri%20Schalek%20Lichtman%20Microsc%20Microanal.pdf>. Anderson JM, Van Itallie CM. Physiology and function of the tight junction. *Cold Spring Harb Perspect Biol*. 2009 Aug;1(2):a002584; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2742087/>. Hayworth KJ. Electron imaging technology for whole brain neural circuit mapping, *Int J Machine Consciousness*, 2012; 4(1):87-108; [http://web.archive.org/web/20150428052029/http://www.brainpreservation.org/sites/default/files/ElectronImagingTechnologyForWholeBrainNeuralCircuitMapping\\_Hayworth2012.pdf](http://web.archive.org/web/20150428052029/http://www.brainpreservation.org/sites/default/files/ElectronImagingTechnologyForWholeBrainNeuralCircuitMapping_Hayworth2012.pdf).

<sup>1352</sup> Hayworth KJ, Xu CS, Lu Z, Knott GW, Fetter RD, Tapia JC, Lichtman JW, Hess HF. Ultrastructurally smooth thick partitioning and volume stitching for large-scale connectomics. *Nat Methods*. 2015 Apr;12(4):319-22; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4382383/>.

<sup>1353</sup> Knott G, Rosset S, Cantoni M. Focussed ion beam milling and scanning electron microscopy of brain tissue. *J Vis Exp*. 2011 Jul 6;(53):e2588; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3196160/>.

resolution has been demonstrated using SEM/TEM techniques.<sup>1354</sup> At least one possible future path to achieve a whole human brain scan to the nanoscale resolution using conventional scanning technologies has been proposed.<sup>1355</sup> But all of these methods destroy the brain and thus could not be used on living AD patients who prefer their original physical brain to be repaired.

Figure 22. 3D reconstruction of rat hippocampal dendrite with synapses located at post-synaptic densities or PSDs (Bourne and Harris 2008).<sup>1356</sup>



<sup>1354</sup> Briggman KL, Bock DD. Volume electron microscopy for neuronal circuit reconstruction. *Curr Opin Neurobiol.* 2012 Feb;22(1):154-61; [http://web.stanford.edu/class/cs379c/calendar\\_invited\\_talks/articles/BriggmanandBockCON-12.pdf](http://web.stanford.edu/class/cs379c/calendar_invited_talks/articles/BriggmanandBockCON-12.pdf).

<sup>1355</sup> Hayworth KJ. Electron imaging technology for whole brain neural circuit mapping, *Int J Machine Consciousness*, 2012; 4(1):87-108; [http://web.archive.org/web/20150428052029/http://www.brainpreservation.org/sites/default/files/ElectronImagingTechnologyForWholeBrainNeuralCircuitMapping\\_Hayworth2012.pdf](http://web.archive.org/web/20150428052029/http://www.brainpreservation.org/sites/default/files/ElectronImagingTechnologyForWholeBrainNeuralCircuitMapping_Hayworth2012.pdf).

<sup>1356</sup> Bourne JN, Harris KM. Balancing structure and function at hippocampal dendritic spines. *Annu Rev Neurosci.* 2008;31:47-67; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2561948/>.

The current *in vivo* MRI radiological protocols for measuring, scoring, and diagnosing neurodegenerative conditions at the millimeter size scale of tissues,<sup>1357</sup> and even so-called “molecular imaging” in which the location of tagged biomolecules are reported at a similar size scale,<sup>1358</sup> will be conveniently extended to measurements at the micron size scale (individual cells) and beyond, via nanorobotics. Here’s the technical challenge, as described by one neuroscientist:<sup>1359</sup>

A typical cortical pyramidal neuron may have a cell body that is only 10 microns in width but will send out an incredibly elaborate dendritic arbor spanning a cubic millimeter in volume. The finest branches of this dendritic arbor are thousands of dendritic spines whose tiny necks can shrink down to just a few tens of nanometers in diameter. This neuron will also send out a long thin axon that in places will shrink down to just a few tens of nanometers in diameter, but will travel many millimeters before branching and arborizing to form synaptic contacts with distant neurons. Any random cubic millimeter of cortical tissue will contain tens of thousands of neurons with overlapping dendritic arbors and will receive tens of thousands of axonal projections whose arbors also overlap. And within this cubic millimeter of densely tangled jungle will be on the order of a few hundred million synaptic connections each with a tiny area of contact on the order of 500 nm in diameter.<sup>1360</sup>

One recent 3D electron microscopy scan of a 6  $\mu\text{m}$  x 6  $\mu\text{m}$  x 5  $\mu\text{m}$  block of hippocampal area CA1 in rats identified 449 synapses, 446 axons, 149 dendrites, 287 spines, and 236 spines and presynaptic boutons that were fully contained within the volume.<sup>1361</sup>

Ideally it would be nice to have a good map of the patient’s original undamaged connectome,<sup>1362</sup> including all the synaptic connections between neurons, and at least one theoretical proposal for

<sup>1357</sup> Hacking C, Gaillard F. Neurodegenerative MRI brain (an approach), Radiopaedia.org, 2016; <http://radiopaedia.org/articles/neurodegenerative-mri-brain-an-approach>.

<sup>1358</sup> “Fact Sheet: Molecular Imaging and Alzheimer’s Disease,” Society of Nuclear Medicine and Molecular Imaging, 2016; <http://www.snmnm.org/AboutSNMMI/Content.aspx?ItemNumber=5651>.

<sup>1359</sup> Hayworth KJ. Electron imaging technology for whole brain neural circuit mapping, *Int J Machine Consciousness*, 2012; 4(1):87-108; [http://web.archive.org/web/20150428052029/http://www.brainpreservation.org/sites/default/files/ElectronImagingTechnologyForWholeBrainNeuralCircuitMapping\\_Hayworth2012.pdf](http://web.archive.org/web/20150428052029/http://www.brainpreservation.org/sites/default/files/ElectronImagingTechnologyForWholeBrainNeuralCircuitMapping_Hayworth2012.pdf).

<sup>1360</sup> Shepherd, GM. *The Synaptic Organization of the Brain*, 3rd edition, Oxford University Press, New York, 1990.

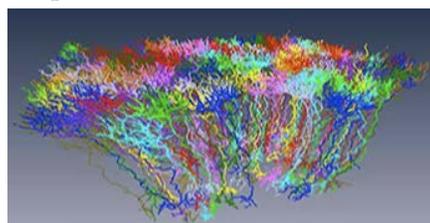
<sup>1361</sup> Bartol TM, Bromer C, Kinney J, Chirillo MA, Bourne JN, Harris KM, Sejnowski TJ. Nanoconnectomic upper bound on the variability of synaptic plasticity. *Elife*. 2015 Nov 30;4. pii: e10778; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4737657/>.

<sup>1362</sup> Bohland JW, *et al.* A proposal for a coordinated effort for the determination of brainwide neuroanatomical connectivity in model organisms at a mesoscopic scale. *PLoS Comput Biol*. 2009 Mar;5(3):e1000334; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2655718/>. Seung S. *Connectome: How the Brain’s Wiring Makes Us Who We Are*. Houghton Mifflin Harcourt, NY, 2012. Plaza SM, Scheffer LK, Chklovskii DB. Toward large-scale connectome reconstructions. *Curr Opin Neurobiol*. 2014 Apr;25:201-10; [https://www.researchgate.net/profile/Dmitri\\_Chklovskii/publication/260562044\\_Toward\\_large-](https://www.researchgate.net/profile/Dmitri_Chklovskii/publication/260562044_Toward_large-)

nondestructively obtaining a whole-brain synaptic map using medical nanorobots has been published.<sup>1363</sup> But much of the extensive arborization and synaptic interconnections within the large blocks of missing neurons in an AD brain will have been permanently destroyed, and many of the interconnections among the surviving neurons will be damaged or seriously degraded. This represents massive brain information loss, possibly unrecoverable even in principle.

Rather than trying to resurrect vast regions of a destroyed connectome using incomplete or missing information, the simplest approach for a satisfactory AD patient recovery is to employ medical nanorobots to create a coarse cytoarchitectural brain map with  $\sim 1 \mu\text{m}$  spatial resolution. This will not yield a detailed synapse-level connectome map because the human neocortex consists of densely packed intertwining neuronal axons, dendrites, and synapses with feature sizes in the 40-50 nm range or smaller. Rather, the procedure described herein will produce a spatial map of neuron body shapes and positions, plus the locations of major neuron processes (neurites), and possibly a crude synapse number density map with a coarse  $\sim 1 \mu\text{m}$  spatial uncertainty. This should be sufficient to (1) identify and diagnose surviving neurons needing *in situ* repair and (2) position newly arriving neural cells for induced growth into vacant spaces (Section 5.3.2) under the directed influence of intensive neural network retraining (Section 5.3.3).

Following this approach, the basic mission will involve targeting one survey nanorobot to every surviving neuron in the AD patient's brain. Each survey nanorobot will record in its onboard memory the spatial positions of the neuron cell body and major neurites, along with the locations and timing of any synaptic activity it detects. Afterwards the nanorobots are extracted from the patient. Their data is offloaded into an external computer and processed to create the desired cytoarchitectural spatial map of all surviving neurons in the AD patient's brain. Software tools have already been developed to automate the computational 3D reconstruction of neuron cell bodies and axons starting from conventional 2D scans, as illustrated by the digital extraction of 114 rod bipolar nerve cells from scans of a piece of mouse retina (image, right).<sup>1364</sup>




---

[scale\\_connectome\\_reconstructions/links/00b49531a2afb51667000000.pdf](http://www.humanconnectomeproject.org/links/00b49531a2afb51667000000.pdf). Human Connectome Project; <http://www.humanconnectomeproject.org/>. See also: Allen Brain Atlas; <http://www.brain-map.org/>.

<sup>1363</sup> Martins NRB, Erlhagen W, Freitas RA Jr. Human connectome mapping and monitoring using neuronanorobots. *J Evol Technol.* 2016 Jan;26:1-25; <http://jetpress.org/v26.1/martins.htm>. Martins NRB. Neuronanorobots: A Proposed Medical Technology to Preserve Human Brain Information. PhD Thesis, University of Minho, Feb 2016.

<sup>1364</sup> Helmstaedter M, Briggman KL, Denk W. High-accuracy neurite reconstruction for high-throughput neuroanatomy. *Nat Neurosci.* 2011 Jul 10;14(8):1081-8; [http://www.tara.tcd.ie/bitstream/handle/2262/61607/PEER\\_stage2\\_10.1038%252Fnn.2868.pdf](http://www.tara.tcd.ie/bitstream/handle/2262/61607/PEER_stage2_10.1038%252Fnn.2868.pdf). Helmstaedter M, Mitra PP. Computational methods and challenges for large-scale circuit mapping. *Curr Opin Neurobiol.* 2012 Feb;22(1):162-9; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3406305/>. Helmstaedter M. Cellular-resolution connectomics: challenges of dense neural circuit reconstruction. *Nat Methods.* 2013 Jun;10(6):501-7; [http://brain.mpg.de/fileadmin/user\\_upload/Documents/Papers/Papers\\_Helmstaedter/2013\\_NatMeth.pdf](http://brain.mpg.de/fileadmin/user_upload/Documents/Papers/Papers_Helmstaedter/2013_NatMeth.pdf). Berning M, Boergens KM, Helmstaedter M. SegEM: Efficient Image Analysis for High-Resolution Connectomics. *Neuron.* 2015 Sep 23;87(6):1193-206; <http://www.ncbi.nlm.nih.gov/pubmed/26402603>.

Software to automate the construction of a cytoarchitectural brain map can be developed to process data supplied from nanorobot brain scans. The computational problem is crudely analogous to the problem domain of jigsaw puzzles, which has already been applied to fields as diverse as biology,<sup>1365</sup> chemistry,<sup>1366</sup> literature,<sup>1367</sup> archeology,<sup>1368</sup> reconstruction of historical statues (e.g., a billion-polygon model),<sup>1369</sup> speech descrambling,<sup>1370</sup> cryptography,<sup>1371</sup> image editing,<sup>1372</sup> and the recovery of shredded documents or photographs.<sup>1373</sup> Numerous automated

<sup>1365</sup> Marande W, Burger G. Mitochondrial DNA as a genomic jigsaw puzzle. *Science*. 2007 Oct 19;318(5849):415; [http://3dinformatics.com/genome\\_in\\_pieces3.pdf](http://3dinformatics.com/genome_in_pieces3.pdf).

<sup>1366</sup> Wang C-SE. Determining molecular conformation from distance or density data . PhD thesis, Massachusetts Institute of Technology, Dept. of Electrical Engineering and Computer Science, 2000; [http://dspace.mit.edu/bitstream/handle/1721.1/16736/45147791.pdf?sequence=1&origin=publication\\_detail](http://dspace.mit.edu/bitstream/handle/1721.1/16736/45147791.pdf?sequence=1&origin=publication_detail)

<sup>1367</sup> Morton AQ, Levison M. The computer in literary studies. *IFIP Congress (2)*, 1968:1072-81.

<sup>1368</sup> Leitao HCG, Stolfi J. Automatic reassembly of irregular fragments. Tech. Report IC-98-06, Univ. of Campinas, 1998; <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.37.6671&rep=rep1&type=pdf>. Leitao HCG. Information Contents of Fracture Lines. Tech. Report, Univ. of Campinas, 1999; <https://dspace.zcu.cz/bitstream/handle/11025/15481/Leitao.pdf?sequence=1>. Levoy M. Digital Forma Urbis Romae Project, 2015; <http://www.graphics.stanford.edu/projects/forma-urbis>. Koller D, Levoy M. Computer-aided reconstruction and new matches in the forma urbis romae. *Bullettino Della Commissione Archeologica Comunale di Roma*, 2006:103-125; <http://graphics.stanford.edu/papers/forma-bullcom/forma-bullcom-text.pdf>. Brown B, Toler-Franklin C, Nehab D, Burns M, Dobkin D, Vlachopoulos A, Doumas C, Rusinkiewicz S, Weyrich T. A system for high-volume acquisition and matching of fresco fragments: Reassembling Theran wall paintings. *ACM Transactions on Graphics* 2008;27(3):84; <http://dl.acm.org/citation.cfm?id=1360683>.

<sup>1369</sup> Levoy M. The Digital Michelangelo Project, 2015; <http://www.graphics.stanford.edu/projects/mich/>.

<sup>1370</sup> Zhao Y, Su M, Chou Z, Lee J. A puzzle solver and its application in speech descrambling. *WSEAS Int. Conf. Comput Eng Appl*, 2007:171-176; <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.499.356&rep=rep1&type=pdf>.

<sup>1371</sup> Farn EJ, Chen CC. Novel steganographic method based on jig swap puzzle images. *J Electronic Imaging*. 2009 Jan-Mar;18(1):013003; <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.154.9766&rep=rep1&type=pdf>.

<sup>1372</sup> Cho TS, Butman M, Avidan S, Freeman WT. The patch transform and its applications to image editing. *IEEE Conference on Computer Vision and Pattern Recognition*, 2008:1-8; <http://people.csail.mit.edu/billf/papers/CVPRPatch.pdf>.

<sup>1373</sup> Justino E, Oliveira LS, Freitas C. Reconstructing shredded documents through feature matching. *Forensic Sci Int*. 2006 Jul 13;160(2-3):140-7; <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.90.2640&rep=rep1&type=pdf>. Zhu L, Zhou Z, Hu D. Globally consistent reconstruction of ripped-up documents. *IEEE Trans Pattern Anal Mach Intell*. 2008 Jan;30(1):1-13; <http://www.ncbi.nlm.nih.gov/pubmed/18000320>. Marques MAO, Freitas COA. Reconstructing strip-shredded documents using color as feature matching. *ACM Symp Appl Comput*. 2009:893-894. Cao S, Liu H, Yan S. Automated assembly of shredded pieces from multiple photos. *IEEE*

jigsaw puzzle assembly algorithms have been published,<sup>1374</sup> including one package that can assemble 10,000 pieces using software that is “fully automatic, requires no manually provided hints, operates on puzzles having thousands of pieces, and does so with near perfect performance.”<sup>1375</sup> More recently, another package that uses a genetic algorithm has solved a 22,834-piece puzzle in only 13.2 hr of runtime on a single modern PC and allegedly could solve “more difficult variations of the jigsaw problem, including unknown piece orientation, missing and excessive puzzle pieces, unknown puzzle dimensions, and **three-dimensional puzzles**.”<sup>1376</sup>

Construction of a cytoarchitectural brain map also has conceptual similarities to the reverse engineering of machines<sup>1377</sup> and to the growing field of computer-aided reverse engineering.<sup>1378</sup>

Int. Conf. Multimedia Expo 2010:358-363; <http://ieeexplore.ieee.org/xpl/articleDetails.jsp?tp=&arnumber=5582544>. Deever A, Gallagher A. Semi-automatic assembly of real cross-cut shredded documents. ICIIP 2012:233-236; <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.362.7366&rep=rep1&type=pdf>. Gallagher A. Jigsaw puzzles with pieces of unknown orientation. IEEE Conf Comput Vision Pattern Recog 2012:382-389; <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.405.927&rep=rep1&type=pdf>. DARPA Shredder Challenge 2011; [https://en.wikipedia.org/wiki/DARPA\\_Shredder\\_Challenge\\_2011](https://en.wikipedia.org/wiki/DARPA_Shredder_Challenge_2011).

<sup>1374</sup> Kosiba DA, Devaux PM, Balasubramanian S, Kasturi R. An automatic jigsaw puzzle solver. Proc. 12th Int. Conf. Pattern Recognition, Vol. 1, 1994:616-618; <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.344.1536&rep=rep1&type=pdf>. Goldberg D, Malon C, Bern M. A global approach to automatic solution of jigsaw puzzles. Proc 18th Ann Symp Comput Geom ACM, 2002 Jun 5;82-87; [http://www.parc.com/content/attachments/global\\_approach\\_automatic\\_4365\\_parc.pdf](http://www.parc.com/content/attachments/global_approach_automatic_4365_parc.pdf). Cho TS, Avidan S, Freeman WT. A probabilistic image jigsaw puzzle solver. Proc. 2010 IEEE CVPR, 2010; <http://people.csail.mit.edu/taegsang/Documents/JigsawSolver.pdf>. Pomeranz D, Shemesh M, Ben-Shahar O. A fully automated greedy square jigsaw puzzle solver. IEEE Conference on Computer Vision and Pattern Recognition, 2011:9-16; <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.298.3227&rep=rep1&type=pdf>. Paikin G, Tal A. Solving multiple square jigsaw puzzles with missing pieces. Proc. IEEE Conf Comput Vision Pattern Recog. 2015:4832-4839; <http://webee.technion.ac.il/~ayellet/Ps/15-PT.pdf>.

<sup>1375</sup> Gallagher A. Jigsaw puzzles with pieces of unknown orientation. IEEE Conf Comput Vision Pattern Recog 2012:382-389; <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.405.927&rep=rep1&type=pdf>. “Computational Jigsaw Puzzle Solving,” Interdisciplinary Computational Vision laboratory, 2012; [http://www.cs.bgu.ac.il/~icvl/icvl\\_projects/automatic-jigsaw-puzzle-solving/](http://www.cs.bgu.ac.il/~icvl/icvl_projects/automatic-jigsaw-puzzle-solving/).

<sup>1376</sup> Sholomon D, David O, Netanyahu NS. A Genetic Algorithm-Based Solver for Very Large Jigsaw Puzzles. 2013 IEEE Conf. Comp. Vision Pattern Recog. 2013 Jun:1767-1774; [http://www.cv-foundation.org/openaccess/content\\_cvpr\\_2013/papers/Sholomon\\_A\\_Genetic\\_Algorithm-Based\\_2013\\_CVPR\\_paper.pdf](http://www.cv-foundation.org/openaccess/content_cvpr_2013/papers/Sholomon_A_Genetic_Algorithm-Based_2013_CVPR_paper.pdf), <https://www.aaai.org/ocs/index.php/AAAI/AAAI14/paper/viewFile/8650/8638>, and <http://www.genetic-programming.org/hc2014/Sh-Paper.pdf>.

<sup>1377</sup> Varady T, Martin R, Cox J. Reverse engineering of geometric models—an introduction. Computer-Aided Design 1997;29(4):255–268; <http://ralph.cs.cf.ac.uk/papers/Geometry/RE.pdf>. “The Rise of Reverse Engineering,” ASME, Feb 2012; <https://www.asme.org/engineering-topics/articles/modeling-computational-methods/the-rise-of-reverse-engineering>. Kumar A, Jain PK, Pathak PM. Reverse

A whole-brain cellular-level wiring diagram for the mouse brain has been published,<sup>1379</sup> and algorithms for automated neuronal arbor analysis<sup>1380</sup> and neuron classification<sup>1381</sup> are available, including a gamified version called EyeWire that elicits help from human volunteers in tracing neuron connectivity.<sup>1382</sup> A statistical inference technique called “maximum likelihood estimation”,<sup>1383</sup> might also be used to estimate the position and composition of original brain structures, based on the position and composition of surviving partial brain structures.<sup>1384</sup>

An initial quantitative scaling analysis of the nanorobotic data collection mission suggests the following parameters:

**Whole-Brain Scanning Time.** The normal adult male human brain has up to  $86.1 \times 10^9$  neurons<sup>1385</sup> and  $2.4 \times 10^{14}$  synapses,<sup>1386</sup> or ~2800 synapses per neuron. An  $\sim 8000 \mu\text{m}^3$  neuron with a roughly cubic shape would have a surface area of  $\sim 2400 \mu\text{m}^2/\text{neuron}$ , but  $\sim 24,000$

engineering in product manufacturing: an overview. DAAAM International Scientific Book 2013, pp. 665-678; [http://www.daaam.info/Downloads/Pdfs/science\\_books\\_pdfs/2013/Sc\\_Book\\_2013-039.pdf](http://www.daaam.info/Downloads/Pdfs/science_books_pdfs/2013/Sc_Book_2013-039.pdf).

<sup>1378</sup> Sarfraz M. Computer-aided reverse engineering using evolutionary heuristics on NURBS. Chapter 14 in: Interactive Curve Modeling, Springer, 2008:297-330; [http://link.springer.com/chapter/10.1007%2F978-1-84628-871-5\\_14#page-1](http://link.springer.com/chapter/10.1007%2F978-1-84628-871-5_14#page-1). Oancea G, Ivan NV, Pescaru R. Computer aided reverse engineering system used for customized products. Acad J Manuf Eng 2013;11(4):p30-35; [http://www.auif.utcluj.ro/images/v11\\_4/6-Oancea%20Gh.pdf](http://www.auif.utcluj.ro/images/v11_4/6-Oancea%20Gh.pdf).

<sup>1379</sup> Oh SW, *et al.* A mesoscale connectome of the mouse brain. Nature. 2014 Apr 10;508(7495):207-14; <http://neuro-s.co.jp/images/product/5-151/5-1515.pdf>.

<sup>1380</sup> Sümbül U, Zlateski A, Vishwanathan A, Masland RH, Seung HS. Automated computation of arbor densities: a step toward identifying neuronal cell types. Front Neuroanat. 2014 Nov 25;8:139; <http://journal.frontiersin.org/article/10.3389/fnana.2014.00139/full>.

<sup>1381</sup> Sümbül U, Song S, McCulloch K, Becker M, Lin B, Sanes JR, Masland RH, Seung HS. A genetic and computational approach to structurally classify neuronal types. Nat Commun. 2014 Mar 24;5:3512; <http://www.nature.com/ncomms/2014/140324/ncomms4512/pdf/ncomms4512.pdf>.

<sup>1382</sup> <https://en.wikipedia.org/wiki/EyeWire>.

<sup>1383</sup> [https://en.wikipedia.org/wiki/Maximum\\_likelihood](https://en.wikipedia.org/wiki/Maximum_likelihood); <http://mathworld.wolfram.com/MaximumLikelihood.html>.

<sup>1384</sup> Merkle RC. Cryonics, Cryptography, and Maximum Likelihood Estimation. Proc 1st Extropy Inst Conf 1994; <http://www.merkle.com/cryo/cryptoCryo.html>.

<sup>1385</sup> Azevedo FA, Carvalho LR, Grinberg LT, Farfel JM, Ferretti RE, Leite RE, Jacob Filho W, Lent R, Herculano-Houzel S. Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. J Comp Neurol. 2009 Apr 10;513(5):532-41; <http://www.sakkyndig.com/psykologi/artvit/frederico2009.pdf>.

<sup>1386</sup> Martins NRB, Erlhagen W, Freitas RA Jr. Non-destructive whole-brain monitoring using nanorobots: Neural electrical data rate requirements. Intl J Machine Consciousness 2012 Jun; 4:109-140; <http://www.nanomedicine.com/Papers/NanoroboticBrainMonitoring2012.pdf>.

$\mu\text{m}^2/\text{neuron}$  seems more typical for the surface area of a neuronal dendritic tree,<sup>1387</sup> giving a mean separation of synapses on the neuron surface of  $\sim[(24,000 \mu\text{m}^2/\text{neuron}) / (2800 \text{ synapses}/\text{neuron})]^{1/2} = 2.9 \mu\text{m}/\text{synapse}$ . Microglial cells, the immune system phagocytes in the brain, have been observed extending and retracting their filopodia-like processes at  $1\text{-}4 \mu\text{m}/\text{min}$  ( $0.02\text{-}0.07 \mu\text{m}/\text{sec}$ ),<sup>1388</sup> but fast axonal transport has been observed at  $3\text{-}5 \mu\text{m}/\text{sec}$ .<sup>1389</sup> Assuming a  $\sim 1 \mu\text{m}/\text{sec}$  synapse seek speed by the nanorobots, each new synapse requires  $(2.9 \mu\text{m}/\text{synapse}) / (1 \mu\text{m}/\text{sec}) = 2.9 \text{ sec}$  to find. If each nanorobot lingers near each synapse for  $\sim 3 \text{ sec}$  (probably long enough to ensure at least one or a few detectable electrical firings),<sup>1390</sup> then each synapse requires  $\sim 6 \text{ sec}$  to find and monitor for activity, requiring  $(6 \text{ sec}/\text{synapse}) (2800 \text{ synapses}/\text{neuron}) = 16,800 \text{ sec}/\text{neuron}$ . A  $1 \mu\text{m}$  contact sensor moving with the nanorobot can contact (and map) the entire neuron surface in  $(24,000 \mu\text{m}^2/\text{neuron}) / [(1 \mu\text{m}) (1 \mu\text{m}/\text{sec})] = 24,000 \text{ sec}/\text{neuron}$ , for a total scanning time of  $40,800 \text{ sec}$  (**11.3 hr**). If we allow another hour for nanorobot entry to and exit from its target neuron, plus an hour for nanorobot entry to and exit from the patient's brain, then the total *in vivo* scan time required to collect the brain map data is **13.3 hr**.

**Nanorobot Onboard Data Storage.** Recording spatial XYZ coordinates to  $\sim 1 \mu\text{m}$  resolution across a  $\sim 10 \text{ cm}$  brain dimension requires a measurement precision of 1 part per 100,000, or  $\log(100,000)/\log(2) = 17 \text{ bits}/\text{dimension}$  or 51 bits per XYZ record at the required precision, or  $(24,000 \mu\text{m}^2/\text{neuron}) (51 \text{ bits}/\text{record}) / (1 \mu\text{m}^2/\text{record}) = \underline{1.22 \times 10^6 \text{ bits}/\text{neuron}}$  for the complete neuron surface map. Recording the spatial positions of up to 2800 synapses requires another  $(2800 \text{ synapses}/\text{neuron}) (1 \text{ record}/\text{synapse}) (51 \text{ bits}/\text{record}) = \underline{0.14 \times 10^6 \text{ bits}/\text{neuron}}$  at  $1 \mu\text{m}$  resolution. Recording time-stamped synaptic firing events may also have some diagnostic value in assessing neuron health. A 10 millisecond time resolution during each 3 sec/synapse scan period requires a  $\log(3 \text{ sec}/0.010 \text{ sec})/\log(2) = 8 \text{ bit}$  time stamp for each record. If each synapse can generate up to 100 spikes/sec, then the additional data requirement for this feature is  $(10 \text{ sec}/\text{synapse}) (2800 \text{ synapses}/\text{neuron}) (100 \text{ spikes}/\text{sec}) (1 \text{ record}/\text{spike}) (8 \text{ bits}/\text{record}) = \underline{22.4 \times 10^6 \text{ bits}/\text{neuron}}$ . This raises the onboard data storage requirement to at least  $23.76 \times 10^6 \text{ bits}$ . Allowing **30 megabits/nanorobot** is well within the design limits of the microbivore (50

---

<sup>1387</sup> Donohue DE, Ascoli GA. A comparative computer simulation of dendritic morphology. PLoS Comput Biol. 2008 Jun 6;4(5):e1000089; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2376061/>.

<sup>1388</sup> Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. Science. 2005 May 27;308(5726):1314-8; <http://www.sciencemag.org/content/308/5726/1314.long>.

<sup>1389</sup> Perrot R, Berges R, Bocquet A, Eyer J. Review of the multiple aspects of neurofilament functions, and their possible contribution to neurodegeneration. Mol Neurobiol. 2008 Aug;38(1):27-65; <http://www.ncbi.nlm.nih.gov/pubmed/18649148>.

<sup>1390</sup> Martins NRB, Erlhagen W, Freitas RA Jr. Action Potential Monitoring Using Neuronanorobots: Neuroelectric Nanosensors. Intl J Nanomater Nanostructures 2015 Jun; 1:20-41; <http://materials.journalspub.info/index.php/IJNN/article/view/107>.

megabits/nanorobot of mass memory)<sup>1391</sup> and the chromalloyocyte (500 megabits/nanorobot of mass memory).<sup>1392</sup>

**Power Consumption.** If each nanorobot has a continuous 200 pW/nanorobot power budget similar to the chromalloyocyte,<sup>1393</sup> then the maximum power demand by the entire nanorobot mapping fleet is  $(86 \times 10^9 \text{ neurons}) (1 \text{ nanorobot/neuron}) (200 \text{ pW/nanorobot}) = \mathbf{17 \text{ watts}}$ , slightly less than the normal ~20 watt power demand of the brain and well within safe thermogenic operating limits.<sup>1394</sup>

**Communications and Navigation.** With  $86 \times 10^9$  nanorobots simultaneously present in a  $1510 \text{ cm}^3$  adult male brain, the average distance between neighboring nanorobots is  $[(1510 \text{ cm}^3/\text{brain}) / (86 \times 10^9 \text{ nanorobots/brain})]^{1/3} = 26 \text{ }\mu\text{m}$  and on average there will be  $[(100 \text{ }\mu\text{m}) / (26 \text{ }\mu\text{m})]^3 = 57$  neighboring robots within a 100  $\mu\text{m}$  radius, the customary maximum range for convenient *in vivo* robot-to-robot acoustic communication/navigation inside biological tissue.<sup>1395</sup>



**Nanorobot Intrusiveness.** If each of the  $86 \times 10^9$  nanorobots infused into a human brain is roughly cubical with a volume of  $10 \text{ }\mu\text{m}^3/\text{nanorobot}$  and an edge length of  $(10 \text{ }\mu\text{m}^3)^{1/3} = 2.15 \text{ }\mu\text{m}$ , then the total nanorobot mapping fleet volume is  $(86 \times 10^9 \text{ nanorobots}) (10 \text{ }\mu\text{m}^3/\text{nanorobot}) = 0.86 \text{ cm}^3$ , which represents only  $(0.86 \text{ cm}^3) / (1510 \text{ cm}^3) = 0.06\%$  of total brain volume – well within the 1%-2% safety limits for brain intrusiveness.<sup>1396</sup>

<sup>1391</sup> Freitas RA Jr. Microbivores: Artificial mechanical phagocytes using digest and discharge protocol. J Evol Technol 2005;14:1-52; <http://www.jetpress.org/volume14/freitas.html>.

<sup>1392</sup> Freitas RA Jr. The ideal gene delivery vector: Chromalloyocytes, cell repair nanorobots for chromosome replacement therapy. J Evol Technol 2007;16:1-97; <http://jetpress.org/v16/freitas.pdf>.

<sup>1393</sup> Freitas RA Jr. The ideal gene delivery vector: Chromalloyocytes, cell repair nanorobots for chromosome replacement therapy. J Evol Technol 2007;16:1-97; <http://jetpress.org/v16/freitas.pdf>.

<sup>1394</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 6.4.1 “Acoustic Power Transmission”, <http://www.nanomedicine.com/NMI/6.4.1.htm>, and Section 6.5.2 “Thermogenic Limits *in vivo*”, <http://www.nanomedicine.com/NMI/6.5.2.htm>.

<sup>1395</sup> Freitas RA Jr. Nanomedicine, Volume I: Basic Capabilities. Landes Bioscience, Georgetown, TX, 1999, Section 7.2.2.2 “Free-Tissue Acoustic Channel Capacity”, <http://www.nanomedicine.com/NMI/7.2.2.2.htm>, and Section 7.3.2 “Mobile Networks”, <http://www.nanomedicine.com/NMI/7.2.2.2.htm>; Hogg T, Freitas RA Jr. Acoustic communication for medical nanorobots. Nano Commun Networks 2012 Jun; 3:83-102; [https://www.researchgate.net/profile/Tad\\_Hogg/publication/221661618\\_Acoustic\\_Communication\\_for\\_Medical\\_Nanorobots/links/0046351757f6312846000000.pdf](https://www.researchgate.net/profile/Tad_Hogg/publication/221661618_Acoustic_Communication_for_Medical_Nanorobots/links/0046351757f6312846000000.pdf).

<sup>1396</sup> Freitas RA Jr. Nanomedicine, Volume IIA: Biocompatibility. Landes Bioscience, Georgetown, TX, 2003, Section 15.6.1 “Somatic Intrusiveness”; <http://www.nanomedicine.com/NMIIA/15.6.1.htm>.

**Map Stability.** How stable is the cytoarchitectural brain map? If normal brains lose ~1 neuron/sec due to aging and AD brains lose ~10 neurons/sec during the progression of the disease, then during the entire ~13.3 hr scan period the AD patient will lose ~500,000 neurons, only 0.0006% of the total, implying a reasonably high map stability during the nanorobotic scanning and treatment period. Neurogenesis is also taking place at a much slower normal pace, with perhaps thousands of new neurons added to the adult brain every day (i.e., ~0.02 neuron/sec),<sup>1397</sup> with up to ~1000 new neurons added during the scan period.

After the 13.3-hr nanorobotic survey scan described above, followed by the external computer reconstruction of the current cytoarchitectural brain map, examination of the map will reveal that significant portions of the brain are lightly synapsified, damaged, or even missing. It is then time to prepare the “delta” file that will guide the brain repair process. For this purpose, a smaller group of nanorobots can be infused into the AD patient’s brain and directed to more closely examine the damaged areas, seeking clues as to what might have been there before the damage occurred.

For example, in AD brains many neurons with tau tangles degenerate, eventually leaving “tombstones” or “**ghost tangles**” behind in the extracellular space where the host neuron has died,<sup>1398</sup> usually within 3-5 years after the neuron becomes affected by neurofibrillary pathology.<sup>1399</sup> These ghost tangles,<sup>1400</sup> aka. extracellular neurofibrillary tangles (eNFTs), appear not to be degraded by tissue proteases along with the rest of the neuron body, but are abnormally phosphorylated and may have been “extensively processed and irreversibly transformed into

---

<sup>1397</sup> Gross CG. Neurogenesis in the adult brain: death of a dogma. *Nat Rev Neurosci.* 2000 Oct;1(1):67-73; [https://www.princeton.edu/~cggross/NatRevNeuro\\_1\\_67\\_2000.pdf](https://www.princeton.edu/~cggross/NatRevNeuro_1_67_2000.pdf).

<sup>1398</sup> Braak E, Braak H, Mandelkow EM. A sequence of cytoskeleton changes related to the formation of neurofibrillary tangles and neuropil threads. *Acta Neuropathol.* 1994;87(6):554-67; <https://www.ncbi.nlm.nih.gov/pubmed/7522386>. Köpke E, Tung YC, Shaikh S, Alonso AC, Iqbal K, Grundke-Iqbal I. Microtubule-associated protein tau. Abnormal phosphorylation of a non-paired helical filament pool in Alzheimer disease. *J Biol Chem.* 1993 Nov 15;268(32):24374-84; <http://www.jbc.org/content/268/32/24374.long>. Kobayashi K, Muramori F, Aoki T, Hayashi M, Miyazu K, Fukutani Y, Mukai M, Koshino F. KP-1 is a marker for extraneuronal neurofibrillary tangles and senile plaques in Alzheimer diseased brains. *Dement Geriatr Cogn Disord.* 1998 Jan-Feb;9(1):13-9; <https://www.ncbi.nlm.nih.gov/pubmed/9469259>.

<sup>1399</sup> Bobinski M, Wegiel J, Tarnawski M, de Leon MJ, Reisberg B, Miller DC, Wisniewski HM. Duration of neurofibrillary changes in the hippocampal pyramidal neurons. *Brain Res.* 1998 Jul 13;799(1):156-8; <https://www.ncbi.nlm.nih.gov/pubmed/9666111>.

<sup>1400</sup> “Initially, the abnormal tau protein is distributed diffusely throughout the cell body and the neuronal processes. Subsequently, it aggregates to form a neurofibrillary tangle, which appears as a spherical somatic inclusion. The cell processes gradually become fragmented. Finally the parent cell dies, leaving behind an extraneuronal ‘**ghost tangle**’.” Sassin I, Schultz C, Thal DR, Rüb U, Arai K, Braak E, Braak H. Evolution of Alzheimer’s disease-related cytoskeletal changes in the basal nucleus of Meynert. *Acta Neuropathol.* 2000 Sep;100(3):259-69; <https://www.ncbi.nlm.nih.gov/pubmed/10965795>.

highly insoluble extracellular deposits.”<sup>1401</sup> The presence of these ghosts may indicate where missing neurons used to reside (as there is no evidence of neuron phagocytosis near eNFTs),<sup>1402</sup> providing useful data that can inform our creation of the AD patient’s notional “pre-AD” brain map. By the late or “isocortical” stage of AD, even the ghost tangles have been degraded and replaced by **glial cell accumulations**,<sup>1403</sup> another informative landmark indicating the past presence of a neuron. Similarly, it is believed that nerve terminal destruction occurs in the immediate vicinity of **beta-amyloid deposits**.<sup>1404</sup> Carefully recording the positions of all beta-amyloid deposits prior to their removal (Section 5.2.1.1) may provide additional clues as to which neural structures have gone missing, and where.

After augmenting the scan map with the aforementioned indirect evidence pertaining to missing neurons, the AD patient’s notional pre-AD brain map is then completed by digitally deleting spurious glial cell accumulations, ghost tangles and other biomaterials occupying desired neuron spaces and adding the inferential positions and cell types of the missing neural “cortical columns.” There are about 0.5 million cortical columns or “hypercolumns” in the human brain, each about 500 µm wide and 2000 µm tall, containing ~60,000 neurons; there are 100-1000 million “minicolumns” in the neocortex with 80-100 neurons each (**Figure 23**).<sup>1405</sup>

---

<sup>1401</sup> Endoh R, Ogawara M, Iwatsubo T, Nakano I, Mori H. Lack of the carboxyl terminal sequence of tau in ghost tangles of Alzheimer’s disease. *Brain Res.* 1993 Jan 22;601(1-2):164-72; <http://www.sciencedirect.com/science/article/pii/000689939391707Y>.

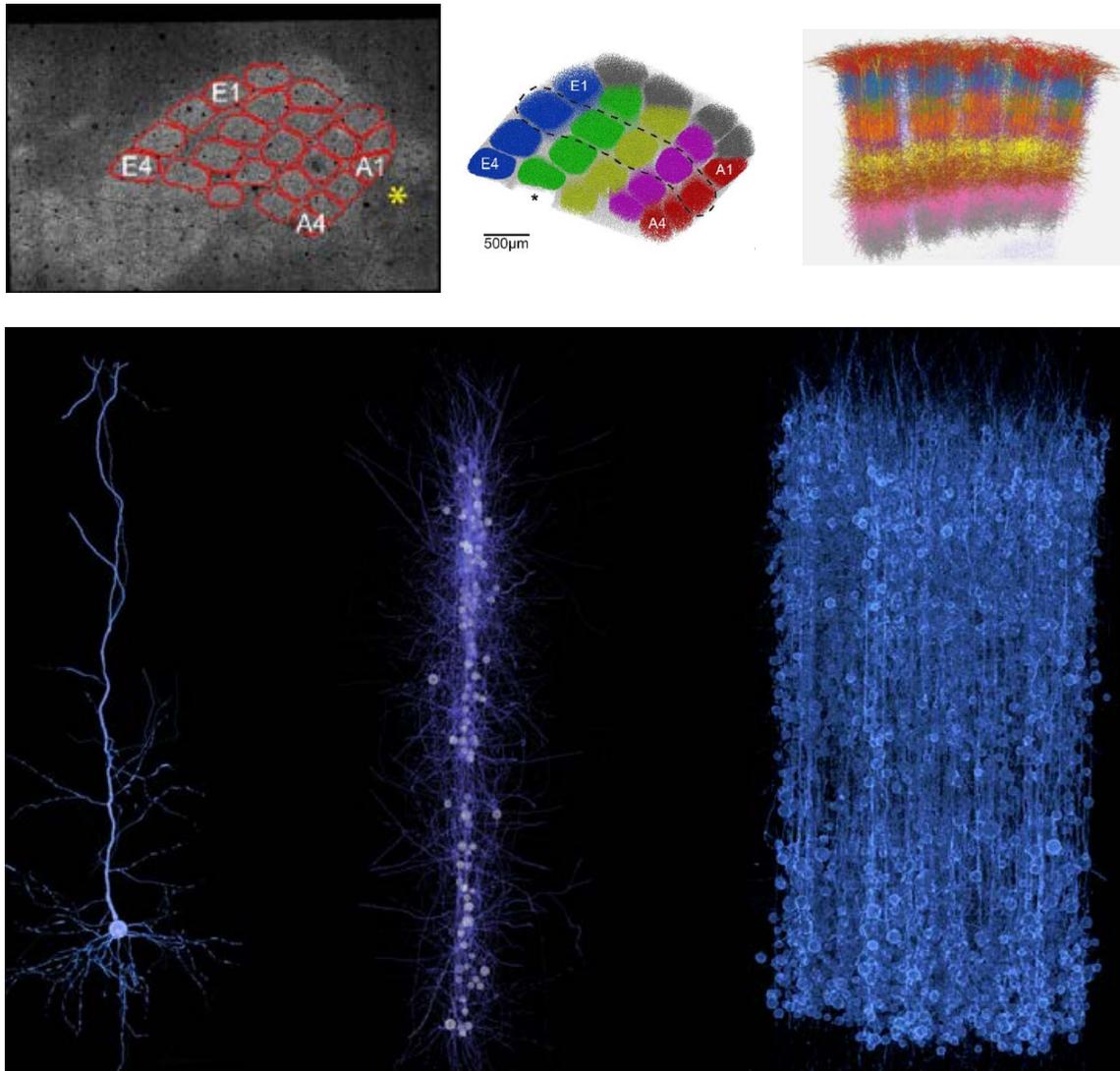
<sup>1402</sup> Schwab C, Steele JC, McGeer PL. Pyramidal neuron loss is matched by ghost tangle increase in Guam parkinsonism-dementia hippocampus. *Acta Neuropathol.* 1998 Oct;96(4):409-16; <https://www.ncbi.nlm.nih.gov/pubmed/9797006>.

<sup>1403</sup> Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol.* 1991;82(4):239-59; [http://info-centre.jenage.de/assets/pdfs/library/braak\\_braak\\_ACTA\\_NEUROPATHOL\\_1991.pdf](http://info-centre.jenage.de/assets/pdfs/library/braak_braak_ACTA_NEUROPATHOL_1991.pdf).

<sup>1404</sup> Wenk GL. Neuropathologic changes in Alzheimer’s disease: potential targets for treatment. *J Clin Psychiatry.* 2006;67 Suppl 3:3-7; quiz 23; <http://faculty.psy.ohio-state.edu/wenk/documents/JClinPsychiatry2006.pdf>.

<sup>1405</sup> Buxhoeveden DP, Casanova MF. The minicolumn hypothesis in neuroscience. *Brain.* 2002 May;125(Pt 5):935-51; <http://brain.oxfordjournals.org/content/125/5/935>. Krueger JM, Rector DM, Roy S, Van Dongen HP, Belenky G, Panksepp J. Sleep as a fundamental property of neuronal assemblies. *Nat Rev Neurosci.* 2008 Dec;9(12):910-9; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2586424/>.

Figure 23. *Top:* Automated detection of neuron somata from high-resolution confocal image stacks (left); assignment of somata to cortical “barrel” columns (center); side view of 3D reconstruction of 6-layer cortical columns (right).<sup>1406</sup> *Bottom:* Image of a single neuron (left), a minicolumn (middle) and a cortical column or “neural microcircuit” (right).<sup>1407</sup>



<sup>1406</sup> Meyer HS, Egger R, Guest JM, Foerster R, Reissl S, Oberlaender M. Cellular organization of cortical barrel columns is whisker-specific. *Proc Natl Acad Sci U S A*. 2013 Nov 19;110(47):19113-8; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3839692/>. Rightmost image 26 Oct 2013 from <http://www.scitechdigest.net/2013/10/scitech-digest-37-27th-oct-2013.html>.

<sup>1407</sup> <http://www.kavlifoundation.org/science-spotlights/it-takes-world-map-brain#.VpdB6VKZPuO>.

These will be digitally added using custom automated software that can determine the characteristic size, cell membership, cell orientation and unit organization of the patient's surviving cortical columns,<sup>1408</sup> and can then insert additions to the pre-AD brain map that are consistent both with the surviving portions of the patient's brain and with generic models of human cortical columns appropriate for the patient's age, weight, gender, genetic profile, and experiential background that have been obtained from other data sources (**Table 3**).<sup>1409</sup> Other brain cells, including microglia (phagocytes) and macroglia (e.g., astrocytes, oligodendrocytes, ependymal cells, and radial glia) can be added to the map in a statistically appropriate distribution. The resulting map should be reviewed and adjusted as necessary by a human digital neurologist who specializes in this activity.

---

<sup>1408</sup> Oberlaender M, Dercksen VJ, Egger R, Gensel M, Sakmann B, Hege HC. Automated three-dimensional detection and counting of neuron somata. *J Neurosci Methods*. 2009 May 30;180(1):147-60; <http://homes.mpimf-heidelberg.mpg.de/~mhelmsta/pdf/2009%20Oberlaender%20.%20JNeurosciMeth.pdf>. Meijering E. Neuron tracing in perspective. *Cytometry A*. 2010 Jul;77(7):693-704; <http://onlinelibrary.wiley.com/doi/10.1002/cyto.a.20895/epdf>. Parekh R, Ascoli GA. Neuronal morphology goes digital: a research hub for cellular and system neuroscience. *Neuron*. 2013 Mar 20;77(6):1017-38; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3653619/>.

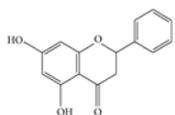
<sup>1409</sup> Kasthuri N, Hayworth KJ, Berger DR, Schalek RL, Conchello JA, Knowles-Barley S, Lee D, Vázquez-Reina A, Kaynig V, Jones TR, Roberts M, Morgan JL, Tapia JC, Seung HS, Roncal WG, Vogelstein JT, Burns R, Sussman DL, Priebe CE, Pfister H, Lichtman JW. Saturated Reconstruction of a Volume of Neocortex. *Cell*. 2015 Jul 30;162(3):648-61; <http://seunglab.org/wp-content/uploads/2015/09/saturatedreconstruction.pdf>. "The Human Connectome Project"; <http://www.humanconnectome.org/>. Allen Brain Atlas; <http://www.brain-map.org/>. "BRAIN 2025: A Scientific Vision," U.S. National Institutes of Health (NIH), 5 Jun 2014; [http://braininitiative.nih.gov/pdf/BRAIN2025\\_508C.pdf](http://braininitiative.nih.gov/pdf/BRAIN2025_508C.pdf) (guiding document for the NIH Brain Initiative; <http://www.braininitiative.nih.gov/>).

**Table 3. A few representative databases and datasets containing information about neuroanatomical connections.**<sup>1410</sup>

Database	Available Connectivity Information	URL
Brain Architecture Management System (BAMS)	Projections in rodent brain, curated manually from existing literature	<a href="http://brancusi.usc.edu/bkms/">http://brancusi.usc.edu/bkms/</a>
Collations of Connectivity Data on the Macaque Brain (CoCoMac)	Projections in macaque brain, curated manually from existing literature	<a href="http://www.cocomac.org">http://www.cocomac.org</a>
Functional Anatomy of the Cerebro–Cerebellar System (FACCS)	3D atlas of axonal tracing data in rat cerebro–cerebellar system	<a href="http://ocelot.uio.no/nesys/">http://ocelot.uio.no/nesys/</a>
BrainMaps.org	Tables of connections from literature and primary data for some tracer injections	<a href="http://brainmaps.org">http://brainmaps.org</a>
BrainPathways.org	Multiscale visualization of connectivity data from collated literature reports	<a href="http://brainpathways.org">http://brainpathways.org</a>
Human Brain Connectivity Database	Curated reports of connectivity studies in postmortem human brain tissue	<a href="http://brainarchitecture.org">http://brainarchitecture.org</a>
Internet Brain Connectivity Database	Estimated connectional data between human cortical gyral areas	<a href="http://www.cma.mgh.harvard.edu/ibcd/">http://www.cma.mgh.harvard.edu/ibcd/</a>
Surface Management System DataBase (SumsDB)	Connection densities from macaque retrograde tracer injections mapped to surface-based atlas	<a href="http://sumsdb.wustl.edu/sums/">http://sumsdb.wustl.edu/sums/</a>
SynapseWeb	Reconstructed volumes and structures from serial section electron microscopy	<a href="http://synapses.clm.utexas.edu/">http://synapses.clm.utexas.edu/</a>
Neocortical Microcircuit Database	Connection data between single cells in mammalian cortex	<a href="http://microcircuit.epfl.ch/">http://microcircuit.epfl.ch/</a>
ICBM DTI-81 Atlas	Probabilistic atlas of human white matter tracts based on diffusion tensor imaging	<a href="http://www.loni.ucla.edu/Atlases/Atlas_Detail.jsp?atlas_id=15">http://www.loni.ucla.edu/Atlases/Atlas_Detail.jsp?atlas_id=15</a>
Anatomy Toolbox Fiber Tracts	Probabilistic atlas of human white matter tracts based on postmortem studies	<a href="http://www.fz-juelich.de/ime/spm_anatomy_toolbox">http://www.fz-juelich.de/ime/spm_anatomy_toolbox</a>
WormAtlas	Full neuronal wiring data for <i>C. elegans</i>	<a href="http://www.wormatlas.org">http://www.wormatlas.org</a>

<sup>1410</sup> Bohland JW, *et al.* A proposal for a coordinated effort for the determination of brainwide neuroanatomical connectivity in model organisms at a mesoscopic scale. PLoS Comput Biol. 2009 Mar;5(3):e1000334; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2655718/>.

Another consideration is the brain vasculature, which can also be damaged by Alzheimer's disease and thus may require repair in some AD patients. More specifically, cerebral amyloid angiopathy is the deposition of  $\beta$ -amyloid in small arteries, arterioles and capillaries of the cerebral cortex and leptomeninges,<sup>1411</sup> and it occurs in almost all Alzheimer's disease patients, with severe cases occurring in about 25% of AD patients and with greater risk for intracerebral hemorrhage among patients (1) with the ApoE2 or ApoE4 alleles<sup>1412</sup> or (2) who have received A $\beta$  immunotherapy for AD.<sup>1413</sup>  $\beta$ -amyloid is thought to have damaging effects on the vasculature resulting in microbleeds and symptomatic intracerebral hemorrhage.<sup>1414</sup> The vascular disruption produced by  $\beta$ -amyloid includes endothelial cell and smooth muscle cell damage, leukocyte adhesion and migration across arteries and venules, and even platelet activation and thrombus formation.<sup>1415</sup>



Apparently the antioxidant flavonoid **pinocembrin** (image, left) can reverse the injurious effects of fibrillar A $\beta_{1-40}$  on human brain microvascular endothelial cells,<sup>1416</sup> and both stem cell therapy<sup>1417</sup> and electromagnetic treatment<sup>1418</sup> have

<sup>1411</sup> Viswanathan A, Greenberg SM. Cerebral amyloid angiopathy in the elderly. *Ann Neurol*. 2011 Dec;70(6):871-80; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4004372/>. Auriel E, Greenberg SM. The pathophysiology and clinical presentation of cerebral amyloid angiopathy. *Curr Atheroscler Rep*. 2012 Aug;14(4):343-50; <http://www.ncbi.nlm.nih.gov/pubmed/22565298>.

<sup>1412</sup> Viswanathan A, Greenberg SM. Cerebral amyloid angiopathy in the elderly. *Ann Neurol*. 2011 Dec;70(6):871-80; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4004372/>.

<sup>1413</sup> Sakai K, Boche D, Carare R, Johnston D, Holmes C, Love S, Nicoll JA. A $\beta$  immunotherapy for Alzheimer's disease: effects on apoE and cerebral vasculopathy. *Acta Neuropathol*. 2014 Dec;128(6):777-89; <http://www.ncbi.nlm.nih.gov/pubmed/25195061>.

<sup>1414</sup> Samarasekera N, Smith C, Al-Shahi Salman R. The association between cerebral amyloid angiopathy and intracerebral haemorrhage: systematic review and meta-analysis. *J Neurol Neurosurg Psychiatry*. 2012 Mar;83(3):275-81; <http://www.ncbi.nlm.nih.gov/pubmed/22056966>.

<sup>1415</sup> Thomas T, Sutton ET, Bryant MW, Rhodin JA. *In vivo* vascular damage, leukocyte activation and inflammatory response induced by beta-amyloid. *J Submicrosc Cytol Pathol*. 1997 Jul;29(3):293-304; <http://www.ncbi.nlm.nih.gov/pubmed/9267037>. Rhodin JA, Thomas TN, Clark L, Garces A, Bryant M. *In vivo* cerebrovascular actions of amyloid beta-peptides and the protective effect of conjugated estrogens. *J Alzheimers Dis*. 2003 Aug;5(4):275-86; <http://www.ncbi.nlm.nih.gov/pubmed/14624023>.

<sup>1416</sup> Liu R, Li JZ, Song JK, Sun JL, Li YJ, Zhou SB, Zhang TT, Du GH. Pinocembrin protects human brain microvascular endothelial cells against fibrillar amyloid- $\beta$ (1-40) injury by suppressing the MAPK/NF- $\kappa$ B inflammatory pathways. *Biomed Res Int*. 2014;2014:470393; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4135138/>.

<sup>1417</sup> Borlongan CV. Recent preclinical evidence advancing cell therapy for Alzheimer's disease. *Exp Neurol*. 2012 Sep;237(1):142-6; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4082718/>.

<sup>1418</sup> Arendash GW, Mori T, Dorsey M, Gonzalez R, Tajiri N, Borlongan C. Electromagnetic treatment to old Alzheimer's mice reverses  $\beta$ -amyloid deposition, modifies cerebral blood flow, and provides selected cognitive benefit. *PLoS One*. 2012;7(4):e35751; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3338462/>.

been proposed as conventional methods for vascular repair. But before vascular repair activities may begin, as part of the assembly of the cytoarchitectural brain map the AD patient's whole-brain vasculature should be mapped using a moderate number of simple bloodborne nanorobots, likely completable in a survey time on the order of ~1000 sec.<sup>1419</sup>

Finally, the possibility of at least some nanorobot-derived neural information recovery from **perineuronal nets** (Section 5.3.2.2) must also be considered, and all such recovered information can be added to the growing data model of the AD patient's brain as it is accumulated during the intracranial debridement phase.

At the end of this process of computational reconstruction, augmenting any available pre- or post-Alzheimer's MRI scans of the patient's brain with finer-grained data provided by the nanorobots, the nanomedical therapist will possess a crude model of the patient's best-estimate pre-Alzheimer's whole-brain cytoarchitecture (**Figure 24**). The target pre-AD model can now be compared to the patient's current cytoarchitectural brain map, with the difference between the two maps serving as the basis for the repair plan to be executed as described in Section 5.3.2.

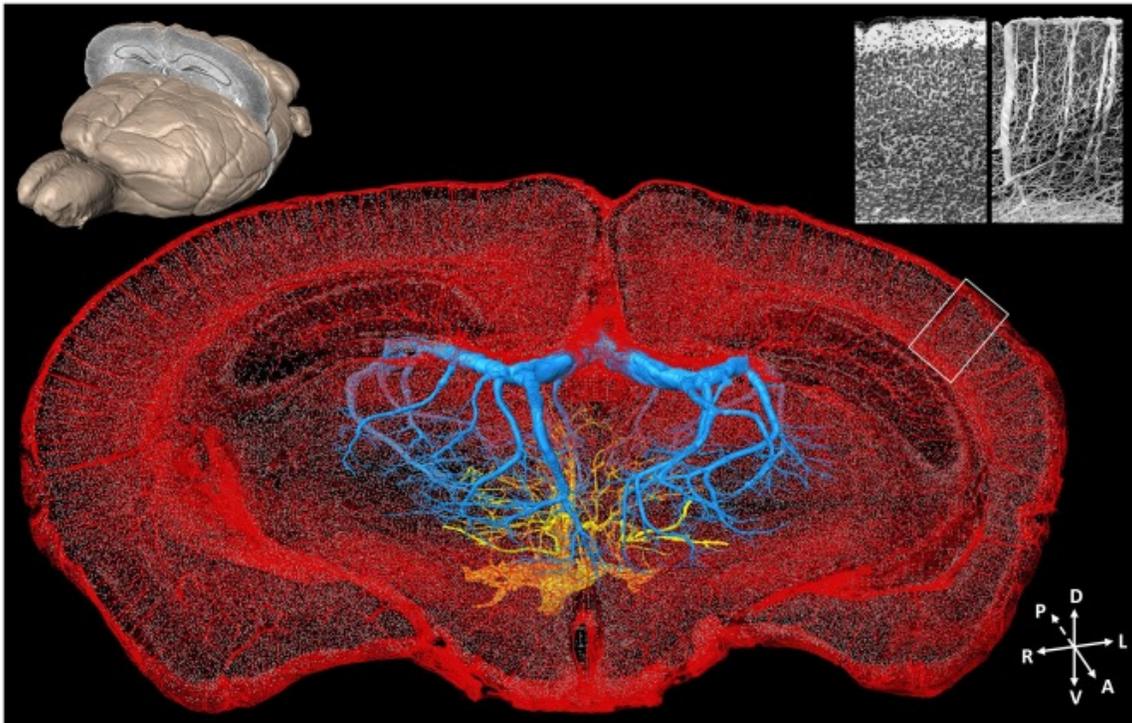
After comparison to the patient's current cytoarchitectural map, the estimated pre-AD map is annotated with both the locations of existing neurons to be repaired *in situ* and the locations of missing neurons to be replaced, along with the desired vectors of axon extension (e.g., spanning an indicated cortical column) and dendrite growth (e.g., regions of below-normal synaptic activity) that are consistent with an adult neuroanatomy. Required repair or replacement of glial cells and related structures will be specified in accord with the extent of the damage (**Figure 25**). Any necessary vascular repairs will also be highlighted, including subsections of the brain indicating the relevant neurovascular units.<sup>1420</sup>

---

<sup>1419</sup> Freitas RA Jr. "Chapter 23. Comprehensive Nanorobotic Control of Human Morbidity and Aging," in Gregory M. Fahy, Michael D. West, L. Stephen Coles, and Steven B. Harris, eds, *The Future of Aging: Pathways to Human Life Extension*, Springer, New York, 2010, pp. 685-805, Section 6.2.2 "Cancer"; <http://www.nanomedicine.com/Papers/Aging.pdf>.

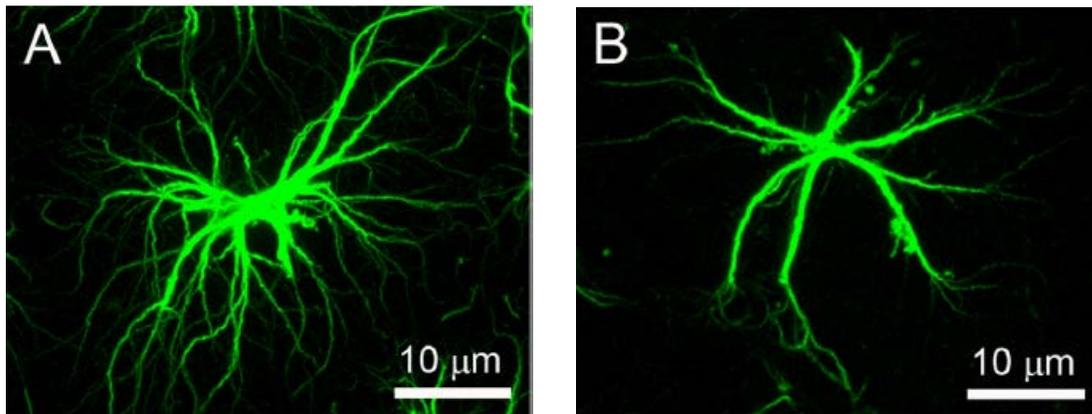
<sup>1420</sup> The elementary component of brain microcirculation is represented by a **neurovascular unit**, in which astrocytes integrate neurons, brain endothelium, pericytes, and vascular smooth muscle cells into a functionally independent entity, establishing the link between neuronal activity and local blood flow. Iadecola C. Neurovascular regulation in the normal brain and in Alzheimer's disease. *Nat Rev Neurosci*. 2004 May;5(5):347-60; [http://ftp.mpib-tuebingen.mpg.de/kyb/bweber/papers\\_db/2004/Iadecola/Nat%20Rev%20Neurosci%202004%20Iadecola.pdf](http://ftp.mpib-tuebingen.mpg.de/kyb/bweber/papers_db/2004/Iadecola/Nat%20Rev%20Neurosci%202004%20Iadecola.pdf). Takano T, Tian GF, Peng W, Lou N, Libionka W, Han X, Nedergaard M. Astrocyte-mediated control of cerebral blood flow. *Nat Neurosci*. 2006 Feb;9(2):260-7; [http://www.cns.nyu.edu/events/spf/SPF\\_papers/takano\\_etal\\_2006.pdf](http://www.cns.nyu.edu/events/spf/SPF_papers/takano_etal_2006.pdf). Iadecola C, Nedergaard M. Glial regulation of the cerebral microvasculature. *Nat Neurosci*. 2007 Nov;10(11):1369-76; <http://www.ncbi.nlm.nih.gov/pubmed/17965657>.

Figure 24. “Whole-brain cytoarchitecture and vascular networks. A 3D reconstruction of the **mouse brain** is in the top left corner; the selected 300  $\mu\text{m}$  slab at the coronal plane presents the spatial location of the data at the center. The center shows the cytoarchitecture and vascular network, simultaneously acquired in the brain. Blue and yellow represents the branches of the longitudinal hippocampal vein and some thalamo-perforating arteries in thalamus, respectively, red represents all else vessels in this data set, and gray dots represent the center of somas. The enlarged views of the cytoarchitecture and vascular architecture of the white rectangle in cortical region in the data at the center are in the top right corner.”<sup>1421</sup>



<sup>1421</sup> Yuan J, Gong H, Li A, Li X, Chen S, Zeng S, Luo Q. Visible rodent brain-wide networks at single-neuron resolution. *Front Neuroanat.* 2015 May 28;9:70; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4446545/>.

Figure 25. Confocal micrographs of atrophy in hippocampal astrocytes (a type of glial cell) in mouse model of AD: (A) normal brain; (B) Alzheimer's brain.<sup>1422</sup>



Nearly full functionality should be restored to most surviving neurons or neural structures by applying the First Alzheimer Protocol (Section 5.1) and the Second Alzheimer Protocol (Section 5.2).

The major therapeutic task remaining for the Third Alzheimer Protocol will be to reconstruct missing or irreparably damaged physical structures – ranging from dendritic trees of individual neurons and glial cells to entire cortical columns and possibly neural microvasculature (Section 5.3.2) – encouraging regrowth of missing axons, dendrites, and synapses under the directed influence of intensive neural network retraining (Section 5.3.3).

---

<sup>1422</sup> Verkhatsky A, Olabarria M, Noristani H, Yeh C, Rodriguez J. Astrocytes in Alzheimer's disease. *Neurotherapeutics* 2010;7(4): 399-412; [http://xa.yimg.com/kq/groups/2051587/638361111/name/Neurotherapeutics\\_2010\\_7\\_399\\_412.pdf](http://xa.yimg.com/kq/groups/2051587/638361111/name/Neurotherapeutics_2010_7_399_412.pdf).

### 5.3.2 Reconstruction of Neural Tissues

With a stepwise neural repair plan in hand (Section 5.3.1), we are now ready to begin the process of neural reconstruction of the patient's AD brain.

Unlike peripheral nervous system injury, injury to the central nervous system is usually not followed by extensive regeneration.<sup>1423</sup> Regeneration is limited by the inhibitory influences of the glial and extracellular environment. The hostile, non-permissive growth environment is, in part, created by the in-migration of myelin-associated inhibitors, astrocytes, oligodendrocytes, oligodendrocyte precursors, and microglia. The environment within the CNS, especially following trauma, counteracts the repair of myelin and neurons. Growth factors are not expressed or re-expressed; for instance, the extracellular matrix is lacking laminins. Glial scars rapidly form, and the glia actually produce factors that inhibit remyelination and axon repair.<sup>1424</sup> The axons themselves also lose the potential for growth with age, in part due to a decrease in GAP 43 expression.

As a result, we will have to manually initiate regeneration using medical nanorobots. The repair plan includes the locations of existing neurons to be repaired *in situ*. Ideally this will include the great majority (>80%) of surviving neurons, and these cells will all have had Alzheimer Protocols #1 and #2 applied to them. After that, the process of neural reconstruction described in this Section can begin. The repair plan will also include the locations of missing neurons to be replaced, along with the desired vectors of axon extension (e.g., spanning an indicated cortical column) and dendrite growth (e.g., regions of below-normal synaptic activity), highlighting any necessary vascular repairs.

Reconstruction of the missing neural tissue requires manufacturing replacement neural cells (Section 5.3.2.1), debridement of neural detritus in heavily damaged or envolded areas (Section 5.3.2.2), insertion and emplacement of replacement cells via a nanocatheter array (Section 5.3.2.3), and incorporation of the replacement cells into the existing neural tissues by establishing conditions that temporarily replicate much of the high neural plasticity of youth (Section 5.3.2.4).

---

<sup>1423</sup> <https://en.wikipedia.org/wiki/Neuroregeneration>.

<sup>1424</sup> Karnezis T, Mandemakers W, McQualter JL, Zheng B, Ho PP, Jordan KA, Murray BM, Barres B, Tessier-Lavigne M, Bernard CC. The neurite outgrowth inhibitor Nogo A is involved in autoimmune-mediated demyelination. *Nat Neurosci*. 2004 Jul;7(7):736-44; [https://www.researchgate.net/profile/Wim\\_Mandemakers/publication/8521983\\_The\\_neurite\\_outgrowth\\_inhibitor\\_Nogo\\_A\\_is\\_involved\\_in\\_autoimmune-mediated\\_demyelination/links/0912f508e8f26ae720000000.pdf](https://www.researchgate.net/profile/Wim_Mandemakers/publication/8521983_The_neurite_outgrowth_inhibitor_Nogo_A_is_involved_in_autoimmune-mediated_demyelination/links/0912f508e8f26ae720000000.pdf). Yiu G, He Z. Glial inhibition of CNS axon regeneration. *Nat Rev Neurosci*. 2006 Aug;7(8):617-27; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2693386/>.

### 5.3.2.1 Replacement Cell Manufacture

A cell mill (Section 4.2.6) is employed to fabricate all needed neural cell types, including differentiated cells such as neurons, microglia, astrocytes, oligodendrocytes, ependymal cells, and radial glia, but also including neural stem cells as required. The exact mix of cell types chosen will depend on the severity and cytoarchitectural distribution of the damage, and the specific requirements of the neural repair plan (Section 5.3.1).

Each cell of each cell type will be manufactured with genomes representing the patient's own neural genome and methylation pattern, and will be primed for maximum growth potential as might be found in neonatal or juvenile human brains. Manufactured neural cells may be pregrown with an appropriate number of generic "starter set" neurites and arborizations, together with axons of the appropriate length as determined by the neural repair plan.

Completed manufactured whole neural cells are inspected, sorted, and transported to a collection depot for export through a nanocatheter delivery system (Section 4.3.5). The cell mill may be required to fabricate up to  $\sim 250 \text{ cm}^3$  of missing grey matter, white matter, vascular and auxiliary cells for an adult male Alzheimer's patient if up to 20% of brain volume needs replacement in the most severe AD cases.

### 5.3.2.2 Intracranial Debridement

Nanocatheters (Section 4.3.5) will be used to insert mobile surgical nanorobots into the brain to perform intracranial debridement, tracking the neural repair plan. These nanorobots will clear out all unwanted irreparable neurons and other biological matter in the spaces where we want missing neurons to be replaced. More specifically, these nanorobots can remove ghost tangles,<sup>1425</sup> extracellular amyloid masses,<sup>1426</sup> glial scars<sup>1427</sup> or spurious glial cell accumulations that have replaced the degraded ghost tangles<sup>1428</sup> or that result from past episodes of reactive astrogliosis,<sup>1429</sup> abnormally infiltrated immune cells,<sup>1430</sup> pathological extracellular matrix materials,<sup>1431</sup> blood components from a leaky AD vasculature,<sup>1432</sup> or other necrotic biomaterial that may be occupying the desired replacement neuron spaces. (Gliosis and glial scarring occur in areas surrounding the amyloid plaques which are hallmarks of Alzheimer's disease, with

---

<sup>1425</sup> Endoh R, Ogawara M, Iwatsubo T, Nakano I, Mori H. Lack of the carboxyl terminal sequence of tau in ghost tangles of Alzheimer's disease. *Brain Res.* 1993 Jan 22;601(1-2):164-72; <http://www.sciencedirect.com/science/article/pii/000689939391707Y>.

<sup>1426</sup> Wenk GL. Neuropathologic changes in Alzheimer's disease: potential targets for treatment. *J Clin Psychiatry.* 2006;67 Suppl 3:3-7; quiz 23; <http://faculty.psy.ohio-state.edu/wenk/documents/JClinPsychiatry2006.pdf>.

<sup>1427</sup> **Glial scars** occur after damage to the central nervous system. Glial scar formation significantly inhibits nerve regeneration, leading to loss of function, with several families of released molecules (transforming growth factors  $\beta 1$  and  $\beta 2$ , interleukins, and cytokines) that promote and drive glial scar formation. The inhibition of nerve regeneration is a result of the accumulation of reactive astrocytes at the site of injury and the up-regulation of molecules that are inhibitory to neurite extension outgrowth. Zhang H, Uchimura K, Kadomatsu K. Brain keratan sulfate and glial scar formation. *Ann N Y Acad Sci.* 2006 Nov;1086:81-90; <http://www.ncbi.nlm.nih.gov/pubmed/17185507>.

<sup>1428</sup> Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol.* 1991;82(4):239-59; [http://info-centre.jenage.de/assets/pdfs/library/braak\\_braak\\_ACTA\\_NEUROPATHOL\\_1991.pdf](http://info-centre.jenage.de/assets/pdfs/library/braak_braak_ACTA_NEUROPATHOL_1991.pdf).

<sup>1429</sup> Eng LF, Ghirnikar RS. GFAP and astrogliosis. *Brain Pathol.* 1994 Jul;4(3):229-37; <http://www.ncbi.nlm.nih.gov/pubmed/7952264>. Sofroniew MV. Molecular dissection of reactive astrogliosis and glial scar formation. *Trends Neurosci.* 2009 Dec;32(12):638-47; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2787735/>.

<sup>1430</sup> Raposo C, Schwartz M. Glial scar and immune cell involvement in tissue remodeling and repair following acute CNS injuries. *Glia.* 2014 Nov;62(11):1895-904; <http://www.ncbi.nlm.nih.gov/pubmed/24756949>.

<sup>1431</sup> Jones EV, Bouvier DS. Astrocyte-secreted matricellular proteins in CNS remodelling during development and disease. *Neural Plast.* 2014;2014:321209; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3914553/>.

<sup>1432</sup> Loitfelder M, Seiler S, Schwingenschuh P, Schmidt R. Cerebral microbleeds: a review. *Panminerva Med.* 2012 Sep;54(3):149-60; <http://www.ncbi.nlm.nih.gov/pubmed/22801432>.

postmortem tissues indicating a correlation between the degree of astrogliosis and cognitive decline.<sup>1433</sup>)

If a  $\sim 1 \text{ cm}^3$  solid mass can be cleared from the brain in  $\sim 5$  min using a fleet volume of  $\sim 10^{10}$  micron<sup>3</sup> of nanorobots (Section 4.3.5), then a slightly larger fleet volume of  $\sim 10^{11}$  micron<sup>3</sup> of debridement nanorobots could debride up to  $\sim 20\%$  of pathological brain volume ( $\sim 250 \text{ cm}^3$ ) in  $\sim 125$  min ( $\sim 2.1$  hr).

The nanocatheter array should also deliver into the brain a modest number of additional support nanorobots. These support devices might include respirocytes to maintain proper oxygenation and nutrient flows, along with microbivores to help guarantee an infection-free environment for the duration of the brain reconstruction process.

Of course, the process of intracranial debridement might itself stimulate an injury response, triggering a new episode of gliosis mediated by astrocytes – specialized glial cells that contiguously tile the entire central nervous system (CNS) and are the main component of the glial scar.<sup>1434</sup> After injury (as also occurs during AD), these cells become reactive and undergo morphological changes, extend their processes, and increase synthesis of glial fibrillary acidic protein (GFAP).<sup>1435</sup> GFAP is an important intermediate filament protein that allows the astrocytes to begin synthesizing more cytoskeletal supportive structures and extend pseudopodia. Ultimately, reactive astrocytes will form a dense web of their plasma membrane extensions that fills the empty space generated by the dead or dying neuronal cells (a process called reactive astrogliosis). The heavy proliferation of astrocytes also modifies the extracellular matrix surrounding the damaged region by secreting many molecules including laminin, fibronectin, tenascin C, and proteoglycans.<sup>1436</sup> These molecules are important modulators of neuronal outgrowth and their presence after injury contributes to the inhibition of regeneration.<sup>1437</sup>

---

<sup>1433</sup> Verkhatsky A, Olabarria M, Noristani H, Yeh C, Rodriguez J. Astrocytes in Alzheimer's disease. *Neurotherapeutics* 2010;7(4): 399-412;

[http://xa.yimg.com/kq/groups/2051587/638361111/name/Neurotherapeutics\\_2010\\_7\\_399\\_412.pdf](http://xa.yimg.com/kq/groups/2051587/638361111/name/Neurotherapeutics_2010_7_399_412.pdf).

Sofroniew MV, Vinters HV. Astrocytes: biology and pathology. *Acta Neuropathol.* 2010 Jan;119(1):7-35; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2799634/>.

<sup>1434</sup> Sofroniew MV, Vinters HV. Astrocytes: biology and pathology. *Acta Neuropathol.* 2010 Jan;119(1):7-35; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2799634/>.

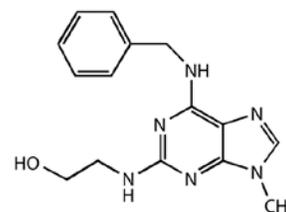
<sup>1435</sup> Eng LF, Ghirnikar RS. GFAP and astrogliosis. *Brain Pathol.* 1994 Jul;4(3):229-37; <http://www.ncbi.nlm.nih.gov/pubmed/7952264>.

<sup>1436</sup> Jones LL, Margolis RU, Tuszynski MH. The chondroitin sulfate proteoglycans neurocan, brevican, phosphacan, and versican are differentially regulated following spinal cord injury. *Exp Neurol.* 2003 Aug;182(2):399-411; <http://www.ncbi.nlm.nih.gov/pubmed/12895450>. Jones LL, Sajed D, Tuszynski MH. Axonal regeneration through regions of chondroitin sulfate proteoglycan deposition after spinal cord injury: a balance of permissiveness and inhibition. *J Neurosci.* 2003 Oct 15;23(28):9276-88; <http://www.jneurosci.org/content/23/28/9276.long>.

<sup>1437</sup> Davies SJ, Fitch MT, Memberg SP, Hall AK, Raisman G, Silver J. Regeneration of adult axons in white matter tracts of the central nervous system. *Nature.* 1997 Dec 18-25;390(6661):680-3; <http://huguenard-lab.stanford.edu/205/1999-2000/silver.pdf>. Silver J, Miller JH. Regeneration beyond the

**Microglia** (nervous system macrophages) are the second most prominent cell type present within the glial scar, rapidly activating near the injury and secreting several cytokines, bioactive lipids, coagulation factors, reactive oxygen intermediates, and neurotrophic factors.<sup>1438</sup> These biologically active molecules recruit **endothelial cells** and **fibroblasts** into the injured area, stimulating collagen secretion and angiogenesis that ultimately doubles the number of capillaries extended into the affected regions after a couple of weeks.<sup>1439</sup> Brain-injury-activated microglia may induce neural stem cell proliferation and promote differentiation into neurons and oligodendrocytes.<sup>1440</sup>

As a result, injury suppression nanorobots should be injected alongside the debridement nanorobots to temporarily suppress any natural injury response that might occur in the regions where rapid nanorobot-mediated debridement is occurring. Glial scar formation may be suppressed via the localized release (and post-operative retrieval) of various bioactive substances by the injury suppression nanorobots. For example, **olomoucine** (image, right), a purine derivative, is a cyclin-dependent kinase (CDK)<sup>1441</sup> inhibitor that has been shown to reduce neuronal cell death, suppress microglial proliferation within the glial scar,<sup>1442</sup> and reduce astroglial proliferation (and therefore reduce astrogliosis), thus decreasing expression of chondroitin sulfate proteoglycans which are major extracellular matrix molecules associated with inhibition of neuroregeneration after trauma to the CNS.<sup>1443</sup>




---

glial scar. *Nat Rev Neurosci.* 2004 Feb;5(2):146-56;  
<http://www.societyns.org/runn/2008/Silver%20and%20Miller.pdf>.

<sup>1438</sup> Elkabes S, DiCicco-Bloom EM, Black IB. Brain microglia/macrophages express neurotrophins that selectively regulate microglial proliferation and function. *J Neurosci.* 1996 Apr 15;16(8):2508-21;  
<http://www.jneurosci.org/content/16/8/2508.long>.

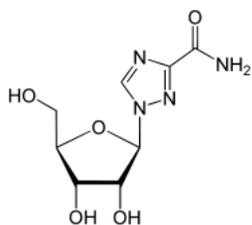
<sup>1439</sup> Jaeger CB, Blight AR. Spinal cord compression injury in guinea pigs: structural changes of endothelium and its perivascular cell associations after blood-brain barrier breakdown and repair. *Exp Neurol.* 1997 Apr;144(2):381-99; <http://www.ncbi.nlm.nih.gov/pubmed/9168838>.

<sup>1440</sup> Deierborg T, Roybon L, Inacio AR, Pesic J, Brundin P. Brain injury activates microglia that induce neural stem cell proliferation *ex vivo* and promote differentiation of neurosphere-derived cells into neurons and oligodendrocytes. *Neuroscience.* 2010 Dec 29;171(4):1386-96;  
<http://lup.lub.lu.se/record/1772436/file/1787847.pdf>

<sup>1441</sup> **CDK** is a cell-cycle promoting protein that is abnormally activated during glial scar formation.

<sup>1442</sup> Tian DS, Xie MJ, Yu ZY, Zhang Q, Wang YH, Chen B, Chen C, Wang W. Cell cycle inhibition attenuates microglia induced inflammatory response and alleviates neuronal cell death after spinal cord injury in rats. *Brain Res.* 2007 Mar 2;1135(1):177-85; <http://www.ncbi.nlm.nih.gov/pubmed/17188663>.

<sup>1443</sup> Tian DS, Yu ZY, Xie MJ, Bu BT, Witte OW, Wang W. Suppression of astroglial scar formation and enhanced axonal regeneration associated with functional recovery in a spinal cord injury rat model by the cell cycle inhibitor olomoucine. *J Neurosci Res.* 2006 Oct;84(5):1053-63;  
<http://www.ncbi.nlm.nih.gov/pubmed/16862564>.



**Ribavirin** (image, left), a purine nucleoside analogue generally used as an anti-viral medication, has been shown to decrease the number of reactive astrocytes.<sup>1444</sup> An **antisense GFAP retrovirus** (PLBskG) reduces GFAP mRNA expression, suppresses growth, and arrests astrocytes in the G1 phase of the cell cycle.<sup>1445</sup> **Monoclonal antibodies** raised to transforming growth factor- $\beta$ <sup>1446</sup> and to interleukin-6<sup>1447</sup> have produced a marked reduction in angiogenesis, inflammation, matrix deposition, the numbers of astrocytes and microglia, and glial scarring in injured brain or spinal cord tissue. **Type I interferon**,<sup>1448</sup> **granulocyte macrophage-colony stimulating factor**,<sup>1449</sup> **pranlukast**,<sup>1450</sup> **hyaluronic acid**,<sup>1451</sup> **curcumin**,<sup>1452</sup> and **triptolide**<sup>1453</sup> have all been found to inhibit glial scar formation.

---

<sup>1444</sup> Peković S, Filipović R, Subasić S, Lavrnja I, Stojkov D, Nedeljković N, Rakić L, Stojiljković M. Downregulation of glial scarring after brain injury: the effect of purine nucleoside analogue ribavirin. *Ann N Y Acad Sci.* 2005 Jun;1048:296-310; <http://www.ncbi.nlm.nih.gov/pubmed/16154942>.

<sup>1445</sup> Huang QL, Cai WQ, Zhang KC. Effect of the control proliferation of astrocyte on the formation of glial scars by antisense GFAP retrovirus. *Chinese Science Bulletin* 2000;45(1):38-44; <http://link.springer.com/article/10.1007%2FBF02884900>.

<sup>1446</sup> Logan A, Green J, Hunter A, Jackson R, Berry M. Inhibition of glial scarring in the injured rat brain by a recombinant human monoclonal antibody to transforming growth factor-beta2. *Eur J Neurosci.* 1999 Jul;11(7):2367-74; <http://www.ncbi.nlm.nih.gov/pubmed/10383626>.

<sup>1447</sup> Okada S, Nakamura M, Mikami Y, Shimazaki T, Mihara M, Ohsugi Y, Iwamoto Y, Yoshizaki K, Kishimoto T, Toyama Y, Okano H. Blockade of interleukin-6 receptor suppresses reactive astrogliosis and ameliorates functional recovery in experimental spinal cord injury. *J Neurosci Res.* 2004 Apr 15;76(2):265-76; <http://www.ncbi.nlm.nih.gov/pubmed/15048924>.

<sup>1448</sup> Ito M, Natsume A, Takeuchi H, Shimato S, Ohno M, Wakabayashi T, Yoshida J. Type I interferon inhibits astrocytic gliosis and promotes functional recovery after spinal cord injury by deactivation of the MEK/ERK pathway. *J Neurotrauma.* 2009 Jan;26(1):41-53; <http://www.ncbi.nlm.nih.gov/pubmed/19196180>.

<sup>1449</sup> Huang X, Kim JM, Kong TH, Park SR, Ha Y, Kim MH, Park H, Yoon SH, Park HC, Park JO, Min BH, Choi BH. GM-CSF inhibits glial scar formation and shows long-term protective effect after spinal cord injury. *J Neurol Sci.* 2009 Feb 15;277(1-2):87-97; <http://www.ncbi.nlm.nih.gov/pubmed/19033079>.

<sup>1450</sup> Yu GL, Wei EQ, Wang ML, Zhang WP, Zhang SH, Weng JQ, Chu LS, Fang SH, Zhou Y, Chen Z, Zhang Q, Zhang LH. Pranlukast, a cysteinyl leukotriene receptor-1 antagonist, protects against chronic ischemic brain injury and inhibits the glial scar formation in mice. *Brain Res.* 2005 Aug 16;1053(1-2):116-25; [http://www.researchgate.net/profile/Shi-Hong-Zhang/publication/7694953\\_Pranlukast\\_a\\_cysteinyl\\_leukotriene\\_receptor-1\\_antagonist\\_protects\\_against\\_chronic\\_ischemic\\_brain\\_injury\\_and\\_inhibits\\_the\\_glial\\_scar\\_formation\\_in\\_mice/links/54b4c8280cf28ebe92e4847e.pdf](http://www.researchgate.net/profile/Shi-Hong-Zhang/publication/7694953_Pranlukast_a_cysteinyl_leukotriene_receptor-1_antagonist_protects_against_chronic_ischemic_brain_injury_and_inhibits_the_glial_scar_formation_in_mice/links/54b4c8280cf28ebe92e4847e.pdf).

<sup>1451</sup> Lin CM, Lin JW, Chen YC, Shen HH, Wei L, Yeh YS, Chiang YH, Shih R, Chiu PL, Hung KS, Yang LY, Chiu WT. Hyaluronic acid inhibits the glial scar formation after brain damage with tissue loss in rats. *Surg Neurol.* 2009 Dec;72 Suppl 2:S50-4; [https://www.researchgate.net/profile/Liang-Yo-Yang/publication/40037971\\_Hyaluronic\\_acid\\_inhibits\\_the\\_glial\\_scar\\_formation\\_after\\_brain\\_damage\\_with\\_tissue\\_loss\\_in\\_rats/links/00b7d530fec9cca62e000000.pdf](https://www.researchgate.net/profile/Liang-Yo-Yang/publication/40037971_Hyaluronic_acid_inhibits_the_glial_scar_formation_after_brain_damage_with_tissue_loss_in_rats/links/00b7d530fec9cca62e000000.pdf).

Another important aspect of neural debridement may be the removal of damaged<sup>1454</sup> or ghost remnants<sup>1455</sup> of **perineuronal nets (PNNs)** (**Figure 26**). In normal healthy brains, PNNs are specialized extracellular matrix structures composed of chondroitin sulfate proteoglycans (CSPGs) – a mesh of proteins attached to carbohydrates – that are found sheathing certain mature neuron cell bodies and proximal neurites in the central nervous system. PNNs comprise a complex of extracellular matrix molecules interposed between the meshwork of glial processes and nerve-cell surfaces.<sup>1456</sup> PNNs appear to be mainly present in the cortex, hippocampus, thalamus, brainstem, and the spinal cord. Studies of the rat brain have shown that the cortex contains high numbers of PNNs in the motor and primary sensory areas and relatively fewer in the association and limbic cortices.<sup>1457</sup> In the cortex, PNNs are associated mostly with inhibitory interneurons and are thought to be responsible for maintaining the excitatory/inhibitory balance in the adult brain.<sup>1458</sup> Recent studies in mammalian systems have implicated CSPGs and PNNs in

<sup>1452</sup> Wang YF, Zu JN, Li J, Chen C, Xi CY, Yan JL. Curcumin promotes the spinal cord repair via inhibition of glial scar formation and inflammation. *Neurosci Lett*. 2014 Feb 7;560:51-6; <http://www.ncbi.nlm.nih.gov/pubmed/24316441>.

<sup>1453</sup> Su Z, Yuan Y, Cao L, Zhu Y, Gao L, Qiu Y, He C. Triptolide promotes spinal cord repair by inhibiting astrogliosis and inflammation. *Glia*. 2010 Jun;58(8):901-15; [http://www.researchgate.net/profile/Zhida\\_Su/publication/41430205\\_Triptolide\\_promotes\\_spinal\\_cord\\_repair\\_by\\_inhibiting\\_astrogliosis\\_and\\_inflammation/links/0c96052efce8ce89c2000000.pdf](http://www.researchgate.net/profile/Zhida_Su/publication/41430205_Triptolide_promotes_spinal_cord_repair_by_inhibiting_astrogliosis_and_inflammation/links/0c96052efce8ce89c2000000.pdf).

<sup>1454</sup> Two post-mortem studies have shown that certain components of the perineuronal net may be lost in the brains of patients with Alzheimer's disease. Kobayashi K, Emson PC, Mountjoy CQ. Vicia villosa lectin-positive neurones in human cerebral cortex. Loss in Alzheimer-type dementia. *Brain Res*. 1989 Sep 25;498(1):170-4; <http://www.ncbi.nlm.nih.gov/pubmed/2790470>. Baig S, Wilcock GK, Love S. Loss of perineuronal net N-acetylgalactosamine in Alzheimer's disease. *Acta Neuropathol*. 2005 Oct;110(4):393-401; <http://www.ncbi.nlm.nih.gov/pubmed/16133543>.

<sup>1455</sup> Two other studies found that perineuronal nets in Alzheimer's brains resist disintegration and may protect neurons from degeneration. Brückner G, Hausen D, Härtig W, Drlicek M, Arendt T, Brauer K. Cortical areas abundant in extracellular matrix chondroitin sulphate proteoglycans are less affected by cytoskeletal changes in Alzheimer's disease. *Neuroscience*. 1999;92(3):791-805; <http://www.ncbi.nlm.nih.gov/pubmed/10426522>. Morawski M, Brückner G, Jäger C, Seeger G, Matthews RT, Arendt T. Involvement of perineuronal and perisynaptic extracellular matrix in Alzheimer's disease neuropathology. *Brain Pathol*. 2012 Jul;22(4):547-61; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3639011/>. However, PNNs are more durable than neurons and it cannot yet be ruled out that "ghost" PNN structures might remain wholly or partially intact even after the thorough degradation of the neuron with which the PNN was originally associated in the AD brain.

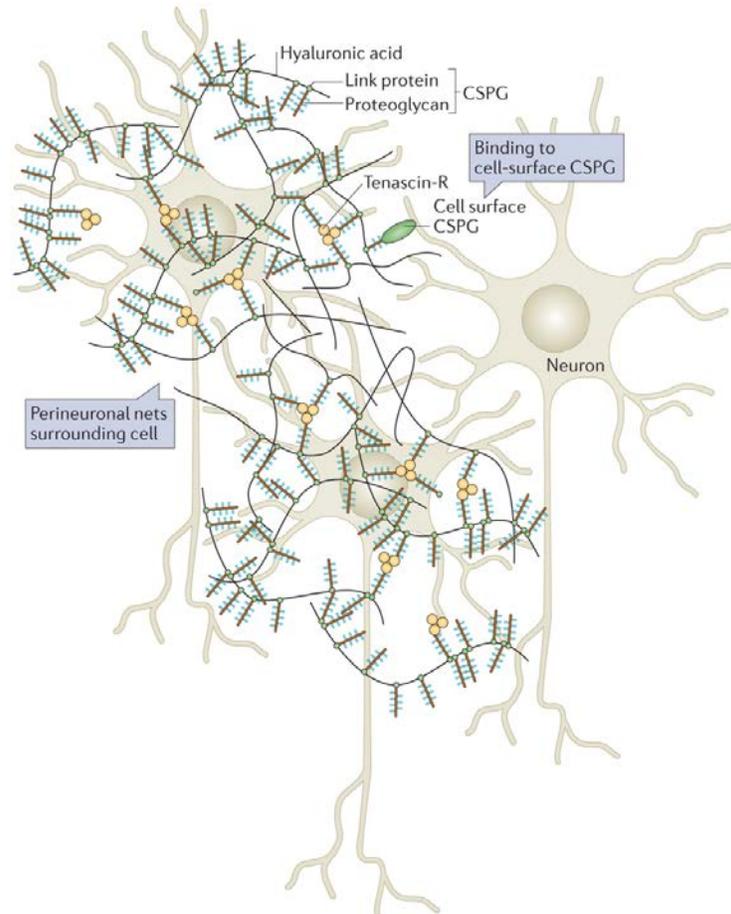
<sup>1456</sup> Celio MR, Spreafico R, De Biasi S, Vitellaro-Zuccarello L. Perineuronal nets: past and present. *Trends Neurosci*. 1998 Dec;21(12):510-5; <http://www.ncbi.nlm.nih.gov/pubmed/9881847>.

<sup>1457</sup> Galtrey CM, Fawcett JW. The role of chondroitin sulfate proteoglycans in regeneration and plasticity in the central nervous system. *Brain Res Rev*. 2007 Apr;54(1):1-18; <http://www.ncbi.nlm.nih.gov/pubmed/17222456>.

<sup>1458</sup> Hensch TK. Critical period plasticity in local cortical circuits. *Nat Rev Neurosci*. 2005 Nov;6(11):877-88; <http://www.bio.brandeis.edu/classes/nbio143/Papers/Plasticity/Hensch05.pdf>.

regulating and restricting structural plasticity – the enzymatic degradation of CSPGs or destabilization of PNNs can allow axons to penetrate the vacated space, enhancing neuronal activity and restoring plasticity after central nervous system injury.<sup>1459</sup> Disruption of existing or degraded PNNs during neural debridement by nanorobots should similarly restore neural plasticity (Section 5.3.2.4).

*Figure 26. Perineuronal nets (PNNs) surround cells of the brain and act as inhibitors of both growth and migration. PNNs are lattices of hyaluronic acid, proteoglycans and tenascin molecules.<sup>1460</sup>*



<sup>1459</sup> Soleman S, Filippov MA, Dityatev A, Fawcett JW. Targeting the neural extracellular matrix in neurological disorders. *Neuroscience*. 2013 Dec 3;253:194-213; <http://www.ncbi.nlm.nih.gov/pubmed/24012743/>.

<sup>1460</sup> Mouw JK, Ou G, Weaver VM. Extracellular matrix assembly: a multiscale deconstruction. *Nat Rev Mol Cell Biol*. 2014 Dec;15(12):771-85; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4682873/>.

While it has long been known that synapses form through gaps in the perineuronal net, Tsien proposed<sup>1461</sup> in 2013 that long-term memories might be stored in patterns of holes created within the lattice-like structure of the PNN, rather like old-fashioned computers that encoded information as holes in punched cards or paper tapes. In 2015, Tsien's graduate student presented supporting evidence<sup>1462</sup> that the perineuronal net is highly stable, but when synapses are strengthened they produce a small amount of an enzyme that chews up the matrix to form a seemingly permanent hole structure in the PNN right next to the synapse. Mice that have been genetically engineered to lack this enzyme have normal short- and medium-term memory but very poor long-term memory.

Since Alzheimer's disease apparently has at most a modest degradatory effect on PNNs, long-term memory information might still exist in an AD brain even after the synapses, axons, or neurons have become seriously damaged or nonexistent. To the greatest extent possible, this information should be collected during debridement to augment the neural repair plan. After debridement, we will be relying mostly on the newly inserted/emplaced (Section 5.3.2.3) and incorporated (Section 5.3.2.4) neurons to self-assemble new PNNs in the debrided volumes as the patient's memories are rebuilt during the neural network retraining process (Section 5.3.3).

---

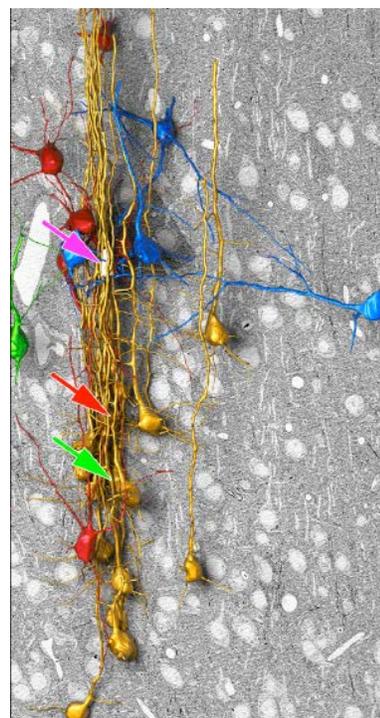
<sup>1461</sup> Tsien RY. Very long-term memories may be stored in the pattern of holes in the perineuronal net. Proc Natl Acad Sci U S A. 2013 Jul 23;110(30):12456-61; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3725115/>.

<sup>1462</sup> Emily Underwood, "Mysterious 'holes' in neuron net may help store long-term memories," 20 Oct 2015; <http://www.sciencemag.org/news/2015/10/mysterious-holes-neuron-net-may-help-store-long-term-memories>. Moheb Costandi, "Do Long-Term Memories Punch Holes in the Brain?" 23 Oct 2015; <https://www.braindecoder.com/memory-perineuronal-nets-1418933042.html>.

### 5.3.2.3 Cell Insertion and Emplacement

After intracranial debridement is complete and the debridement nanorobots have been retrieved from the brain, nanocatheters (Section 4.3.5) will again be used to insert into the brain a significant population of mobile surgical “shepherd” nanorobots having two important purposes: (1) to chaperone each newly manufactured neural cell in its journey from the cell mill through the nanocatheter into the brain, and (2) upon arrival in the brain, to physically reposition those replacement neural cells as desired within brain tissue voids or other spaces that have previously been cleared of unwanted cellular or biomaterial detritus.

The process of cell insertion begins as each manufactured cell is picked up by two or more shepherd nanorobots at the cell mill export depot (Section 4.2.6) and carried into, through, and out of a nanocatheter, inserting each prefabricated neural cell into the neuropil in the correct location and orientation (image, right)<sup>1463</sup> needed to construct the desired neurovascular units and cortical columns in accordance with the brain repair plan. Shepherd nanorobots are capable of locomotion through extracellular matrix and tissue spaces,<sup>1464</sup> carrying a cargo.



Some basic mission quantification: A nanocatheter array for the cell insertion mission might consist of  $N_{\text{cath}} = 10,000$  individually-positioned nanocatheters of diameter  $D_{\text{cath}} = 100 \mu\text{m}$  inserted in a 3D pattern throughout the brain such that the active tip of each nanocatheter is responsible for a service volume of  $V_{\text{service}} = 100 \text{ mm}^3$  into which it alone will deliver neural cells and shepherd nanorobots. Within this service volume, we assume that each active tip must visit and make deliveries of cells and nanorobots into  $V_{\text{voxel}} = 1 \text{ mm}^3$  tissue voxels, each of which may incorporate up to tens of thousands of neurons with overlapping dendritic arbors and ultimately may also receive up to tens of thousands of axonal projections whose arbors also overlap.<sup>1465</sup> With an average distance between neighboring voxels of  $d_{\text{voxel}} = V_{\text{voxel}}^{1/3} = 1 \text{ mm/voxel}$ , an ability to maneuver the nanocatheter active delivery tip through the neuropil at an assumed speed of  $v_{\text{tip}}$

<sup>1463</sup> Kasthuri N, Hayworth KJ, Berger DR, Schalek RL, Conchello JA, Knowles-Barley S, Lee D, Vázquez-Reina A, Kaynig V, Jones TR, Roberts M, Morgan JL, Tapia JC, Seung HS, Roncal WG, Vogelstein JT, Burns R, Sussman DL, Priebe CE, Pfister H, Lichtman JW. Saturated Reconstruction of a Volume of Neocortex. *Cell*. 2015 Jul 30;162(3):648-61; <http://seunglab.org/wp-content/uploads/2015/09/saturatedreconstruction.pdf>.

<sup>1464</sup> Freitas RA Jr. *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown, TX, 1999, Section 9.4.4 “Histonatation”; <http://www.nanomedicine.com/NMI/9.4.4.htm>.

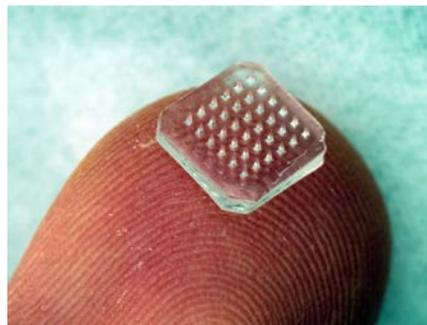
<sup>1465</sup> Shepherd, GM. *The Synaptic Organization of the Brain*, 3rd edition, Oxford University Press, New York, 1990; <http://www.amazon.com/Synaptic-Organization-Brain-Gordon-Shepherd/dp/019515956X>.

$\sim 1 \mu\text{m}/\text{sec}$  ( $>1000$ -fold slower than the estimated  $\sim 2300 \mu\text{m}/\text{sec}$  nanocatheter buckling velocity; Section 4.3.5) implies a dwell time within each voxel of  $t_{\text{voxel}} = d_{\text{voxel}} / v_{\text{tip}} = 1000 \text{ sec}/\text{voxel}$  (to complete deliveries of all cells and nanorobots targeted for that one tissue voxel), and a total service delivery time for the nanocatheter active tip to visit all voxels in its service volume of  $t_{\text{service}} = (t_{\text{voxel}}) (V_{\text{service}}) / (V_{\text{voxel}}) = 100,000 \text{ sec}$  (27.8 hr).

Although 1000 sec/voxel are allotted to complete deliveries of all cells and nanorobots targeted for each individual tissue voxel, subsequent cell repositioning and final cell emplacement does not require the presence of the delivering nanocatheter and could take much longer if necessary, mediated by the shepherd nanorobots, and still complete the emplacement task in all tissue voxels within the total service volume delivery time of  $\sim 1$  day as estimated above. For example, a shepherd nanorobot that is emplacing a cell and is towing a neuron or its axonal process a distance  $d_{\text{voxel}}$  across an entire voxel at a tissue transit speed of  $v_{\text{nanorobot}} \sim 1 \mu\text{m}/\text{sec}$  would require a towing time of  $t_{\text{towing}} = d_{\text{voxel}} / v_{\text{nanorobot}} = 1000 \text{ sec}$  (0.3 hr). After task completion, a shepherd nanorobot seeking exit from the brain tissue can migrate to a common retrieval site (e.g., the nanocatheter's last stop before being retracted from the brain) located at most  $x_{\text{retrieval}} = V_{\text{service}}^{1/3} = 4.64 \text{ mm}$  across the entire width of an individual nanocatheter's service volume in a migration time of at most  $t_{\text{migrate}} = x_{\text{retrieval}} / v_{\text{nanorobot}} = 4640 \text{ sec}$  ( $\sim 1.3$  hr).

If all 10,000 nanocatheters are inserted simultaneously and must travel an average of  $x_{\text{depth}} = 0.5V_{\text{brain}}^{1/3} = 5.74 \text{ cm}$  into the brain (taking brain volume as  $V_{\text{brain}} = 1510 \text{ cm}^3$ ), and conservatively assuming the nanocatheter tips must be pushed through brain tissue at only  $v_{\text{nanocath}} \sim 1 \mu\text{m}/\text{sec}$ , then the nanocatheter insertion time is  $t_{\text{insert}} = x_{\text{depth}} / v_{\text{nanocath}} = 57,400 \text{ sec}$  (15.9 hr). We assume that nanorobot collection at the retrieval site and subsequent nanocatheter removal from the brain can be done at much higher speed without causing tissue damage, e.g.,  $t_{\text{retract}} \sim 1000 \text{ sec}$  (0.3 hr). Thus the entire cell insertion/emplacement mission can be completed in a time period on the order of  $t_{\text{mission}} = t_{\text{insert}} + t_{\text{service}} + t_{\text{towing}} + t_{\text{migrate}} + t_{\text{retract}} \sim 45.6 \text{ hr}$  (**1.9 days**).

The nanocatheter array and nanorobot fleet are not unduly intrusive in the brain. For areal intrusiveness, taking the brain as roughly spherical with exterior surface area  $A_{\text{brain}} \sim (4\pi)^{1/3} (3V_{\text{brain}})^{2/3} = 636.5 \text{ cm}^2$  with only the top hemisphere of available for penetration by the nanocatheter array, then the distance between adjacent nanocatheters penetrating the surface of the brain is  $d_{\text{nanocath}} = [A_{\text{brain}} / (2 N_{\text{cath}})]^{1/2} = 1.78 \text{ mm}$ . That's equivalent to  $\sim 31$  nanocatheters/ $\text{cm}^2$ , much like today's very low-density microneedle arrays (image, right)<sup>1466</sup> and almost 1000 times less densely packed than the densest microneedle arrays currently available (e.g., 18,750 microneedles/ $\text{cm}^2$ ; Section 4.3.5). The cross-sectional area of each nanocatheter is  $A_{\text{nanocath}} = \pi (D_{\text{cath}} / 2)^2 = 7853 \mu\text{m}^2$ , so the fraction of exterior brain upper-hemisphere surface area occupied by the entire nanocatheter array is a modest  $N_{\text{cath}} A_{\text{nanocath}} / 0.5A_{\text{brain}} = \mathbf{0.25\%}$ . Regarding volumetric intrusiveness, the volume of each nanocatheter is



<sup>1466</sup> Shana Leonard, "Dissolving microneedles could eliminate use of sharps, spreading of disease," QMed MedTech Pulse Blog, 23 Jul 2010; <http://www.qmed.com/mpmn/medtechpulse/dissolving-microneedles-could-eliminate-use-sharps-spreading-disease>.

$V_{\text{nanocath}} = x_{\text{depth}} A_{\text{nanocath}} = 0.45 \text{ mm}^3$ , so the fraction of brain volume occupied by the entire fully-inserted nanocatheter array is only  $N_{\text{cath}} V_{\text{nanocath}} / V_{\text{brain}} = \mathbf{0.30\%}$ . If we assume that: (1) nanorobot volume is  $V_{\text{nanorobot}} = 69 \mu\text{m}^3$  (the same as chromalloyocytes; Section 4.2.3), (2)  $n_{\text{bot}} = 2$  shepherd nanorobots are required per emplaced brain cell, (3) there are  $N_{\text{braincells}} = 170.7 \times 10^9$  neural cells in the adult male brain (Section 5.2.6), and (4) we must emplace  $f_{\text{replace}} = 20\%$  of all brain cells in a severely damaged AD brain, then the mission includes  $N_{\text{nanorobot}} = n_{\text{bot}} f_{\text{replace}} N_{\text{braincells}} = 68.28 \times 10^9$  shepherd nanorobots of total volume  $V_{\text{fleet}} = N_{\text{nanorobot}} V_{\text{nanorobot}} = 4.71 \text{ cm}^3$ , so the fraction of brain volume occupied by the entire inserted nanorobotic fleet is only  $V_{\text{fleet}} / V_{\text{brain}} = \mathbf{0.31\%}$ . All of these intrusiveness figures are well within the **~1-10% safety limits** previously estimated elsewhere.<sup>1467</sup>

The emplacement process will be accomplished using manufacturing systems analogous to additive 3D printing, but employing nanorobots to achieve dexterous final emplacement rather than a static nozzle-jet.<sup>1468</sup> Shepherd nanorobots will unfurl and position neuron processes as each cell arrives, packing each one into place in approximately the desired location and orientation indicated by the repair plan (**Figure 27**). Non-neuron cells such as astrocytes and microglia, along with some elements of the brain ECM, might also be included in this procedure. The fact that microglia in the healthy brain are highly motile, actively surveying the brain parenchyma,<sup>1469</sup> provides reassurance that shepherd nanorobots should be capable of similar in-tissue mobility even within the tightly-packed tissues of the brain.

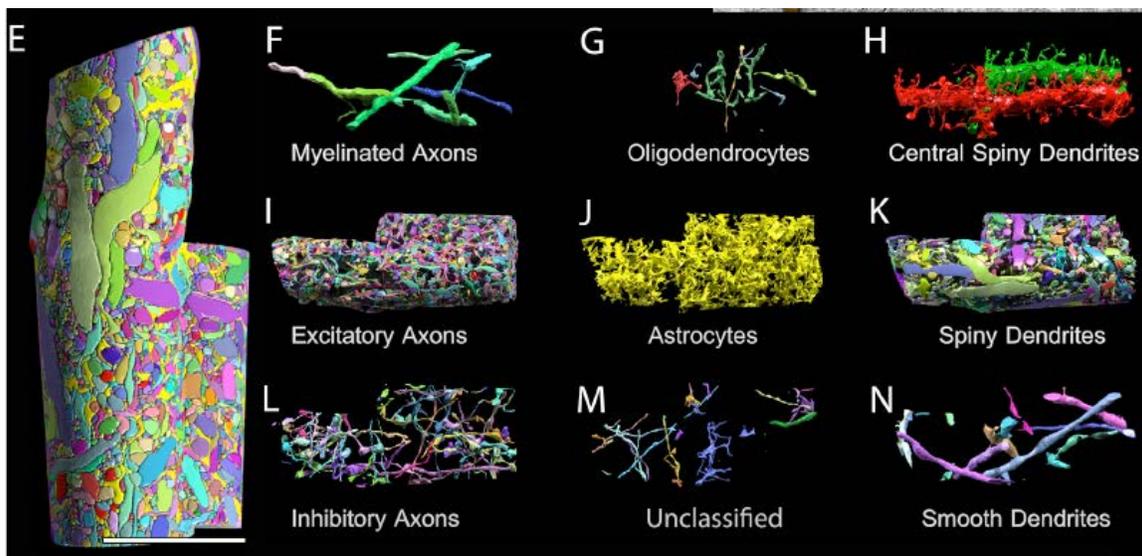
---

<sup>1467</sup> Freitas RA Jr. Nanomedicine, Volume IIA: Biocompatibility. Landes Bioscience, Georgetown, TX, 2003, Section 15.6.1 "Somatic Intrusiveness"; <http://www.nanomedicine.com/NMIIA/15.6.1.htm>.

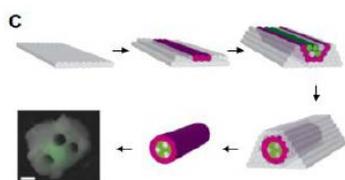
<sup>1468</sup> Mandrycky C, Wang Z, Kim K, Kim DH. 3D bioprinting for engineering complex tissues. *Biotechnol Adv.* 2015 Dec 23. pii: S0734-9750(15)30066-5; [https://www.researchgate.net/profile/Zongjie\\_Wang/publication/288040709\\_3D\\_Bioprinting\\_for\\_Engineering\\_Complex\\_Tissues/links/5684bb4b08aebccc4e0fecal.pdf](https://www.researchgate.net/profile/Zongjie_Wang/publication/288040709_3D_Bioprinting_for_Engineering_Complex_Tissues/links/5684bb4b08aebccc4e0fecal.pdf).

<sup>1469</sup> Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. *Science*. 2005 May 27;308(5726):1314-8; <http://science.sciencemag.org/content/308/5726/1314.long>. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, Gan WB. ATP mediates rapid microglial response to local brain injury *in vivo*. *Nat Neurosci*. 2005 Jun;8(6):752-8; <http://www.med.nyu.edu/skirball-lab/dustinlab/pdfs/davalos2005-NN.pdf>.

Figure 27. Multiscale reconstruction of generic cortical neuronal somata for mouse brain: (E) small section of the entire cytoarchitectural map (scale bar = 7  $\mu\text{m}$ ); (F-N) the “parts list” comprising the map (different scale).<sup>1470</sup>



As an analogous proof of principle, simple bioprinting of nerve tissue has been preliminarily explored by researchers. Large synthetic tissues must be integrated with the host nervous system, but 3D bioprinting is already considered as a means to generate new nerve tissue or to enhance the innervation of tissue engineered constructs.

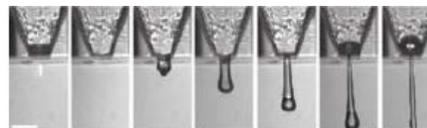


For example, one research group printed a synthetic nerve graft using cells alone.<sup>1471</sup> In this early work, isolated mouse bone marrow stem cells and Schwann cells were cast into 500  $\mu\text{m}$  diameter tubes and then loaded into a bioprinter which extruded discrete tubes to form a dense nerve conduit of Schwann cell tubes surrounded by mouse bone marrow stem cell tubes for use in animal studies (image, above). These early-stage proof-of-principle printed grafts performed similarly to control tissues and remain promising as the methodology is refined and improved. Another research group provided important validation on the feasibility of printing cells of the

<sup>1470</sup> Kasthuri N, Hayworth KJ, Berger DR, Schalek RL, Conchello JA, Knowles-Barley S, Lee D, Vázquez-Reina A, Kaynig V, Jones TR, Roberts M, Morgan JL, Tapia JC, Seung HS, Roncal WG, Vogelstein JT, Burns R, Sussman DL, Priebe CE, Pfister H, Lichtman JW. Saturated Reconstruction of a Volume of Neocortex. *Cell*. 2015 Jul 30;162(3):648-61; <http://seunglab.org/wp-content/uploads/2015/09/saturatedreconstruction.pdf>.

<sup>1471</sup> Owens CM, Marga F, Forgacs G, Heesch CM. Biofabrication and testing of a fully cellular nerve graft. *Biofabrication*. 2013 Dec;5(4):045007; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4007150/>.

nervous system, showing that rat retinal ganglion cells and glia could be used in inkjet printing systems (image, right).<sup>1472</sup>



3D bioprinting with stem cells has been demonstrated experimentally.<sup>1473</sup> Artificial tissues can be seeded by printing stem cells,<sup>1474</sup> including embryonic stem cells,<sup>1475</sup> for further differentiation. Extrusion-based 3D bioprinting technology has been used for biofabricating embryonic stem cells into a 3D cell-laden construct, printing a grid-like 3D structure laden with stem cells.<sup>1476</sup>

Another more conventional approach is an effort to create lab-grown neural networks, called micro-tissue engineered neural networks (micro-TENNS), which are hoped to have the ability to replace lost axonal tracks in the brains of patients with severe head injuries, strokes, or neurodegenerative diseases while being safely delivered with minimal disruption to brain tissue.<sup>1477</sup> In this experiment, “the micro-TENNS formed synaptic connections to existing neural networks in the cerebral cortex and the thalamus – involved in sensory and motor processing –

<sup>1472</sup> Lorber B, Hsiao WK, Hutchings IM, Martin KR. Adult rat retinal ganglion cells and glia can be printed by piezoelectric inkjet printing. *Biofabrication*. 2014 Mar;6(1):015001; <http://www.3d4surgeons.com/ganglion-cells-printing.pdf>.

<sup>1473</sup> “3D-Printed Human Embryonic Stem Cells Created for First Time,” 5 Feb 2013; <http://www.livescience.com/26865-3d-printed-embryonic-stem-cells.html>. “Scientists have found a way to 3D-print embryonic stem cell ‘building blocks’,” 9 Nov 2015; <http://www.sciencealert.com/scientists-have-found-a-way-to-3d-print-embryonic-stem-cell-building-blocks>.

<sup>1474</sup> Gruene M, Pflaum M, Deiwick A, Koch L, Schlie S, Unger C, Wilhelmi M, Haverich A, Chichkov BN. Adipogenic differentiation of laser-printed 3D tissue grafts consisting of human adipose-derived stem cells. *Biofabrication*. 2011 Mar;3(1):015005; <http://www.ncbi.nlm.nih.gov/pubmed/21358040>. Duarte Campos DF, Blaeser A, Weber M, Jäkel J, Neuss S, Jahnen-Dechent W, Fischer H. Three-dimensional printing of stem cell-laden hydrogels submerged in a hydrophobic high-density fluid. *Biofabrication*. 2013 Mar;5(1):015003; <http://www.ncbi.nlm.nih.gov/pubmed/23172592>. Hong S, Song SJ, Lee JY, Jang H, Choi J, Sun K, Park Y. Cellular behavior in micropatterned hydrogels by bioprinting system depended on the cell types and cellular interaction. *J Biosci Bioeng*. 2013 Aug;116(2):224-30; <http://www.ncbi.nlm.nih.gov/pubmed/23562089>. Owens CM, Marga F, Forgacs G, Heesch CM. Biofabrication and testing of a fully cellular nerve graft. *Biofabrication*. 2013 Dec;5(4):045007; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4007150/>.

<sup>1475</sup> Xu F, Sridharan B, Wang S, Gurkan UA, Syverud B, Demirci U. Embryonic stem cell bioprinting for uniform and controlled size embryoid body formation. *Biomicrofluidics*. 2011 Jun;5(2):22207; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3145232/>.

<sup>1476</sup> Ouyang L, Yao R, Mao S, Chen X, Na J, Sun W. Three-dimensional bioprinting of embryonic stem cells directs highly uniform embryoid body formation. *Biofabrication* 2015;7:044101; <http://iopscience.iop.org/article/10.1088/1758-5090/7/4/044101>.

<sup>1477</sup> Struzyna LA, Wolf JA, Mietus CJ, Adewole DO, Chen HI, Smith DH, Cullen DK. Rebuilding brain circuitry with living micro-tissue engineered neural networks. *Tissue Eng Part A*. 2015 Nov;21(21-22):2744-56; [http://www.med.upenn.edu/cullenlab/user\\_documents/TEA.pdf](http://www.med.upenn.edu/cullenlab/user_documents/TEA.pdf).

and maintained their axonal architecture for several weeks to structurally emulate long-distance axon connections.”

When all neural, stem, and appurtenant cells and gross ECM brain structures have been properly emplaced and positioned by nanorobots in the AD patient’s brain in approximately the correct positions as specified by the neural repair plan, and when the endothelial cells lining the walls of all leaky capillaries have been refurbished or replaced (possibly with the assistance of vasculocytes,<sup>1478</sup> support nanorobots such as respirocytes, and related nanorobots capable of controlled cell transport through vascular spaces), the shepherd nanorobots can migrate to predetermined retrieval sites to be collected and exported out of the brain, through the nanocatheter array. The injury suppression nanorobots are similarly removed after reversing the effects of their suppression activities. All nanocatheters are then retracted from the brain, completing the cell insertion and emplacement process.

---

<sup>1478</sup> Freitas RA Jr. “Chapter 23. Comprehensive Nanorobotic Control of Human Morbidity and Aging,” in Gregory M. Fahy, Michael D. West, L. Stephen Coles, and Steven B. Harris, eds, *The Future of Aging: Pathways to Human Life Extension*, Springer, New York, 2010, pp. 685-805; Section 6.2.3 “Heart and Vascular Disease”; <http://www.nanomedicine.com/Papers/Aging.pdf>.

### 5.3.2.4 Neural Incorporation

At this point in the reconstruction process, all necessary neural, stem, and non-neural cells are present in the patient's brain, occupying approximately the positions and at least partial branching structures which they either used to have, or which they plausibly might have had, before those parts of the patient's brain were destroyed by the advance of AD. Existing neural cells are rejuvenated, and all new cells are primed for maximum growth potential (i.e., with the appropriate neonatal- and juvenile-active gene circuits engaged) during their manufacture in the cell mill. A few remaining nanorobots provide logistical support and can encourage controlled synaptogenesis, axonal growth, and stem cell differentiation while the patient is subjected to an intense barrage of sensory inputs representing the memories comprising the patient's missing life history (Section 5.3.3). This allows normal memory-reinforcing cognitive processes to provide continuous network retraining of the newly installed neural cells, in concert with existing cells.

The key to successful neural reconstruction includes (1) synaptogenesis, (2) axonal growth and extension, and (3) microglia and neuroplasticity, as discussed at length in this Section.

#### Synaptogenesis

Synaptogenesis is the formation of synapses between neurons in the nervous system. Studies of postnatal synaptic development in human prefrontal cortex have shown that synaptic density rises after birth, reaches a plateau in childhood, and then decreases to adult levels by late adolescence.<sup>1479</sup> Postnatal cortical synaptic development is characterized by stages of “exuberant synaptogenesis”<sup>1480</sup> or “synaptic exuberance”,<sup>1481</sup> followed by synaptic pruning during adolescence, and finally stabilization during adulthood. Modest rates of synaptic growth persist in adulthood, but this is counterbalanced by increasing rates of synaptic elimination, resulting in stable synaptic number and a slow ongoing synaptic turnover in the human adult cortex.<sup>1482</sup> Exactly how gene expression orchestrates these stages of synaptic development is currently under detailed investigation.<sup>1483</sup> Beyond their genetic determination, the major mechanism for

---

<sup>1479</sup> Glantz LA, Gilmore JH, Hamer RM, Lieberman JA, Jarskog LF. Synaptophysin and postsynaptic density protein 95 in the human prefrontal cortex from mid-gestation into early adulthood. *Neuroscience*. 2007 Nov 9;149(3):582-91; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2128709/>.

<sup>1480</sup> Huttenlocher PR, Dabholkar AS. Regional differences in synaptogenesis in human cerebral cortex. *J Comp Neurol*. 1997 Oct 20;387(2):167-78; <https://www.ncbi.nlm.nih.gov/pubmed/9336221>.

<sup>1481</sup> Eckenhoff MF, Rakic P. A quantitative analysis of synaptogenesis in the molecular layer of the dentate gyrus in the rhesus monkey. *Brain Res Dev Brain Res*. 1991 Dec 17;64(1-2):129-35; <https://www.ncbi.nlm.nih.gov/pubmed/1786637>.

<sup>1482</sup> Goyal MS, Raichle ME. Gene expression-based modeling of human cortical synaptic density. *Proc Natl Acad Sci U S A*. 2013 Apr 16;110(16):6571-6; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3631628/>.

<sup>1483</sup> Kang HJ, *et al.* Spatiotemporal transcriptome of the human brain. *Nature*. 2011 Oct 26; 478(7370): 483-9; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3566780/>. Goyal MS, Raichle ME. *Gene*

generating diversity of neuronal connections and a specific wiring pattern is the activity-dependent stabilization and selective elimination of the initially overproduced synapses.<sup>1484</sup> The objective of the Third Alzheimer Protocol is to recapitulate this process on an accelerated basis.

Beyond artificially activated genes known to be involved in neurite and synaptic growth<sup>1485</sup> during neural cell manufacture in cell mills (Section 4.2.6), local application of a high concentration (1 mM) of N-methyl-D-aspartate (**NMDA**) causes cortical neural cells to respond to NMDA receptor<sup>1486</sup> activation with an increase in mRNA for genes (BDNF, VGF, NARP, and IEGs such as Ania-3) known to be involved in synaptogenesis.<sup>1487</sup> The majority of these genes are still upregulated after 24 hr even following a brief (10 min) activation of NMDA receptors, indicating that a short event is enough to activate intracellular signal transduction pathways leading to gene transcription. Another study found that exposure to 10 nM of **estradiol** for 48 hr mediates subcellular changes of synaptic proteins to induce new synapses in the hippocampus (a brain area important for learning and memory) via an estrogen-receptor-mediated process; treatment with **PPT**, an estrogen-receptor-specific agonist, also induces significant increases in synapse density that mimic treatment with estradiol.<sup>1488</sup> As yet another example, brain-derived neurotrophic factor (**BDNF**) directly promotes synaptic plasticity, neurite growth, glutamate dependent spine and dendritic formation and disassembly,<sup>1489</sup> axonal filopodia formation,<sup>1490</sup> synaptic growth (synaptogenesis) and possibly synaptic repair.<sup>1491</sup>

expression-based modeling of human cortical synaptic density. Proc Natl Acad Sci U S A. 2013 Apr 16;110(16):6571-6; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3631628/>.

<sup>1484</sup> Changeux JP, Danchin A. Selective stabilisation of developing synapses as a mechanism for the specification of neuronal networks. Nature. 1976 Dec 23-30;264(5588):705-12; [https://books.google.com/books?hl=en&lr=&id=AqnzXFRU\\_BoC&oi=fnd&pg=PA229&ots=hQxwNRriS u&sig=4WvrEQZouQnYzFVChvAcKZ263Tw](https://books.google.com/books?hl=en&lr=&id=AqnzXFRU_BoC&oi=fnd&pg=PA229&ots=hQxwNRriS u&sig=4WvrEQZouQnYzFVChvAcKZ263Tw).

<sup>1485</sup> e.g., HOMER1, MAP1A, CAMK2A, SYP, SYN1, BAIAP2.

<sup>1486</sup> The NMDA receptor is a glutamate receptor and ion channel protein found in nerve cells that is activated when glutamate (an excitatory neurotransmitter) and either glycine or D-serine bind to it. When activated, the receptor allows positively charged ions to flow through the cell membrane. The NMDA receptor is very important for controlling synaptic plasticity and memory function; see [https://en.wikipedia.org/wiki/NMDA\\_receptor](https://en.wikipedia.org/wiki/NMDA_receptor).

<sup>1487</sup> Ghiani CA, Beltran-Parrazal L, Sforza DM, Malvar JS, Seksenyan A, Cole R, Smith DJ, Charles A, Ferchmin PA, de Vellis J. Genetic program of neuronal differentiation and growth induced by specific activation of NMDA receptors. Neurochem Res. 2007 Feb;32(2):363-76; [http://www.researchgate.net/profile/Cristina\\_Ghiani/publication/6609713\\_Genetic\\_program\\_of\\_neuronal\\_differentiation\\_and\\_growth\\_induced\\_by\\_specific\\_activation\\_of\\_NMDA\\_receptors/links/0fcfd50a3f2cb6d6b9000000.pdf](http://www.researchgate.net/profile/Cristina_Ghiani/publication/6609713_Genetic_program_of_neuronal_differentiation_and_growth_induced_by_specific_activation_of_NMDA_receptors/links/0fcfd50a3f2cb6d6b9000000.pdf).

<sup>1488</sup> Jelks KB, Wylie R, Floyd CL, McAllister AK, Wise P. Estradiol targets synaptic proteins to induce glutamatergic synapse formation in cultured hippocampal neurons: critical role of estrogen receptor-alpha. J Neurosci. 2007 Jun 27;27(26):6903-13; <http://www.jneurosci.org/content/27/26/6903.long>.

<sup>1489</sup> Yoshii A, Constantine-Paton M. Postsynaptic BDNF-TrkB signaling in synapse maturation, plasticity, and disease. Dev Neurobiol. 2010 Apr;70(5):304-22; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2923204/>.

Networks of signaling molecules called **morphogens** govern the pattern of tissue development and the positions of the various specialized cell types within a tissue, acting directly on cells to produce specific cellular responses depending on local concentration. In particular, the secreted glycoprotein **Wingless-Int (Wnt)** family of embryonic morphogens contribute to early neural pattern formation in the developing embryo, but recent work has demonstrated that the Wnt family has roles in the later development of synapse formation and plasticity in the central nervous system, including the differentiation of synaptic specializations, microtubule dynamics, architecture of synaptic protein organization, modulation of synaptic efficacy, and regulation of gene expression. **Wnt** morphogens mediate the synaptic changes induced by patterned neuronal activity or sensory experience in mature neurons, as for example:

- **Axon Remodeling.** In the mouse cerebellum (a brain region whose three main neuronal cell types include Purkinje cells, granule cells and mossy fiber cells), **Wnt7a** regulates axon terminal remodeling and synaptic assembly.<sup>1492</sup> Mossy fiber axons form terminal and en passant synapses with cerebellar granule cell dendrites resulting in the assembly of a complex multisynaptic structure.<sup>1493</sup> Axon remodeling is characterized by the formation of large growth cones and lamellipodia in the axon shaft, a process that is linked to the formation of synaptic boutons. Loss and gain of function studies in mice demonstrate that **Wnt7a** acts as a retrograde signal to regulate the remodeling of mossy fibers and synaptic assembly.<sup>1494</sup> Wnt morphogens decrease growth cone translocation but increase growth cone size and branching.<sup>1495</sup> Granule

<sup>1490</sup> Menna E, Disanza A, Cagnoli C, Schenk U, Gelsomino G, Frittoli E, Hertzog M, Offenhauser N, Sawallisch C, Kreienkamp HJ, Gertler FB, Di Fiore PP, Scita G, Matteoli M. Eps8 regulates axonal filopodia in hippocampal neurons in response to brain-derived neurotrophic factor (BDNF). *PLoS Biol.* 2009 Jun 30;7(6):e1000138; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2696597/>.

<sup>1491</sup> Lu B, Nagappan G, Guan X, Nathan PJ, Wren P. BDNF-based synaptic repair as a disease-modifying strategy for neurodegenerative diseases. *Nat Rev Neurosci.* 2013 Jun;14(6):401-16; [https://www.researchgate.net/profile/Pradeep\\_Nathan/publication/236837118\\_BDNF-based\\_synaptic\\_repair\\_as\\_a\\_disease-modifying\\_strategy\\_for\\_neurodegenerative\\_diseases/links/00b4952fe85ff3e70a000000.pdf](https://www.researchgate.net/profile/Pradeep_Nathan/publication/236837118_BDNF-based_synaptic_repair_as_a_disease-modifying_strategy_for_neurodegenerative_diseases/links/00b4952fe85ff3e70a000000.pdf).

<sup>1492</sup> Hall AC, Lucas FR, Salinas PC. Axonal remodeling and synaptic differentiation in the cerebellum is regulated by WNT-7a signaling. *Cell.* 2000 Mar 3;100(5):525-35; [http://www.cell.com/cell/pdf/S0092-8674\(00\)80689-3.pdf](http://www.cell.com/cell/pdf/S0092-8674(00)80689-3.pdf).

<sup>1493</sup> Hámori J, Somogyi J. Differentiation of cerebellar mossy fiber synapses in the rat: a quantitative electron microscope study. *J Comp Neurol.* 1983 Nov 10;220(4):365-77; <http://www.ncbi.nlm.nih.gov/pubmed/6643733/>.

<sup>1494</sup> Hall AC, Lucas FR, Salinas PC. Axonal remodeling and synaptic differentiation in the cerebellum is regulated by WNT-7a signaling. *Cell.* 2000 Mar 3;100(5):525-35; [http://www.cell.com/cell/pdf/S0092-8674\(00\)80689-3.pdf](http://www.cell.com/cell/pdf/S0092-8674(00)80689-3.pdf).

<sup>1495</sup> Purro SA, Ciani L, Hoyos-Flight M, Stamatakou E, Siomou E, Salinas PC. Wnt regulates axon behavior through changes in microtubule growth directionality: a new role for adenomatous polyposis coli. *J Neurosci.* 2008 Aug 20;28(34):8644-54; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2832753/>.

cells express Wnt7a to promote axon spreading and branching in their synaptic partner (mossy fiber cells), and retrograde secretion of Wnt7a to mossy fiber cells causes growth cone enlargement by spreading microtubules.<sup>1496</sup>

• **Synaptic Assembly.** Wnt morphogens promote the assembly of central synapses by stimulating the recruitment of pre and postsynaptic components, and can both destabilize synapses and enhance synaptogenesis.<sup>1497</sup> Wnt7a stimulates the recruitment of synaptic vesicles, active zone proteins and the formation of numerous presynaptic recycling sites.<sup>1498</sup> In addition, other Wnt morphogens like Wnt5a preferentially stimulate postsynaptic assembly<sup>1499</sup> suggesting that different Wnts might regulate synapse formation by promoting either presynaptic or postsynaptic assembly. Adding Wnt7a to cultured cerebral granule cells (GCs) increased axonal spreading and branching, as well as enhancing the clustering of the synaptic vesicle protein synapsin-I, particularly in spread axonal areas or in growth cones.<sup>1500</sup> Wnt3 is expressed in Purkinje cells in the developing cerebellum during neurite outgrowth and synapse formation.<sup>1501</sup> Wnt7a retrograde signaling recruits synaptic vesicles and presynaptic proteins to the synaptic active zone; Wnt5a performs a similar function on postsynaptic granule cells, stimulating receptor assembly and clustering of the scaffolding protein PSD-95.<sup>1502</sup> Wnt7a also supports postsynaptic function and increases the density and maturity of dendritic spines.<sup>1503</sup>

---

<sup>1496</sup> Speese SD, Budnik V. Wnts: up-and-coming at the synapse. *Trends Neurosci.* 2007 Jun;30(6):268-75; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3499976/>.

<sup>1497</sup> Klassen MP, Shen K. Wnt signaling positions neuromuscular connectivity by inhibiting synapse formation in *C. elegans*. *Cell.* 2007 Aug 24;130(4):704-16; [http://www.keck.ucsf.edu/neurograd/files/ns221\\_0809\\_materials/Ullian/120208\\_paper1.pdf](http://www.keck.ucsf.edu/neurograd/files/ns221_0809_materials/Ullian/120208_paper1.pdf).

<sup>1498</sup> Ahmad-Annuar A, Ciani L, Simeonidis I, Herreros J, Fredj NB, Rosso SB, Hall A, Brickley S, Salinas PC. Signaling across the synapse: a role for Wnt and Dishevelled in presynaptic assembly and neurotransmitter release. *J Cell Biol.* 2006 Jul 3;174(1):127-39; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2064170/>.

<sup>1499</sup> Farías GG, Alfaro IE, Cerpa W, Grabowski CP, Godoy JA, Bonansco C, Inestrosa NC. Wnt-5a/JNK signaling promotes the clustering of PSD-95 in hippocampal neurons. *J Biol Chem.* 2009 Jun 5;284(23):15857-66; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2708882/>.

<sup>1500</sup> Lucas FR, Salinas PC. WNT-7a induces axonal remodeling and increases synapsin I levels in cerebellar neurons. *Dev Biol.* 1997 Dec 1;192(1):31-44; <http://www.sciencedirect.com/science/article/pii/S0012160697987340/pdf?md5=6b09816409994c72f4b084a42bb325a5&pid=1-s2.0-S0012160697987340-main.pdf>.

<sup>1501</sup> Salinas PC, Nusse R. Regional expression of the Wnt-3 gene in the developing mouse forebrain in relationship to diencephalic neuromeres. *Mech Dev.* 1992 Dec;39(3):151-60; <http://www.ncbi.nlm.nih.gov/pubmed/1363370/>. Speese SD, Budnik V. Wnts: up-and-coming at the synapse. *Trends Neurosci.* 2007 Jun;30(6):268-75; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3499976/>. Budnik V, Salinas PC. Wnt signaling during synaptic development and plasticity. *Curr Opin Neurobiol.* 2011 Feb;21(1):151-9; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3499977/>.

<sup>1502</sup> Budnik V, Salinas PC. Wnt signaling during synaptic development and plasticity. *Curr Opin Neurobiol.* 2011 Feb;21(1):151-9; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3499977/>.

• **Hippocampal Arborization.** Wnt family members promote synapse formation in conjunction with cell electrical activity. Wnt7b is expressed in maturing dendrites,<sup>1504</sup> and the expression of the Wnt7a receptor Frizzled (Fz) increases highly with synapse formation in the hippocampus, regulating activity-mediated synaptogenesis.<sup>1505</sup> NMDA glutamate receptor activation increases Wnt2 expression, with long-term potentiation (LTP) due to NMDA activation and subsequent Wnt expression leading to Fz5 localization at the postsynaptic active zone.<sup>1506</sup> Wnt7a and Wnt2 signaling after NMDA receptor mediated LTP leads to increased dendritic arborization and regulates activity induced synaptic plasticity.<sup>1507</sup>

• **Activity-Dependent Synaptic Plasticity.** Neuronal activity plays a key role in several aspects of neuronal circuit generation by modulating synapse structure and function. A proper balance between synapse assembly and disassembly is crucial for the formation of functional neuronal circuits and synaptic plasticity in the adult brain.<sup>1508</sup> Recent studies<sup>1509</sup> have suggested

<sup>1503</sup> Ciani L, Boyle KA, Dickins E, Sahores M, Anane D, Lopes DM, Gibb AJ, Salinas PC. Wnt7a signaling promotes dendritic spine growth and synaptic strength through Ca<sup>2+</sup>/Calmodulin-dependent protein kinase II. *Proc Natl Acad Sci U S A*. 2011 Jun 28;108(26):10732-7; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3127879/>.

<sup>1504</sup> Speese SD, Budnik V. Wnts: up-and-coming at the synapse. *Trends Neurosci*. 2007 Jun;30(6):268-75; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3499976/>.

<sup>1505</sup> Sahores M, Gibb A, Salinas PC. Frizzled-5, a receptor for the synaptic organizer Wnt7a, regulates activity-mediated synaptogenesis. *Development*. 2010 Jul;137(13):2215-25; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2882138/>. Budnik V, Salinas PC. Wnt signaling during synaptic development and plasticity. *Curr Opin Neurobiol*. 2011 Feb;21(1):151-9; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3499977/>.

<sup>1506</sup> Sahores M, Gibb A, Salinas PC. Frizzled-5, a receptor for the synaptic organizer Wnt7a, regulates activity-mediated synaptogenesis. *Development*. 2010 Jul;137(13):2215-25; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2882138/>.

<sup>1507</sup> Park M, Shen K. WNTs in synapse formation and neuronal circuitry. *EMBO J*. 2012 Jun 13;31(12):2697-704; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3380216/>.

<sup>1508</sup> Dickins EM, Salinas PC. Wnts in action: from synapse formation to synaptic maintenance. *Front Cell Neurosci*. 2013 Nov 5;7:162; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3819050/>.

<sup>1509</sup> Chen J, Park CS, Tang SJ. Activity-dependent synaptic Wnt release regulates hippocampal long term potentiation. *J Biol Chem*. 2006 Apr 28;281(17):11910-6; <http://www.jbc.org/content/281/17/11910.long>. Wayman GA, Impey S, Marks D, Saneyoshi T, Grant WF, Derkach V, Soderling TR. Activity-dependent dendritic arborization mediated by CaM-kinase I activation and enhanced CREB-dependent transcription of Wnt-2. *Neuron*. 2006 Jun 15;50(6):897-909; <https://courses.cit.cornell.edu/bionb4110/2012/secure/Reprints/Activity%20dependent%20dendritic%20arborization%20Neuron%202006.pdf>. Beaumont V, Thompson SA, Choudhry F, Nuthall H, Glantschnig H, Lipfert L, David GR, Swain CJ, McAllister G, Munoz-Sanjuan I. Evidence for an enhancement of excitatory transmission in adult CNS by Wnt signaling pathway modulation. *Mol Cell Neurosci*. 2007 Aug;35(4):513-24; <http://www.ncbi.nlm.nih.gov/pubmed/17588772>. Ataman B, Ashley J, Gorczyca M, Ramachandran P, Fouquet W, Sigrist SJ, Budnik V. Rapid activity-dependent modifications in synaptic

that Wnt signaling is involved in synaptic plasticity. Taken together, these findings support the idea that neuronal activity regulates Fz5 trafficking to and out of synapses, contributing to activity-mediated synapse remodeling. Enriched-environment training (Section 5.3.3) increases the level of Wnt7a and Wnt7b in postsynaptic CA3 pyramidal neurons and the complexity and number of large mossy fiber terminals in the CA3 region.<sup>1510</sup>

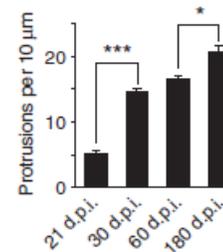
By exploiting nanorobotically-assisted neural cell gene activations and positional targeting of neurotrophic factors and morphogens in living brain tissue,<sup>1511</sup> and perhaps other means as well, we can artificially induce a temporary “exuberant synaptogenesis” phase into those sections of the AD patient’s brain tissues that incorporate the newly-emplaced cell-mill-manufactured neural cells, thus enabling easier re-learning of forgotten memories by the AD patient (Section 5.3.3) and quicker re-establishment of neural wiring patterns that may not be too dissimilar from the original ones that were destroyed during the neurodegenerative processes of Alzheimer’s disease.

structure and function require bidirectional Wnt signaling. *Neuron*. 2008 Mar 13;57(5):705-18; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2435264/>. Cerpa W, Godoy JA, Alfaro I, Fariás GG, Metcalfe MJ, Fuentealba R, Bonansco C, Inestrosa NC. Wnt-7a modulates the synaptic vesicle cycle and synaptic transmission in hippocampal neurons. *J Biol Chem*. 2008 Feb 29;283(9):5918-27; <http://www.jbc.org/content/283/9/5918.long>. Gogolla N, Galimberti I, Deguchi Y, Caroni P. Wnt signaling mediates experience-related regulation of synapse numbers and mossy fiber connectivities in the adult hippocampus. *Neuron*. 2009 May 28;62(4):510-25; <http://www.sciencedirect.com/science/article/pii/S0896627309003468>. Avila ME, Sepúlveda FJ, Burgos CF, Moraga-Cid G, Parodi J, Moon RT, Aguayo LG, Opazo C, De Ferrari GV. Canonical Wnt3a modulates intracellular calcium and enhances excitatory neurotransmission in hippocampal neurons. *J Biol Chem*. 2010 Jun 11;285(24):18939-47; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2881816/>. Sahores M, Gibb A, Salinas PC. Frizzled-5, a receptor for the synaptic organizer Wnt7a, regulates activity-mediated synaptogenesis. *Development*. 2010 Jul;137(13):2215-25; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2882138/>. Varela-Nallar L, Alfaro IE, Serrano FG, Parodi J, Inestrosa NC. Wingless-type family member 5A (Wnt-5a) stimulates synaptic differentiation and function of glutamatergic synapses. *Proc Natl Acad Sci U S A*. 2010 Dec 7;107(49):21164-9; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3000271/>. Jensen M, Hoerndli FJ, Brockie PJ, Wang R, Johnson E, Maxfield D, Francis MM, Madsen DM, Maricq AV. Wnt signaling regulates acetylcholine receptor translocation and synaptic plasticity in the adult nervous system. *Cell*. 2012 Mar 30;149(1):173-87; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3375111/>. Salinas PC. Wnt signaling in the vertebrate central nervous system: from axon guidance to synaptic function. *Cold Spring Harb Perspect Biol*. 2012 Feb 1;4(2). pii: a008003; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3281574/>. Stamatakou E, Salinas PC. Postsynaptic assembly: a role for Wnt signaling. *Dev Neurobiol*. 2014 Aug;74(8):818-27; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4237178/>.

<sup>1510</sup> Gogolla N, Galimberti I, Deguchi Y, Caroni P. Wnt signaling mediates experience-related regulation of synapse numbers and mossy fiber connectivities in the adult hippocampus. *Neuron*. 2009 May 28;62(4):510-25; <http://www.sciencedirect.com/science/article/pii/S0896627309003468>.

<sup>1511</sup> E.g., mature neurons, in response to disease, can produce morphogens that alter gene expression in astrocytes, a response that could be locally elicited or altered by nanorobots. Farmer WT, *et al.* Neurons diversify astrocytes in the adult brain through sonic hedgehog signaling. *Science* 2016 Feb 19; 351(6275):849-854; <http://science.sciencemag.org/content/351/6275/849.full>.

There is some evidence that this approach to neural restoration may have a good likelihood of success. Recently, more has become known about how new functional neurons that are continually produced in the adult brain<sup>1512</sup> are integrated into existing mature networks of older established neurons. One study<sup>1513</sup> attempted to learn more by exploring mechanisms of synaptogenesis in new neurons born into an adult mouse hippocampus. Combining data from confocal microscopy, electron microscopy, and live imaging, the researchers found that the new neurons, similar to mature granule neurons, became contacted by axosomatic, axodendritic and axospinous synapses. Consistent with their putative role in synaptogenesis, dendritic filopodia<sup>1514</sup> were more abundant during the early stages of maturation, the tips of all filopodia were found within 200 nm of preexisting boutons that already synapsed on other neurons, and dendritic spines<sup>1515</sup> primarily synapsed on multiple-synapse boutons, suggesting that initial contacts were preferentially made with pre-existing boutons already involved in a synapse. The first glutamatergic synapses were likely to appear during the first month after the arrival of the new neuron, but full maturation of the connectivity of new neurons was reached only after 60-180 days (image, right; “d.p.i.” = “days post injection”).



Similar results have been obtained for new neurons introduced into surgically innervated brain tissue. For example, transcommissural olivocerebellar axons reinnervate surgically denervated Purkinje cells (large heavily-arborized neurons in the cerebellum) in the rat cerebellum, reestablishing the original pattern of climbing fiber bands within a few days after lesioning,<sup>1516</sup> and immature Purkinje cells in the rat cerebellum can be multi-reinnervated during postlesional

<sup>1512</sup> Galvan V, Bredesen DE. Neurogenesis in the adult brain: implications for Alzheimer’s disease. *CNS Neurol Disord Drug Targets*. 2007 Oct;6(5):303-10; <http://www.ncbi.nlm.nih.gov/pubmed/18045158>.

<sup>1513</sup> Toni N, Teng EM, Bushong EA, Aimone JB, Zhao C, Consiglio A, van Praag H, Martone ME, Ellisman MH, Gage FH. Synapse formation on neurons born in the adult hippocampus. *Nat Neurosci*. 2007 Jun;10(6):727-34; [https://www.researchgate.net/profile/Nicolas\\_Toni/publication/6345504\\_Synapse\\_formation\\_on\\_neurons\\_born\\_in\\_the\\_adult\\_hippocampus/links/0912f4fabe2f56b79c000000.pdf](https://www.researchgate.net/profile/Nicolas_Toni/publication/6345504_Synapse_formation_on_neurons_born_in_the_adult_hippocampus/links/0912f4fabe2f56b79c000000.pdf).

<sup>1514</sup> **Dendritic filopodia** are small membranous protrusions found primarily on dendritic stretches of developing neurons, able to receive synaptic input and to develop into dendritic spines; [https://en.wikipedia.org/wiki/Dendritic\\_filopodia](https://en.wikipedia.org/wiki/Dendritic_filopodia).

<sup>1515</sup> **Dendritic spines** are small membranous protrusions from a neuron’s dendrite that typically receive input from a single synapse of an axon, serving as a storage site for synaptic strength and helping transmit electrical signals to the neuron’s cell body; [https://en.wikipedia.org/wiki/Dendritic\\_spine](https://en.wikipedia.org/wiki/Dendritic_spine).

<sup>1516</sup> Zagrebelsky M, Strata P, Hawkes R, Rossi F. Reestablishment of the olivocerebellar projection map by compensatory transcommissural reinnervation following unilateral transection of the inferior cerebellar peduncle in the newborn rat. *J Comp Neurol*. 1997 Mar 10;379(2):283-99; [https://www.researchgate.net/publication/227929899\\_Reestablishment\\_of\\_the\\_olivocerebellar\\_projection\\_map\\_by\\_compensatory\\_transcommissural\\_reinnervation\\_following\\_unilateral\\_transection\\_of\\_the\\_inferior\\_cerebellar\\_peduncle\\_in\\_the\\_newborn\\_rat](https://www.researchgate.net/publication/227929899_Reestablishment_of_the_olivocerebellar_projection_map_by_compensatory_transcommissural_reinnervation_following_unilateral_transection_of_the_inferior_cerebellar_peduncle_in_the_newborn_rat)

neosynaptogenesis.<sup>1517</sup> A study of embryonic nerve cells introduced into a relatively mature surgically deinnervated rat cerebellum found that many morphological characteristics of developmental synaptogenesis are recapitulated when synapse formation is induced onto a mature Purkinje cell, including single re-innervation but not multiple innervation,<sup>1518</sup> which suggests that some nanorobotically-delivered modification to the mature cells at the interface between new and old might be required to enable full multi-innervation. Stem cells grafted into the intact mouse/rat brains were mostly followed by their incorporation into the host parenchyma and differentiation into functional neural lineages, stem cells exhibiting targeted migration towards the damaged regions of lesioned brain, where they engrafted, proliferated and matured into functional neurons. Intravenously administered neural precursor cells (NPCs) migrate into brain-damaged areas and induce functional recovery, with animal models of AD providing evidence that transplanted stem cells or NPCs survive, migrate, and differentiate into cholinergic neurons, astrocytes, and oligodendrocytes with amelioration of learning/memory deficits.<sup>1519</sup>

Stem cell implants (Section 3.2.14) have also given promising results. For example, human stem cell-derived neurons can functionally integrate into rat hippocampal network and drive endogenous neuronal network activity.<sup>1520</sup> Embryonic interneuron progenitor cells that were transplanted into the hippocampal hilus of aged mice developed into mature interneurons, functionally integrated into the hippocampal circuitry, and restored normal learning, memory and cognitive function in two widely used AD-related mouse models.<sup>1521</sup> Transplanted human spinal neural stem cells integrate into the spinal cord, forming synapses with the host neurons in rats.<sup>1522</sup>

---

<sup>1517</sup> Lohof AM, Mariani J, Sherrard RM. Afferent-target interactions during olivocerebellar development: transcommissural reinnervation indicates interdependence of Purkinje cell maturation and climbing fibre synapse elimination. *Eur J Neurosci.* 2005 Dec;22(11):2681-8; [https://www.researchgate.net/profile/Rachel\\_Sherrard/publication/7445287\\_Afferent-target\\_interactions\\_during\\_olivocerebellar\\_development\\_transcommissural\\_reinnervation\\_indicates\\_interdependence\\_of\\_Purkinje\\_cell\\_maturation\\_and\\_climbing\\_fibre\\_synapse\\_elimination/links/02e7e520d1fed52eae000000.pdf](https://www.researchgate.net/profile/Rachel_Sherrard/publication/7445287_Afferent-target_interactions_during_olivocerebellar_development_transcommissural_reinnervation_indicates_interdependence_of_Purkinje_cell_maturation_and_climbing_fibre_synapse_elimination/links/02e7e520d1fed52eae000000.pdf).

<sup>1518</sup> Letellier M, Bailly Y, Demais V, Sherrard RM, Mariani J, Lohof AM. Reinnervation of late postnatal Purkinje cells by climbing fibers: neosynaptogenesis without transient multi-innervation. *J Neurosci.* 2007 May 16;27(20):5373-83; <http://www.jneurosci.org/content/27/20/5373.long>.

<sup>1519</sup> Abdel-Salam OM. Stem cell therapy for Alzheimer's disease. *CNS Neurol Disord Drug Targets.* 2011 Jun;10(4):459-85; <http://www.ncbi.nlm.nih.gov/pubmed/21495961>.

<sup>1520</sup> Piña-Crespo JC, Talantova M, Cho EG, Soussou W, Dolatabadi N, Ryan SD, Ambasudhan R, McKercher S, Deisseroth K, Lipton SA. High-frequency hippocampal oscillations activated by optogenetic stimulation of transplanted human ESC-derived neurons. *J Neurosci.* 2012 Nov 7;32(45):15837-42; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3513396/>.

<sup>1521</sup> Tong LM, Djukic B, Arnold C, Gillespie AK, Yoon SY, Wang MM, Zhang O, Knoferle J, Rubenstein JL, Alvarez-Buylla A, Huang Y. Inhibitory interneuron progenitor transplantation restores normal learning and memory in ApoE4 knock-in mice without or with A $\beta$  accumulation. *J Neurosci.* 2014 Jul 16;34(29):9506-15; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4099537/>.

<sup>1522</sup> Hefferan MP, Galik J, Kakinohana O, Sekerkova G, Santucci C, Marsala S, Navarro R, Hruska-Plochan M, Johe K, Feldman E, Cleveland DW, Marsala M. Human neural stem cell replacement therapy for

Neural stem cells have regenerated lost tissue in damaged corticospinal tracts of rats, resulting in functional benefit.<sup>1523</sup>

### Axonal Growth and Extension

The replacement neural cells should be manufactured with appropriate gene expression,<sup>1524</sup> and these and existing neurons should be situated in a biochemical environment (see below) encouraging facile axonal growth<sup>1525</sup> and extension. It was once thought that the adult mammalian central nervous system was unable to regenerate axons, but it has since been shown, for example, that adult CNS white matter can support long-distance regeneration of adult axons in the absence of glial scarring. In one experiment using a microtransplantation technique that minimized scarring, minute volumes of dissociated adult rat dorsal root ganglia were atraumatically injected directly into adult rat CNS pathways, allowing considerable numbers of regenerating adult axons immediate access to the host glial terrain, where they rapidly extended for long distances in white matter, eventually invading grey matter.<sup>1526</sup>

Neurotrophic factors promote the initial growth and development of neurons in the central nervous system and peripheral nervous system and can regrow damaged neurons in test tubes and animal models. In one study of neurotrophin nerve growth factor (NGF), glial cell-line derived neurotrophic factor (GDNF) and neurotrophic cytokine ciliary neurotrophic factor (CNTF), the combination of 50 ng/ml NGF and 10 ng/ml each of GDNF and CNTF induced a 752% increase

amyotrophic lateral sclerosis by spinal transplantation. PLoS One. 2012;7(8):e42614;  
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3423406/>.

<sup>1523</sup> Kadoya K, Lu P, Nguyen K, Lee-Kubli C, Kumamaru H, Yao L, Knackert J, Poplawski G, Dulin JN, Strobl H, Takashima Y, Biane J, Conner J, Zhang SC, Tuszynski MH. Spinal cord reconstitution with homologous neural grafts enables robust corticospinal regeneration. Nat Med. 2016 Mar 28;  
<http://www.ncbi.nlm.nih.gov/pubmed/27019328>.

<sup>1524</sup> Lindner R, Puttagunta R, Di Giovanni S. Epigenetic regulation of axon outgrowth and regeneration in CNS injury: the first steps forward. Neurotherapeutics. 2013 Oct;10(4):771-81;  
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3805867/>. Carmel JB, Young W, Hart RP. Flipping the transcriptional switch from myelin inhibition to axon growth in the CNS. Front Mol Neurosci. 2015 Jul 17;8:34; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4505142/>. Li C, Hisamoto N, Matsumoto K. Axon Regeneration Is Regulated by Ets-C/EBP Transcription Complexes Generated by Activation of the cAMP/Ca2+ Signaling Pathways. PLoS Genet. 2015 Oct 20;11(10):e1005603;  
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4618690/>.

<sup>1525</sup> Cafferty WB, McGee AW, Strittmatter SM. Axonal growth therapeutics: regeneration or sprouting or plasticity? Trends Neurosci. 2008 May;31(5):215-20;  
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2678051/>.

<sup>1526</sup> Davies SJ, Fitch MT, Memberg SP, Hall AK, Raisman G, Silver J. Regeneration of adult axons in white matter tracts of the central nervous system. Nature. 1997 Dec 18-25;390(6661):680-3;  
<https://huguenard-lab.stanford.edu/205/1999-2000/silver.pdf>.

in neurite outgrowth over untreated rat dorsal root ganglion explants and increased the longest neurite length to 2031  $\mu\text{m}$  (~2 mm) compared to 916  $\mu\text{m}$  (~1 mm) for untreated neurons.<sup>1527</sup>

There are also repulsive axonal guidance molecules that inhibit axonal growth and regeneration.<sup>1528</sup> Axon growth has been amplified in the presence of NGF-secreting grafts, with axons growing best along substrates co-expressing both inhibitory and permissive molecules, suggesting that regeneration is successful when local permissive signals balance and exceed inhibitory signals.<sup>1529</sup> (Clinical trials of NGF on Alzheimer's patients have reported some improvement in cognitive deficits,<sup>1530</sup> though at present the record is mixed.<sup>1531</sup>)

Increased intracellular cAMP levels in neurons have also been shown to induce axonal growth.<sup>1532</sup> Of course, too much axonal sprouting too fast in random directions is also undesirable, even if self-pruning occurs later.<sup>1533</sup> Excessive artificial neurogenesis can induce memory loss if existing neuronal or synaptic populations are overwhelmed by the new

---

<sup>1527</sup> Deister C, Schmidt CE. Optimizing neurotrophic factor combinations for neurite outgrowth. *J Neural Eng.* 2006 Jun;3(2):172-9; <http://www.ncbi.nlm.nih.gov/pubmed/16705273>.

<sup>1528</sup> Satoh J, Tabunoki H, Ishida T, Saito Y, Arima K. Accumulation of a repulsive axonal guidance molecule RGMa in amyloid plaques: a possible hallmark of regenerative failure in Alzheimer's disease brains. *Neuropathol Appl Neurobiol.* 2013 Feb;39(2):109-20; <http://www.ncbi.nlm.nih.gov/pubmed/22582881>.

<sup>1529</sup> Jones LL, Sajed D, Tuszynski MH. Axonal regeneration through regions of chondroitin sulfate proteoglycan deposition after spinal cord injury: a balance of permissiveness and inhibition. *J Neurosci.* 2003 Oct 15;23(28):9276-88; <http://www.jneurosci.org/content/23/28/9276.long>.

<sup>1530</sup> Ferreira D, Westman E, Eyjolfsdottir H, Almqvist P, Lind G, Linderöth B, Seiger A, Blennow K, Karami A, Darreh-Shori T, Wiberg M, Simmons A, Wahlund LO, Wahlberg L, Eriksson M. Brain changes in Alzheimer's disease patients with implanted encapsulated cells releasing nerve growth factor. *J Alzheimers Dis.* 2015;43(3):1059-72; [https://www.researchgate.net/profile/Daniel\\_Ferreira9/publication/264047939\\_Brain\\_changes\\_in\\_Alzheimer\\_patients\\_with\\_implanted\\_encapsulated\\_cells\\_releasing\\_Nerve\\_Growth\\_Factor/links/54b97c0cf253b50e2a96fa.pdf](https://www.researchgate.net/profile/Daniel_Ferreira9/publication/264047939_Brain_changes_in_Alzheimer_patients_with_implanted_encapsulated_cells_releasing_Nerve_Growth_Factor/links/54b97c0cf253b50e2a96fa.pdf).

<sup>1531</sup> Budni J, Bellettini-Santos T, Mina F, Garcez ML, Zugno AI. The involvement of BDNF, NGF and GDNF in aging and Alzheimer's disease. *Aging Dis.* 2015 Oct 1;6(5):331-41; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4567216/>.

<sup>1532</sup> Neumann S, Bradke F, Tessier-Lavigne M, Basbaum AI. Regeneration of sensory axons within the injured spinal cord induced by intraganglionic cAMP elevation. *Neuron.* 2002 Jun 13;34(6):885-93; [http://www.cell.com/neuron/pdf/S0896-6273\(02\)00702-X.pdf](http://www.cell.com/neuron/pdf/S0896-6273(02)00702-X.pdf).

<sup>1533</sup> Klimaschewski L, Hausott B, Angelov DN. The pros and cons of growth factors and cytokines in peripheral axon regeneration. *Int Rev Neurobiol.* 2013;108:137-71; <http://www.ncbi.nlm.nih.gov/pubmed/24083434>.

arrivals.<sup>1534</sup> Nanorobots can help time and maintain the ideal *in vivo* biochemical environment for axonal regeneration, growth, and extension consistent with optimal AD patient recovery.

Axons cannot normally regenerate beyond glial scars which consist predominately of reactive astrocytes and proteoglycans.<sup>1535</sup> As already noted (Section 5.3.2.2), glial scars will be targeted for elimination by the debridement nanorobots.

Autologous nerve grafting, aka. nerve autograft, is commonly used clinically to repair large lesion gaps in the peripheral nervous system. Gene-therapy induced expression of neurotrophic factors within a target muscle<sup>1536</sup> or muscle stem cells,<sup>1537</sup> or local delivery of soluble neurotrophic factors such as IGF-1 (insulin-like growth factor),<sup>1538</sup> BDNF (brain-derived neurotrophic factor),<sup>1539</sup> GDNF,<sup>1540</sup> CNTF,<sup>1541</sup> PSA on NCAM (neural cell adhesion molecule),<sup>1542</sup> or VEGF

<sup>1534</sup> Akers KG, Martinez-Canabal A, Restivo L, Yiu AP, De Cristofaro A, Hsiang HL, Wheeler AL, Guskjolen A, Niibori Y, Shoji H, Ohira K, Richards BA, Miyakawa T, Josselyn SA, Frankland PW. Hippocampal neurogenesis regulates forgetting during adulthood and infancy. *Science*. 2014 May 9;344(6184):598-602; <http://science.sciencemag.org/content/344/6184/598.full>.

<sup>1535</sup> Silver J, Miller JH. Regeneration beyond the glial scar. *Nat Rev Neurosci*. 2004 Feb;5(2):146-56; <http://www.societyns.org/runn/2008/Silver%20and%20Miller.pdf>.

<sup>1536</sup> Shiotani A, O'Malley BW Jr, Coleman ME, Alila HW, Flint PW. Reinnervation of motor endplates and increased muscle fiber size after human insulin-like growth factor I gene transfer into the paralyzed larynx. *Hum Gene Ther*. 1998 Sep 20;9(14):2039-47; <http://www.ncbi.nlm.nih.gov/pubmed/9759931>. Flint PW, Shiotani A, O'Malley BW Jr. IGF-1 gene transfer into denervated rat laryngeal muscle. *Arch Otolaryngol Head Neck Surg*. 1999 Mar;125(3):274-9; <http://archinte.jamanetwork.com/data/Journals/OTOL/13035/oa8048.pdf>.

<sup>1537</sup> Halum SL, McRae B, Bijangi-Vishehsaraei K, Hiatt K. Neurotrophic factor-secreting autologous muscle stem cell therapy for the treatment of laryngeal denervation injury. *Laryngoscope*. 2012 Nov;122(11):2482-96; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3484207/>.

<sup>1538</sup> Tiangco DA, Papakonstantinou KC, Mullinax KA, Terzis JK. IGF-I and end-to-side nerve repair: a dose-response study. *J Reconstr Microsurg*. 2001 May;17:247-56; <http://www.ncbi.nlm.nih.gov/pubmed/11396586>.

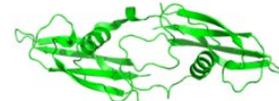
<sup>1539</sup> Pajenda G, Hercher D, Márton G, Pajer K, Feichtinger GA, Maléth J, Redl H, Nógrádi A. Spatiotemporally limited BDNF and GDNF overexpression rescues motoneurons destined to die and induces elongative axon growth. *Exp Neurol*. 2014 Nov;261:367-76; <http://www.ncbi.nlm.nih.gov/pubmed/24873730>.

<sup>1540</sup> Marquardt LM, Ee X, Iyer N, Hunter D, Mackinnon SE, Wood MD, Sakiyama-Elbert SE. Finely tuned temporal and spatial delivery of GDNF promotes enhanced nerve regeneration in a long nerve defect model. *Tissue Eng Part A*. 2015 Dec;21(23-24):2852-64; <http://www.ncbi.nlm.nih.gov/pubmed/26466815>.

<sup>1541</sup> Dubový P, Raška O, Klusáková I, Stejskal L, Celakovský P, Haninec P. Ciliary neurotrophic factor promotes motor reinnervation of the musculocutaneous nerve in an experimental model of end-to-side neurorrhaphy. *BMC Neurosci*. 2011 Jun 22;12:58; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3224149/>.

(vascular endothelial growth factor)<sup>1543</sup> – potentially by medical nanorobots – to the site of autologous nerve grafting enhances reinnervation and axon regeneration within the graft. Recovery after a spinal cord injury often requires that axons restore synaptic connectivity with denervated targets located as much as several centimeters away from the site of injury.

One recent study<sup>1544</sup> found that systemic ARTN (artemin, aka. enovin, neublastin; image, right) treatment promotes the robust regeneration of large myelinated sensory axons to the brainstem after brachial dorsal root crush in adult rats, and also promotes functional reinnervation of the appropriate target region (the cuneate nucleus). Regeneration to the brainstem requires more than 3 months but ARTN was delivered for just 2 weeks, suggesting that “brief trophic support may initiate intrinsic growth programs that remain active until targets are reached.”



### Microglia and Neuroplasticity

The formation and removal of the physical connections between neurons is a critical part of maintaining a healthy brain. The process of creating new pathways and networks among brain cells enables us to absorb, learn, and memorize new information. This constant reorganization of neural networks is called neuroplasticity. It is already known that microglia (motile cells normally associated with protecting the brain from infection and injury) dynamically interact with neuronal circuits, making direct contacts with synaptic elements in a way that is modulated by experience-dependent changes in neuronal activity.<sup>1545</sup> Microglia actively shape maturing neuronal networks within the first few weeks of life in the mouse via the complement and fractalkine systems.<sup>1546</sup> But microglial contributions to network maintenance and plasticity at

<sup>1542</sup> Gravvanis AI, Lavdas AA, Papalois A, Tsoutsos DA, Matsas R. The beneficial effect of genetically engineered Schwann cells with enhanced motility in peripheral nerve regeneration: review. *Acta Neurochir Suppl.* 2007;100:51-6; <http://www.ncbi.nlm.nih.gov/pubmed/17985545>.

<sup>1543</sup> Haninec P, Kaiser R, Bobek V, Dubový P. Enhancement of musculocutaneous nerve reinnervation after vascular endothelial growth factor (VEGF) gene therapy. *BMC Neurosci.* 2012 Jun 6;13:57; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3441459/>.

<sup>1544</sup> Wong LE, Gibson ME, Arnold HM, Pepinsky B, Frank E. Artemin promotes functional long-distance axonal regeneration to the brainstem after dorsal root crush. *Proc Natl Acad Sci U S A.* 2015 May 12;112(19):6170-5; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4434726/>.

<sup>1545</sup> Wake H, Moorhouse AJ, Jinno S, Kohsaka S, Nabekura J. Resting microglia directly monitor the functional state of synapses *in vivo* and determine the fate of ischemic terminals. *J Neurosci.* 2009 Apr 1;29(13):3974-80; <http://www.jneurosci.org/content/29/13/3974.long>. Tremblay ME, Lowery RL, Majewska AK. Microglial interactions with synapses are modulated by visual experience. *PLoS Biol.* 2010 Nov 2;8(11):e1000527; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2970556/>.

<sup>1546</sup> Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR, Yamasaki R, Ransohoff RM, Greenberg ME, Barres BA, Stevens B. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron.* 2012 May 24;74(4):691-705; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3528177/>. Zhan Y, Paolicelli RC, Sforzini F, Weinhard L, Bolasco G, Pagani F, Vyssotski AL, Bifone A, Gozzi A, Ragozzino D, Gross CT. Deficient neuron-

later developmental stages, including the basic mechanisms by which connections between brain cells are made and broken, have remained poorly understood.

Recent research<sup>1547</sup> now suggests that microglia play an even more important direct role in rewiring the connections between adult nerve cells:

Performing experiments in mice, the researchers employed a well-established model of measuring neuroplasticity by observing how cells reorganize their connections when visual information received by the brain is reduced from two eyes to one.

The researchers found that in the mice's brains microglia responded rapidly to changes in neuronal activity as the brain adapted to processing information from only one eye. They observed that the microglia targeted the synaptic cleft – the business end of the connection that transmits signals between neurons. The microglia “pulled up” the appropriate connections, physically disconnecting one neuron from another, while leaving other important connections intact.

This is similar to what occurs during an infection or injury, in which microglia are activated, quickly navigate towards the injured site, and remove dead or diseased tissue while leaving healthy tissue untouched.

The researchers also pinpointed one of the key molecular mechanisms in this process and observed that when a single receptor – called P2Y12 – was turned off the microglia ceased removing the connections between neurons. It is possible that when the microglia's synapse pruning function is interrupted or when the cells mistakenly remove the wrong connections – perhaps due to genetic factors or because the cells are too occupied elsewhere fighting an infection or injury – the result is impaired signaling between brain cells.<sup>1548</sup>

Immunohistochemical and electron microscopic studies had long revealed that activated microglia closely appose neurons. The role of microglia has been investigated in the removal of synapses that occurs after nerve axotomy and during development,<sup>1549</sup> and astrocyte-microglia

microglia signaling results in impaired functional brain connectivity and social behavior. *Nat Neurosci.* 2014 Mar;17(3):400-6; <http://www.vyssotski.ch/Publications/NatNeurosci2014.pdf>.

<sup>1547</sup> Sipe GO, Lowery RL, Tremblay MÈ, Kelly EA, Lamantia CE, Majewska AK. Microglial P2Y12 is necessary for synaptic plasticity in mouse visual cortex. *Nat Commun.* 2016 Mar 7;7:10905; <http://www.nature.com/ncomms/2016/160307/ncomms10905/full/ncomms10905.html>.

<sup>1548</sup> “The Brain’s Gardeners: Immune Cells ‘Prune’ Connections Between Neurons,” University of Rochester Medical Center News, 7 Mar 2016; <https://www.urmc.rochester.edu/news/story/4516/the-brains-gardeners-immune-cells-prune-connections-between-neurons.aspx>.

<sup>1549</sup> Blinzinger K, Kreutzberg G. Displacement of synaptic terminals from regenerating motoneurons by microglial cells. *Z Zellforsch Mikrosk Anat.* 1968;85(2):145-57; <http://www.springerlink.com/index/P148MN3604241036.pdf>. Bruce-Keller AJ. Microglial-neuronal interactions in synaptic damage and recovery. *J Neurosci Res.* 1999 Oct 1;58(1):191-201; <http://www.ncbi.nlm.nih.gov/pubmed/10491582>. Bessis A, Béchade C, Bernard D, Roumier A. Microglial control of neuronal death and synaptic properties. *Glia.* 2007 Feb;55(3):233-8; <http://www.ncbi.nlm.nih.gov/pubmed/17106878>. Trapp BD, Wujek JR, Criste GA, Jalabi W, Yin X, Kidd GJ, Stohlman S, Ransohoff R. Evidence for synaptic stripping by cortical microglia. *Glia.* 2007 Mar;55(4):360-8;

communication may dictate which synaptic elements should be pruned during thalamo-cortical network refinement.<sup>1550</sup> But Sipe *et al.*<sup>1551</sup> have demonstrated that “microglia react rapidly to changes in cortical activity, increasing process arborization, reducing process motility and increasing their interactions with synaptic elements. These findings implicate microglia as key players in the execution of plastic changes in cortical networks during experience-driven plasticity, and have implications for the understanding of microglial contributions to cognitive disorders where such functions are compromised.”

Microglia manufactured in cell mills can be emplaced in reconstructed brain tissue in the locations and numbers required to ensure maximum neuroplasticity consistent with a successful regeneration outcome, thus preparing the AD patient for the neural network retraining regimen that is next to come (Section 5.3.3).

---

[https://www.researchgate.net/profile/Richard\\_Ransohoff2/publication/6661586\\_Evidence\\_for\\_synaptic\\_stripping\\_by\\_cortical\\_microglia/links/5573a97008ae7536374fd67d.pdf](https://www.researchgate.net/profile/Richard_Ransohoff2/publication/6661586_Evidence_for_synaptic_stripping_by_cortical_microglia/links/5573a97008ae7536374fd67d.pdf). Cullheim S, Thams S. The microglial networks of the brain and their role in neuronal network plasticity after lesion. *Brain Res Rev.* 2007 Aug;55(1):89-96; <http://www.ncbi.nlm.nih.gov/pubmed/17509690>.

<sup>1550</sup> Bialas AR, Stevens B. TGF- $\beta$  signaling regulates neuronal C1q expression and developmental synaptic refinement. *Nat Neurosci.* 2013 Dec;16(12):1773-82; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3973738/>.

<sup>1551</sup> Sipe GO, Lowery RL, Tremblay MÈ, Kelly EA, Lamantia CE, Majewska AK. Microglial P2Y12 is necessary for synaptic plasticity in mouse visual cortex. *Nat Commun.* 2016 Mar 7;7:10905; <http://www.nature.com/ncomms/2016/160307/ncomms10905/full/ncomms10905.html>.

### 5.3.3 Neural Network Retraining

With brain mapping completed, the neural repair plan compiled, and the neural tissue repaired or reconstructed and placed in a receptive state of high mental plasticity over a minimum physical treatment time of 4-6 days (**Table 4**), the AD patient can now be retrained to strengthen existing memories or to reacquire missing memories over a period of weeks or months using aggressive “environmental enrichment” (Section 5.3.3.1). Axonal growth, dendritic re-arborization, and exuberant synaptogenesis can be elicited and then guided by an intensive program of mental and physical activities, allowing normal memory-reinforcing cognitive processes to provide continuous neural network retraining with significant restoration of function to the AD patient.

<b>Table 4. Nanorobot count and treatment time to execute nanorobotic tasks for all three Alzheimer Protocols.</b>		
<b>Nanorobotic Protocol Task</b>	<b># of Nanorobots</b>	<b>Treatment Time</b>
<b><u>Alzheimer Protocol 1: Genetic Derisking</u></b>		
1A. Genotyping	external computers	1 hr
1B. Chromosome manufacture	external computers	1 hr
1Ca. Genetic derisking (brain)	86 billion	7 hr
1Cb. Genetic derisking (whole body)	1000 billion x 10 cycles	48 hr
<b><u>Alzheimer Protocol 2: Tissue Rejuvenation</u></b>		
2A. Remove amyloid/glutamate	86 billion	1 hr
2B. Remove tau	86 billion	1 hr
2C. Remove lipofuscin	86 billion	1 hr
2D. Remove $\alpha$ -synuclein	86 billion	1 hr
2E. Correct cancer, mutations	(done in 1C)	
2F. Mutant mitochondria	86 billion	7 hr
2G. Senescent cells	(done in 1C)	
2H. Lost cells	(done in 3E)	
<b><u>Alzheimer Protocol 3: Neural Reconstruction</u></b>		
3A. Brain mapping	86 billion	13 hr
3B. Compile neural repair plan	external computers	24 hr
Damaged area close inspection	20 billion	
3C. Manufacture replacement cells	external cell mill	1 hr
3D. Intracranial debridement		2 hr
Debridement	10-100 billion	
Support	1000 billion	
Injury suppression	86 billion	
3E. Cell emplacement/incorporation		46 hr
Shepherd	68 billion	
Support	86 billion	
<b>TOTALS</b>	<b>1958-3048 billion</b>	<b>106-154 hr</b> (4.4-6.4 days)

There are two sorts of information that are stored as memories in the human brain and must be restored in the AD patient – **data** (the facts of someone's life; Section 5.3.3.2) and **algorithms** (the way someone processes those facts; Section 5.3.3.3). This is crudely analogous to the role of data and software in a computer memory.

### 5.3.3.1 Environmental Enrichment

It has long been established that a rich sensory and cognitive environment will maximally stimulate additional synaptogenesis. Rats raised with “environmental enrichment” have 3%-7% thicker cerebral cortices and 25% more synapses than controls.<sup>1552</sup> This effect occurs whether a more stimulating environment is experienced immediately following birth,<sup>1553</sup> after weaning,<sup>1554</sup> or during maturity (with increased synapse numbers in adults remaining high even after leaving the enriched environment for 30 days).<sup>1555</sup> Environmental stimulation increases synaptogenesis upon both pyramidal neurons (the main projecting neurons in the cerebral cortex) and stellate neurons,<sup>1556</sup> and in the hippocampus,<sup>1557</sup> as well as in retinal neurons outside of the brain (long considered less plastic than cortex or hippocampus, the very sites of experience-dependent plasticity).<sup>1558</sup> Animals placed in enriched environments show evidence of larger synapses,<sup>1559</sup>

---

<sup>1552</sup> Diamond MC, Krech D, Rosenzweig MR. The Effects of an Enriched Environment on the Histology of the Rat Cerebral Cortex. *J Comp Neurol*. 1964 Aug;123:111-20; <http://onlinelibrary.wiley.com/doi/10.1002/cne.901230110/abstract>. Diamond MC, Law F, Rhodes H, Lindner B, Rosenzweig MR, Krech D, Bennett EL. Increases in cortical depth and glia numbers in rats subjected to enriched environment. *J Comp Neurol*. 1966 Sep;128(1):117-26; <http://onlinelibrary.wiley.com/doi/10.1002/cne.901280110/abstract>.

<sup>1553</sup> Schapiro S, Vukovich KR. Early experience effects upon cortical dendrites: a proposed model for development. *Science*. 1970 Jan 16;167(3916):292-4; <http://classic.sciencemag.org/content/167/3916/292.long>.

<sup>1554</sup> Bennett EL, Diamond MC, Krech D, Rosenzweig MR. Chemical and Anatomical Plasticity of Brain. *Science*. 1964 Oct 30;146(3644):610-9; <http://classic.sciencemag.org/content/146/3644/610.long>.

<sup>1555</sup> Briones TL, Klintsova AY, Greenough WT. Stability of synaptic plasticity in the adult rat visual cortex induced by complex environment exposure. *Brain Res*. 2004 Aug 20;1018(1):130-5; <http://www.ncbi.nlm.nih.gov/pubmed/15262214>.

<sup>1556</sup> Greenough WT, Volkmar FR. Pattern of dendritic branching in occipital cortex of rats reared in complex environments. *Exp Neurol*. 1973 Aug;40(2):491-504; <http://www.ncbi.nlm.nih.gov/pubmed/4730268>.

<sup>1557</sup> Kumazawa-Manita N, Hama H, Miyawaki A, Iriki A. Tool use specific adult neurogenesis and synaptogenesis in rodent (*Octodon degus*) hippocampus. *PLoS One*. 2013;8(3):e58649; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3596278/>.

<sup>1558</sup> Landi S, Sale A, Berardi N, Viegi A, Maffei L, Cenni MC. Retinal functional development is sensitive to environmental enrichment: a role for BDNF. *FASEB J*. 2007 Jan;21(1):130-9; <http://www.fasebj.org/content/21/1/130.long>.

<sup>1559</sup> Sirevaag AM, Greenough WT. Differential rearing effects on rat visual cortex synapses. II. Synaptic morphometry. *Brain Res*. 1985 Apr;351(2):215-26; <http://www.ncbi.nlm.nih.gov/pubmed/3995348>.

increased synapse activation,<sup>1560</sup> and larger-amplitude “gamma oscillations”<sup>1561</sup> in the hippocampus.<sup>1562</sup> Environmental enrichment that includes motor skills practiced for 7-10 days also raises synapse numbers in rats.<sup>1563</sup>

Exposure to “enriched environment” conditions can lead to increases in cortical brain weight,<sup>1564</sup> in cortical thickness,<sup>1565</sup> in dendritic branching and synaptic density (including the complexity and length of the dendrite arbors upon which synapses form),<sup>1566</sup> in the number of synaptic

<sup>1560</sup> Sirevaag AM, Greenough WT. Differential rearing effects on rat visual cortex synapses. III. Neuronal and glial nuclei, boutons, dendrites, and capillaries. *Brain Res.* 1987 Oct 27;424(2):320-32; <https://www.ncbi.nlm.nih.gov/pubmed/3676831>.

<sup>1561</sup> Gamma rhythms at 25-140 Hz serve to synchronize neurons and form cell assemblies in mammalian cortical structures.

<sup>1562</sup> Shinohara Y, Hosoya A, Hirase H. Experience enhances gamma oscillations and interhemispheric asymmetry in the hippocampus. *Nat Commun.* 2013;4:1652; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3644069/>.

<sup>1563</sup> Black JE, Isaacs KR, Anderson BJ, Alcantara AA, Greenough WT. Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in cerebellar cortex of adult rats. *Proc Natl Acad Sci U S A.* 1990 Jul;87(14):5568-72; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC54366/>. Kleim JA, Hogg TM, VandenBerg PM, Cooper NR, Bruneau R, Rempel M. Cortical synaptogenesis and motor map reorganization occur during late, but not early, phase of motor skill learning. *J Neurosci.* 2004 Jan 21;24(3):628-33; <http://www.jneurosci.org/content/24/3/628.long>.

<sup>1564</sup> Rosenzweig MR, Krech D, Bennett EL, Diamond MC. Effects of environmental complexity and training on brain chemistry and anatomy: a replication and extension. *J Comp Physiol Psychol.* 1962 Aug;55:429-37; <http://psycnet.apa.org/journals/com/55/4/429/>. Bennett EL, Diamond MC, Krech D, Rosenzweig MR. Chemical and Anatomical Plasticity of Brain. *Science.* 1964 Oct 30;146(3644):610-9; <http://classic.sciencemag.org/content/146/3644/610.long>.

<sup>1565</sup> Krech D, Rosenzweig MR, Bennett EL. Effects of environmental complexity and training on brain chemistry. *J Comp Physiol Psychol.* 1960 Dec;53:509-19; <http://psycnet.apa.org/journals/com/53/6/509/>. Rosenzweig MR, Krech D, Bennett EL, Diamond MC. Effects of environmental complexity and training on brain chemistry and anatomy: a replication and extension. *J Comp Physiol Psychol.* 1962 Aug;55:429-37; <http://psycnet.apa.org/journals/com/55/4/429/>. Bennett EL, Diamond MC, Krech D, Rosenzweig MR. Chemical and Anatomical Plasticity of Brain. *Science.* 1964 Oct 30;146(3644):610-9; <http://classic.sciencemag.org/content/146/3644/610.long>. Cummins RA, Livesy PJ, Bell JA. Cortical depth changes in enriched and isolated mice. *Dev Psychobiol.* 1982 May;15(3):187-95; <http://www.ncbi.nlm.nih.gov/pubmed/7095285>.

<sup>1566</sup> Holloway RL Jr. Dendritic branching: some preliminary results of training and complexity in rat visual cortex. *Brain Res.* 1966 Oct;2(4):393-6; <http://www.sciencedirect.com/science/article/pii/0006899366900096>. Globus A, Rosenzweig MR, Bennett EL, Diamond MC. Effects of differential experience on dendritic spine counts in rat cerebral cortex. *J Comp Physiol Psychol.* 1973 Feb;82(2):175-81; <http://psycnet.apa.org/journals/com/82/2/175/>. Greenough WT, Volkmar FR. Pattern of dendritic branching in occipital cortex of rats reared in complex environments. *Exp Neurol.* 1973 Aug;40(2):491-504; <http://www.ncbi.nlm.nih.gov/pubmed/4730268>. Uylings HB, Kuypers K, Diamond MC, Veltman WAM. Effects of differential environments on plasticity of dendrites of cortical pyramidal neurons in adult rats. *Exp Neurol.* 1978 Dec;62(3):658-77;

contacts,<sup>1567</sup> in higher-order dendrite branch complexity<sup>1568</sup> and the length of distal branches,<sup>1569</sup> and in neurogenesis,<sup>1570</sup> plus changes in the regulation of neural plasticity<sup>1571</sup> along with improved memory and learning.<sup>1572</sup>

More specifically, enriched environments have been observed to cause 12%-14% increase in glial cells per neuron,<sup>1573</sup> 19% expansion of glial cell direct appositional area,<sup>1574</sup> 37% higher volume

[https://www.researchgate.net/profile/Harry\\_Uylings/publication/22392534\\_Effects\\_of\\_differential\\_environments\\_on\\_plasticity\\_of\\_dendrites\\_of\\_cortical\\_pyramidal\\_neurons\\_in\\_adult\\_rats/links/02e7e52e8ce9f6011e000000.pdf](https://www.researchgate.net/profile/Harry_Uylings/publication/22392534_Effects_of_differential_environments_on_plasticity_of_dendrites_of_cortical_pyramidal_neurons_in_adult_rats/links/02e7e52e8ce9f6011e000000.pdf). Faherty CJ, Kerley D, Smeyne RJ. A Golgi-Cox morphological analysis of neuronal changes induced by environmental enrichment. *Brain Res Dev Brain Res*. 2003 Mar 14;141(1-2):55-61; [https://www.researchgate.net/profile/Richard\\_Smeyne/publication/10850263\\_Faherty\\_CJ\\_Kerley\\_D\\_Smeyne\\_RJ\\_A\\_Golgi-Cox\\_morphological\\_analysis\\_of\\_neuronal\\_changes\\_induced\\_by\\_environmental\\_enrichment\\_Brain\\_Res\\_Dev\\_Brain\\_Res\\_141\\_55-61/links/00b7d51c09ba67524e000000.pdf](https://www.researchgate.net/profile/Richard_Smeyne/publication/10850263_Faherty_CJ_Kerley_D_Smeyne_RJ_A_Golgi-Cox_morphological_analysis_of_neuronal_changes_induced_by_environmental_enrichment_Brain_Res_Dev_Brain_Res_141_55-61/links/00b7d51c09ba67524e000000.pdf).

<sup>1567</sup> Hosseiny S, Pietri M, Petit-Paitel A, Zarif H, Heurteaux C, Chabry J, Guyon A. Differential neuronal plasticity in mouse hippocampus associated with various periods of enriched environment during postnatal development. *Brain Struct Funct*. 2015 Nov;220(6):3435-48; <http://www.ncbi.nlm.nih.gov/pubmed/25096287>.

<sup>1568</sup> Greenough WT, Volkmar FR. Pattern of dendritic branching in occipital cortex of rats reared in complex environments. *Exp Neurol*. 1973 Aug;40(2):491-504; <http://www.ncbi.nlm.nih.gov/pubmed/4730268>. Volkmar FR, Greenough WT. Rearing complexity affects branching of dendrites in the visual cortex of the rat. *Science*. 1972 Jun 30;176(4042):1445-7; <http://classic.sciencemag.org/content/176/4042/1445.long>.

<sup>1569</sup> Wallace CS, Kilman VL, Withers GS, Greenough WT. Increases in dendritic length in occipital cortex after 4 days of differential housing in weanling rats. *Behav Neural Biol*. 1992 Jul;58(1):64-8; <http://www.ncbi.nlm.nih.gov/pubmed/1417672>.

<sup>1570</sup> Kempermann G, Kuhn HG, Gage FH. More hippocampal neurons in adult mice living in an enriched environment. *Nature*. 1997 Apr 3;386(6624):493-5; <http://zeta.math.utsa.edu/~yxk833/METROPOLIS/REFERENCES/Kempermann.pdf>. van Praag H, Kempermann G, Gage FH. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat Neurosci*. 1999 Mar;2(3):266-70; [http://www.neurogenesisresearch.com/Runing\\_and\\_Neurogenesis\\_Nat.pdf](http://www.neurogenesisresearch.com/Runing_and_Neurogenesis_Nat.pdf).

<sup>1571</sup> Li S, Tian X, Hartley DM, Feig LA. The environment versus genetics in controlling the contribution of MAP kinases to synaptic plasticity. *Curr Biol*. 2006 Dec 5;16(23):2303-13; [http://www.cell.com/current-biology/pdf/S0960-9822\(06\)02365-7.pdf](http://www.cell.com/current-biology/pdf/S0960-9822(06)02365-7.pdf).

<sup>1572</sup> Hebb DO. *The Organization of Behavior*. John Wiley and Sons, NY, 1949. Williams BM, Luo Y, Ward C, Redd K, Gibson R, Kuczaj SA, McCoy JG. Environmental enrichment: effects on spatial memory and hippocampal CREB immunoreactivity. *Physiol Behav*. 2001 Jul;73(4):649-58; [https://www.researchgate.net/profile/Stan\\_Kuczaj/publication/11848470\\_Environmental\\_enrichment\\_Effects\\_on\\_spatial\\_memory\\_and\\_hippocampal\\_CREB\\_immunoreactivity/links/02bfe50f4632ff17e6000000.pdf](https://www.researchgate.net/profile/Stan_Kuczaj/publication/11848470_Environmental_enrichment_Effects_on_spatial_memory_and_hippocampal_CREB_immunoreactivity/links/02bfe50f4632ff17e6000000.pdf).

<sup>1573</sup> Diamond MC, Krech D, Rosenzweig MR. The Effects of an Enriched Environment on the Histology of the Rat Cerebral Cortex. *J Comp Neurol*. 1964 Aug;123:111-20; <http://onlinelibrary.wiley.com/doi/10.1002/cne.901230110/abstract>. Diamond MC, Law F, Rhodes H,

of glial cell nuclei per synapse (63% higher per neuron) and 20% higher mitochondrial mean volume per neuron,<sup>1575</sup> and wider capillaries (4.35  $\mu\text{m}$  vs. 4.15  $\mu\text{m}$ ) along with increased capillary density with shorter maximum distance from neuropil to nearest capillary (27.6  $\mu\text{m}$  vs. 34.6  $\mu\text{m}$ ).<sup>1576</sup>

There is also some preliminary evidence for neurological rehabilitation. Environmental enrichment has enhanced and partially repaired memory deficits in Alzheimer's-model mice between the ages of 2-7 months.<sup>1577</sup> Environmental enrichment for 7 weeks efficiently ameliorates early hippocampal-dependent spatial learning and memory deficits in Alzheimer's-model mice.<sup>1578</sup> Multiple studies have reported that environmental enrichment for adult mice helps relieve neuronal death, beneficial for Parkinson's disease.<sup>1579</sup> In Parkinson's, environmental enrichment affects the nigrostriatal pathway, which is important for managing dopamine and acetylcholine levels that are critical for motor deficits.<sup>1580</sup> Environmental

Lindner B, Rosenzweig MR, Krech D, Bennett EL. Increases in cortical depth and glia numbers in rats subjected to enriched environment. *J Comp Neurol.* 1966 Sep;128(1):117-26; <http://onlinelibrary.wiley.com/doi/10.1002/cne.901280110/abstract>.

<sup>1574</sup> Jones TA, Greenough WT. Ultrastructural evidence for increased contact between astrocytes and synapses in rats reared in a complex environment. *Neurobiol Learn Mem.* 1996 Jan;65(1):48-56; <http://www.ncbi.nlm.nih.gov/pubmed/8673406>.

<sup>1575</sup> Sirevaag AM, Greenough WT. Differential rearing effects on rat visual cortex synapses. III. Neuronal and glial nuclei, boutons, dendrites, and capillaries. *Brain Res.* 1987 Oct 27;424(2):320-32; <https://www.ncbi.nlm.nih.gov/pubmed/3676831>.

<sup>1576</sup> Borowsky IW, Collins RC. Metabolic anatomy of brain: a comparison of regional capillary density, glucose metabolism, and enzyme activities. *J Comp Neurol.* 1989 Oct 15;288(3):401-13; <http://www.ncbi.nlm.nih.gov/pubmed/2551935>. Sirevaag AM, Greenough WT. Differential rearing effects on rat visual cortex synapses. III. Neuronal and glial nuclei, boutons, dendrites, and capillaries. *Brain Res.* 1987 Oct 27;424(2):320-32; <https://www.ncbi.nlm.nih.gov/pubmed/3676831>.

<sup>1577</sup> Berardi N, Braschi C, Capsoni S, Cattaneo A, Maffei L. Environmental enrichment delays the onset of memory deficits and reduces neuropathological hallmarks in a mouse model of Alzheimer-like neurodegeneration. *J Alzheimers Dis.* 2007 Jun;11(3):359-70; <https://www.ncbi.nlm.nih.gov/pubmed/17851186>.

<sup>1578</sup> Valero J, España J, Parra-Damas A, Martín E, Rodríguez-Álvarez J, Saura CA. Short-term environmental enrichment rescues adult neurogenesis and memory deficits in APP(Sw,Ind) transgenic mice. *PLoS One.* 2011 Feb 9;6(2):e16832; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3036721/>.

<sup>1579</sup> Faherty CJ, Raviie Shepherd K, Herasimtschuk A, Smeyne RJ. Environmental enrichment in adulthood eliminates neuronal death in experimental Parkinsonism. *Brain Res Mol Brain Res.* 2005 Mar 24;134(1):170-9; [https://www.researchgate.net/profile/Richard\\_Smeyne/publication/7944480\\_Environmental\\_enrichment\\_in\\_adulthood\\_eliminate\\_neuronal\\_death\\_in\\_experimental\\_Parkinsonism/links/0c96051c09b4b54ffa000000.pdf](https://www.researchgate.net/profile/Richard_Smeyne/publication/7944480_Environmental_enrichment_in_adulthood_eliminate_neuronal_death_in_experimental_Parkinsonism/links/0c96051c09b4b54ffa000000.pdf).

<sup>1580</sup> Goldberg NR, Fields V, Pflibsen L, Salvatore MF, Meshul CK. Social enrichment attenuates nigrostriatal lesioning and reverses motor impairment in a progressive 1-methyl-2-phenyl-1,2,3,6-

enrichment also increases neurogenesis in aged rodents (that are exhibiting decreased hippocampal neurogenesis) by potentiating neuronal differentiation and new cell survival, giving the old rodents a superior ability to retain existing levels of spatial and learning memory.<sup>1581</sup> Of course, nanorobotic intracranial debridement and cell insertion/emplacement might stimulate an injury response (Section 5.3.2.2) that must be suppressed – reactive synaptogenesis induced by ordinary lesions can subsequently preclude the beneficial effects of environmental enrichment.<sup>1582</sup>

While environmental enrichment research has been done mostly on rodents, comparable effects are known to occur in primates<sup>1583</sup> and appear likely to affect the human brain similarly.<sup>1584</sup>

Collectively, these results suggest that an adult brain into which new neurons have been introduced might successfully form new synapses, expand neural network connections, and create fresh memories (potentially of similar content to the lost old memories) among AD patients who are subjected to a sensorily-intensive retraining environment over a therapeutic retraining period of several months. Environmental enrichment appears to stimulate neural regrowth to roughly the same percentage extent as the percentage declines that are observed in the brains of AD patients.

---

tetrahydropyridine (MPTP) mouse model of Parkinson's disease. *Neurobiol Dis.* 2012 Mar;45(3):1051-67; <https://www.ncbi.nlm.nih.gov/pubmed/22198503>.

<sup>1581</sup> Speisman RB, Kumar A, Rani A, Pastoriza JM, Severance JE, Foster TC, Ormerod BK. Environmental enrichment restores neurogenesis and rapid acquisition in aged rats. *Neurobiol Aging.* 2013 Jan;34(1):263-74; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3480541/>.

<sup>1582</sup> Kolb B, Gibb R. Environmental enrichment and cortical injury: behavioral and anatomical consequences of frontal cortex lesions. *Cereb Cortex.* 1991 Mar-Apr;1(2):189-98; <http://www.ncbi.nlm.nih.gov/pubmed/1822732>.

<sup>1583</sup> Bryan GK, Riesen AH. Deprived somatosensory-motor experience in stump-tailed monkey neocortex: dendritic spine density and dendritic branching of layer IIIb pyramidal cells. *J Comp Neurol.* 1989 Aug 8;286(2):208-17; <http://www.ncbi.nlm.nih.gov/pubmed/2794116>. Kozorovitskiy Y, Gross CG, Kopil C, Battaglia L, McBreen M, Stranahan AM, Gould E. Experience induces structural and biochemical changes in the adult primate brain. *Proc Natl Acad Sci U S A.* 2005 Nov 29;102(48):17478-82; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1297690/>.

<sup>1584</sup> Jacobs B, Schall M, Scheibel AB. A quantitative dendritic analysis of Wernicke's area in humans. II. Gender, hemispheric, and environmental factors. *J Comp Neurol.* 1993 Jan 1;327(1):97-111; <http://amyalexander.wiki.westga.edu/file/view/dendritic+analysis.pdf>.

### 5.3.3.2 Brain Memory: Data Recovery

Data are the facts of one's life – the names of friends and loved ones, the dates of birthdays, the details of the patient's favorite sports team, the multiplication tables memorized in grade school, and so forth. The treatment specified in the first two Alzheimer Protocols ensures that memories still possessed by the AD patient at the time of treatment will not be lost, and some memories that are on the brink of being lost will be recovered due to redundancy. If neuron losses due to aging are more or less randomly distributed and do not exceed the 10% figure mentioned in Section 5.2.6, then it seems unlikely that surviving connectivity networks will have become so thoroughly disrupted by aging alone as to produce complete information loss, assuming that estimates of from 10-fold to 1000-fold redundancy in human neocortical memory patterns<sup>1585</sup> are essentially correct.

It may also be the case that some memories lost to conscious awareness will still retain enough physical traces to be recoverable by the experientially-stimulated regrowth of a few dendrites or synapses guided by the remains of the perineuronal net – a possibility that should constrain and guide the extent of the debridement procedure (Section 5.3.2.2).

But what about the data that is completely lost? With the neural reconstruction completed as described in Section 5.3.2, the new neurons now present in the AD patient's brain should be almost as plastic as when the patient was a child – voracious for information and forgetting almost nothing that seems interesting. The majority of the patient's mature original brain is still present even in cases of advanced AD, and these surviving (rejuvenated) neurons and their connections can help guide the process of data reacquisition, possibly assisted by other means.<sup>1586</sup>

For example, the patient might recall that they have children, but not the exact number, their names, faces, or birthdays. This is just data, and it can be presented to the patient as text or audiovisual information during rehabilitation, allowing the brain to rapidly “fill in the blanks”. Such personal data can be gathered and presented intensively to the patient – indeed, this is what the first course of retraining should entail. Other memory stimulators may include long talks with friends and relatives who visit the patient and repeat the traditional family stories or who can recount childhood experiences of the patient, or may include looking through family photo albums and watching old films or videos featuring the patient and his friends, family, and former associates, and significant events in the patient's life. Patients can travel to places or buildings that once were meaningful to them (e.g., a favorite beach retreat, the old family farm, a favorite bar or restaurant in the city), and can be re-exposed to their personal collections of favorite music, plays, videos, movies, TV shows, books, poems, articles, diaries, recollections of pets, boxes of

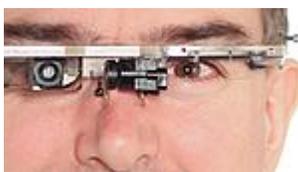
---

<sup>1585</sup> Kurzweil R. *How to Create a Mind*. Viking Press, 2012, pp. 39-40, 48. See also: <https://www.quora.com/Is-there-any-redundancy-in-human-memory>.

<sup>1586</sup> Roy DS, Arons A, Mitchell TI, Pignatelli M, Ryan TJ, Tonegawa S. Memory retrieval by activating engram cells in mouse models of early Alzheimer's disease. *Nature*. 2016 Mar 16; <http://www.nature.com/nature/journal/vaop/ncurrent/full/nature17172.html>.

memorabilia,<sup>1587</sup> and other personal effects. With input from friends and relatives, the patient could be exposed to virtual reality movies depicting key events in the patient's life, recreated using automated digital cinematographic techniques and featuring virtual actors<sup>1588</sup> who look like the patient and his family or associates. The patient should also read or view anything they themselves wrote, sang, performed, or created, as well as their own CVs, resumes, LinkedIn and Facebook pages, including their Wikipedia entry or articles and biographies written describing them or their activities, to restore the patient's memories of their own personal history and accomplishments. Of course, friends and relatives who are no longer alive cannot directly offer information to the recovering patient, but recordings of the departed can at least provide some personal data that might help the patient recreate lost memories. Early adulthood autobiographical memories are important in retaining one's sense of personal identity.<sup>1589</sup>

Memory recovery can be further enhanced if the patient has proactively attempted to actively record important elements of their life history. Many people may have saved decades of personal emails and other correspondence, but only a few have taken this even further into the realm of complete life recording or "lifelogging".<sup>1590</sup> For example, for several decades Steven Mann has worn a video camera (image, left)<sup>1591</sup> that records everything he sees and hears. Gordon Bell's MyLifeBits project<sup>1592</sup> accumulates about 1 gigabyte/month while recording all the physical events of a person's daily life using the Microsoft SenseCam,<sup>1593</sup> creating a "digital diary or e-memory continuously."<sup>1594</sup> The LifeNaut project<sup>1595</sup> allows people to (1) upload biographical pictures,



<sup>1587</sup> "Memorabilia Storage Agreement"; <http://www.cryonics.org/resources/memorabilia-storage-agreement>.

<sup>1588</sup> [https://en.wikipedia.org/wiki/Virtual\\_actor](https://en.wikipedia.org/wiki/Virtual_actor)

<sup>1589</sup> Addis DR, Tippett LJ. Memory of myself: autobiographical memory and identity in Alzheimer's disease. *Memory*. 2004 Jan;12(1):56-74; <http://www.ncbi.nlm.nih.gov/pubmed/15098621>.

<sup>1590</sup> "Lifelogging: Digital locker looks after your stuff," *Technology News*, 8 Jan 2014; <http://www.newscientist.com/article/mg22129514.400-lifelogging-digital-locker-looks-after-your-stuff.html>

<sup>1591</sup> [https://en.wikipedia.org/wiki/Steve\\_Mann](https://en.wikipedia.org/wiki/Steve_Mann).

<sup>1592</sup> Bell has captured a lifetime's worth of articles, books, cards, CDs, letters, memos, papers, photos, pictures, presentations, home movies, videotaped lectures, and voice recordings and stored them digitally. He is now paperless, and is beginning to capture phone calls, IM transcripts, television, and radio. <http://research.microsoft.com/en-us/projects/mylifebits/>. Gemmell J, Bell G, Lueder R. MyLifeBits: a personal database for everything. *Commun ACM* 2006 Jan;49(1):88-95; <http://research.srv.microsoft.com/pubs/64157/tr-2006-23.pdf>.

<sup>1593</sup> SenseCam is a badge-sized wearable camera that captures up to 2000 VGA images per day into 128 MB flash memory. Sensor data such as movement, light level and temperature is recorded every second, similar to an aircraft "black box" accident recorder but miniaturized for the human body. This could help with memory recall (e.g., where did I leave my spectacles or keys? Who did I meet last week?) by allowing a quick rewind of the day's events. If a person has an accident, the events and images leading up to this will be recorded (possibly useful to medical staff). The device could also be used for automatic diary

videos, and documents to a digital archive that will be preserved for generations; (2) organize through geo-mapping, timelines, and tagging, a rich portrait of information about themselves, including the places they've been and the people they've met; and (3) create a computer-based avatar to interact and respond with their attitudes, values, mannerisms and beliefs. The foresighted patient who creates these kinds of records in advance of need stands a much better chance of extensive memory recovery than one who does not.

The SenseCam has already been used to assist dementia patients with serious autobiographical memory defects. For example, in the case of one patient who could only recall ~2% of events that happened the previous week, using the SenseCam dramatically improved her memory, allowing her to recall ~80% percent of events six weeks after they happened.<sup>1596</sup> According to one of the study authors: "Not only does SenseCam allow people to recall memories while they are looking at the images, but after an initial period of consolidation, it appears to lead to long-term retention of memories over many months, without the need to view the images repeatedly."

One interesting issue is the restoration of unpleasant memories. Memories of being a victim of violence or other crimes, or of being cheated by a business partner or spouse, or of undergoing very painful medical procedures or even the death of a loved one, might be very painful to remember but undoubtedly contribute significantly to the patient's personality. Perhaps such memories, if forgotten, should be offered to the patient upon request and with informed consent, with full knowledge that a person with an edited memory is a different person than someone with all of their memories intact. Less troublesome are conflicting but non-painful memories<sup>1597</sup> – these also make the pre-AD patient the unique person who they were, and should be restored if possible.

---

generation. Originally conceived as a personal "black box" accident recorder, it soon became evident that looking through images previously recorded tends to elicit quite vivid remembering of the original event. This exciting effect has formed the basis of a great deal of research around the world using SenseCam and the device is now available to buy as the Vicon Revue. <http://research.microsoft.com/en-us/um/cambridge/projects/sensecam/>.

<sup>1594</sup> Bell G, Gemmell J. Total Recall: How the E-Memory Revolution Will Change Everything, Dutton, 2009; <http://www.amazon.com/Total-Recall-E-Memory-Revolution-Everything/dp/0525951342>.

<sup>1595</sup> LifeNaut Mindfile Accounts; <https://www.lifenaut.com/learn-more/>.

<sup>1596</sup> Hodges S, Berry E, Wood K. SenseCam: a wearable camera that stimulates and rehabilitates autobiographical memory. *Memory*. 2011 Oct;19(7):685-96; [http://msr-waypoint.com/en-us/um/cambridge/projects/sensecam/pdf\\_files/Memory.pdf](http://msr-waypoint.com/en-us/um/cambridge/projects/sensecam/pdf_files/Memory.pdf). See also: <https://www.technologyreview.com/s/409170/a-camera-to-help-dementia-patients/>.

<sup>1597</sup> For example: "Suppose you enjoy[ed] bird watching at age 20, and leave extensive records on this hobby, but lose interest later in life. Should these records be used for repair even though they are obsolete, and could result in a mixture of memories and preferences from different times in your life?" Hogg T. Information storage and computational aspects of repair. *Cryonics* 1996 3rd Qtr;16(3):18-25; <http://www.alcor.org/cryonics/cryonics1996-3.pdf#page=20>.

The availability of detailed records of these types by the AD patient or his associates will likely improve the patient's memory data recovery efforts. More specifically, this intensive retraining may allow the reconstitution of the patient's brain network configuration<sup>1598</sup> to a state closely resembling its original functional state.

---

<sup>1598</sup> Foster PP. Role of physical and mental training in brain network configuration. *Front Aging Neurosci.* 2015 Jun 23;7:117; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4477154/>.

### 5.3.3.3 Brain Memory: Algorithm Recovery

The more difficult area for AD patient recovery is the reconstruction of lost algorithms. An algorithm is what a person's brain does with the data it knows. An algorithm is also a piece of data, but it is data that describes or embodies a procedure for processing data, as distinct from the data upon which the process acts.<sup>1599</sup> Of course, there may exist multiple redundant external sources for data (e.g., photographs, videos, books, or testimonials from living people) that survive outside of the AD patient's own mind, and thus can be retrieved from the external world. In the case of algorithms, a difficulty arises when there are no external sources of the patient's internal algorithms available to be retrieved. There will often, perhaps even usually, be no external records of the specific mental processes that the patient once employed in his internal mental life.

Algorithms pertaining to physical activities (e.g., playing tennis or football, repairing a car or piloting an airplane, dancing or having sex, painting or knitting, singing or affixing a signature to a document, or even simple activities like using a knife and fork to eat, etc.) might be recovered by repeating the relevant physical activities, especially in the company of a colleague or partner who once shared the same activities with the patient. If the patient has forgotten the relevant mental processes, the missing algorithms can only be partially inferred and recreated by examining the patient's "output" and then attempting to reconstruct what internal mental processes the patient must have used to proceed from the known input data (i.e., his factual memories and experiential knowledge base) to his output (i.e., his writings, utterances, and deeds). Unfortunately there will usually be many ways to obtain a given output from a given set of inputs, so any such reconstructed algorithm, while perhaps accurate for the data at hand, might differ significantly from the lost original. These inferences are in principle possible with enough data, sufficient external computational capacity, and an exact knowledge of brain structure, but it is likely to be a difficult task and still may produce at best an approximation of the patient's original internal algorithms.

If the patient has taken psychological tests such as personality tests,<sup>1600</sup> personality inventories,<sup>1601</sup> the Myers-Briggs test,<sup>1602</sup> or aptitude tests such as for mechanical aptitude,<sup>1603</sup> financial aptitude,<sup>1604</sup> language-learning ability,<sup>1605</sup> or vocational interest inventories,<sup>1606</sup> this may

---

<sup>1599</sup> Since algorithms are stored as data, they can be applied recursively to themselves.

<sup>1600</sup> [https://en.wikipedia.org/wiki/Personality\\_test](https://en.wikipedia.org/wiki/Personality_test).

<sup>1601</sup> [https://en.wikipedia.org/wiki/Minnesota\\_Multiphasic\\_Personality\\_Inventory](https://en.wikipedia.org/wiki/Minnesota_Multiphasic_Personality_Inventory);  
[https://en.wikipedia.org/wiki/Revised\\_NEO\\_Personality\\_Inventory](https://en.wikipedia.org/wiki/Revised_NEO_Personality_Inventory).

<sup>1602</sup> [https://en.wikipedia.org/wiki/Myers%E2%80%93Briggs\\_Type\\_Indicator](https://en.wikipedia.org/wiki/Myers%E2%80%93Briggs_Type_Indicator).

<sup>1603</sup> [https://en.wikipedia.org/wiki/Mechanical\\_aptitude](https://en.wikipedia.org/wiki/Mechanical_aptitude);  
[https://en.wikipedia.org/wiki/Wiesen\\_Test\\_of\\_Mechanical\\_Aptitude](https://en.wikipedia.org/wiki/Wiesen_Test_of_Mechanical_Aptitude).

<sup>1604</sup> [https://en.wikipedia.org/wiki/Bloomberg\\_Aptitude\\_Test](https://en.wikipedia.org/wiki/Bloomberg_Aptitude_Test).

<sup>1605</sup> [https://en.wikipedia.org/wiki/Language-learning\\_aptitude](https://en.wikipedia.org/wiki/Language-learning_aptitude).

provide some useful algorithmic information although such tests are often considered to have poor reliability by psychological professionals. One hopeful sign is that because the majority of the AD patient's brain is still physically intact, and because memory is highly redundant and what remains has by this time been thoroughly rejuvenated, it should be possible for the patient to guide the process of reconstructing his internal algorithms in a direction that "feels" natural or at least vaguely correct.

One useful exercise along these lines might be to present the patient with a song, poem, speech, painting, article, book, or other artistic object that they created in the past, let them study it for awhile, then ask them to independently duplicate it without looking at it again. The duplicated work can then be compared to the recorded original, providing insights to the patient as to what elements of his internal algorithms might still be missing or be slightly altered. Repeating this admittedly labor-intensive process on multiple example tasks might allow the patient to more closely approximate his original process algorithms. Similar processes could be used to test and recover missing skills such as mathematical analysis, poker playing, or foreign languages.

In some cases, close enough might be good enough. For example, if the AD patient has lost the ability to read, an attempt could be made to re-teach literacy as closely as possible to the way the patient was originally taught. However, it may not be possible to use the same books and teaching methods that the patient experienced when they were a child, and the exact childhood environment in which those lessons were originally learned will be nearly impossible to precisely recreate. To the extent that the particular way the patient originally learned to speak and write made a significant difference in the expression of their literacy, and to the extent this learning was further shaped and modified by unique experiences over the patient's many decades of life, the resulting complex expression of these abilities might be lost forever. In such cases a substitute teaching method may have to suffice.

Another minor area that might provide challenging is the recovery of unique sensory associations. For example, many people might associate a particular sound (e.g., a song), taste or smell with certain important life events like a death, a birth, or a first kiss. If these associations are still vaguely remembered by the patient, or if they are at least partially physiologically based, or if there is a reliable witness still alive who can recall them, then it might be possible to recreate or re-strengthen the association – either by experiential means or by direct physical intervention in the brain. But if the patient has forgotten and there is no reliable external record or physiological basis for a particular association, then it is probably unrecoverable.

There is already some mainstream interest in neuroplasticity-enhanced learning as a means to enhance the speed of learning new cognitive skills. In 2016, DARPA launched a new research program called Targeted Neuroplasticity Training (TNT).<sup>1607</sup> TNT "seeks to advance the pace and effectiveness of a specific kind of learning – cognitive skills training – through the precise activation of peripheral nerves that can in turn promote and strengthen neuronal connections in

<sup>1606</sup> [https://en.wikipedia.org/wiki/Strong\\_Interest\\_Inventory](https://en.wikipedia.org/wiki/Strong_Interest_Inventory).

<sup>1607</sup> "Boosting Synaptic Plasticity to Accelerate Learning," Defense Advanced Research Projects Agency, 16 Mar 2016; <http://www.darpa.mil/news-events/2016-03-16>.

the brain. TNT will pursue development of a platform technology to enhance learning of a wide range of cognitive skills. If successful, TNT could accelerate learning and reduce the time needed to train foreign language specialists, intelligence analysts, cryptographers, and others.”

## **Chapter 6. Treating Parkinson's and Related Neurodegenerative Conditions**

Alzheimer's disease is perhaps the most challenging neurodegenerative condition known to modern medicine. With a nanorobotic cure for AD in hand (Chapter 5), other less-challenging neurodegenerative diseases can most likely be completely reversed and cured using an appropriate subset of the methods developed for treating Alzheimer's disease.

In this Chapter, we review the nature and current treatment status of Parkinson's disease (Section 6.1) and a number of related neurodegenerative diseases (Section 6.2), providing, in each case, a brief summary of the nanorobotic approach for curing these conditions.

## 6.1 Parkinson's Disease

Parkinson's disease (PD) is a progressive and neurodegenerative brain disorder that leads to motor symptoms such as shaking (tremors) and difficulty with walking, movement, and coordination, and often many non-motor symptoms such as changes in mood or cognition, constipation, incontinence, sexual dysfunction, sleep disorders, visual changes, depression, anxiety, and skin changes. The precise causes of PD remain unknown, and there is currently no cure for Parkinson's disease.<sup>1608</sup>

PD is the second most common neurodegenerative disorder after Alzheimer's disease, affecting approximately seven million people globally and one million people in the United States.<sup>1609</sup> The prevalence of PD in industrialized countries is ~0.3% of the entire population and ~1% in people over 60 years of age,<sup>1610</sup> rising to 4% of the population over age 80.<sup>1611</sup> The mean age of onset is around 60 years, although 5-10% of cases, classified as young-onset PD, begin between the ages of 20-50 years.<sup>1612</sup> The costs of PD to society are high, probably around \$10,000 per patient per year in the U.S. with an estimated total national burden around \$23 billion in 2007.<sup>1613</sup> The largest share of direct cost comes from inpatient care and nursing homes with a much lower share from medications, but indirect costs are high due to reduced productivity, the burden on caregivers, and the reduced quality of life of PD patients.

In this Section we review some genetic causes of Parkinson's disease (Section 6.1.1), a few details of the PD neuropathology (Section 6.1.2), and current treatments for PD (Section 6.1.3). The proposed nanorobotic treatment for Parkinson's disease is then outlined in Section 6.1.4.

---

<sup>1608</sup> Palfreman J. Brain Storms: The Race to Unlock the Mysteries of Parkinson's Disease. SciAm/Farrar, Straus and Giroux, 2015; <http://www.amazon.com/Brain-Storms-Mysteries-Parkinsons-Disease/dp/0374116172>.

<sup>1609</sup> "Fact Sheet: Molecular Imaging and Parkinson's Disease," Society of Nuclear Medicine and Molecular Imaging, 2016; <http://www.snmni.org/AboutSNMMI/Content.aspx?ItemNumber=12770>.

<sup>1610</sup> Nussbaum RL, Ellis CE. Alzheimer's disease and Parkinson's disease. N Engl J Med 2003;348:1356-64; <http://www.nejm.org/doi/full/10.1056/NEJM2003ra020003>.

<sup>1611</sup> de Lau LM, Breteler MM. Epidemiology of Parkinson's disease. Lancet Neurol. 2006 Jun;5(6):525-35; [http://dtfosburgh.pbworks.com/f/Genetic\\_5.pdf](http://dtfosburgh.pbworks.com/f/Genetic_5.pdf).

<sup>1612</sup> Samii A, Nutt JG, Ransom BR. Parkinson's disease. Lancet. 2004 May 29;363(9423):1783-93; <https://www.ncbi.nlm.nih.gov/pubmed/15172778>.

<sup>1613</sup> Findley LJ. The economic impact of Parkinson's disease. Parkinsonism Relat Disord. 2007 Sep;13 Suppl:S8-S12; <https://www.ncbi.nlm.nih.gov/pubmed/17702630>.

### 6.1.1 Genetic Causes of Parkinson's Disease

Most people with PD have “idiopathic” Parkinson's disease (having no specific known cause).<sup>1614</sup> Environmental toxins, herbicides, pesticides, and fungicides have been associated with the risk of developing PD, but no causal relationships have been definitively proven.<sup>1615</sup> Traditionally, Parkinson's was considered a non-genetic disorder. However, ~15% of individuals with PD have a first-degree relative who has the disease,<sup>1616</sup> and at least 5-15% of cases are known to occur because of a mutation in one of several specific genes, transmitted in either an autosomal-dominant or autosomal-recessive pattern.<sup>1617</sup>

Mutations in a few specific genes have been conclusively shown to cause Parkinson's disease. Genes which have been implicated in autosomal-dominant PD include PARK1 and PARK4, PARK5, PARK8, PARK11 and GIGYF2 and PARK13 which code for SNCA ( $\alpha$ -synuclein), UCHL1, LRRK2 (leucine-rich repeat kinase 2, aka. the protein dardarin), and Htra2.<sup>1618</sup> Genes such as PARK2, PARK6, PARK7 and PARK9 – which code for PRKN (parkin), PINK1 (PTEN-induced putative kinase 1), DJ-1 and ATP13A2, respectively – have been implicated in the development of autosomal-recessive PD.<sup>1619</sup>

---

<sup>1614</sup> Interestingly, recent findings indicate that “idiopathic” PD patients may share a previously unknown defect in the function of a specific protein (PLA2g6) that causes dysfunction in calcium homeostasis linked to neuron death. Zhou Q, Yen A, Rymarczyk G, Asai H, Trengrove C, Aziz N, Kirber MT, Mostoslavsky G, Ikezu T, Wolozin B, Bolotina VM. Impairment of PARK14-dependent Ca(2+) signalling is a novel determinant of Parkinson's disease. *Nat Commun.* 2016 Jan 12;7:10332; <http://www.nature.com/ncomms/2016/160112/ncomms10332/full/ncomms10332.html>.

<sup>1615</sup> Goldman SM. Environmental toxins and Parkinson's disease. *Annu Rev Pharmacol Toxicol.* 2014;54:141-64; <https://www.ncbi.nlm.nih.gov/pubmed/24050700>.

<sup>1616</sup> Samii A, Nutt JG, Ransom BR. Parkinson's disease. *Lancet.* 2004 May 29;363(9423):1783-93; <https://www.ncbi.nlm.nih.gov/pubmed/15172778>.

<sup>1617</sup> Lesage S, Brice A. Parkinson's disease: from monogenic forms to genetic susceptibility factors. *Hum Mol Genet.* 2009 Apr 15;18(R1):R48-59; <http://hmg.oxfordjournals.org/content/18/R1/R48.long>.

<sup>1618</sup> Lesage S, Brice A. Parkinson's disease: from monogenic forms to genetic susceptibility factors. *Hum Mol Genet.* 2009 Apr 15;18(R1):R48-59; <http://hmg.oxfordjournals.org/content/18/R1/R48.long>.

<sup>1619</sup> Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature.* 1998 Apr 9;392(6676):605-8; <https://wiki.harvard.edu/confluence/download/attachments/7078049/Kitada+et+al+Nature+1998.pdf>.  
Davie CA. A review of Parkinson's disease. *Br Med Bull.* 2008;86:109-27; <http://bmb.oxfordjournals.org/content/86/1/109.long>.

Mutations in genes including those that code for SNCA, LRRK2 and GBA (glucocerebrosidase) have been found to be risk factors for sporadic PD.<sup>1620</sup> In most cases, people with these mutations will develop PD, though with the exception of LRRK2 they account for only a small minority of cases of PD.<sup>1621</sup>

LRRK2 is perhaps the most extensively studied PD-related gene. Mutations in PARK8 (LRRK2) are the most common known cause of familial and sporadic PD, accounting for ~5% of individuals with a family history of the disease and 3% of sporadic cases. Several Parkinson-related genes – including PARK8 (LRRK2) – are involved in the function of lysosomes, and their mutations might lead to lysosomal dysfunctions that reduce the ability of cells to break down  $\alpha$ -synuclein.<sup>1622</sup>

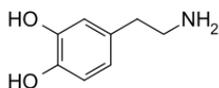
---

<sup>1620</sup> Neumann J, Bras J, Deas E, O'Sullivan SS, Parkkinen L, Lachmann RH, Li A, Holton J, Guerreiro R, Paudel R, Segarane B, Singleton A, Lees A, Hardy J, Houlden H, Revesz T, Wood NW. Glucocerebrosidase mutations in clinical and pathologically proven Parkinson's disease. *Brain*. 2009 Jul;132(Pt 7):1783-94; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2702833/>.

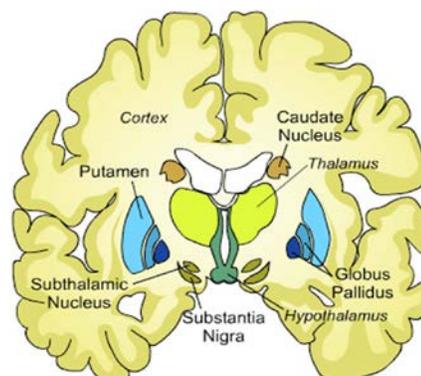
<sup>1621</sup> Davie CA. A review of Parkinson's disease. *Br Med Bull*. 2008;86:109-27; <http://bmb.oxfordjournals.org/content/86/1/109.long>.

<sup>1622</sup> Gan-Or Z, Dion PA, Rouleau GA. Genetic perspective on the role of the autophagy-lysosome pathway in Parkinson disease. *Autophagy*. 2015;11(9):1443-57; <https://www.ncbi.nlm.nih.gov/pubmed/26207393>.

## 6.1.2 Some Details of the Neuropathology



Dopamine (image, left) is one of the major neurotransmitter molecules in the brain, chemically mediating signals between neurons involved in motor control, hormone release, and reward-motivated behavior. The motor symptoms of Parkinson's disease result from the death of dopamine-generating cells in the basal ganglia (a group of brain structures innervated by the dopaminergic system) in the human midbrain.<sup>1623</sup> The main pathological characteristic of PD is cell death in the substantia nigra (image, right) and, more specifically, the ventral or front part of the pars compacta of the substantia nigra, affecting up to 70% of the cells by the time death occurs.<sup>1624</sup> The histopathology (microscopic anatomy) of the substantia nigra and several other brain regions shows neuronal loss and Lewy bodies (Section 5.2.2.3) in the cytoplasm of many of the remaining nerve cells, with thread-like proteinaceous inclusions within neurites (Lewy neurites).<sup>1625</sup> Neuronal loss is accompanied by the death of astrocytes (star-shaped glial cells) and activation of the microglia (another type of glial cell). Lewy bodies are a key pathological feature of PD.<sup>1626</sup>



There is speculation on multiple molecular mechanisms by which the brain cells could be lost, including the accumulation of aberrant or misfolded proteins,<sup>1627</sup> ubiquitin-proteasome and lysosomal system dysfunction,<sup>1628</sup> reduced or impaired mitochondrial function,<sup>1629</sup> oxidative and

<sup>1623</sup> Obeso JA, Rodríguez-Oroz MC, Benitez-Temino B, Blesa FJ, Guridi J, Marin C, Rodriguez M. Functional organization of the basal ganglia: therapeutic implications for Parkinson's disease. *Mov Disord.* 2008;23 Suppl 3:S548-59; <http://xa.yimg.com/kq/groups/17470070/1603603583/name/Functional+Organization+of+the+Basal+Ganglia.pdf>.

<sup>1624</sup> Davie CA. A review of Parkinson's disease. *Br Med Bull.* 2008;86:109-27; <http://bmb.oxfordjournals.org/content/86/1/109.long>.

<sup>1625</sup> Braak H, Sandmann-Keil D, Gai W, Braak E. Extensive axonal Lewy neurites in Parkinson's disease: a novel pathological feature revealed by alpha-synuclein immunocytochemistry. *Neurosci Lett.* 1999 Apr 9;265(1):67-9; <http://www.ncbi.nlm.nih.gov/pubmed/10327208>.

<sup>1626</sup> Dickson DV. *Neuropathology of movement disorders.* Tolosa E, Jankovic JJ. Parkinson's disease and movement disorders. Hagerstown, MD: Lippincott Williams & Wilkins. 2007:271-83.

<sup>1627</sup> Tenreiro S, Eckermann K, Outeiro TF. Protein phosphorylation in neurodegeneration: friend or foe? *Front Mol Neurosci.* 2014 May 13;7:42; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4026737/>.

<sup>1628</sup> Moore DJ, West AB, Dawson VL, Dawson TM. Molecular pathophysiology of Parkinson's disease. *Annu Rev Neurosci.* 2005;28:57-87; <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.321.4079&rep=rep1&type=pdf>. Obeso JA,

nitrosative stress,<sup>1630</sup> glutamate receptor mediated excitotoxicity and inflammation,<sup>1631</sup> the misregulation of calcium and potassium ion channels,<sup>1632</sup> excess lipofuscin,<sup>1633</sup> and aging.<sup>1634</sup>

Perhaps the most popular proposed specific mechanism is the abnormal accumulation of the protein  $\alpha$ -synuclein bound to ubiquitin in the damaged cells. This insoluble protein accumulates inside neurons, forming inclusions called Lewy bodies.<sup>1635</sup> According to the Braak staging, a

Rodriguez-Oroz MC, Goetz CG, Marin C, Kordower JH, Rodriguez M, Hirsch EC, Farrer M, Schapira AH, Halliday G. Missing pieces in the Parkinson's disease puzzle. *Nat Med*. 2010 Jun;16(6):653-61; <http://www.its.caltech.edu/~bio156/Papers/PDFs/PD%20rev%2010%20copy.pdf>.

<sup>1629</sup> Perier C, Vila M. Mitochondrial biology and Parkinson's disease. *Cold Spring Harb Perspect Med*. 2012 Feb;2(2):a009332; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3281591/>. Subramaniam SR, Chesselet MF. Mitochondrial dysfunction and oxidative stress in Parkinson's disease. *Prog Neurobiol*. 2013 Jul-Aug;106-107:17-32; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3742021/>. Paoli A, Bianco A, Damiani E, Bosco G. Ketogenic diet in neuromuscular and neurodegenerative diseases. *Biomed Res Int*. 2014;2014:474296; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4101992/>.

<sup>1630</sup> Tsang AH, Chung KK. Oxidative and nitrosative stress in Parkinson's disease. *Biochim Biophys Acta*. 2009 Jul;1792(7):643-50; <http://www.sciencedirect.com/science/article/pii/S092544390800269X>. Chinta SJ, Andersen JK. Nitrosylation and nitration of mitochondrial complex I in Parkinson's disease. *Free Radic Res*. 2011 Jan;45(1):53-8; [https://www.researchgate.net/profile/Shankar\\_Chinta/publication/46147149\\_Nitrosylation\\_and\\_nitration\\_of\\_mitochondrial\\_complex\\_I\\_in\\_Parkinson's\\_disease/links/00463535818f145f61000000.pdf](https://www.researchgate.net/profile/Shankar_Chinta/publication/46147149_Nitrosylation_and_nitration_of_mitochondrial_complex_I_in_Parkinson's_disease/links/00463535818f145f61000000.pdf).

<sup>1631</sup> Gallagher DA, Schapira AH. Etiopathogenesis and treatment of Parkinson's disease. *Curr Top Med Chem*. 2009;9(10):860-8; <http://www.ncbi.nlm.nih.gov/pubmed/19754402>.

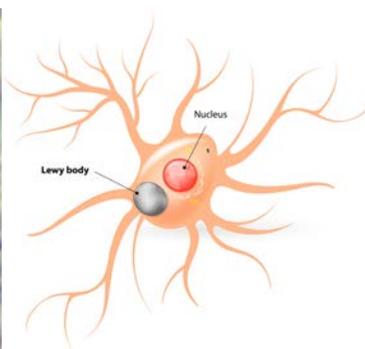
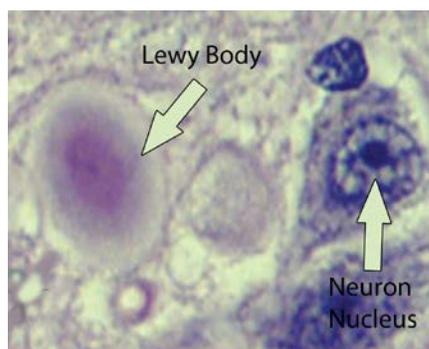
<sup>1632</sup> Dragicevic E, Schiemann J, Liss B. Dopamine midbrain neurons in health and Parkinson's disease: emerging roles of voltage-gated calcium channels and ATP-sensitive potassium channels. *Neuroscience*. 2015 Jan 22;284:798-814; <http://www.sciencedirect.com/science/article/pii/S0306452214009099>.

<sup>1633</sup> Ulfing N. Altered lipofuscin pigmentation in the basal nucleus (Meynert) in Parkinson's disease. *Neurosci Res*. 1989 Jun;6(5):456-62; <http://www.ncbi.nlm.nih.gov/pubmed/2771203>.

<sup>1634</sup> Chinta SJ, Lieu CA, Demaria M, Laberge RM, Campisi J, Andersen JK. Environmental stress, ageing and glial cell senescence: a novel mechanistic link to Parkinson's disease? *J Intern Med*. 2013 May;273(5):429-36; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3633085/>. Tan FC, Hutchison ER, Eitan E, Mattson MP. Are there roles for brain cell senescence in aging and neurodegenerative disorders? *Biogerontology*. 2014 Dec;15(6):643-60; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4264619/>. Liu G, Chen M, Mi N, Yang W, Li X, Wang P, Yin N, Li Y, Yue F, Chan P, Yu S. Increased oligomerization and phosphorylation of  $\alpha$ -synuclein are associated with decreased activity of glucocerebrosidase and protein phosphatase 2A in aging monkey brains. *Neurobiol Aging*. 2015 Sep;36(9):2649-59; <http://www.ncbi.nlm.nih.gov/pubmed/26149921>.

<sup>1635</sup> Davie CA. A review of Parkinson's disease. *Br Med Bull*. 2008;86:109-27; <http://bmb.oxfordjournals.org/content/86/1/109.long>. Schulz-Schaeffer WJ. The synaptic pathology of alpha-synuclein aggregation in dementia with Lewy bodies, Parkinson's disease and Parkinson's disease dementia. *Acta Neuropathol*. 2010 Aug;120(2):131-43; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2892607/>.

classification of Parkinson's disease based on pathological findings, Lewy bodies (image, below) first appear in the olfactory bulb, medulla oblongata and pontine tegmentum, with these patients still asymptomatic. As the disease progresses, Lewy bodies develop in the substantia nigra, areas of the midbrain and basal forebrain, and finally in the neocortex.<sup>1636</sup> These brain sites are the main locales of neuronal degeneration in PD – although, like amyloid plaques in AD, Lewy bodies may not themselves cause cell death and might even be protective.<sup>1637</sup> In people with dementia, a generalized presence of Lewy bodies is common in cortical areas.



### **Lewy body dementia**

(LBD), aka. dementia with Lewy bodies (DLB), diffuse Lewy body disease, cortical Lewy body disease, or senile dementia of Lewy type, is a type of progressive neurodegenerative dementia closely associated with Parkinson's disease

primarily affecting older adults, whose primary feature is cognitive decline.<sup>1638</sup> Lewy body dementia is the third-most-common form of dementia (15% of all cases) after Alzheimer's disease (50%-70%) and vascular dementia (25%) in older adults.<sup>1639</sup> The prognosis, molecular details, and palliative treatment options for LBD are very similar to PD and will not be discussed further here.

---

<sup>1636</sup> Davie CA. A review of Parkinson's disease. *Br Med Bull.* 2008;86:109-27; <http://bmb.oxfordjournals.org/content/86/1/109.long>.

<sup>1637</sup> Schulz-Schaeffer WJ. The synaptic pathology of alpha-synuclein aggregation in dementia with Lewy bodies, Parkinson's disease and Parkinson's disease dementia. *Acta Neuropathol.* 2010 Aug;120(2):131-43; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2892607/>. Obeso JA, Rodriguez-Oroz MC, Goetz CG, Marin C, Kordower JH, Rodriguez M, Hirsch EC, Farrer M, Schapira AH, Halliday G. Missing pieces in the Parkinson's disease puzzle. *Nat Med.* 2010 Jun;16(6):653-61; <http://www.its.caltech.edu/~bio156/Papers/PDFs/PD%20rev%2010%20copy.pdf>.

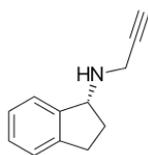
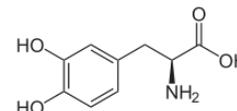
<sup>1638</sup> [https://en.wikipedia.org/wiki/Dementia\\_with\\_Lewy\\_bodies](https://en.wikipedia.org/wiki/Dementia_with_Lewy_bodies).

<sup>1639</sup> Burns A, Iliffe S. Dementia. *BMJ.* 2009 Feb 5;338:b75; <https://www.ncbi.nlm.nih.gov/pubmed/19196746>. Battistin L, Cagnin A. Vascular cognitive disorder. A biological and clinical overview. *Neurochem Res.* 2010 Dec;35(12):1933-8; <https://www.ncbi.nlm.nih.gov/pubmed/21127967>.

### 6.1.3 Current Treatments for Parkinson's Disease

There is no known cure for Parkinson's disease. However, PD is one of the few neurodegenerative disorders with an effective pharmaceutical treatment that can at least temporarily halt the symptoms.

Most notably, in PD the neurons that supply the brain with dopamine neurotransmitter degenerate. So patients can be given a pill containing **levodopa** (aka. L-DOPA, L-3,4-dihydroxyphenylalanine; image, right);<sup>1640</sup> also sold as Atamet, Carbilev, or Sinemet when combined with the enzyme inhibitor carbidopa<sup>1641</sup>), a drug that can be converted into the missing dopamine in the brain. This "dopamine replacement therapy" treats only the symptoms of Parkinson's – the tremors, the stiffness, and the slowness of movements – but not its root causes. Even with the drug, the death of dopaminergic neurons continues. The levodopa pills typically become ineffective after ~7 years, half the patients experience occasional relapses of symptoms despite taking the drug, and there are unwanted side effects (e.g., nausea) to deal with. Dopamine agonists (Pramipexole, Ropinerole, Bromocriptine) bind to dopaminergic post-synaptic receptors in the brain and have similar effects to levodopa, but are not as reliable for symptom control<sup>1642</sup> and may lead to pathological behaviors.<sup>1643</sup>



MAO-B inhibitors such as **rasagiline** (aka. Azilect, TVP-1012, N-propargyl-1(R)-aminoindan; image, left) increase the level of dopamine in the basal ganglia by blocking its metabolism<sup>1644</sup> but produce more adverse effects and are less effective than levodopa. COMT inhibitors (Entacapone/Comtan, Tolcapone/Tasmar) mildly prolong the effect of levodopa therapy by blocking an enzyme that breaks down dopamine.<sup>1645</sup> Neurotransmitters other than dopamine, such as norepinephrine, 5-hydroxytryptamine, glutamate, adenosine and acetylcholine also contribute to

<sup>1640</sup> Salat D, Tolosa E. Levodopa in the treatment of Parkinson's disease: current status and new developments. *J Parkinsons Dis.* 2013 Jan 1;3(3):255-69; <http://content.iospress.com/download/journal-of-parkinsons-disease/jpd130186?id=journal-of-parkinsons-disease%2Fjpd130186>.

<sup>1641</sup> <https://en.wikipedia.org/wiki/Carbidopa/levodopa>.

<sup>1642</sup> Stowe RL, Ives NJ, Clarke C, van Hilten J, Ferreira J, Hawker RJ, Shah L, Wheatley K, Gray R. Dopamine agonist therapy in early Parkinson's disease. *Cochrane Database Syst Rev.* 2008 Apr 16;(2):CD006564; <http://www.ncbi.nlm.nih.gov/pubmed/18425954>.

<sup>1643</sup> Ahlskog JE. Pathological behaviors provoked by dopamine agonist therapy of Parkinson's disease. *Physiol Behav.* 2011 Jul 25;104(1):168-72; <http://www.ncbi.nlm.nih.gov/pubmed/21557955>.

<sup>1644</sup> Schapira AH. Monoamine oxidase B inhibitors for the treatment of Parkinson's disease: a review of symptomatic and potential disease-modifying effects. *CNS Drugs.* 2011 Dec 1;25(12):1061-71; <http://www.leavesite.com/usr/uploads/2015/08/2170251236.pdf>.

<sup>1645</sup> Müller T. Catechol-O-methyltransferase inhibitors in Parkinson's disease. *Drugs.* 2015 Feb;75(2):157-74; <http://www.ncbi.nlm.nih.gov/pubmed/25559423>.

PD symptomatology, so non-dopaminergic therapies have attracted interest, especially caffeine<sup>1646</sup> and adenosine A2A antagonists such as tozadenant, PBF-509, ST1535, ST4206, V81444, and istradefylline (now licensed as an anti-Parkinsonian drug in Japan).<sup>1647</sup>

In some cases, surgery may be appropriate for PD patients whose disease no longer responds well to drugs. The most common procedure, called **deep brain stimulation** (DBS),<sup>1648</sup> is FDA-approved and involves implanting electrodes in the brain and sending high frequency electrical impulses into specific areas of the organ to mitigate symptoms,<sup>1649</sup> though adverse effects have been reported.<sup>1650</sup> More recently, transcranial direct current stimulation (tDTS) using a headband-type device with two electrodes is being investigated as a noninvasive variant of this approach,<sup>1651</sup> and a gyroscopically-stabilized glove is claimed to halt symptomatic hand tremors

<sup>1646</sup> Prediger RD. Effects of caffeine in Parkinson's disease: from neuroprotection to the management of motor and non-motor symptoms. *J Alzheimers Dis.* 2010;20 Suppl 1:S205-20; [https://www.researchgate.net/profile/Rui\\_Prediger/publication/41562326\\_Effects\\_of\\_Caffeine\\_in\\_Parkinson's\\_Disease\\_From\\_Neuroprotection\\_to\\_the\\_Management\\_of\\_Motor\\_and\\_Non-Motor\\_Symptoms/links/5628c21508aef25a243d2004.pdf](https://www.researchgate.net/profile/Rui_Prediger/publication/41562326_Effects_of_Caffeine_in_Parkinson's_Disease_From_Neuroprotection_to_the_Management_of_Motor_and_Non-Motor_Symptoms/links/5628c21508aef25a243d2004.pdf). Petzer JP, Petzer A. Caffeine as a lead compound for the design of therapeutic agents for the treatment of Parkinson's disease. *Curr Med Chem.* 2015;22(8):975-88; <http://www.ncbi.nlm.nih.gov/pubmed/25544641>. Nehlig A. Effects of coffee/caffeine on brain health and disease: What should I tell my patients? *Pract Neurol.* 2015 Dec 16; <http://www.ncbi.nlm.nih.gov/pubmed/26677204>.

<sup>1647</sup> Pinna A. Adenosine A2A receptor antagonists in Parkinson's disease: progress in clinical trials from the newly approved istradefylline to drugs in early development and those already discontinued. *CNS Drugs.* 2014 May;28(5):455-74; <http://www.ncbi.nlm.nih.gov/pubmed/24687255>.

<sup>1648</sup> Perlmutter JS, Mink JW. Deep brain stimulation. *Annu Rev Neurosci.* 2006;29:229-57; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4518728/>. Lyons MK. Deep brain stimulation: current and future clinical applications. *Mayo Clin Proc.* 2011 Jul;86(7):662-72; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3127561/>.

<sup>1649</sup> deSouza RM, Moro E, Lang AE, Schapira AH. Timing of deep brain stimulation in Parkinson disease: a need for reappraisal? *Ann Neurol.* 2013 May;73(5):565-75; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4065356/>.

<sup>1650</sup> Smeding HM, Speelman JD, Koning-Haanstra M, Schuurman PR, Nijssen P, van Laar T, Schmand B. Neuropsychological effects of bilateral STN stimulation in Parkinson disease: a controlled study. *Neurology* 2006;66(12):1830-6; <http://europepmc.org/abstract/med/16801645>.

<sup>1651</sup> Broeder S, Nackaerts E, Heremans E, Vervoort G, Meesen R, Verheyden G, Nieuwboer A. Transcranial direct current stimulation in Parkinson's disease: Neurophysiological mechanisms and behavioral effects. *Neurosci Biobehav Rev.* 2015 Oct;57:105-17; [https://www.researchgate.net/profile/Sanne\\_Broeder/publication/281166331\\_Title\\_Transcranial\\_direct\\_current\\_stimulation\\_in\\_Parkinsons\\_disease\\_Neurophysiological\\_mechanisms\\_and\\_behavioral\\_effects/links/55e082ae08ae2fac471bc8df.pdf](https://www.researchgate.net/profile/Sanne_Broeder/publication/281166331_Title_Transcranial_direct_current_stimulation_in_Parkinsons_disease_Neurophysiological_mechanisms_and_behavioral_effects/links/55e082ae08ae2fac471bc8df.pdf). Ferrucci R, Cortese F, Bianchi M, Pittera D, Turrone R, Bocci T, Borroni B, Vergari M, Cogiamanian F, Ardolino G, Di Fonzo A, Padovani A, Priori A. Cerebellar and motor cortical transcranial stimulation decrease levodopa-induced dyskinesias in Parkinson's disease. *Cerebellum.* 2016 Feb;15(1):43-7; [https://www.researchgate.net/profile/Roberta\\_Ferrucci/publication/283540310\\_Cerebellar\\_and\\_Motor\\_Cor](https://www.researchgate.net/profile/Roberta_Ferrucci/publication/283540310_Cerebellar_and_Motor_Cor)

in PD patients.<sup>1652</sup> Ultrasound thermal lesioning of selected brain tissue<sup>1653</sup> is another form of non-invasive surgery now in clinical trials for treating Parkinson's symptoms,<sup>1654</sup> a modernized version of the old ablative thalamotomy procedures for PD.<sup>1655</sup>

The prospect for future cures for Parkinson's disease is much the same as for Alzheimer's disease: many potential conventional approaches are being tried,<sup>1656</sup> but nothing is working yet and a genuine complete cure still looks a long way off.<sup>1657</sup> The dim prospects for a true PD cure – much as we saw in the case of Alzheimer's disease (e.g., comments by Whitehouse; Chapter 1) – have led at least a few researchers to question the value of large investments in seeking a cure, while urging that resources should be directed toward improved palliative treatments.<sup>1658</sup>

The following is a brief and non-comprehensive summary of current efforts to find a PD cure.

---

[tical Transcranial Stimulation Decrease Levodopa-Induced Dyskinesias in Parkinson's Disease/links/564061ea08ae45b5d28d3cce.pdf](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4266014/).

<sup>1652</sup> “Could this glove be the solution to Parkinson's tremors?” CNN Health, 16 Feb 2016; <http://www.cnn.com/2016/02/16/health/gyroglove-parkinsons-tremors-feat/>.

<sup>1653</sup> Magara A, Bühler R, Moser D, Kowalski M, Pourtehrani P, Jeanmonod D. First experience with MR-guided focused ultrasound in the treatment of Parkinson's disease. *J Ther Ultrasound*. 2014 May 31;2:11; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4266014/>. Metman LV, Slavin KV. Advances in functional neurosurgery for Parkinson's disease. *Mov Disord*. 2015 Sep 15;30(11):1461-70; <http://www.ncbi.nlm.nih.gov/pubmed/26274629>. Na YC, Chang WS, Jung HH, Kweon EJ, Chang JW. Unilateral magnetic resonance-guided focused ultrasound pallidotomy for Parkinson disease. *Neurology*. 2015 Aug 11;85(6):549-51; <http://www.ncbi.nlm.nih.gov/pubmed/26180137>.

<sup>1654</sup> “Metabolic Imaging Center Uses New Ultrasound Technology to Target Deep Structures of the Brain,” Univ. of Maryland, 1 Sep 2015; <http://umm.edu/news-and-events/news-releases/2015/university-of-maryland-medicine-tests-novel-treatment-for-parkinsons> and <http://parkinsonsnewstoday.com/2015/09/04/new-ultrasound-technology-for-treating-parkinsons-disease-patients-is-under-clinical-trial/>. “Parkinson's Disease: Focused Ultrasound Treatment,” Focused Ultrasound Foundation, 2016; <http://www.fusfoundation.org/diseases-and-conditions/brain-disorders/parkinsonian-tremor>.

<sup>1655</sup> Perry VL, Lenz FA. Ablative therapy for movement disorders. Thalamotomy for Parkinson's disease. *Neurosurg Clin N Am*. 1998 Apr;9(2):317-24; <http://www.ncbi.nlm.nih.gov/pubmed/9495894>.

<sup>1656</sup> “Medicines in Development: Parkinson's Disease,” 2014 Report; <http://www.phrma.org/sites/default/files/pdf/2014-parkinsons-report.pdf>.

<sup>1657</sup> Dimond PF. No New Parkinson Disease Drug Expected Anytime Soon. *GEN News Highlights*. GEN-Genetic Engineering and Biotechnology News, 16 Aug 2010; <http://www.genengnews.com/analysis-and-insight/no-new-parkinson-disease-drug-expected-anytime-soon/77899336/>.

<sup>1658</sup> “Are we too focused on the search for a Parkinson's cure?” *Parkinson's Life*, 7 Jan 2016; <http://parkinsonslife.eu/sara-riggare-tom-isaacs-jon-stamford-parkinsons-disease-cure-debate/>.

Both active and passive immunization strategies against  $\alpha$ -synuclein are being investigated,<sup>1659</sup> so far without conclusive success, but are continuing.<sup>1660</sup> In 2015 at least three biotech companies – AFFiRiS, Prothena, and Biogen – were running competing trials of immunotherapy against  $\alpha$ -synuclein targets for PD.<sup>1661</sup>

The search for neuroprotective agents (to delay or halt cell death) is of high priority among PD researchers, but no proven neuroprotective agents or treatments are currently available. Numerous drug molecules have been proposed and are being investigated as potential treatments – including anti-apoptotics (omigapil, CEP-1347), anti-glutamatergics, monoamine oxidase inhibitors (selegiline, rasagiline), pro-mitochondrials (coenzyme Q10, creatine), immunosuppressants (tacrolimus/FK506),<sup>1662</sup> calcium channel blockers (isradipine), anti-convulsants (zonisamide),<sup>1663</sup> anti-inflammatory agents (CU-CPT22,<sup>1664</sup> aspirin,<sup>1665</sup> NSAIDs<sup>1666</sup>),

---

<sup>1659</sup> Brundin P, Atkin G, Lamberts JT. Basic science breaks through: New therapeutic advances in Parkinson's disease. *Mov Disord.* 2015 Sep 15;30(11):1521-7; <http://www.ncbi.nlm.nih.gov/pubmed/26177603>. Lee JS, Lee SJ. Mechanism of anti- $\alpha$ -synuclein immunotherapy. *J Mov Disord.* 2016 Jan;9(1):14-9; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4734990/>. Bergström AL, Kallunki P, Fog K. Development of passive immunotherapies for synucleinopathies. *Mov Disord.* 2016 Feb;31(2):203-13; <http://www.ncbi.nlm.nih.gov/pubmed/26704735>.

<sup>1660</sup> “Antibody Against  $\alpha$ -Synuclein Looks Safe In Phase 1,” 2015 Intl. Conf. on Alzheimer's and Parkinson's Diseases, 31 Mar 2015; <http://www.alzforum.org/news/conference-coverage/antibody-against-synuclein-looks-safe-early-trials>.

<sup>1661</sup> Bret Schulte, “The beginning of the end? The race for a Parkinson's cure,” *Boston Globe*, 27 Sep 2015; <https://www.bostonglobe.com/ideas/2015/09/26/the-beginning-end-the-race-for-parkinson-cure/ZcOup0USufAhaD4tTOF9XL/story.html>. “Two more alpha-synuclein drugs in clinical trials,” MJFF, 14 Aug 2015; <https://www.michaeljfox.org/foundation/news-detail.php?two-more-alpha-synuclein-drugs-in-clinical-trials>.

<sup>1662</sup> Van der Perren A, Macchi F, Toelen J, Carlon MS, Maris M, de Loor H, Kuypers DR, Gijsbers R, Van den Haute C, Debyser Z, Baekelandt V. FK506 reduces neuroinflammation and dopaminergic neurodegeneration in an  $\alpha$ -synuclein-based rat model for Parkinson's disease. *Neurobiol Aging.* 2015 Mar;36(3):1559-68; [https://www.researchgate.net/profile/Anke\\_Van\\_Der\\_Perren/publication/272096266\\_FK506\\_reduces\\_neuroinflammation\\_and\\_dopaminergic\\_neurodegeneration\\_in\\_an\\_alpha-synuclein-based\\_rat\\_model\\_for\\_Parkinson's\\_disease/links/54e3023d0cf2c3e7d2d52c14.pdf](https://www.researchgate.net/profile/Anke_Van_Der_Perren/publication/272096266_FK506_reduces_neuroinflammation_and_dopaminergic_neurodegeneration_in_an_alpha-synuclein-based_rat_model_for_Parkinson's_disease/links/54e3023d0cf2c3e7d2d52c14.pdf)

<sup>1663</sup> Arawaka S, Fukushima S, Sato H, Sasaki A, Koga K, Koyama S, Kato T. Zonisamide attenuates  $\alpha$ -synuclein neurotoxicity by an aggregation-independent mechanism in a rat model of familial Parkinson's disease. *PLoS One.* 2014 Feb 20;9(2):e89076; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3930669/>.

<sup>1664</sup> Daniele SG, Béraud D, Davenport C, Cheng K, Yin H, Maguire-Zeiss KA. Activation of MyD88-dependent TLR1/2 signaling by misfolded  $\alpha$ -synuclein, a protein linked to neurodegenerative disorders. *Sci Signal.* 2015 May 12;8(376):ra45; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4601639/>.

<sup>1665</sup> Choi HW, Tian M, Manohar M, Harraz MM, Park SW, Schroeder FC, Snyder SH, Klessig DF. Human GAPDH Is a Target of Aspirin's Primary Metabolite Salicylic Acid and Its Derivatives. *PLoS One.* 2015 Nov 25;10(11):e0143447; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4659538/>.

anti-malarials (chloroquine, amodiaquine),<sup>1667</sup> LRRK2 kinase inhibitors (MLi-2),<sup>1668</sup> cholesterol medications (simvastatin),<sup>1669</sup> respiratory drugs (ambroxol) and diabetes drugs (exenatide),<sup>1670</sup> nicotine,<sup>1671</sup> and growth factors (GDNF).<sup>1672</sup> None of these has been conclusively demonstrated to reduce degeneration in PD.<sup>1673</sup>

<sup>1666</sup> Gagne JJ, Power MC. Anti-inflammatory drugs and risk of Parkinson disease: a meta-analysis. *Neurology*. 2010 Mar 23;74(12):995-1002; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2848103/>.

<sup>1667</sup> Kim CH, Han BS, Moon J, Kim DJ, Shin J, Rajan S, Nguyen QT, Sohn M, Kim WG, Han M, Jeong I, Kim KS, Lee EH, Tu Y, Naffin-Olivos JL, Park CH, Ringe D, Yoon HS, Petsko GA, Kim KS. Nuclear receptor Nurr1 agonists enhance its dual functions and improve behavioral deficits in an animal model of Parkinson's disease. *Proc Natl Acad Sci U S A*. 2015 Jul 14;112(28):8756-61; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4507186/>.

<sup>1668</sup> Fell MJ, Mirescu C, Basu K, Cheewatrakoolpong B, DeMong DE, Ellis JM, Hyde LA, Lin Y, Markgraf CG, Mei H, Miller M, Poulet FM, Scott JD, Smith MD, Yin Z, Zhou X, Parker EM, Kennedy ME, Morrow JA. MLI-2, a Potent, Selective, and Centrally Active Compound for Exploring the Therapeutic Potential and Safety of LRRK2 Kinase Inhibition. *J Pharmacol Exp Ther*. 2015 Dec;355(3):397-409; <http://www.ncbi.nlm.nih.gov/pubmed/26407721>.

<sup>1669</sup> "Cholesterol Medication Simvastatin Tested As Potential Treatment For Parkinson's Disease In New Clinical Trial," *Medical Daily*, 12 Jan 2016; <http://www.medicaldaily.com/cholesterol-medication-simvastatin-tested-potential-treatment-parkinsons-disease-new-369008>.

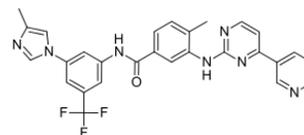
<sup>1670</sup> "New clinical trial focuses on respiratory drug ambroxol for treatment of Parkinson's disease," *Parkinson's News*, 23 Feb 2016; <https://nwpf.org/stay-informed/news/2016/02/new-clinical-trial-focuses-on-respiratory-drug-ambroxol-for-treatment-of-parkinsons-disease/>.

<sup>1671</sup> Quik M, Zhang D, McGregor M, Bordia T. Alpha7 nicotinic receptors as therapeutic targets for Parkinson's disease. *Biochem Pharmacol*. 2015 Oct 15;97(4):399-407; [https://www.researchgate.net/profile/Tanuja\\_Bordia/publication/278743589\\_Alpha7\\_nicotinic\\_receptors\\_as\\_therapeutic\\_targets\\_for\\_Parkinson%27s\\_disease/links/56aa8fd808ae8f3865664233.pdf?origin=publication\\_detail&ev=pub\\_int\\_prw\\_xdl&msrp=MpJqcYY2TxjdVmSgCdOCgB8as9Yfqo9DS1UVkCM8HSn25ONwvdzvzsgbpbOdvTf8qkmz6rekQwb\\_sVtsHL9-A.MW0VsLhJ7eEm2PazJRcAGpI1ry3JnJs1DhO7I4PIaA64r2EVeEzKAJpIMX0-kEyAo2r4XIZzT9zt7V\\_n3L8ynQ.04XZuVNGTuG-F5GmOTtCl3bRBCpk\\_26g9\\_ihRQYPI3UtCpeV3G2GXHDpv6R\\_OrAyyCzaDEWLjEIPAPU0aE1FQ](https://www.researchgate.net/profile/Tanuja_Bordia/publication/278743589_Alpha7_nicotinic_receptors_as_therapeutic_targets_for_Parkinson%27s_disease/links/56aa8fd808ae8f3865664233.pdf?origin=publication_detail&ev=pub_int_prw_xdl&msrp=MpJqcYY2TxjdVmSgCdOCgB8as9Yfqo9DS1UVkCM8HSn25ONwvdzvzsgbpbOdvTf8qkmz6rekQwb_sVtsHL9-A.MW0VsLhJ7eEm2PazJRcAGpI1ry3JnJs1DhO7I4PIaA64r2EVeEzKAJpIMX0-kEyAo2r4XIZzT9zt7V_n3L8ynQ.04XZuVNGTuG-F5GmOTtCl3bRBCpk_26g9_ihRQYPI3UtCpeV3G2GXHDpv6R_OrAyyCzaDEWLjEIPAPU0aE1FQ).

<sup>1672</sup> d'Anglemont de Tassigny X, Pascual A, López-Barneo J. GDNF-based therapies, GDNF-producing interneurons, and trophic support of the dopaminergic nigrostriatal pathway. Implications for Parkinson's disease. *Front Neuroanat*. 2015 Feb 13;9:10; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4327623/>. Fan CH, Ting CY, Lin CY, Chan HL, Chang YC, Chen YY, Liu HL, Yeh CK. Noninvasive, targeted, and non-viral ultrasound-mediated GDNF-plasmid delivery for treatment of Parkinson's disease. *Sci Rep*. 2016 Jan 20;6:19579; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4726227/>. NsGene; <http://nsgene.dk/NsGene-447.aspx>.

<sup>1673</sup> Obeso JA, Rodriguez-Oroz MC, Goetz CG, Marin C, Kordower JH, Rodriguez M, Hirsch EC, Farrer M, Schapira AH, Halliday G. Missing pieces in the Parkinson's disease puzzle. *Nat Med*. 2010 Jun;16(6):653-61; <http://www.its.caltech.edu/~bio156/Papers/PDFs/PD%20rev%2010%20copy.pdf>.

A small trial of a cancer drug called **nilotinib** (aka. AMN107, Tasigna; image, right), has shown promise for PD,<sup>1674</sup> but the effects don't last (when volunteers are taken off the drug, deterioration resumes), the drug costs \$10,000/month, and there are many adverse side effects as is typical of most cancer drugs.<sup>1675</sup>



Microparticle-, nanoparticle-, and implant-based drug delivery systems are being investigated.<sup>1676</sup> “Natural” remedies (e.g., mucuna seed powder, bacopa monnieri herb, creatine monohydrate, coca flavonols, fish oil, anthocyanins, CoQ10, etc.),<sup>1677</sup> herbal medicines,<sup>1678</sup> and cannabis<sup>1679</sup> are as yet of unproven benefit, and purely diet-based approaches to PD have shown uncertain

---

<sup>1674</sup> “People with Parkinson’s walk again after promising drug trial,” New Scientist, 17 Oct 2015; <https://www.newscientist.com/article/dn28357-people-with-parkinsons-walk-again-after-promising-drug-trial/>. “Can A Cancer Drug Reverse Parkinson’s Disease And Dementia?” NPR Health News, 20 Oct 2015; <http://www.npr.org/sections/health-shots/2015/10/17/448323916/can-a-cancer-drug-reverse-parkinsons-disease-and-dementia>.

<sup>1675</sup> <https://en.wikipedia.org/wiki/Nilotinib>.

<sup>1676</sup> Garbayo E, Ansorena E, Blanco-Prieto MJ. Drug development in Parkinson’s disease: from emerging molecules to innovative drug delivery systems. *Maturitas*. 2013 Nov;76(3):272-8; [https://www.researchgate.net/profile/Elisa\\_Garbayo/publication/245537589\\_Drug\\_development\\_in\\_Parkinson's\\_disease\\_From\\_emerging\\_molecules\\_to\\_innovative\\_drug\\_delivery\\_systems/links/00463529cd205e7fc2000000.pdf](https://www.researchgate.net/profile/Elisa_Garbayo/publication/245537589_Drug_development_in_Parkinson's_disease_From_emerging_molecules_to_innovative_drug_delivery_systems/links/00463529cd205e7fc2000000.pdf).

<sup>1677</sup> “5 Natural Remedies To Treat Parkinson’s Disease Symptoms,” Elite Daily, 20 Apr 2015; <http://elitedaily.com/life/natural-remedies-for-parkinsons-disease/999278/>. Ray Sahelian, “Parkinson’s Disease herbs vitamins and supplements - natural and alternative treatment, diet, food and home remedy,” 20 Jan 2016; <http://www.raysahelian.com/parkinson.html>.

<sup>1678</sup> Li XZ, Zhang SN, Liu SM, Lu F. Recent advances in herbal medicines treating Parkinson’s disease. *Fitoterapia*. 2013 Jan;84:273-85; <http://www.sciencedirect.com/science/article/pii/S0367326X1200336X>.

<sup>1679</sup> Arjmand S, Vaziri Z, Behzadi M, Abbassian H, Stephens GJ, Shabani M. Cannabinoids and tremor induced by motor-related disorders: friend or foe? *Neurotherapeutics*. 2015 Oct;12(4):778-87; <http://www.ncbi.nlm.nih.gov/pubmed/26152606>.

results.<sup>1680</sup> Gene therapy approaches to PD<sup>1681</sup> over the last 10 years have provided useful information with as yet no useful treatment outcomes,<sup>1682</sup> but trials continue.<sup>1683</sup>

Cell transplant therapy<sup>1684</sup> and stem cell therapies<sup>1685</sup> are also being investigated, and companies have been formed to pursue this approach.<sup>1686</sup> A few early cell transplant experiments, once

<sup>1680</sup> Vanitallie TB, Nonas C, Di Rocco A, Boyar K, Hyams K, Heymsfield SB. Treatment of Parkinson disease with diet-induced hyperketonemia: a feasibility study. *Neurology*. 2005 Feb 22;64(4):728-30; <http://www.ncbi.nlm.nih.gov/pubmed/15728303>. Stafstrom CE, Rho JM. The ketogenic diet as a treatment paradigm for diverse neurological disorders. *Front Pharmacol*. 2012 Apr 9;3:59; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3321471/>.

<sup>1681</sup> Allen PJ, Feigin A. Gene-based therapies in Parkinson's disease. *Neurotherapeutics*. 2014 Jan;11(1):60-7; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3899481/>. Bartus RT, Weinberg MS, Samulski RJ. Parkinson's disease gene therapy: success by design meets failure by efficacy. *Mol Ther*. 2014 Mar;22(3):487-97; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3944322/>.

<sup>1682</sup> Bartus RT. Gene therapy for Parkinson's disease: a decade of progress supported by posthumous contributions from volunteer subjects. *Neural Regen Res*. 2015 Oct;10(10):1586-8; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4660746/>.

<sup>1683</sup> "Gene Therapy for Parkinson's Disease to be Tested by Pitt, UPMC Experts," UPMC, 22 Feb 2016; <http://www.upmc.com/media/NewsReleases/2016/Pages/pd-gene-tx.aspx>.

<sup>1684</sup> Barker RA, Drouin-Ouellet J, Parmar M. Cell-based therapies for Parkinson disease – past insights and future potential. *Nat Rev Neurol*. 2015 Sep;11(9):492-503; [http://soundchoice.s3.amazonaws.com/soundchoice/wp-content/uploads/20151006222427/Barker\\_cell-based-therapies-for-parkinson-disease\\_2015.pdf](http://soundchoice.s3.amazonaws.com/soundchoice/wp-content/uploads/20151006222427/Barker_cell-based-therapies-for-parkinson-disease_2015.pdf). Tronci E, Fidalgo C, Carta M. Foetal cell transplantation for Parkinson's disease: focus on graft-induced dyskinesia. *Parkinsons Dis*. 2015 Dec; 2015:563820; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4736211/>.

<sup>1685</sup> Lindvall O, Kokaia Z, Martinez-Serrano A. Stem cell therapy for human neurodegenerative disorders-how to make it work. *Nat Med*. 2004 Jul;10 Suppl:S42-50; <http://www.keck.bioimaging.wisc.edu/neuro670/requreading/StemCellTherapyForHumanNeurodegenerativeDisorders-HowToMakeItWork.pdf>. Fu YS, Cheng YC, Lin MY, Cheng H, Chu PM, Chou SC, Shih YH, Ko MH, Sung MS. Conversion of human umbilical cord mesenchymal stem cells in Wharton's jelly to dopaminergic neurons *in vitro*: potential therapeutic application for Parkinsonism. *Stem Cells*. 2006 Jan;24(1):115-24; <http://onlinelibrary.wiley.com/doi/10.1634/stemcells.2005-0053/epdf>. van den Berge SA, van Strien ME, Hol EM. Resident adult neural stem cells in Parkinson's disease – the brain's own repair system? *Eur J Pharmacol*. 2013 Nov 5;719(1-3):117-27; [http://www.researchgate.net/profile/Eilly\\_Hol/publication/250923573\\_Resident\\_adult\\_neural\\_stem\\_cells\\_in\\_Parkinson's\\_disease\\_-\\_the\\_brain's\\_own\\_repair\\_system/links/0deec5288b95b50395000000.pdf](http://www.researchgate.net/profile/Eilly_Hol/publication/250923573_Resident_adult_neural_stem_cells_in_Parkinson's_disease_-_the_brain's_own_repair_system/links/0deec5288b95b50395000000.pdf). Battista D, Ganat Y, El Maarouf A, Studer L, Rutishauser U. Enhancement of polysialic acid expression improves function of embryonic stem-derived dopamine neuron grafts in Parkinsonian mice. *Stem Cells Transl Med*. 2014 Jan;3(1):108-13; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3902288/>. Chou CH, Fan HC, Hueng DY. Potential of Neural Stem Cell-based therapy for Parkinson's disease. *Parkinsons Dis*. 2015;2015:571475; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4664819/>. Gonzalez C, Bonilla S, Flores AI, Cano E, Liste I. Gonzalez C, Bonilla S, Flores AI, Cano E, Liste II. *Curr Stem Cell Res Ther*. 2015 May 31; <http://www.ncbi.nlm.nih.gov/pubmed/26027681>.

<sup>1686</sup> <http://sage.buckinstitute.org/interview-with-dr-xianmin-zeng-stem-cell-treatment-for-parkinsons-disease/>.

thought to be negative<sup>1687</sup> but later recognized as more positive,<sup>1688</sup> have resumed with clinical trials expected to begin in 2017.

Most recently on the stem cell front, International Stem Cell Corporation, a California-based biotechnology company, is starting a Phase I trial in which neural stem cells will be injected directly into the brains of 12 Australian sufferers with moderate to severe Parkinson's.<sup>1689</sup> The trial, to be run by the company's Australian subsidiary (Cyto Therapeutics) and conducted at Royal Melbourne Hospital, will evaluate three different doses of stem cells that can transform into dopamine-producing neurons. (Following trials on rats and monkeys that appeared promising,<sup>1690</sup> the company is using "parthenogenetic" stem cells obtained from unfertilized human eggs, reducing the risk of rejection and avoiding the ethical complications of embryonic stem cells.) If the Phase I trial is successful, larger Phase II trials will begin in 2017 or 2018 in Australia, Europe and the US.

---

<sup>1687</sup> "Location of brain grafts blamed for 'devastating' results," New Scientist Daily News, 13 Nov 2001; <https://www.newscientist.com/article/dn1556-location-of-brain-grafts-blamed-for-devastating-results/>.

<sup>1688</sup> "Fetal cells injected into a man's brain to cure his Parkinson's," New Scientist Daily News, 26 May 2015; <https://www.newscientist.com/article/dn27593-fetal-cells-injected-into-a-mans-brain-to-cure-his-parkinsons/>.

<sup>1689</sup> John Ross, "Australian stem cell bid to cure Parkinson's disease," The Australian News, 15 Dec 2015; <http://www.theaustralian.com.au/news/health-science/australian-stem-cell-bid-to-cure-parkinsons-disease/news-story/dd4e796d51aa86f76b794f14db5c26e4>.

<sup>1690</sup> Gonzalez R, Garitaonandia I, Poustovoitov M, Abramihina T, McEntire C, Culp B, Attwood J, Noskov A, Christiansen-Weber T, Khater M, Mora-Castilla S, To C, Crain A, Sherman G, Semechkin A, Laurent LC, Elsworth JD, Sladek J, Snyder EY, Jr DE, Kern RA. Neural Stem Cells derived from human parthenogenetic stem cells engraft and promote recovery in a nonhuman primate model of Parkinson's disease. *Cell Transplant*. 2016 May 20; <http://www.ncbi.nlm.nih.gov/pubmed/27213850>; <http://www.ingentaconnect.com/content/cog/ct/pre-prints/content-CT-1518> Gonzalez et al.

### 6.1.4 Nanorobotic Treatment for Parkinson's Disease

In general, the nanorobotic treatment for Parkinson's disease closely mirrors the previously described treatment protocols for Alzheimer's disease (Chapter 5), but with the genetics, tissues and cell types altered appropriately for the PD target.

For asymptomatic PD patients without Lewy bodies but having some genetic risk factor, an application of PD-modified nanorobotic **Alzheimer Protocol #1** (Section 5.1) should suffice to reduce that individual's propensity for PD to the much lower probability typical of the general population. In the PD-modified nanorobotic Alzheimer Protocol #1, all genes with undesirable mutations, including PARK1 (SNCA), PARK8 (LRRK2), and the rest of the PARK genes (Section 6.1), will be replaced throughout the PD patient's body with unmutated versions and correct numbers of these genes.

For asymptomatic PD patients with or without Lewy bodies, PD-modified Alzheimer Protocol #1 will eliminate any genetic risk and **Alzheimer Protocol #2** (Section 5.2) will eliminate excess  $\alpha$ -synuclein and Lewy bodies (Section 5.2.2.3) and restore most neural cells to youthful vigor, essentially reducing to zero the probability of the patient progressing to symptomatic PD. Pathological tau oligomers may be present along with the  $\alpha$ -synuclein<sup>1691</sup> and will be cleaned out at the same time using a similar process (Section 5.2.2.1). Even if asymptomatic, the cautious PD patient might nonetheless wish to apply the neuron replacement procedures of a PD-modified Alzheimer Protocol #3 (Section 5.3), since substantial dopaminergic neuron damage and cell death can occur before the symptoms of PD appear.<sup>1692</sup>

For PD patients who are already exhibiting symptoms of the disease, the application of PD-modified Alzheimer Protocol #1 and Alzheimer Protocol #2 should halt the progress of neural damage and should reverse PD-related pathologies for neural cells that are not yet damaged beyond the point of rejuvenatable self-repair.

For dopamine-producing cells in the substantia nigra (and elsewhere) that are so damaged as to preclude self-repair even after attempted rejuvenation, these cells may be remanufactured in a cell mill (Section 4.2.6) and replaced in the affected tissue in much the same way as for missing neural tissues in AD, using a PD-modified application of the more aggressive **Alzheimer Protocol #3**. There are only 400,000-600,000 dopaminergic neurons in the entire human brain, although their axons project into ~1000 times this number of postsynaptic neurons in the striatum,

---

<sup>1691</sup> Sengupta U, Guerrero-Muñoz MJ, Castillo-Carranza DL, Lasagna-Reeves CA, Gerson JE, Paulucci-Holthausen AA, Krishnamurthy S, Farhed M, Jackson GR, Kaye R. Pathological interface between oligomeric alpha-synuclein and tau in synucleinopathies. *Biol Psychiatry*. 2015 Nov 15;78(10):672-83; [https://www.researchgate.net/profile/Urmi\\_Sengupta/publication/270650947\\_Pathological\\_Interface\\_between\\_Oligomeric\\_Alpha-Synuclein\\_and\\_Tau\\_in\\_Synucleinopathies/links/551c55e90cf2fe6cbf78fd52.pdf](https://www.researchgate.net/profile/Urmi_Sengupta/publication/270650947_Pathological_Interface_between_Oligomeric_Alpha-Synuclein_and_Tau_in_Synucleinopathies/links/551c55e90cf2fe6cbf78fd52.pdf).

<sup>1692</sup> Kordower JH, Olanow CW, Dodiya HB, Chu Y, Beach TG, Adler CH, Halliday GM, Bartus RT. Disease duration and the integrity of the nigrostriatal system in Parkinson's disease. *Brain*. 2013 Aug;136(Pt 8):2419-31; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3722357/>.

cortex, amygdala, and other structures,<sup>1693</sup> possibly including the claustrum.<sup>1694</sup> The dopaminergic neurons and their processes are mostly located in two areas of the brain: the substantia nigra, where dopamine neurons live, and the main brain area where these neurons release their dopamine – called the striatum or putamen.

Because the substantia nigra is relatively small in volume, the neural reconstruction task in PD patients should be considerably less challenging than in AD brains where potentially tens of billions of neurons might need to be replaced, requiring tens of billions of shepherd nanorobots and related devices. By comparison, at the onset of PD symptoms when  $\geq 50\%$  of substantia nigra neurons are gone, only  $\sim 425,000$  missing neurons will need to be replaced (e.g., the substantia nigra has 810,000 neurons in controls vs. 385,000 neurons in PD patients, according to one study),<sup>1695</sup> possibly rising to as many as  $\sim 570,000$  missing neurons if there is 70% neuron loss in the substantia nigra just prior to PD death. Replacing so few neurons might require thousands of times fewer nanorobots than would be needed for AD repair.

The task of replacement should also be somewhat easier for Parkinson's than for Alzheimer's patients because in PD the missing axonal and synaptic connections are more readily inferred from the larger amount of surviving tissue, and because replicating the precise original connectivity may be less important for PD patients than in AD where actual memory data may have become unrecoverable. For reversal of PD, it might suffice to re-establish "typical" connectivity using previously assembled comprehensive maps of nigral neuronal processes and their connections in normal brains.

---

<sup>1693</sup> Schultz W. Multiple dopamine functions at different time courses. *Annu Rev Neurosci.* 2007;30:259-88; <http://w3.pdn.cam.ac.uk/staff/schultz/pdfs%20website/2007%20AnnRevNeurosci.pdf>.

<sup>1694</sup> Kalaitzakis ME, Pearce RK, Gentleman SM. Clinical correlates of pathology in the claustrum in Parkinson's disease and dementia with Lewy bodies. *Neurosci Lett.* 2009 Sep 11;461(1):12-5; [https://www.researchgate.net/profile/Steve\\_Gentleman/publication/26289306\\_Clinical\\_correlates\\_of\\_pathology\\_in\\_the\\_claustrum\\_in\\_Parkinsons\\_disease\\_and\\_dementia\\_with\\_Lewy\\_bodies. Neurosci Lett/links/0046352de462939541000000.pdf](https://www.researchgate.net/profile/Steve_Gentleman/publication/26289306_Clinical_correlates_of_pathology_in_the_claustrum_in_Parkinsons_disease_and_dementia_with_Lewy_bodies. Neurosci Lett/links/0046352de462939541000000.pdf).

<sup>1695</sup> Pakkenberg B, Møller A, Gundersen HJ, Mouritzen Dam A, Pakkenberg H. The absolute number of nerve cells in substantia nigra in normal subjects and in patients with Parkinson's disease estimated with an unbiased stereological method. *J Neurol Neurosurg Psychiatry.* 1991 Jan;54(1):30-3; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1014294/pdf/jnnpsyc00499-0040.pdf>.

## 6.2 Other Neurodegenerative Conditions

Other presently-incurable neurodegenerative diseases appear amenable to treatment and cure using our previously-described nanorobot-based protocols (Chapter 5). Specific examples include:

- **trinucleotide repeat disorders** (e.g., Huntington’s disease, spinocerebellar ataxia, Kennedy’s disease, Naito-Oyanagi disease, fragile X-associated tremor/ataxia syndrome, and Friedreich’s ataxia; Section 6.2.1),
- **amyotrophic lateral sclerosis** (Section 6.2.2),
- **vascular dementia** (Section 6.2.3),
- **cerebral amyloidosis** (e.g., cerebral amyloid angiopathy, prion disease, Creutzfeldt-Jakob disease, and familial amyloid polyneuropathy; Section 6.2.4),
- **neurodegenerative tauopathies** (e.g., Pick’s disease, corticobasal degeneration, progressive supranuclear palsy, and chronic traumatic encephalopathy; Section 6.2.5),
- **neuronal ceroid lipofuscinoses** (Section 6.2.6),
- **neural autoimmune disorders** (Section 6.2.7), and
- **multiple system atrophy** (Section 6.2.8).

A very few neurodegenerative conditions already have effective conventional treatments today.

For example, **neurosyphilis**<sup>1696</sup> is a neurodegenerative infection of the brain or spinal cord caused by the spirochete *Treponema pallidum*, usually occurring in people who have had chronic, untreated syphilis 10-20 years after first infection and developing in about 25%-40% of persons who are not treated with penicillin (which is an effective cure).<sup>1697</sup>

**Normal pressure hydrocephalus** (NPH), aka. symptomatic hydrocephalus, is a type of brain malfunction caused by decreased absorption of cerebrospinal fluid (CSF), with typical symptoms of gait disturbance, urinary incontinence, and dementia or mental decline.<sup>1698</sup> It can be treated by

---

<sup>1696</sup> <https://en.wikipedia.org/wiki/Neurosyphilis>.

<sup>1697</sup> Mehrabian S, Raycheva M, Traykova M, Stankova T, Penev L, Grigorova O, Traykov L. Neurosyphilis with dementia and bilateral hippocampal atrophy on brain magnetic resonance imaging. BMC Neurol. 2012 Sep 20;12:96; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3517431/>.

<sup>1698</sup> [https://en.wikipedia.org/wiki/Normal\\_pressure\\_hydrocephalus](https://en.wikipedia.org/wiki/Normal_pressure_hydrocephalus).

installing a shunt to drain excess CSF into another part of the body, reversing the symptoms and restoring normal functioning in 86% of all patients.<sup>1699</sup>

In **low-pressure hydrocephalus** (LPH), brain ventricles are enlarged and the patient experiences severe dementia, inability to walk, and incontinence despite very low intracranial pressure (apparently a more acute form of normal pressure hydrocephalus).<sup>1700</sup> LPH is a rare clinical condition that can also be treated using an external ventricular drain.<sup>1701</sup>

Other usually reversible causes of neurodegeneration currently include **encephalitis**,<sup>1702</sup> **Guillain-Barré syndrome** (GBS),<sup>1703</sup> **Lyme disease**,<sup>1704</sup> various **polyneuropathies**,<sup>1705</sup> **progressive inflammatory neuropathy**,<sup>1706</sup> **vitamin B12 deficiency**,<sup>1707</sup> and **Whipple's disease**.<sup>1708</sup>

---

<sup>1699</sup> Poca MA, Mataró M, Del Mar Matarín M, Arian F, Junqué C, Sahuquillo J. Is the placement of shunts in patients with idiopathic normal-pressure hydrocephalus worth the risk? Results of a study based on continuous monitoring of intracranial pressure. *J Neurosurg*. 2004 May;100(5):855-66; <https://www.ncbi.nlm.nih.gov/pubmed/15137605>.

<sup>1700</sup> [https://en.wikipedia.org/wiki/Low\\_pressure\\_hydrocephalus](https://en.wikipedia.org/wiki/Low_pressure_hydrocephalus).

<sup>1701</sup> Pang D, Altschuler E. Low-pressure hydrocephalic state and viscoelastic alterations in the brain. *Neurosurgery*. 1994 Oct;35(4):643-55; <http://www.ncbi.nlm.nih.gov/pubmed/7808607>. Oowler BK, Jacobson EE, Johnston IH. Low pressure hydrocephalus: issues of diagnosis and treatment in five cases. *Br J Neurosurg*. 2001 Aug;15(4):353-9; <https://www.ncbi.nlm.nih.gov/pubmed/11599454>. Lesniak MS, Clatterbuck RE, Rigamonti D, Williams MA. Low pressure hydrocephalus and ventriculomegaly: hysteresis, non-linear dynamics, and the benefits of CSF diversion. *Br J Neurosurg*. 2002 Dec;16(6):555-61; <https://www.ncbi.nlm.nih.gov/pubmed/12617236>.

<sup>1702</sup> <https://en.wikipedia.org/wiki/Encephalitis>.

<sup>1703</sup> [https://en.wikipedia.org/wiki/Guillain-Barré\\_syndrome](https://en.wikipedia.org/wiki/Guillain-Barré_syndrome).

<sup>1704</sup> [https://en.wikipedia.org/wiki/Lyme\\_disease](https://en.wikipedia.org/wiki/Lyme_disease).

<sup>1705</sup> <https://en.wikipedia.org/wiki/Polyneuropathy>.

<sup>1706</sup> [https://en.wikipedia.org/wiki/Progressive\\_inflammatory\\_neuropathy](https://en.wikipedia.org/wiki/Progressive_inflammatory_neuropathy).

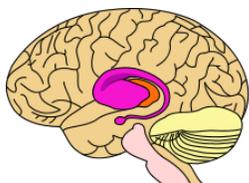
<sup>1707</sup> [https://en.wikipedia.org/wiki/Vitamin\\_B12\\_deficiency](https://en.wikipedia.org/wiki/Vitamin_B12_deficiency).

<sup>1708</sup> [https://en.wikipedia.org/wiki/Whipple's\\_disease](https://en.wikipedia.org/wiki/Whipple's_disease).

## 6.2.1 Trinucleotide Repeat Disorders (including Huntington's Disease)

Trinucleotide repeat disorders (also known as trinucleotide repeat expansion disorders, triplet repeat expansion disorders, codon reiteration disorders, and polyglutamine disease<sup>1709</sup>) are a set of at least 9 genetic neurodegenerative disorders caused by trinucleotide repeat expansion, a special mutation where the number of trinucleotide repeats in certain genes exceeds the normal stable threshold, which differs per gene. Here are a few examples of such disorders.

**Huntington's disease**,<sup>1710</sup> aka. Huntington's chorea, is a neurodegenerative genetic disorder that affects muscle coordination and leads to mental decline and behavioral symptoms.<sup>1711</sup> All humans have two copies of the Huntingtin gene (HTT), which codes for the protein Huntingtin (Htt).<sup>1712</sup> Part of this gene is a repeated section of the nucleotides CAG, called a trinucleotide repeat (e.g., ...CAGCAGCAG...), which varies in length between individuals. If the CAG repeat is present in a healthy gene, a dynamic mutation may increase the repeat count and result in a defective gene. When the length of this repeated section exceeds a threshold of 36-40 copies, it produces an altered form of the protein, called mutant Huntingtin protein (mHtt). The mHtt molecule strands can make hydrogen bonds with one another, forming a protein aggregate rather than folding into functional proteins. Over time, the aggregates accumulate, misfold and coalesce in a process called protein aggregation, forming neuronal intranuclear inclusion bodies called "aggresomes"<sup>1713</sup> within cells that interfere with neuron function.<sup>1714</sup> Inclusion bodies are found in both cell nucleus and cytoplasm, ultimately leading to a loss of GABAergic neurons in a part of the basal ganglia called the neostriatum (purple area in image, left) with atrophy of the caudate nucleus and putamen. Other areas affected include the substantia nigra, layers 3, 5 and 6 of the cerebral



<sup>1709</sup> Yamada M, Shimohata M, Sato T, Tsuji S, Takahashi H. Polyglutamine disease: recent advances in the neuropathology of dentatorubral-pallidoluysian atrophy. *Neuropathology*. 2006 Aug;26(4):346-51; <http://www.ncbi.nlm.nih.gov/pubmed/16961072>. Chopra R, Shakkottai VG. The role for alterations in neuronal activity in the pathogenesis of polyglutamine repeat disorders. *Neurotherapeutics*. 2014 Oct;11(4):751-63; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4391381/>.

<sup>1710</sup> [https://en.wikipedia.org/wiki/Huntington's\\_disease](https://en.wikipedia.org/wiki/Huntington's_disease).

<sup>1711</sup> Walker FO. Huntington's disease. *Lancet*. 2007 Jan 20;369(9557):218-28; <http://www.hdreach.org/HDRReachblog/wp-content/uploads/2013/04/Walker-HDpaperinlancet.pdf>.

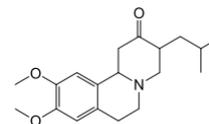
<sup>1712</sup> Saudou F, Humbert S. The biology of Huntingtin. *Neuron*. 2016 Mar 2;89(5):910-26; <http://www.ncbi.nlm.nih.gov/pubmed/26938440>.

<sup>1713</sup> Shimohata T, Sato A, Burke JR, Strittmatter WJ, Tsuji S, Onodera O. Expanded polyglutamine stretches form an 'aggresome'. *Neurosci Lett*. 2002 May 3;323(3):215-8; <http://www.ncbi.nlm.nih.gov/pubmed/11959423>.

<sup>1714</sup> Rubinsztein DC, Carmichael J. Huntington's disease: molecular basis of neurodegeneration. *Expert Rev Mol Med*. 2003 Aug 22;5(20):1-21; <https://www.ncbi.nlm.nih.gov/pubmed/14585171>.

cortex, the hippocampus, Purkinje cells in the cerebellum, lateral tuberal nuclei of the hypothalamus and parts of the thalamus.<sup>1715</sup> HD also causes an abnormal increase in astrocytes and activation of microglia, the brain's immune cells.<sup>1716</sup>

There is no cure for HD, but there are treatments available to reduce the severity of some of its symptoms,<sup>1717</sup> including tetrabenazine (image, right), neuroleptics and benzodiazepines that help to reduce the symptoms of chorea (abnormal involuntary writhing movements).



**Spinocerebellar ataxia,**<sup>1718</sup> aka. spinocerebellar atrophy (SCA) or spinocerebellar degeneration, is a progressive and irreversible degenerative genetic disease with multiple (~60) types, characterized by degeneration of the spinal cord and cerebellum, with slowly progressive incoordination of gait often associated with poor coordination of hands, speech, and eye movements.<sup>1719</sup> Like Huntington's disease, the repeated codon is CAG which codes for glutamine (Q) – the reason why these diseases are commonly referred to as polyglutamine (or PolyQ) diseases. The threshold for symptoms in most forms of SCA is around 35 copies of CAG. Like Huntington's disease, there is no cure for spinocerebellar ataxia although research continues on possible future treatment directions.<sup>1720</sup>

**Kennedy's disease,** aka. spinal and bulbar muscular atrophy (SBMA),<sup>1721</sup> spinobulbar muscular atrophy, bulbo-spinal atrophy, X-linked bulbospinal neuropathy (XBSN), or X-linked

<sup>1715</sup> Waldvogel HJ, Kim EH, Tippett LJ, Vonsattel JP, Faull RL. The neuropathology of Huntington's Disease. *Curr Top Behav Neurosci.* 2015;22:33-80; [https://www.researchgate.net/profile/Henry\\_Waldvogel/publication/266744716\\_The\\_Neuropathology\\_of\\_Huntington's\\_Disease/links/5472879a0cf2d67fc035c80a.pdf](https://www.researchgate.net/profile/Henry_Waldvogel/publication/266744716_The_Neuropathology_of_Huntington's_Disease/links/5472879a0cf2d67fc035c80a.pdf).

<sup>1716</sup> Lobsiger CS, Cleveland DW. Glial cells as intrinsic components of non-cell-autonomous neurodegenerative disease. *Nat Neurosci.* 2007 Nov;10(11):1355-60; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3110080/>.

<sup>1717</sup> Frank S, Jankovic J. Advances in the pharmacological management of Huntington's disease. *Drugs.* 2010 Mar 26;70(5):561-71; <https://www.ncbi.nlm.nih.gov/pubmed/20329804>. Shannon KM, Frait A. Therapeutic advances in Huntington's Disease. *Mov Disord.* 2015 Sep 15;30(11):1539-46; <http://www.ncbi.nlm.nih.gov/pubmed/26226924>.

<sup>1718</sup> [https://en.wikipedia.org/wiki/Spinocerebellar\\_ataxia](https://en.wikipedia.org/wiki/Spinocerebellar_ataxia).

<sup>1719</sup> Rossi M, Perez-Lloret S, Doldan L, Cerquetti D, Balej J, Millar Vernetti P, Hawkes H, Cammarota A, Merello M. Autosomal dominant cerebellar ataxias: a systematic review of clinical features. *Eur J Neurol.* 2014 Apr;21(4):607-15; <https://www.ncbi.nlm.nih.gov/pubmed/24765663>.

<sup>1720</sup> Evers MM, Toonen LJ, van Roon-Mom WM. Ataxin-3 protein and RNA toxicity in spinocerebellar ataxia type 3: current insights and emerging therapeutic strategies. *Mol Neurobiol.* 2014 Jun;49(3):1513-31; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4012159/>. Wu Y, Peng Y, Wang Y. An insight into advances in the pathogenesis and therapeutic strategies of spinocerebellar ataxia type 3. *Rev Neurosci.* 2015;26(1):95-104; <http://www.ncbi.nlm.nih.gov/pubmed/25222595>.

<sup>1721</sup> [https://en.wikipedia.org/wiki/Spinal\\_and\\_bulbar\\_muscular\\_atrophy](https://en.wikipedia.org/wiki/Spinal_and_bulbar_muscular_atrophy).

spinal muscular atrophy type 1 (SMAX1),<sup>1722</sup> is another debilitating neurodegenerative disorder resulting in muscle cramps and progressive weakness due to degeneration of motor neurons in the brain stem and spinal cord. The condition is associated with mutation of the androgen receptor gene and is inherited in an X-linked recessive manner.<sup>1723</sup> SBMA is caused by expansion of a CAG repeat (i.e., trinucleotide repeats) in the first exon of the androgen receptor gene.<sup>1724</sup> The CAG repeat encodes a polyglutamine tract in the androgen receptor protein – the greater the expansion of the CAG repeat, the earlier the disease onset and the more severe the manifestation of the disease. Neuromuscular management is supportive – the disease progresses very slowly but can eventually lead to extreme disability, and there is currently no treatment or cure for this disease.<sup>1725</sup>

**Naito-Oyanagi disease,**<sup>1726</sup> aka. Haw River syndrome or dentatorubral-pallidolusian atrophy (DRPLA),<sup>1727</sup> is an autosomal dominant spinocerebellar degeneration caused by an expansion of a CAG repeat encoding a polyglutamine tract in the atrophin-1 protein.<sup>1728</sup> Naito-Oyanagi is characterized clinically by involuntary muscle twitching, epilepsy, loss of voluntary coordination of muscle movements including gait abnormality and choreoathetosis (twisting and writhing), generalized brain atrophy, and dementia. There are also neuronal intranuclear inclusions in both neurons and glial cells in the striatum, pontine nuclei, inferior olive, cerebellar cortex and dentate nucleus,<sup>1729</sup> though the incidence of neurons with such inclusions is only 1-

<sup>1722</sup> Arvin S. Analysis of inconsistencies in terminology of spinal and bulbar muscular atrophy and its effect on retrieval of research. *J Med Libr Assoc.* 2013 Apr;101(2):147-50; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3634378/>.

<sup>1723</sup> Fischbeck KH, Lieberman A, Bailey CK, Abel A, Merry DE. Androgen receptor mutation in Kennedy's disease. *Philos Trans R Soc Lond B Biol Sci.* 1999 Jun 29;354(1386):1075-8; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1692603/>.

<sup>1724</sup> La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature.* 1991 Jul 4;352(6330):77-9; [https://www.researchgate.net/profile/Elizabeth\\_Wilson25/publication/21094409\\_Androgen\\_Receptor\\_Gene\\_Mutations\\_in\\_X-linked\\_Spinal\\_and\\_Bulbar\\_Muscular\\_Atrophy/links/55b79b8108ae9289a08bf678.pdf](https://www.researchgate.net/profile/Elizabeth_Wilson25/publication/21094409_Androgen_Receptor_Gene_Mutations_in_X-linked_Spinal_and_Bulbar_Muscular_Atrophy/links/55b79b8108ae9289a08bf678.pdf).

<sup>1725</sup> Grunseich C, Rinaldi C, Fischbeck KH. Spinal and bulbar muscular atrophy: pathogenesis and clinical management. *Oral Dis.* 2014 Jan;20(1):6-9; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4284073/>.

<sup>1726</sup> Naito H, Oyanagi S. Familial myoclonus epilepsy and choreoathetosis: hereditary dentatorubral-pallidolusian atrophy. *Neurology.* 1982 Aug;32(8):798-807; <https://www.ncbi.nlm.nih.gov/pubmed/6808417>.

<sup>1727</sup> [https://en.wikipedia.org/wiki/Dentatorubral-pallidolusian\\_atrophy](https://en.wikipedia.org/wiki/Dentatorubral-pallidolusian_atrophy).

<sup>1728</sup> Kanazawa I. Molecular pathology of dentatorubral-pallidolusian atrophy. *Philos Trans R Soc Lond B Biol Sci.* 1999 Jun 29;354(1386):1069-74; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1692599/>.

<sup>1729</sup> Hayashi Y, Kakita A, Yamada M, Koide R, Igarashi S, Takano H, Ikeuchi T, Wakabayashi K, Egawa S, Tsuji S, Takahashi H. Hereditary dentatorubral-pallidolusian atrophy: detection of widespread ubiquitinated neuronal and glial intranuclear inclusions in the brain. *Acta Neuropathol.* 1998 Dec;96(6):547-52; <https://www.ncbi.nlm.nih.gov/pubmed/9845282>.

3%.<sup>1730</sup> These inclusions are non-membrane-bound spherical eosinophilic structures of various sizes, composed of both granular and filamentous structures that are ubiquitinated and often paired or in doublet form within the nucleus.<sup>1731</sup> Filamentous atrophin-1-positive inclusions are also observed exclusively in the cytoplasm of the dentate nucleus, which are extremely similar to the inclusions observed in the motor neurons in amyotrophic lateral sclerosis.<sup>1732</sup> Seizures can be treated with anticonvulsants and psychiatric disturbances can be treated with psychotropic medications,<sup>1733</sup> but there is currently no known cure for this disease.

**Fragile X-associated tremor/ataxia syndrome (FXTAS)**<sup>1734</sup> is a late-onset neurodegenerative disorder associated with problems of movement, memory, and the autonomic nervous system.<sup>1735</sup> As distinct from fragile X syndrome, in FXTAS the fragile X mental retardation 1 gene, FMR1, is overexpressed and interferes with brain function, due to a trinucleotide (CGG) repeat expansion in FMR1. The physical symptoms of FXTAS<sup>1736</sup> include an intention tremor, ataxia, and Parkinsonism with small shuffling steps, muscle rigidity, slowed speech, and neuropathic symptoms. In advanced stages, FXTAS patients are also at risk of autonomic dysfunction including hypertension, bowel and bladder dysfunction, and impotence. Psychological symptoms may include a decrease in cognition (with diminishing short-term memory and executive function skills) and declining math, spelling, and decision-making abilities. FXTAS may also cause personality changes due to limbic alterations in the brain,

---

<sup>1730</sup> Yamada M, Sato T, Tsuji S, Takahashi H. CAG repeat disorder models and human neuropathology: similarities and differences. *Acta Neuropathol.* 2008 Jan;115(1):71-86; <https://www.ncbi.nlm.nih.gov/pubmed/17786457>.

<sup>1731</sup> Yamada M, Sato T, Shimohata T, Hayashi S, Igarashi S, Tsuji S, Takahashi H. Interaction between neuronal intranuclear inclusions and promyelocytic leukemia protein nuclear and coiled bodies in CAG repeat diseases. *Am J Pathol.* 2001 Nov;159(5):1785-95; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1867069/>.

<sup>1732</sup> Yamada M, Piao YS, Toyoshima Y, Tsuji S, Takahashi H. Ubiquitinated filamentous inclusions in cerebellar dentate nucleus neurons in dentatorubral-pallidolusian atrophy contain expanded polyglutamine stretches. *Acta Neuropathol.* 2000 Jun;99(6):615-8; <https://www.ncbi.nlm.nih.gov/pubmed/10867794>.

<sup>1733</sup> Hamada S, Shimakawa S, Satomura S, Naito E, Hashimoto T. Successful treatment of epilepsy and circadian rhythm disturbance with levetiracetam in a patient with dentatorubral-pallidolusian atrophy (DRPLA). *No To Hattatsu.* 2014 Nov;46(6):439-42, in Japanese; <http://www.ncbi.nlm.nih.gov/pubmed/25558587>.

<sup>1734</sup> [https://en.wikipedia.org/wiki/Fragile\\_X-associated\\_tremor/ataxia\\_syndrome](https://en.wikipedia.org/wiki/Fragile_X-associated_tremor/ataxia_syndrome).

<sup>1735</sup> Amiri K, Hagerman RJ, Hagerman PJ. Fragile X-associated tremor/ataxia syndrome: an aging face of the fragile X gene. *Arch Neurol.* 2008 Jan;65(1):19-25; <http://archneur.jamanetwork.com/article.aspx?articleid=794994>.

<sup>1736</sup> Hagerman PJ, Hagerman RJ. Fragile X-associated tremor/ataxia syndrome (FXTAS). *Ment Retard Dev Disabil Res Rev.* 2004;10(1):25-30; <http://fragilex.se/doc/FXTAS.pdf>.

including increased irritability, angry outbursts, and impulsive behavior.<sup>1737</sup> There is no cure for FXTAS, but several of the symptoms can be managed with medication.<sup>1738</sup>

**Friedreich's ataxia (FA)**<sup>1739</sup> is another example of a “trinucleotide repeat expansion” wherein the FXN gene on Chromosome 9, which encodes the mitochondrial matrix protein frataxin, develops multiple (>27) repeats of the nucleotide triplet GAA in the first intron.<sup>1740</sup> FA is an autosomal recessive inherited disease that causes progressive damage to the nervous system, manifesting initially as poor coordination such as gait disturbance, progressing to scoliosis, heart disease and diabetes until a wheelchair is required for mobility, though without affecting cognitive function. The particular genetic mutation leads to reduced expression of frataxin, a deficiency that over time causes the aforementioned damage along with frequent fatigue due to effects on cellular metabolism.<sup>1741</sup> There is currently no cure for FA.<sup>1742</sup>

For all six diseases described in this Section, the condition can be cured by first applying nanorobotic Alzheimer Protocol #1 (Section 5.1) to the nucleus of every cell in the patient's body to reduce the number of CAG, CGG, or GAA repeats to the minimum 6-12 normal number, thus eliminating all genetic propensity for the disease. Suitably modified simplified versions of Alzheimer Protocol #2 (Section 5.2) and Alzheimer Protocol #3 (Section 5.3) can then be applied to extract intranuclear and cytoplasmic inclusion bodies, rejuvenate surviving neurons, and to replace missing neurons.

---

<sup>1737</sup> Berry-Kravis E, Abrams L, Coffey SM, Hall DA, Greco C, Gane LW, Grigsby J, Bourgeois JA, Finucane B, Jacquemont S, Brunberg JA, Zhang L, Lin J, Tassone F, Hagerman PJ, Hagerman RJ, Leehey MA. Fragile X-associated tremor/ataxia syndrome: clinical features, genetics, and testing guidelines. *Mov Disord.* 2007 Oct 31;22(14):2018-30; <https://fragilex.org/wp-content/uploads/2012/01/Fragile-X-Associated-TremorAtaxia-Syndrome-Clinical-Features-Genetics-and-Testing-Guidelines.pdf>.

<sup>1738</sup> Hagerman RJ, Hall DA, Coffey S, Leehey M, Bourgeois J, Gould J, Zhang L, Seritan A, Berry-Kravis E, Olichney J, Miller JW, Fong AL, Carpenter R, Bodine C, Gane LW, Rainin E, Hagerman H, Hagerman PJ. Treatment of fragile X-associated tremor ataxia syndrome (FXTAS) and related neurological problems. *Clin Interv Aging.* 2008;3(2):251-62; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2546470/>.

<sup>1739</sup> [https://en.wikipedia.org/wiki/Friedreich's\\_ataxia](https://en.wikipedia.org/wiki/Friedreich's_ataxia).

<sup>1740</sup> Montermini L, Andermann E, Labuda M, Richter A, Pandolfo M, Cavalcanti F, Pianese L, Iodice L, Farina G, Monticelli A, Turano M, Filla A, De Michele G, Coccozza S. The Friedreich ataxia GAA triplet repeat: premutation and normal alleles. *Hum Mol Genet.* 1997 Aug;6(8):1261-6; <http://hmg.oxfordjournals.org/content/6/8/1261.long>. Bayot A, Rustin P. Friedreich's ataxia, frataxin, PIP5K1B: echo of a distant fracas. *Oxid Med Cell Longev.* 2013;2013:725635; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3806116/>.

<sup>1741</sup> Pandolfo M. Friedreich ataxia: the clinical picture. *J Neurol.* 2009 Mar;256 Suppl 1:3-8; [http://www.neuro.it/documents/materiale%20didattico\\_Siena\\_2011/Filla\\_3.pdf](http://www.neuro.it/documents/materiale%20didattico_Siena_2011/Filla_3.pdf).

<sup>1742</sup> Richardson TE, Kelly HN, Yu AE, Simpkins JW. Therapeutic strategies in Friedreich's ataxia. *Brain Res.* 2013 Jun 13;1514:91-7; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4461031/>.

## 6.2.2 Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS),<sup>1743</sup> aka. Lou Gehrig's disease or Charcot disease, is the most common form of motor neuron disease, resulting in progressive weakness and loss of motor strength, death of the neurons that control voluntary muscles, wasting of the muscles of the face, limbs and diaphragm, paralysis, and eventual death of the patient. Dementia, usually of the frontotemporal lobar type, may occur in some ALS cases. There is no known cure for ALS and the cause is not known in 90%-95% of cases.<sup>1744</sup>

About 5%-10% of cases are inherited from parents and may involve any of hundreds of mutations in at least a couple of dozen genes. The main feature of ALS is the death of both upper and lower motor neurons in the motor cortex of the brain, the brain stem, and the spinal cord.<sup>1745</sup> Prior to their destruction, motor neurons develop protein-rich inclusions in their cell bodies and axons, possibly in part due to defects in protein degradation.<sup>1746</sup> These inclusions often contain ubiquitin, and generally incorporate one of the ALS-associated proteins: SOD1 (superoxide dismutase 1),<sup>1747</sup> TARDBP (TAR DNA binding protein or TDP-43),<sup>1748</sup> and FUS (fused in sarcoma) protein.<sup>1749</sup> There is some evidence of mutations in these genes in astrocytes, which might then exhibit toxic effects on the motor neurons.<sup>1750</sup> The cause of neuronal cell death is

<sup>1743</sup> [https://en.wikipedia.org/wiki/Amyotrophic\\_lateral\\_sclerosis](https://en.wikipedia.org/wiki/Amyotrophic_lateral_sclerosis).

<sup>1744</sup> Kiernan MC, Vucic S, Cheah BC, Turner MR, Eisen A, Hardiman O, Burrell JR, Zoing MC. Amyotrophic lateral sclerosis. *Lancet*. 2011 Mar 12;377(9769):942-55; <http://www.sciencedirect.com/science/article/pii/S0140673610611567>.

<sup>1745</sup> Wijesekera LC, Leigh PN. Amyotrophic lateral sclerosis. *Orphanet J Rare Dis*. 2009 Feb 3;4:3; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2656493/>.

<sup>1746</sup> Deng HX, Chen W, Hong ST, Boycott KM, Gorrie GH, Siddique N, Yang Y, Fecto F, Shi Y, Zhai H, Jiang H, Hirano M, Rampersaud E, Jansen GH, Donkervoort S, Bigio EH, Brooks BR, Ajroud K, Sufit RL, Haines JL, Mugnaini E, Pericak-Vance MA, Siddique T. Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. *Nature*. 2011 Aug 21;477(7363):211-5; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3169705/>.

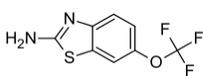
<sup>1747</sup> Rakhit R, Chakrabartty A. Structure, folding, and misfolding of Cu,Zn superoxide dismutase in amyotrophic lateral sclerosis. *Biochim Biophys Acta*. 2006 Nov-Dec;1762(11-12):1025-37; <http://www.sciencedirect.com/science/article/pii/S0925443906000846>.

<sup>1748</sup> Chen-Plotkin AS, Lee VM, Trojanowski JQ. TAR DNA-binding protein 43 in neurodegenerative disease. *Nat Rev Neurol*. 2010 Apr;6(4):211-20; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2892118/>.

<sup>1749</sup> Deng HX, Zhai H, Bigio EH, Yan J, Fecto F, Ajroud K, Mishra M, Ajroud-Driss S, Heller S, Sufit R, Siddique N, Mugnaini E, Siddique T. FUS-immunoreactive inclusions are a common feature in sporadic and non-SOD1 familial amyotrophic lateral sclerosis. *Ann Neurol*. 2010 Jun;67(6):739-48; <http://www.warons.com/uploaded/Research%20Northwestern/Annals.pdf>.

<sup>1750</sup> Di Giorgio FP, Carrasco MA, Siao MC, Maniatis T, Eggan K. Non-cell autonomous effect of glia on motor neurons in an embryonic stem cell-based ALS model. *Nat Neurosci*. 2007 May;10(5):608-14;

uncertain but may involve genetic and environmental factors including excessive oxidation damage, generation of free radicals, neurofilament accumulation, excitotoxicity and mitochondrial membrane dysfunction.<sup>1751</sup>



There is no known cure for ALS.<sup>1752</sup> A medication called **riluzole** (image, left) may extend life expectancy by about two to three months.<sup>1753</sup> A ketogenic diet<sup>1754</sup> might offer some neuroprotection, and non-invasive ventilation may provide improved quality and length of life.<sup>1755</sup> Peak age at onset is 58-63 years for sporadic disease and 47-52 years for familial disease, and the lifetime risk of ALS is 1:400 for women and 1:350 for men.<sup>1756</sup> The average survival from onset to death is 3-4 years, though about 10% survive longer than 10 years.

Application of suitably-modified Alzheimer Protocol #1 (Section 5.1) can eliminate the ALS genetic risk factors. Alzheimer Protocol #2 (Section 5.2) may rejuvenate surviving neurons by extracting the protein-rich intracellular inclusions containing ubiquitin and ALS-associated proteins, and also by removing any problematic lipofuscin<sup>1757</sup> (Section 5.2.2.2). Alzheimer

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3139463/>. Nagai M, Re DB, Nagata T, Chalazonitis A, Jessell TM, Wichterle H, Przedborski S. Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. *Nat Neurosci*. 2007 May;10(5):615-22; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3799799/>.

<sup>1751</sup> Zhao Z, Lange DJ, Voustantiyouk A, MacGrogan D, Ho L, Suh J, Humala N, Thiyagarajan M, Wang J, Pasinetti GM. A ketogenic diet as a potential novel therapeutic intervention in amyotrophic lateral sclerosis. *BMC Neurosci*. 2006 Apr 3;7:29; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1488864/>.

<sup>1752</sup> “Amyotrophic Lateral Sclerosis (ALS) Fact Sheet,” National Institute of Neurological Disorders and Stroke, 3 Mar 2016; [http://www.ninds.nih.gov/disorders/amyotrophiclateralsclerosis/detail\\_ALS.htm](http://www.ninds.nih.gov/disorders/amyotrophiclateralsclerosis/detail_ALS.htm).

<sup>1753</sup> Miller RG, Mitchell JD, Moore DH. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *Cochrane Database Syst Rev*. 2012 Mar 14;3:CD001447; <https://www.ncbi.nlm.nih.gov/pubmed/22419278>.

<sup>1754</sup> Zhao Z, Lange DJ, Voustantiyouk A, MacGrogan D, Ho L, Suh J, Humala N, Thiyagarajan M, Wang J, Pasinetti GM. A ketogenic diet as a potential novel therapeutic intervention in amyotrophic lateral sclerosis. *BMC Neurosci*. 2006 Apr 3;7:29; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1488864/>. Maalouf M, Sullivan PG, Davis L, Kim DY, Rho JM. Ketones inhibit mitochondrial production of reactive oxygen species production following glutamate excitotoxicity by increasing NADH oxidation. *Neuroscience*. 2007 Mar 2;145(1):256-64; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1865572/>.

<sup>1755</sup> McDermott CJ, Shaw PJ. Diagnosis and management of motor neurone disease. *BMJ*. 2008 Mar 22;336(7645):658-62; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2270983/>.

<sup>1756</sup> Kiernan MC, Vucic S, Cheah BC, Turner MR, Eisen A, Hardiman O, Burrell JR, Zoing MC. Amyotrophic lateral sclerosis. *Lancet*. 2011 Mar 12;377(9769):942-55; [http://www.thelancet.com/pdfs/journals/lancet/PIIS0140-6736\(10\)61156-7.pdf](http://www.thelancet.com/pdfs/journals/lancet/PIIS0140-6736(10)61156-7.pdf).

<sup>1757</sup> McHolm GB, Aguilar MJ, Norris FH. Lipofuscin in amyotrophic lateral sclerosis. *Arch Neurol*. 1984 Nov;41(11):1187-8; <http://www.ncbi.nlm.nih.gov/pubmed/6487103>. Bajaj NP, Al-Sarraj ST, Anderson V, Kibble M, Leigh N, Miller CC. Cyclin-dependent kinase-5 is associated with lipofuscin in motor neurones

Protocol #3 (Section 5.3) replaces any missing neurons, thus reversing and curing ALS using medical nanorobotics.

---

in amyotrophic lateral sclerosis. *Neurosci Lett*. 1998 Mar 27;245(1):45-8;  
<http://www.ncbi.nlm.nih.gov/pubmed/9596352>.

### 6.2.3 Vascular Dementia

Vascular dementia (VaD),<sup>1758</sup> aka. multi-infarct dementia (MID) or vascular cognitive impairment (VCI) – and closely related to Binswanger’s disease<sup>1759</sup> and what used to be called “cerebral arteriosclerosis” – is dementia caused by problems in the supply of blood to the brain, typically a series of minor strokes leading to stepwise cognitive decline.<sup>1760</sup>

VaD is a syndrome involving a complex interaction of cerebrovascular disease and risk factors that lead to changes in brain structure due to strokes and lesions, with resulting changes in cognition.<sup>1761</sup> A brain with VaD may have noticeable lesions and damage to blood vessels, and accumulation of lipid deposits and clotted blood may appear in microscopic views. White matter shows noticeable atrophy (tissue loss) with calcification of the arteries in VaD, along with microinfarcts in the gray matter (cerebral cortex), sometimes in large numbers, and atheroma of the major cerebral arteries (though smaller vessels and arterioles are mainly affected). Vascular dementia is the second-most-common form of dementia (25% of all cases) after Alzheimer’s disease (50%-70%) in older adults.<sup>1762</sup> The prevalence of the illness is 1.5% in Western countries and 2.2% in Japan – it accounts for 50% of all dementias in Japan, 20%-40% in Europe, and 15% in Latin America.



There are no medications that have been approved specifically for the prevention or treatment of vascular dementia. Alzheimer’s medications such as cholinesterase inhibitors and memantine have yielded only small improvements of cognition in vascular dementia. In short, there is

---

<sup>1758</sup> [https://en.wikipedia.org/wiki/Vascular\\_dementia](https://en.wikipedia.org/wiki/Vascular_dementia).

<sup>1759</sup> Libon DJ, Scanlon M, Swenson R, Coslet HB. Binswanger’s disease: some neuropsychological considerations. *J Geriatr Psychiatry Neurol*. 1990 Jan-Mar;3(1):31-40; <http://www.ncbi.nlm.nih.gov/pubmed/2346585>. Goldsmith HS. Binswanger disease may benefit from omental arteries. *Surg Neurol Int*. 2015 Jan 7;6:4; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4310055/>. See also [https://en.wikipedia.org/wiki/Binswanger's\\_disease](https://en.wikipedia.org/wiki/Binswanger's_disease).

<sup>1760</sup> Lee AY. Vascular dementia. *Chonnam Med J*. 2011 Aug;47(2):66-71; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3214877/>. O’Brien JT, Thomas A. Vascular dementia. *Lancet*. 2015 Oct 24;386(10004):1698-706; <http://www.ncbi.nlm.nih.gov/pubmed/26595643>. Venkat P, Chopp M, Chen J. Models and mechanisms of vascular dementia. *Exp Neurol*. 2015 Oct;272:97-108; <http://www.ncbi.nlm.nih.gov/pubmed/25987538>.

<sup>1761</sup> Cunningham EL, McGuinness B, Herron B, Passmore AP. Dementia. *Ulster Med J*. 2015 May;84(2):79-87; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4488926/>.

<sup>1762</sup> Burns A, Iliffe S. Dementia. *BMJ*. 2009 Feb 5;338:b75; <https://www.ncbi.nlm.nih.gov/pubmed/19196746>. Battistin L, Cagnin A. Vascular cognitive disorder. A biological and clinical overview. *Neurochem Res*. 2010 Dec;35(12):1933-8; <https://www.ncbi.nlm.nih.gov/pubmed/21127967>.

currently no specific treatment or cure for VaD,<sup>1763</sup> and existing treatments aim at merely improving symptomatology.<sup>1764</sup>

Nanorobotic treatment using the Alzheimer Protocols (Chapter 5) can effectuate blood vessel repair, then reconstitute the missing white matter and repair all infarcts in the grey matter, rejuvenating surviving neurons and replacing missing neurons, thus curing VaD.

---

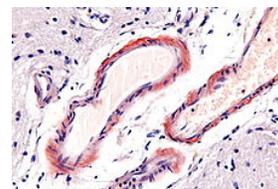
<sup>1763</sup> Igoumenou A, Ebmeier KP. Diagnosing and managing vascular dementia. *Practitioner*. 2012 Jan;256(1747):13-6, 2; <http://www.ncbi.nlm.nih.gov/pubmed/22720454>.

<sup>1764</sup> Versijpt J. Effectiveness and cost-effectiveness of the pharmacological treatment of Alzheimer's disease and vascular dementia. *J Alzheimers Dis*. 2014;42 Suppl 3:S19-25; <http://www.ncbi.nlm.nih.gov/pubmed/25061049>

## 6.2.4 Cerebral Amyloidosis

A number of neurodegenerative diseases other than Alzheimer's (Section 2.4.1) involve the buildup of misfolded proteins known as amyloids. About 60 amyloid proteins have been identified so far,<sup>1765</sup> of which at least 36 are associated with a human disease,<sup>1766</sup> many of them neurodegenerative. Here are a few examples of such disorders.

**Cerebral amyloid angiopathy (CAA),**<sup>1767</sup> aka. congophilic angiopathy,<sup>1768</sup> is a disorder in which amyloid deposits form in the walls of the blood vessels of the brain (image, right), often causing bleeding within the brain though usually confined to a particular lobe. Under normal physiology A $\beta$  is cleared from the brain by four pathways: (1) endocytosis by astrocytes and microglial cells, (2) enzymatic degradation by neprilysin or insulin, (3) clearance by the blood-brain barrier, or (4) drained along periarterial spaces.<sup>1769</sup> Abnormalities in each of these identified clearance pathways have been linked to CAA.



**Transmissible spongiform encephalopathy (TSE),**<sup>1770</sup> aka. prion disease, scrapie and “mad cow disease,” are a group of progressive conditions that affect the brain (encephalopathies) and nervous system of many animals, including humans. According to the most widespread hypothesis, they are transmitted by prions, with possible involvement of a *Spiroplasma* infection.<sup>1771</sup> Prions are misfolded proteins that replicate by converting their properly folded

---

<sup>1765</sup> Mok KH, Pettersson J, Orrenius S, Svanborg C. HAMLET, protein folding, and tumor cell death. *Biochem Biophys Res Commun.* 2007 Mar 2;354(1):1-7; <https://www.ncbi.nlm.nih.gov/pubmed/17223074>.

<sup>1766</sup> Pettersson-Kastberg J, Aits S, Gustafsson L, Mossberg A, Storm P, Trulsson M, Persson F, Mok KH, Svanborg C. Can misfolded proteins be beneficial? The HAMLET case. *Ann Med.* 2009;41(3):162-76; <https://www.ncbi.nlm.nih.gov/pubmed/18985467>.

<sup>1767</sup> [https://en.wikipedia.org/wiki/Cerebral\\_amyloid\\_angiopathy](https://en.wikipedia.org/wiki/Cerebral_amyloid_angiopathy).

<sup>1768</sup> Exley C, Esiri MM. Severe cerebral congophilic angiopathy coincident with increased brain aluminium in a resident of Camelford, Cornwall, UK. *J Neurol Neurosurg Psychiatry.* 2006 Jul;77(7):877-9; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2117501/>.

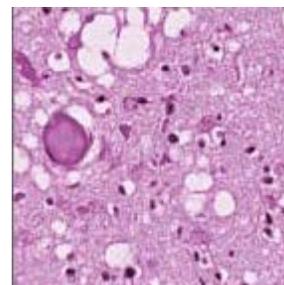
<sup>1769</sup> Morris AW, Carare RO, Schreiber S, Hawkes CA. The cerebrovascular basement membrane: role in the clearance of  $\beta$ -amyloid and Cerebral Amyloid Angiopathy. *Front Aging Neurosci.* 2014 Sep 19;6:251; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4168721/>.

<sup>1770</sup> [https://en.wikipedia.org/wiki/Transmissible\\_spongiform\\_encephalopathy](https://en.wikipedia.org/wiki/Transmissible_spongiform_encephalopathy).

<sup>1771</sup> Bastian FO, Sanders DE, Forbes WA, Hagius SD, Walker JV, Henk WG, Enright FM, Elzer PH. *Spiroplasma* spp. from transmissible spongiform encephalopathy brains or ticks induce spongiform encephalopathy in ruminants. *J Med Microbiol.* 2007 Sep;56(Pt 9):1235-42; <https://www.ncbi.nlm.nih.gov/pubmed/17761489>.

counterparts, in their host, to the same misfolded structure that they possess. Mental and physical abilities deteriorate and myriad tiny holes appear in the cortex causing it to appear like a sponge (hence spongiform) when afflicted brain tissue obtained at autopsy is examined under a microscope. The degenerative tissue damage caused by human prion diseases includes spongiform change, neuronal loss, astrocytosis, and amyloid plaque formation.<sup>1772</sup> The disorders cause impairment of brain function, including memory changes, personality changes and problems with movement that worsen over time. Prion diseases of humans include Creutzfeldt-Jakob disease (see below), Gerstmann-Sträussler-Scheinker syndrome, fatal familial insomnia, and kuru. No validated treatments exist for TSE or prion diseases but the search continues.<sup>1773</sup>

**Creutzfeldt-Jakob disease (CJD)** is a degenerative neurological disease that is incurable and invariably fatal.<sup>1774</sup> The symptoms of CJD are caused by the progressive death of the brain's nerve cells, which is associated with the build-up of abnormal prion proteins forming amyloids. The classic histologic appearance is spongiform change in the gray matter: the presence of many round vacuoles from 1-50  $\mu\text{m}$  in the neuropil (image, right),<sup>1775</sup> in all six cortical layers in the cerebral cortex or with diffuse involvement of the cerebellar molecular layer. These vacuoles appear glassy or eosinophilic and may coalesce. Neuronal loss and gliosis are also seen.<sup>1776</sup> People can also acquire CJD genetically through a mutation of the gene that codes for the prion protein, but this occurs in only ~8% of all CJD cases, with 87% of cases sporadic and 5% iatrogenic.<sup>1777</sup> There is currently no specific treatment or cure for CJD.<sup>1778</sup>



<sup>1772</sup> Jeffrey M, Goodbrand IA, Goodsir CM. Pathology of the transmissible spongiform encephalopathies with special emphasis on ultrastructure. *Micron*. 1995;26(3):277-98; <https://www.ncbi.nlm.nih.gov/pubmed/7788281>.

<sup>1773</sup> Caughey B, Caughey WS, Kocisko DA, Lee KS, Silveira JR, Morrey JD. Prions and transmissible spongiform encephalopathy (TSE) chemotherapeutics: A common mechanism for anti-TSE compounds? *Acc Chem Res*. 2006 Sep;39(9):646-53; <http://www.ncbi.nlm.nih.gov/pubmed/16981681>. Wisniewski T, Chabalgoity JA, Goni F. Is vaccination against transmissible spongiform encephalopathy feasible? *Rev Sci Tech*. 2007 Apr;26(1):243-51; <http://www.ncbi.nlm.nih.gov/pubmed/17633306>.

<sup>1774</sup> Brown P. Bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease. *BMJ*. 2001 Apr 7;322(7290):841-4; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1120011/>.

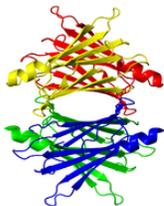
<sup>1775</sup> [http://cjdawareness.blogspot.com/2014\\_04\\_01\\_archive.html](http://cjdawareness.blogspot.com/2014_04_01_archive.html).

<sup>1776</sup> [https://en.wikipedia.org/wiki/Creutzfeldt%E2%80%933Jakob\\_disease](https://en.wikipedia.org/wiki/Creutzfeldt%E2%80%933Jakob_disease).

<sup>1777</sup> Will RG, Alperovitch A, Poser S, Pocchiari M, Hofman A, Mitrova E, de Silva R, D'Alessandro M, Delasnerie-Laupretre N, Zerr I, van Duijn C. Descriptive epidemiology of Creutzfeldt-Jakob disease in six European countries, 1993-1995. EU Collaborative Study Group for CJD. *Ann Neurol*. 1998 Jun;43(6):763-7; <http://www.ncbi.nlm.nih.gov/pubmed/9629846>.

<sup>1778</sup> de Villemeur TB. Creutzfeldt-Jakob disease. *Handb Clin Neurol*. 2013;112:1191-3; <http://www.ncbi.nlm.nih.gov/pubmed/23622328>.

**Familial amyloid polyneuropathy (FAP)**, aka. transthyretin-related hereditary amyloidosis,<sup>1779</sup> transthyretin amyloidosis (ATTR) or TTR amyloidosis,<sup>1780</sup> or Corino de Andrade's disease,<sup>1781</sup> is an autosomal dominant fatal neurodegenerative disease.<sup>1782</sup> Usually manifesting between 20-40 years of age, it is characterized by pain, paresthesia, muscular weakness and autonomic dysfunction, with kidneys and heart affected in its terminal state. FAP



is characterized by the systemic deposition of amyloidogenic variants of the transthyretin protein (TTR; image, left), especially in the peripheral nervous system, causing a progressive sensory and motor polyneuropathy. FAP is caused by a mutation of the TTR gene on human Chromosome 18, encoding the tetrameric transthyretin protein. The tetramer dissociates into misfolded monomers and then aggregates into a variety of structures including amyloid fibrils.<sup>1783</sup> Liver transplantation, although invasive, can sometimes halt the progression of the neuropathy in early-onset patients, and several disease-modifying treatments are now available or in clinical development, but there are as yet no cures for FAP.<sup>1784</sup>

Our nanorobotic approach to the cerebral amyloidosis disorders: An appropriately-modified Alzheimer Protocol #1 (Section 5.1) can be applied to eliminate any genetic susceptibility to FAP. Alzheimer Protocol #2 (Section 5.2) can effectuate blood vessel repair in CAA, extract misfolded prion proteins from cells in TSE and CJD, remove amyloid deposits, plaques and fibrils (Section 5.2.1.1) and rejuvenate surviving brain cells in all cases. Alzheimer Protocol #3 (Section 5.3) is then used to replace missing neural tissue in TSE and CJD, and to replace missing astrocytes and microglial cells in CAA or TSE, completing the nanorobotic cure.

---

<sup>1779</sup> [https://en.wikipedia.org/wiki/Transthyretin-related\\_hereditary\\_amyloidosis](https://en.wikipedia.org/wiki/Transthyretin-related_hereditary_amyloidosis).

<sup>1780</sup> Ando Y, Coelho T, Berk JL, Cruz MW, Ericzon BG, Ikeda S, Lewis WD, Obici L, Planté-Bordeneuve V, Rapezzi C, Said G, Salvi F. Guideline of transthyretin-related hereditary amyloidosis for clinicians. Orphanet J Rare Dis. 2013 Feb 20;8:31; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3584981/>.

<sup>1781</sup> Andrade C. A peculiar form of peripheral neuropathy; familiar atypical generalized amyloidosis with special involvement of the peripheral nerves. Brain. 1952 Sep;75(3):408-27; <https://www.ncbi.nlm.nih.gov/pubmed/12978172>.

<sup>1782</sup> Ando Y, Ueda M. Novel methods for detecting amyloidogenic proteins in transthyretin related amyloidosis. Front Biosci. 2008 May 1;13:5548-58; <https://www.ncbi.nlm.nih.gov/pubmed/18508604>.

<sup>1783</sup> Coelho T. Familial amyloid polyneuropathy: new developments in genetics and treatment. Curr Opin Neurol. 1996 Oct;9(5):355-9; <https://www.ncbi.nlm.nih.gov/pubmed/8894411>.

<sup>1784</sup> Plante-Bordeneuve V. Update in the diagnosis and management of transthyretin familial amyloid polyneuropathy. J Neurol. 2014 Jun;261(6):1227-33; <http://www.ncbi.nlm.nih.gov/pubmed/24888313>.

## 6.2.5 Neurodegenerative Tauopathies

Besides Alzheimer's disease, a number of other neurodegenerative conditions are caused by excessive tau protein or tau aggregation, including Pick's disease, corticobasal degeneration, progressive supranuclear palsy, and chronic traumatic encephalopathy, as briefly described below.

**Pick's disease (PiD)**,<sup>1785</sup> aka. frontotemporal dementia (FTD), frontal lobe dementia, or frontotemporal lobar degeneration (FTLD), is a rare neurodegenerative disease that causes progressive destruction of nerve cells in the brain. PiD usually strikes adults between the ages of 40-60 years, producing symptoms including dementia and loss of language (aphasia). While some of the symptoms can initially be alleviated, the disease progresses and patients often die within 2-10 years after diagnosis. It is not yet clear if classical Pick's disease pathology has a direct genetic link or runs in families or certain ethnic or gender specific subgroups, but mutations in the tau gene (MAPT) have been associated with this disease.<sup>1786</sup>



The disease often produces a build-up of tau proteins in neurons, accumulating into spherical aggregations of tau and ubiquitin known as Pick bodies<sup>1787</sup> that are almost universally present in patients with PiD,<sup>1788</sup> and by insoluble tau proteins with predominantly three microtubule-binding repeat tau isoforms.<sup>1789</sup> Proteins associated with Pick's disease are present in all nerve

<sup>1785</sup> [https://en.wikipedia.org/wiki/Pick's\\_disease](https://en.wikipedia.org/wiki/Pick's_disease).

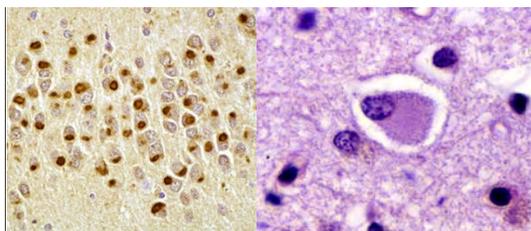
<sup>1786</sup> Tacik P, DeTure M, Hinkle KM, Lin WL, Sanchez-Contreras M, Carlomagno Y, Pedraza O, Rademakers R, Ross OA, Wszolek ZK, Dickson DW. A novel tau mutation in exon 12, p.Q336H, causes hereditary Pick disease. *J Neuropathol Exp Neurol*. 2015 Nov;74(11):1042-52; <http://www.ncbi.nlm.nih.gov/pubmed/26426266>. Kowalska A. The genetics of dementias. Part 1: Molecular basis of frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). *Postepy Hig Med Dosw (Online)*. 2009 Jun 15;63:278-86 (in Polish); <http://www.phmd.pl/fulltxt.php?ICID=888261>.

<sup>1787</sup> Wang LN, Zhu MW, Feng YQ, Wang JH. Pick's disease with Pick bodies combined with progressive supranuclear palsy without tuft-shaped astrocytes: a clinical, neuroradiologic and pathological study of an autopsied case. *Neuropathology*. 2006 Jun;26(3):222-30; <https://www.ncbi.nlm.nih.gov/pubmed/16771179>.

<sup>1788</sup> Armstrong RA, Cairns NJ, Lantos PL. A comparison of histological and immunohistochemical methods for quantifying the pathological lesions of Pick's disease. *Neuropathology: J Japanese Soc Neuropathology* 1998 Sep 1;18(3):295-300; <http://onlinelibrary.wiley.com/doi/10.1111/j.1440-1789.1998.tb00118.x/abstract>.

<sup>1789</sup> Zhukareva V, Mann D, Pickering-Brown S, Uryu K, Shuck T, Shah K, Grossman M, Miller BL, Hulette CM, Feinstein SC, Trojanowski JQ, Lee VM. Sporadic Pick's disease: a tauopathy characterized by a spectrum of pathological tau isoforms in gray and white matter. *Ann Neurol*. 2002 Jun;51(6):730-9; <https://www.ncbi.nlm.nih.gov/pubmed/12112079>.

cells, but patients with the disease have an abnormal amount. PiD brains, which have tau protein tangles (Section 2.4.2) present in many affected neurons, contain only one or (rarely) two of the six isoforms of the tau protein,<sup>1790</sup> all of which result from alternative splicing of the same gene.<sup>1791</sup> (In Alzheimer's disease, all six tau isoforms are abnormally phosphorylated and aggregate into paired helical filaments.) Pick bodies with tangles have predominantly isoform 3R-tau protein and a characteristically round-body shape, often with an indentation in the area facing the cell nucleus.<sup>1792</sup> Less than half of FTD cases are tauopathies, the majority having been discovered to have a TDP-43 and FUS proteinopathy, shared with ALS.<sup>1793</sup>



Pick bodies (images, left)<sup>1794</sup> are almost always found in several places in the PiD brain, including the dentate gyrus, the pyramidal cells of the CA1 sector and subiculum of the hippocampus, and the neocortex as well as a plurality of other nuclei. Other involved regions include the caudate and hypothalamic lateral tuberal nucleus (both severely affected), the dorsomedial region of the putamen, the globus pallidus, and locus coeruleus.<sup>1795</sup> In the neocortex, Pick bodies reside in the II and IV layers (which send neurons within the cortex and to thalamic synapses, respectively). Layers III and V have very few if any Pick bodies but show extreme neuronal loss that can, in some cases, be so severe as to leave a

<sup>1790</sup> Iskei E, Arai H. Progress in the classification of non-Alzheimer-type degenerative dementias. *Psychogeriatrics* 2006;6(1):41-2; <http://onlinelibrary.wiley.com/doi/10.1111/j.1479-8301.2006.00166.x/abstract>.

<sup>1791</sup> Arai T, Ikeda K, Akiyama H, Tsuchiya K, Iritani S, Ishiguro K, Yagishita S, Oda T, Odawara T, Iseki E. Different immunoreactivities of the microtubule-binding region of tau and its molecular basis in brains from patients with Alzheimer's disease, Pick's disease, progressive supranuclear palsy and corticobasal degeneration. *Acta Neuropathol.* 2003 May;105(5):489-98; <https://www.ncbi.nlm.nih.gov/pubmed/12677450>.

<sup>1792</sup> Zhukareva V, Mann D, Pickering-Brown S, Uryu K, Shuck T, Shah K, Grossman M, Miller BL, Hulette CM, Feinstein SC, Trojanowski JQ, Lee VM. Sporadic Pick's disease: a tauopathy characterized by a spectrum of pathological tau isoforms in gray and white matter. *Ann Neurol.* 2002 Jun;51(6):730-9; <https://www.ncbi.nlm.nih.gov/pubmed/12112079>. Munoz DG, Dickson DW, Bergeron C, Mackenzie IR, Delacourte A, Zhukareva V. The neuropathology and biochemistry of frontotemporal dementia. *Ann Neurol.* 2003;54 Suppl 5:S24-8; <https://www.ncbi.nlm.nih.gov/pubmed/12833365>. Uematsu M, Adachi E, Nakamura A, Tsuchiya K, Uchihara T. Atomic identification of fluorescent Q-dots on tau-positive fibrils in 3D-reconstructed Pick bodies. *Am J Pathol.* 2012 Apr;180(4):1394-7; [http://ajp.amjpathol.org/article/S0002-9440\(12\)00032-6/pdf](http://ajp.amjpathol.org/article/S0002-9440(12)00032-6/pdf).

<sup>1793</sup> Kertesz A. Frontotemporal dementia, Pick's disease. *Ideggyogy Sz.* 2010 Jan 30;63(1-2):4-12; <http://www.ncbi.nlm.nih.gov/pubmed/20420119>.

<sup>1794</sup> <http://neuropathology-web.org/chapter9/chapter9cFTD.html>.

<sup>1795</sup> Wang LN, Zhu MW, Feng YQ, Wang JH. Pick's disease with Pick bodies combined with progressive supranuclear palsy without tuft-shaped astrocytes: a clinical, neuroradiologic and pathological study of an autopsied case. *Neuropathology.* 2006 Jun;26(3):222-30; <https://www.ncbi.nlm.nih.gov/pubmed/16771179>.

void in the brain altogether. Myelinated fibers in the white matter (severely atrophied) as well as cerebral neurons are primarily affected in Pick's disease.<sup>1796</sup> There is currently no cure for PiD.

**Corticobasal degeneration (CBD)**, aka. corticobasal ganglionic degeneration (CBGD), is a rare progressive neurodegenerative disease involving the cerebral cortex and the basal ganglia.<sup>1797</sup> CBD symptoms typically begin at 50-70 years of age (average duration 6 years) and include marked disorders in movement and cognitive dysfunction. The micropathology includes astroglial inclusions and excessive tau protein.<sup>1798</sup> Astroglial inclusions in CBD are identified as astrocytic plaques, which present as annular displays of blurry outgrowths from the astrocyte. CBD produces a high density of astrocytic plaques in the anterior portion of the frontal lobe and in the premotor area of the cerebral cortex.<sup>1799</sup> Unnaturally high-level expression of tau protein in astrocytes and glial cells appears responsible for the astrocytic plaques prominently noted in histological CBD examinations, although their precise effect in the progress of CBD remains a mystery.<sup>1800</sup> 4R-tau isoforms aggregate into twisted filaments in CBD.<sup>1801</sup> There is currently no treatment for CBD.

**Progressive supranuclear palsy (PSP)**,<sup>1802</sup> aka. Steele-Richardson-Olszewski syndrome,<sup>1803</sup> is a degenerative tauopathy<sup>1804</sup> involving the gradual deterioration and death of

<sup>1796</sup> Yamakawa K, Takanashi M, Watanabe M, Nakamura N, Kobayashi T, Hasegawa M, Mizuno Y, Tanaka S, Mori H. Pathological and biochemical studies on a case of Pick disease with severe white matter atrophy. *Neuropathology*. 2006 Dec;26(6):586-91; <https://www.ncbi.nlm.nih.gov/pubmed/17203597>.

<sup>1797</sup> [https://en.wikipedia.org/wiki/Corticobasal\\_degeneration](https://en.wikipedia.org/wiki/Corticobasal_degeneration).

<sup>1798</sup> Komori T. Tau-positive glial inclusions in progressive supranuclear palsy, corticobasal degeneration and Pick's disease. *Brain Pathol*. 1999 Oct;9(4):663-79; <http://www.ncbi.nlm.nih.gov/pubmed/10517506>.

<sup>1799</sup> Hattori M, Hashizume Y, Yoshida M, Iwasaki Y, Hishikawa N, Ueda R, Ojika K. Distribution of astrocytic plaques in the corticobasal degeneration brain and comparison with tuft-shaped astrocytes in the progressive supranuclear palsy brain. *Acta Neuropathol*. 2003 Aug;106(2):143-9; <https://www.ncbi.nlm.nih.gov/pubmed/12732936>.

<sup>1800</sup> Komori T. Tau-positive glial inclusions in progressive supranuclear palsy, corticobasal degeneration and Pick's disease. *Brain Pathol*. 1999 Oct;9(4):663-79; <http://www.ncbi.nlm.nih.gov/pubmed/10517506>.

<sup>1801</sup> Buée L, Delacourte A. Comparative biochemistry of tau in progressive supranuclear palsy, corticobasal degeneration, FTDP-17 and Pick's disease. *Brain Pathol*. 1999 Oct;9(4):681-93; <http://www.alzheimer-adna.com/pdf/1999/1999BueeBrainPathol.pdf>.

<sup>1802</sup> [https://en.wikipedia.org/wiki/Progressive\\_supranuclear\\_palsy](https://en.wikipedia.org/wiki/Progressive_supranuclear_palsy).

<sup>1803</sup> Steele JC, Richardson JC, Olszewski J. Progressive supranuclear palsy. A heterogeneous degeneration involving the brain stem, basal ganglia and cerebellum with vertical gaze and pseudobulbar palsy, nuchal dystonia and dementia. *Arch Neurol*. 1964 Apr;10:333-59; <https://www.ncbi.nlm.nih.gov/pubmed/14107684>.

<sup>1804</sup> Rizzo G, Martinelli P, Manners D, Scaglione C, Tonon C, Cortelli P, Malucelli E, Capellari S, Testa C, Parchi P, Montagna P, Barbiroli B, Lodi R. Diffusion-weighted brain imaging study of patients with

specific volumes of the brain. About 6 people per 100,000 population have PSP. Early symptoms are loss of balance, lunging forward when mobilizing, fast walking, bumping into objects or people, and falls, followed later by dementia (esp. loss of inhibition and lost ability to organize information), slurring of speech, difficulty swallowing, and difficulty moving the eyes. The average age at onset is 63 years and an average survival time of 7 years with a wide variance, and pneumonia is a frequent cause of death.<sup>1805</sup>

A variant in the gene for tau protein called the H1 haplotype, located on Chromosome 17, has been linked to PSP, but the H1 haplotype appears to be necessary but not sufficient to cause PSP.<sup>1806</sup> Both neurons and glial cells are affected. PSP neurons display neurofibrillary tangles of tau protein which may be structurally similar to those seen in Alzheimer's disease when they occur in the cerebral cortex.<sup>1807</sup> The chemical composition of PSP tangles is usually different from AD, more similar to that of tangles seen in corticobasal degeneration with only 4R-tau isoforms aggregating into straight filaments in PSP.<sup>1808</sup> Tufts of tau protein in astrocytes, or tufted astrocytes, are also considered diagnostic and may be more widespread in the cortex.<sup>1809</sup>

clinical diagnosis of corticobasal degeneration, progressive supranuclear palsy and Parkinson's disease. *Brain*. 2008 Oct;131(Pt 10):2690-700; <http://brain.oxfordjournals.org/content/131/10/2690.long>.

<sup>1805</sup> Tomita S, Oeda T, Umemura A, Kohsaka M, Park K, Yamamoto K, Sugiyama H, Mori C, Inoue K, Fujimura H, Sawada H. Impact of Aspiration Pneumonia on the Clinical Course of Progressive Supranuclear Palsy: A Retrospective Cohort Study. *PLoS One*. 2015 Aug 13;10(8):e0135823; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4536232/>.

<sup>1806</sup> Pittman AM, Myers AJ, Abou-Sleiman P, Fung HC, Kaleem M, Marlowe L, Duckworth J, Leung D, Williams D, Kilford L, Thomas N, Morris CM, Dickson D, Wood NW, Hardy J, Lees AJ, de Silva R. Linkage disequilibrium fine mapping and haplotype association analysis of the tau gene in progressive supranuclear palsy and corticobasal degeneration. *J Med Genet*. 2005 Nov;42(11):837-46; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1735957/>. Rademakers R, Melquist S, Cruts M, Theuns J, Del-Favero J, Poorkaj P, Baker M, Sleegers K, Crook R, De Pooter T, Bel Kacem S, Adamson J, Van den Bossche D, Van den Broeck M, Gass J, Corsmit E, De Rijk P, Thomas N, Engelborghs S, Heckman M, Litvan I, Crook J, De Deyn PP, Dickson D, Schellenberg GD, Van Broeckhoven C, Hutton ML. High-density SNP haplotyping suggests altered regulation of tau gene expression in progressive supranuclear palsy. *Hum Mol Genet*. 2005 Nov 1;14(21):3281-92; <http://hmg.oxfordjournals.org/content/14/21/3281.long>.

<sup>1807</sup> Amano N, Iwabuchi K, Yokoi S, Yagishita S, Itoh Y, Saitoh A, Nagatomo H, Matsushita M. The reappraisal study of the ultrastructure of Alzheimer's neurofibrillary tangles in three cases of progressive supranuclear palsy. *No To Shinkei*. 1989 Jan;41(1):35-44 (in Japanese); <https://www.ncbi.nlm.nih.gov/pubmed/2655673>.

<sup>1808</sup> Buée L, Delacourte A. Comparative biochemistry of tau in progressive supranuclear palsy, corticobasal degeneration, FTDP-17 and Pick's disease. *Brain Pathol*. 1999 Oct;9(4):681-93; <http://www.alzheimer-adna.com/pdf/1999/1999BueeBrainPathol.pdf>.

<sup>1809</sup> Feany MB, Mattiace LA, Dickson DW. Neuropathologic overlap of progressive supranuclear palsy, Pick's disease and corticobasal degeneration. *J Neuropathol Exp Neurol*. 1996 Jan;55(1):53-67; <http://jnen.oxfordjournals.org/content/55/1/53.long>.

There is currently no effective treatment or cure for PSP, although some of the symptoms can respond to nonspecific measures.<sup>1810</sup>

**Chronic traumatic encephalopathy (CTE)**,<sup>1811</sup> aka. dementia pugilistica (DP) or “punch drunk,” is a form of tauopathy and a progressive degenerative disease found in people who have suffered a severe blow to the head.<sup>1812</sup> CTE is most commonly found in professional athletes participating in American football, association football, boxing, ice hockey, professional wrestling, stunt performing, bull riding, bicycle motocross, rodeo, and other contact sports who have experienced repeated concussions or other brain trauma. The primary physical manifestations of CTE include a reduction in brain weight, associated with atrophy of the frontal and temporal cortices and medial temporal lobe. The lateral ventricles and the third ventricle are often enlarged, with rare instances of dilation of the fourth ventricle.<sup>1813</sup> As CTE progresses, there may be marked atrophy of the hippocampus, entorhinal cortex, and amygdala.<sup>1814</sup> The micropathology of CTE includes neuronal loss, tau deposition, TDP-43 (TAR DNA-binding protein 43) immunoreactive inclusions, white matter changes, and other abnormalities.<sup>1815</sup> The tau deposition occurs as dense neurofibrillary tangles (NFT), neurites, and glial tangles, which are made up of astrocytes and other glial cells.<sup>1816</sup> There is currently no treatment or cure for CTE, beyond simple prevention.<sup>1817</sup>

---

<sup>1810</sup> Ling H. Clinical approach to progressive supranuclear palsy. *J Mov Disord.* 2016 Jan;9(1):3-13; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4734991/>.

<sup>1811</sup> [https://en.wikipedia.org/wiki/Chronic\\_traumatic\\_encephalopathy](https://en.wikipedia.org/wiki/Chronic_traumatic_encephalopathy).

<sup>1812</sup> Saulle M, Greenwald BD. Chronic traumatic encephalopathy: a review. *Rehabil Res Pract.* 2012;2012:816069; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3337491/>.

<sup>1813</sup> Baugh CM, Stamm JM, Riley DO, Gavett BE, Shenton ME, Lin A, Nowinski CJ, Cantu RC, McKee AC, Stern RA. Chronic traumatic encephalopathy: neurodegeneration following repetitive concussive and subconcussive brain trauma. *Brain Imaging Behav.* 2012 Jun;6(2):244-54; [http://www.bu.edu/cte/files/2012/05/Baugh\\_Chronic-Traumatic-Encephalopathy\\_2012.pdf](http://www.bu.edu/cte/files/2012/05/Baugh_Chronic-Traumatic-Encephalopathy_2012.pdf).

<sup>1814</sup> McKee AC, Cantu RC, Nowinski CJ, Hedley-Whyte ET, Gavett BE, Budson AE, Santini VE, Lee HS, Kubilus CA, Stern RA. Chronic traumatic encephalopathy in athletes: progressive tauopathy after repetitive head injury. *J Neuropathol Exp Neurol.* 2009 Jul;68(7):709-35; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2945234/>.

<sup>1815</sup> McKee AC, Stern RA, Nowinski CJ, Stein TD, Alvarez VE, Daneshvar DH, Lee HS, Wojtowicz SM, Hall G, Baugh CM, Riley DO, Kubilus CA, Cormier KA, Jacobs MA, Martin BR, Abraham CR, Ikezu T, Reichard RR, Wolozin BL, Budson AE, Goldstein LE, Kowall NW, Cantu RC. The spectrum of disease in chronic traumatic encephalopathy. *Brain.* 2013 Jan;136(Pt 1):43-64; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624697/>.

<sup>1816</sup> Baugh CM, Stamm JM, Riley DO, Gavett BE, Shenton ME, Lin A, Nowinski CJ, Cantu RC, McKee AC, Stern RA. Chronic traumatic encephalopathy: neurodegeneration following repetitive concussive and subconcussive brain trauma. *Brain Imaging Behav.* 2012 Jun;6(2):244-54; [http://www.bu.edu/cte/files/2012/05/Baugh\\_Chronic-Traumatic-Encephalopathy\\_2012.pdf](http://www.bu.edu/cte/files/2012/05/Baugh_Chronic-Traumatic-Encephalopathy_2012.pdf).

<sup>1817</sup> Antonius D, Mathew N, Picano J, Hinds A, Cogswell A, Olympia J, Brooks T, DiGiacomo M, Baker J, Willer B, Leddy J. Behavioral health symptoms associated with chronic traumatic encephalopathy: a

Our nanorobotic approach to the neurodegenerative tauopathies: Application of a suitably-modified Alzheimer Protocol #1 (Section 5.1) can eliminate tau gene mutations in PiD, replace the H1 haplotype tau protein gene variant in PSP, and correct any other genetic abnormalities that may lead to neurodegenerative tauopathy. Surviving neurons and glial cells can be rejuvenated using Alzheimer Protocol #2 by extracting Pick bodies in PiD, TDP-43 inclusions in CTE, astroglial inclusions and astrocytic plaques in CBD, and excess tau proteins and neurofibrillary tangles in nearly all cases (Section 5.2.2.1). Alzheimer Protocol #3 (Section 5.3) is used to replace missing neurons and glial cells, fully reversing and curing these neurodegenerative conditions.

---

critical review of the literature and recommendations for treatment and research. *J Neuropsychiatry Clin Neurosci*. 2014 Fall;26(4):313-22; <http://www.ncbi.nlm.nih.gov/pubmed/26037854>. Jordan BD. Chronic traumatic encephalopathy and other long-term sequelae. *Continuum (Minneap Minn)*. 2014 Dec;20(6 Sports Neurology):1588-604; <http://www.ncbi.nlm.nih.gov/pubmed/25470162>. Montenegro PH, Corp DT, Stein TD, Cantu RC, Stern RA. Chronic traumatic encephalopathy: historical origins and current perspective. *Annu Rev Clin Psychol*. 2015;11:309-30; <http://www.annualreviews.org/eprint/zT2NHRNBhXyJXuD6vqkd/full/10.1146/annurev-clinpsy-032814-112814>.

## 6.2.6 Neuronal Ceroid Lipofuscinoses

Neuronal ceroid lipofuscinoses (NCL)<sup>1818</sup> is the collective name for a family of at least eight genetically separate neurodegenerative lysosomal storage disorders (e.g., Batten disease<sup>1819</sup>) that result from excessive accumulation of lipopigments (e.g., lipofuscin) in the body's tissues.<sup>1820</sup> These lipofuscin materials (Section 2.4.7) build up mainly in neuronal cells and to a lesser extent in other organs including the liver, spleen, myocardium, and kidneys. Between 1.3% and 10% of cases are the adult variant NCL (ANCL, aka. Kuf's disease)<sup>1821</sup> with symptoms typically appearing around 30 years of age and death occurring ~10 years later. Symptoms include the progressive permanent loss of motor and psychological ability, and a shortened life expectancy. The autosomal dominant ANCL disorder may be caused by a single nucleotide variation (c.344T>G) in the DNAJC5 gene on Chromosome 20, and over the last 20 years at least 300 mutations in 10 genes have been associated with NCLs.<sup>1822</sup>

Seizures may be controlled or reduced with use of anti-epileptic drugs. Physical, speech, and occupational palliative therapies may help affected patients retain functioning for awhile. But currently there is no widely accepted treatment that can cure or halt the progress of NCL.

---

<sup>1818</sup> [https://en.wikipedia.org/wiki/Neuronal\\_ceroid\\_lipofuscinosis](https://en.wikipedia.org/wiki/Neuronal_ceroid_lipofuscinosis).

<sup>1819</sup> Elleder, M, Drahotá, Z, Lisá V, Mares V, Mandys V, Müller J, Palmer DN. Tissue culture loading test with storage granules from animal models of neuronal ceroid-lipofuscinosis (Batten disease): testing their lysosomal degradability by normal and Batten cells. *Am J Med Genet.* 1995 Jun 5;57(2):213-21; <http://www.ncbi.nlm.nih.gov/pubmed/7668332>.

<sup>1820</sup> Haltia M. The neuronal ceroid-lipofuscinoses. *J Neuropathol Exp Neurol.* 2003 Jan;62(1):1-13; <http://jnen.oxfordjournals.org/content/62/1/1.long>.

<sup>1821</sup> Goebel HH, Braak H. Adult neuronal ceroid-lipofuscinosis. *Clin Neuropathol.* 1989 May-Jun;8(3):109-19; <http://www.ncbi.nlm.nih.gov/pubmed/2663281>. Sadzot B, Reznik M, Arrese-Estrada JE, Franck G. Familial Kufs' disease presenting as a progressive myoclonic epilepsy. *J Neurol.* 2000 Jun;247(6):447-54; <https://www.ncbi.nlm.nih.gov/pubmed/10929274/>. Babacan-Yildiz G, Hanagasi H, Gurvit H, Sirin G, Solakoglu S, Kucuk OS. A rare dementing disease: adult neuronal ceroid lipofuscinoses. *J Neuropsychiatry Clin Neurosci.* 2012 Fall;24(4):493-8; [https://www.researchgate.net/profile/Hasmet\\_Hanagasi/publication/233887037\\_A\\_Rare\\_Dementing\\_Disease\\_Adult\\_Neuronal\\_Ceroid\\_Lipofuscinoses/links/55e33d2f08ae2fac47212459.pdf](https://www.researchgate.net/profile/Hasmet_Hanagasi/publication/233887037_A_Rare_Dementing_Disease_Adult_Neuronal_Ceroid_Lipofuscinoses/links/55e33d2f08ae2fac47212459.pdf).

<sup>1822</sup> Nosková L, Stránecký V, Hartmannová H, Přistoupilová A, Barešová V, Ivánek R, Hůlková H, Jahnová H, van der Zee J, Staropoli JF, Sims KB, Tyynelä J, Van Broeckhoven C, Nijssen PC, Mole SE, Elleder M, Kmoch S. Mutations in DNAJC5, encoding cysteine-string protein alpha, cause autosomal-dominant adult-onset neuronal ceroid lipofuscinosis. *Am J Hum Genet.* 2011 Aug 12;89(2):241-52; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3155175/>. Benitez BA, Alvarado D, Cai Y, Mayo K, Chakraverty S, Norton J, Morris JC, Sands MS, Goate A, Cruchaga C. Exome-sequencing confirms DNAJC5 mutations as cause of adult neuronal ceroid-lipofuscinosis. *PLoS One.* 2011;6(11):e26741; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3208569/>.

Nanorobots may effectuate a complete cure by starting with Alzheimer Protocol #1 (Section 5.1) to eliminate all causative genetic mutations by replacing these mutations with normal nucleotide sequences in the chromosomes of every cell in the patient's body. Alzheimer Protocol #2 (Section 5.2) is then applied to rejuvenate all affected cells, with a special focus on removing lipofuscin granules from all cells (Section 5.2.2.2). Alzheimer Protocol #3 (Section 5.3) is used to replace any missing neurons, completing the cure.

## 6.2.7 Neural Autoimmune Disorders

**Multiple sclerosis (MS)**<sup>1823</sup> is the most common autoimmune disorder<sup>1824</sup> of the central nervous system.<sup>1825</sup> As of 2010, the number of people with MS was 2-2.5 million (approximately 30 per 100,000) globally, with rates varying widely in different regions.<sup>1826</sup> MS is an inflammatory-mediated demyelinating disease in which the insulating covers of nerve cells in the brain and spinal cord are damaged, disrupting the ability of parts of the nervous system to communicate. The symptoms of MS can include double vision, blindness in one eye, muscle weakness, trouble with sensation, or trouble with coordination, and over time the pathological changes become dominated by widespread microglial activation associated with extensive and chronic neurodegeneration.<sup>1827</sup> The pathology begins when T cells gain entry into the brain via disruptions in the blood-brain barrier (BBB; Section 4.3.1), recognize myelin as foreign, and attack it. The myelin attack initiates inflammatory processes, which trigger other immune cells and the release of soluble factors like cytokines and antibodies. Further BBB breakdown elicits other damaging effects such as swelling, activation of macrophages, and more activation of cytokines and other destructive proteins.

The clinical course of multiple sclerosis usually starts with reversible episodes of neurological disability in the third or fourth decade of life, transforming into a disease of continuous and irreversible neurological decline by the sixth or seventh decade. MS is not considered a hereditary disease but several genetic variations have been shown to increase the risk.<sup>1828</sup> There are many treatment approaches and a significant number of disease-modifying therapies and drugs exist,<sup>1829</sup> with aggressive immunoablation methods showing good hope in at least one

---

<sup>1823</sup> [https://en.wikipedia.org/wiki/Multiple\\_sclerosis](https://en.wikipedia.org/wiki/Multiple_sclerosis).

<sup>1824</sup> though questioned by some, e.g., Nakahara J, Maeda M, Aiso S, Suzuki N. Current concepts in multiple sclerosis: autoimmunity versus oligodendroglialopathy. *Clin Rev Allergy Immunol*. 2012 Feb;42(1):26-34; <http://www.ncbi.nlm.nih.gov/pubmed/22189514>.

<sup>1825</sup> Berer K, Krishnamoorthy G. Microbial view of central nervous system autoimmunity. *FEBS Lett*. 2014 Nov 17;588(22):4207-13; <http://onlinelibrary.wiley.com/doi/10.1016/j.febslet.2014.04.007/full>.

<sup>1826</sup> Milo R, Kahana E. Multiple sclerosis: geoeidemiology, genetics and the environment. *Autoimmun Rev*. 2010 Mar;9(5):A387-94; [https://www.researchgate.net/profile/Ron\\_Milo/publication/40025561\\_Multiple\\_sclerosis\\_Geoeidemiology\\_genetics\\_and\\_the\\_environment/links/0912f514637d4d6dbc000000.pdf](https://www.researchgate.net/profile/Ron_Milo/publication/40025561_Multiple_sclerosis_Geoeidemiology_genetics_and_the_environment/links/0912f514637d4d6dbc000000.pdf).

<sup>1827</sup> Compston A, Coles A. Multiple sclerosis. *Lancet*. 2008 Oct 25;372(9648):1502-17; <https://www.ncbi.nlm.nih.gov/pubmed/18970977>.

<sup>1828</sup> Dyment DA, Ebers GC, Sadovnick AD. Genetics of multiple sclerosis. *Lancet Neurol*. 2004 Feb;3(2):104-10; <https://www.ncbi.nlm.nih.gov/pubmed/14747002>.

<sup>1829</sup> [https://en.wikipedia.org/wiki/Multiple\\_sclerosis\\_research](https://en.wikipedia.org/wiki/Multiple_sclerosis_research).

recent small-scale clinical trial.<sup>1830</sup> But no treatment has yet been proven to change the course of primary progressive MS<sup>1831</sup> and there is still no known cure for MS.

Other neural autoimmune disorders are much rarer, and include **acute disseminated encephalomyelitis** (aka. acute demyelinating encephalomyelitis),<sup>1832</sup> **acute motor axonal neuropathy** (aka. Chinese paralytic syndrome),<sup>1833</sup> **anti-NMDA receptor encephalitis** (aka. NMDA receptor antibody encephalitis),<sup>1834</sup> **autoimmune encephalitis**,<sup>1835</sup> **Balo concentric sclerosis**,<sup>1836</sup> **chronic inflammatory demyelinating polyneuropathy**,<sup>1837</sup> **diffuse myelinoclastic sclerosis** (aka. Schilder's disease),<sup>1838</sup> **Lambert-Eaton myasthenic syndrome**,<sup>1839</sup> **Morvan's fibrillary chorea** (aka. Morvan's syndrome),<sup>1840</sup> **myasthenia gravis**,<sup>1841</sup> **neuromyelitis optica** (aka. Devic's syndrome),<sup>1842</sup> **neuromyotonia**,<sup>1843</sup> **progressive encephalomyelitis**,<sup>1844</sup> **Sydenham's chorea** (aka. chorea minor, St. Vitus dance),<sup>1845</sup> and **transverse myelitis**.<sup>1846</sup>

Alzheimer Protocol #1 (Section 5.1) can eliminate the minor genetic risks of MS and related conditions. Alzheimer Protocol #2 (Section 5.2) can be used to eliminate autoreactive immune cells (Section 5.2.5) – in particular, autoimmune T and B cells can be selectively deleted by medical nanorobots, somewhat analogously to clonal deletion.<sup>1847</sup> Serious tissue degradation can be repaired with Alzheimer Protocol #3 (Section 5.3).

<sup>1830</sup> Atkins HL, Bowman M, Allan D, Anstee G, Arnold DL, Bar-Or A, Bence-Bruckler I, Birch P, Bredeson C, Chen J, Fergusson D, Halpenny M, Hamelin L, Huebsch L, Hutton B, Laneville P, Lapierre Y, Lee H, Martin L, McDiarmid S, O'Connor P, Ramsay T, Sabloff M, Walker L, Freedman MS. Immunoablation and autologous haemopoietic stem-cell transplantation for aggressive multiple sclerosis: a multicentre single-group phase 2 trial. *Lancet*. 2016 Jun 8. pii: S0140-6736(16)30169-6; <http://www.ncbi.nlm.nih.gov/pubmed/27291994>.

<sup>1831</sup> Tsang BK, Macdonell R. Multiple sclerosis – diagnosis, management and prognosis. *Aust Fam Physician*. 2011 Dec;40(12):948-55; <http://www.racgp.org.au/download/documents/AFP/2011/December/201112tsang.pdf>.

<sup>1832</sup> [https://en.wikipedia.org/wiki/Acute\\_disseminated\\_encephalomyelitis](https://en.wikipedia.org/wiki/Acute_disseminated_encephalomyelitis).

<sup>1833</sup> [https://en.wikipedia.org/wiki/Acute\\_motor\\_axonal\\_neuropathy](https://en.wikipedia.org/wiki/Acute_motor_axonal_neuropathy).

<sup>1834</sup> [https://en.wikipedia.org/wiki/Anti-NMDA\\_receptor\\_encephalitis](https://en.wikipedia.org/wiki/Anti-NMDA_receptor_encephalitis).

<sup>1835</sup> [https://en.wikipedia.org/wiki/Autoimmune\\_encephalitis](https://en.wikipedia.org/wiki/Autoimmune_encephalitis).

<sup>1836</sup> [https://en.wikipedia.org/wiki/Balo\\_concentric\\_sclerosis](https://en.wikipedia.org/wiki/Balo_concentric_sclerosis).

<sup>1837</sup> [https://en.wikipedia.org/wiki/Chronic\\_inflammatory\\_demyelinating\\_polyneuropathy](https://en.wikipedia.org/wiki/Chronic_inflammatory_demyelinating_polyneuropathy).

<sup>1838</sup> [https://en.wikipedia.org/wiki/Diffuse\\_myelinoclastic\\_sclerosis](https://en.wikipedia.org/wiki/Diffuse_myelinoclastic_sclerosis).

<sup>1839</sup> [https://en.wikipedia.org/wiki/Lambert%E2%80%93Eaton\\_myasthenic\\_syndrome](https://en.wikipedia.org/wiki/Lambert%E2%80%93Eaton_myasthenic_syndrome).

<sup>1840</sup> [https://en.wikipedia.org/wiki/Morvan's\\_syndrome](https://en.wikipedia.org/wiki/Morvan's_syndrome).

<sup>1841</sup> [https://en.wikipedia.org/wiki/Myasthenia\\_gravis](https://en.wikipedia.org/wiki/Myasthenia_gravis).

<sup>1842</sup> [https://en.wikipedia.org/wiki/Neuromyelitis\\_optica](https://en.wikipedia.org/wiki/Neuromyelitis_optica).

<sup>1843</sup> <https://en.wikipedia.org/wiki/Neuromyotonia>.

<sup>1844</sup> [https://en.wikipedia.org/wiki/Stiff\\_person\\_syndrome](https://en.wikipedia.org/wiki/Stiff_person_syndrome).

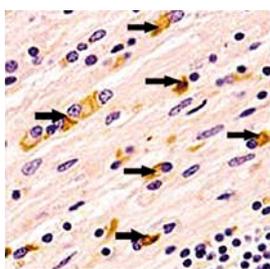
<sup>1845</sup> [https://en.wikipedia.org/wiki/Sydenham's\\_chorea](https://en.wikipedia.org/wiki/Sydenham's_chorea).

<sup>1846</sup> [https://en.wikipedia.org/wiki/Transverse\\_myelitis](https://en.wikipedia.org/wiki/Transverse_myelitis).

<sup>1847</sup> Zhang GX, Liu TT, Ventura ES, Chen Y, Rostami A. Reversal of spontaneous progressive autoimmune encephalomyelitis by myelin basic protein-induced clonal deletion. *Autoimmunity*. 1999;31(4):219-27; <http://www.ncbi.nlm.nih.gov/pubmed/10789987>.

## 6.2.8 Multiple System Atrophy

Multiple system atrophy (MSA)<sup>1848</sup> is a degenerative neurological disorder associated with the degeneration of nerve cells in specific areas of the brain, affecting an estimated 3-4 per 100,000 individuals.<sup>1849</sup> This cell degeneration causes problems with movement, balance, and autonomic functions of the body such as bladder control or blood-pressure regulation, with additional symptoms including constipation, impotence, dysphagia, and sleep disorders.<sup>1850</sup> The cause of MSA is unclear and no specific risk factors have been identified, although research indicates that a misfolded prion form of the  $\alpha$ -synuclein protein could be the cause of the disease.<sup>1851</sup> Oxidative stress, proteasomal and mitochondrial dysfunction, excitotoxicity, neuroinflammation, metabolic changes, and energy failure may also be important contributors to the pathogenesis of MSA.<sup>1852</sup>



Multiple system atrophy can be explained as cell loss and gliosis or a proliferation of astrocytes in damaged areas of the central nervous system, forming a glial scar.<sup>1853</sup> The presence of these glial cytoplasmic inclusions (also known as GCIs or Papp-Lantos bodies; image, left) in the movement, balance, and autonomic-control centers of the brain are the defining histopathologic hallmark of MSA.<sup>1854</sup> Recent studies have shown that the major filamentous component of glial and neuronal cytoplasmic inclusions is hyperphosphorylated  $\alpha$ -synuclein, ubiquitin,

<sup>1848</sup> [https://en.wikipedia.org/wiki/Multiple\\_system\\_atrophy](https://en.wikipedia.org/wiki/Multiple_system_atrophy).

<sup>1849</sup> Federoff M, Schottlaender LV, Houlden H, Singleton A. Multiple system atrophy: the application of genetics in understanding etiology. *Clin Auton Res*. 2015 Feb;25(1):19-36; <https://www.ncbi.nlm.nih.gov/pubmed/25687905>.

<sup>1850</sup> <https://www.consumerhealthdigest.com/health-awareness/multiple-system-atrophy-awareness-month.html>.

<sup>1851</sup> Prusiner SB, Woerman AL, Mordes DA, Watts JC, Rampersaud R, Berry DB, Patel S, Oehler A, Lowe JK, Kravitz SN, Geschwind DH, Glidden DV, Halliday GM, Middleton LT, Gentleman SM, Grinberg LT, Giles K. Evidence for  $\alpha$ -synuclein prions causing multiple system atrophy in humans with parkinsonism. *Proc Natl Acad Sci U S A*. 2015 Sep 22;112(38):E5308-17; <https://www.ncbi.nlm.nih.gov/pubmed/26324905>.

<sup>1852</sup> Jellinger KA. Neuropathology of multiple system atrophy: new thoughts about pathogenesis. *Mov Disord*. 2014 Dec;29(14):1720-41; <https://www.ncbi.nlm.nih.gov/pubmed/25297524>.

<sup>1853</sup> Wenning GK, Colosimo C, Geser F, Poewe W. Multiple system atrophy. *Lancet Neurol*. 2004 Feb;3(2):93-103; <https://www.ncbi.nlm.nih.gov/pubmed/14747001>.

<sup>1854</sup> Brück D, Wenning GK, Stefanova N, Fellner L. Glia and alpha-synuclein in neurodegeneration: A complex interaction. *Neurobiol Dis*. 2016 Jan;85:262-74; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4730552/>.

LRRK2, and many other proteins.<sup>1855</sup> Tau proteins have also been found in some glial cytoplasmic inclusions.<sup>1856</sup> Genetic variants of the  $\alpha$ -synuclein gene, SNCA, may play a role in the disease.<sup>1857</sup>

Current treatment options are very limited and are mainly focused on symptomatic relief, whereas disease modifying options are lacking. Despite extensive testing, no neuroprotective drug treatment for MSA has been identified up to now.<sup>1858</sup> There is currently no cure for MSA.<sup>1859</sup>

Nanorobotic treatment using Alzheimer Protocol #1 (Section 5.1) can correct any genetic variants of the SNCA gene. Alzheimer Protocol #2 (Section 5.2) extracts all misfolded  $\alpha$ -synuclein proteins and Papp-Lantos bodies (Section 5.2.2.3), rejuvenating surviving neurons and eliminating cellular oxidative stress, proteasomal and mitochondrial dysfunction, excitotoxicity, neuroinflammation, and pathological metabolic changes. Alzheimer Protocol #3 (Section 5.3) removes glial scars and replaces missing neurons and glial cells, thus effecting a complete cure for MSA.

---

<sup>1855</sup> Arima K, Uéda K, Sunohara N, Arakawa K, Hirai S, Nakamura M, Tonzuka-Uehara H, Kawai M. NACP/alpha-synuclein immunoreactivity in fibrillary components of neuronal and oligodendroglial cytoplasmic inclusions in the pontine nuclei in multiple system atrophy. *Acta Neuropathol.* 1998 Nov;96(5):439-44; <https://www.ncbi.nlm.nih.gov/pubmed/9829806>. Jellinger KA, Lantos PL. Papp-Lantos inclusions and the pathogenesis of multiple system atrophy: an update. *Acta Neuropathol.* 2010 Jun;119(6):657-67; <https://www.ncbi.nlm.nih.gov/pubmed/20309568>.

<sup>1856</sup> Piao YS, Hayashi S, Hasegawa M, Wakabayashi K, Yamada M, Yoshimoto M, Ishikawa A, Iwatsubo T, Takahashi H. Co-localization of alpha-synuclein and phosphorylated tau in neuronal and glial cytoplasmic inclusions in a patient with multiple system atrophy of long duration. *Acta Neuropathol.* 2001 Mar;101(3):285-93; <https://www.ncbi.nlm.nih.gov/pubmed/11307630>.

<sup>1857</sup> Al-Chalabi A, Dürr A, Wood NW, Parkinson MH, Camuzat A, Hulot JS, Morrison KE, Renton A, Sussmuth SD, Landwehrmeyer BG, Ludolph A, Agid Y, Brice A, Leigh PN, Bensimon G; NNIPPS Genetic Study Group. Genetic variants of the alpha-synuclein gene SNCA are associated with multiple system atrophy. *PLoS One.* 2009 Sep 22;4(9):e7114; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2743996/>.

<sup>1858</sup> Kuzdas-Wood D, Stefanova N, Jellinger KA, Seppi K, Schlossmacher MG, Poewe W, Wenning GK. Towards translational therapies for multiple system atrophy. *Prog Neurobiol.* 2014 Jul;118:19-35; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4068324/>. Palma JA, Kaufmann H. Novel therapeutic approaches in multiple system atrophy. *Clin Auton Res.* 2015 Feb;25(1):37-45; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4265580/>.

<sup>1859</sup> Stefanova N, Wenning GK. Review: Multiple system atrophy: emerging targets for interventional therapies. *Neuropathol Appl Neurobiol.* 2016 Feb;42(1):20-32; <http://www.ncbi.nlm.nih.gov/pubmed/26785838>.

## Chapter 7. Conclusions

The advent of the nanofactory – a proposed new technology for atomically precise manufacturing – will make possible a revolutionary new paradigm in human health care: medical nanorobotics.

Using atomically precise manufacturing, nanofactories the size of a desktop appliance will fabricate kilogram-per-day batches of medical nanorobots at a raw manufacturing cost of \$1-\$10 per treatment dose (a few  $\text{cm}^3$ ). These nanorobots will be bacterium-scale artificial mechanical devices with onboard sensors, manipulators, pumps, motility mechanisms, communication facilities, programmable computers, and biocompatible external hulls, tasked with medical missions of diagnosis and therapy. These devices will make it possible to treat and to cure previously untreatable and incurable diseases.

Alzheimer's disease (AD), along with many other neurodegenerative disorders, is presently incurable despite many decades of research and many billions of dollars invested in the effort. Future developments of conventional technologies now on the long-term R&D horizon – including pharmaceuticals, nanoparticles, gene therapies, stem cells, and anti-aging drugs – will require huge investments, many decades of further development, and (if history is a guide) seem highly likely still to fail to provide a complete cure. A 2015 survey of the neuropharmaceuticals industry reported that treatments for Alzheimer's with a big impact “are unlikely anytime soon” and that the situation has grown so bleak that the research community is now considering “targeting Alzheimer's symptoms but not trying to reverse the disease.”

Alzheimer's disease is the Mount Everest of medical challenges that will require a mature nanotechnology to surmount. AD is a highly complex, highly multifactorial disorder with dense causal networks that will probably resist any attempt to find a single “magic bullet” drug or simple curative treatment. In AD there are many pathologies happening simultaneously at many different sites within the brain. It is likely impossible to correct them all without using medical nanorobots – the ultimate “big hammer” in the 21st century medical technology toolkit.

Medical nanorobotics will provide a single powerful general-purpose therapeutic platform that can simultaneously address many different kinds of biological malfunctions, using platform variants specifically and efficiently targeted to each of the multifactorial pathologies comprising Alzheimer's disease. For example, one class of medical nanorobots will target genetic risk factors, replacing mutant genes that present a high risk for AD with normal genes that present no elevated risk for AD. Other variants of the same nanorobotic therapeutic platform could bind and remove extracellular amyloid plaques or intracellular tau protein tangles, correct cancer-prone mutant genes, replace dysfunctional mitochondria, eliminate toxic cells, and rejuvenate surviving but damaged neural tissues. Still other variants of the same nanorobotic platform would perform *in vivo* brain mapping at subcellular resolution, conduct intracranial debridement of diseased neural tissue, manufacture replacement biological neural cells, and incorporate these replacement neural cells into the patient's brain where the ravages of Alzheimer's disease have left large cell-free gaps within the tissues. Medical nanorobots can also be used as research tools to further study the cellular and biochemical details of the disease, and to refine and perfect the therapeutic protocols described in Chapter 5 of this book.

The nanorobotic treatment for AD proposed here, called the Alzheimer Protocols, can be conceptually summarized as a series of three specific protocols aimed at three distinct clinical objectives: (1) genetic derisking, (2) tissue rejuvenation, and (3) neural reconstruction. The

outcome of these simultaneous nanomedical interventions, perhaps performed over a period of about one week using a few trillion nanorobots in an appropriate clinical setting, will be a halt to the continuing damage of AD and a reversal of existing damage caused by AD, resulting in an effective cure for Alzheimer's disease. Even more advanced protocols can later be developed. What has been presented here is a conservative proof-of-concept "first generation" approach that will likely improve as we gain practical experience with nanorobots and their precise behavior inside the human body, and can test and refine the protocols using real robots and real patients.

Finally, with a nanorobotic cure for AD in hand, other less-challenging neurodegenerative diseases – such as Parkinson's disease, Huntington's disease, ALS, prion diseases, and multiple sclerosis – can likely also be completely reversed and cured using an appropriate subset of the methods developed for defeating Alzheimer's disease.

Our key final conclusion:

**Medical nanorobotics will almost certainly provide a complete cure for Alzheimer's disease and related neurodegenerative conditions. All that remains is to execute the technical plan for implementation.**

## Appendix A. Normal Physiological Roles of Amyloid Beta (A $\beta$ )

It is now over three decades since amyloid  $\beta$  (A $\beta$ ), a 39-43 residue polypeptide or small protein, was first sequenced and recognized as a potential marker of Alzheimer's disease.<sup>1860</sup> A $\beta$  is a cleavage product of a large, transmembrane protein, termed APP (amyloid precursor protein). APP can undergo cleavage down one of at least two major pathways. In the first pathway, cleavage by the enzyme  $\alpha$ -secretase prevents A $\beta$  formation, and instead produces the neuroprotective sAPP $\alpha$  fragment. However, if sequential cleavage by  $\beta$ - and then  $\gamma$ -secretases predominates, A $\beta$  is formed.

While the historical "amyloid hypothesis" implicated A $\beta$  in the progression of Alzheimer's disease, it is now recognized that both cleavage pathways represent normal physiological processes. The physiological activities of A $\beta$  are numerous and are yet to be fully elucidated. What is emerging from recent studies is a steadily growing body of data suggesting that normal levels of A $\beta$  have important physiological functions and may even be crucial for neuronal cell survival.<sup>1861</sup>

As a consequence, A $\beta$  should only be regarded as toxic when its natural production and degradation processes become imbalanced, allowing A $\beta$  concentrations to rise abnormally high. For quantification, abnormally high extracellular levels of A $\beta$  are neurotoxic in monomers, in oligomers,<sup>1862</sup> and in fibrils,<sup>1863</sup> with A $\beta_{1-40}$  (aka. "A $\beta_{40}$ ") dimers ~3-fold more toxic than A $\beta_{1-40}$  monomers and A $\beta_{1-40}$  tetramers ~13-fold more toxic than A $\beta_{1-40}$  monomers, with a range of EC<sub>50</sub> (50% maximum toxicity) for A $\beta_{1-40}$  of ~20-67  $\mu$ M in these three cases.<sup>1864</sup> Extracellular A $\beta_{1-42}$  (aka. "A $\beta_{42}$ ") is about twice as neurotoxic as A $\beta_{1-40}$ .<sup>1865</sup> The normal extracellular A $\beta_{1-40}$

<sup>1860</sup> Glenner GG, Wong CW. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem Biophys Res Commun.* 1984 May 16;120(3):885-90; <http://www.ncbi.nlm.nih.gov/pubmed/6375662>.

<sup>1861</sup> Pearson HA, Peers C. Physiological roles for amyloid beta peptides. *J Physiol.* 2006 Aug 15;575(Pt 1):5-10; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1819417/>.

<sup>1862</sup> Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, Selkoe DJ. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation *in vivo*. *Nature.* 2002 Apr 4;416(6880):535-9; <http://www.ncbi.nlm.nih.gov/pubmed/11932745/>.

<sup>1863</sup> Morgan C, Colombres M, Nuñez MT, Inestrosa NC. Structure and function of amyloid in Alzheimer's disease. *Prog Neurobiol.* 2004 Dec;74(6):323-49; <http://www.ncbi.nlm.nih.gov/pubmed/15649580/>.

<sup>1864</sup> Ono K, Condrón MM, Teplow DB. Structure-neurotoxicity relationships of amyloid beta-protein oligomers. *Proc Natl Acad Sci U S A.* 2009 Sep 1;106(35):14745-50; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2736424/>.

<sup>1865</sup> Liao MQ, Tzeng YJ, Chang LY, Huang HB, Lin TH, Chyan CL, Chen YC. The correlation between neurotoxicity, aggregative ability and secondary structure studied by sequence truncated A $\beta$  peptides. *FEBS Lett.* 2007 Mar 20;581(6):1161-5; <http://www.sciencedirect.com/science/article/pii/S0014579307001822>.

monomer concentration in AD patient cerebrospinal fluid is 0.013  $\mu\text{M}$ , the same as in normal brain, but 0.0024  $\mu\text{M}$  for  $\text{A}\beta_{1-42}$  in AD patient cerebrospinal fluid and 0.0033  $\mu\text{M}$  in normal brain.<sup>1866</sup> The toxicity of intracellular  $\text{A}\beta_{1-42}$  is perhaps 100,000 times greater than for extracellular  $\text{A}\beta_{1-42}$ . Directly microinjecting as little as 1-100 pM (0.005-0.5 ng/ml) of intracellular  $\text{A}\beta_{1-42}$  induces 50% (1 pM) to 90% (100 pM) cell death in some cultured human neurons; yet these neurons do not undergo cell death even up to a 10  $\mu\text{M}$  (4500 ng/ml) dose of extracellular  $\text{A}\beta_{1-42}$ .<sup>1867</sup>

Given the presence of specific enzymatic pathways for the constitutive generation of  $\text{A}\beta$ , coupled with the existence of selective uptake, breakdown and clearance pathways for its removal, it seems clear that  $\text{A}\beta$  has a role to play in the normal function of the nervous system, including essential modulation of synaptic activity and neuronal survival. Thus  $\text{A}\beta$  cannot be regarded simplistically as a neurotoxic factor that requires eradication from the brain to avoid dementia. A large number of natural and normal physiological roles for  $\text{A}\beta$ , many of which lie outside the brain and distributed throughout the human body (**Table 5**), are now known or suspected. A few of these functions are summarized below the Table.

---

<sup>1866</sup> Oe T, Ackermann BL, Inoue K, Berna MJ, Garner CO, Gelfanova V, Dean RA, Siemers ER, Holtzman DM, Farlow MR, Blair IA. Quantitative analysis of amyloid beta peptides in cerebrospinal fluid of Alzheimer's disease patients by immunoaffinity purification and stable isotope dilution liquid chromatography/negative electrospray ionization tandem mass spectrometry. *Rapid Commun Mass Spectrom.* 2006;20(24):3723-35; <http://www.ncbi.nlm.nih.gov/pubmed/17117458>.

<sup>1867</sup> Zhang Y, McLaughlin R, Goodyer C, LeBlanc A. Selective cytotoxicity of intracellular amyloid beta peptide 1-42 through p53 and Bax in cultured primary human neurons. *J Cell Biol.* 2002 Feb 4;156(3):519-29; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2173346/>.

**Table 5. Distribution of A $\beta$  in the human body, ages 57-85 years  
(AD = Alzheimer's disease).<sup>1868</sup>**

Human Fluid or Tissue	Average A $\beta$ <sub>1-40</sub> Component	Average A $\beta$ <sub>1-42</sub> Component
Blood plasma	185-629 pg/ml	61-177 pg/ml
Blood plasma (AD)	218-702 pg/ml	107-310 pg/ml
Brain – gray matter	209 ng/gm	784 ng/gm
Brain – gray matter (AD)	608 ng/gm	6096 ng/gm
Brain – white matter	111 ng/gm	418 ng/gm
Brain – white matter (AD)	1069 ng/gm	1135 ng/gm
Platelets (quiescent)	83.8 ng/gm	1.7 ng/gm
Platelets (activated)	56.8 ng/gm	1.6 ng/gm
Aorta (minimal lesions)	31.4 ng/gm	1.0 ng/gm
Aorta (severe lesions)	75.3 ng/gm	0.7 ng/gm
Leptomenigeal arteries (minimal lesions)	17.3 ng/gm	5.3 ng/gm
Leptomenigeal arteries (severe lesions)	113.1 ng/gm	28.0 ng/gm
Skeletal muscle	29.8 ng/gm	10.2 ng/gm
Skeletal muscle (AD)	37.8 ng/gm	15.7 ng/gm
Liver	67.5 ng/gm	15.5 ng/gm
Liver (AD)	8.6 ng/gm	1.7 ng/gm

Known or suspected roles physiological roles for A $\beta$ :

---

<sup>1868</sup> Roher AE, Esh CL, Kokjohn TA, Castaño EM, Van Vickle GD, Kalback WM, Patton RL, Luehrs DC, Dausgs ID, Kuo YM, Emmerling MR, Soares H, Quinn JF, Kaye J, Connor DJ, Silverberg NB, Adler CH, Seward JD, Beach TG, Sabbagh MN. Amyloid beta peptides in human plasma and tissues and their significance for Alzheimer's disease. *Alzheimers Dement.* 2009 Jan;5(1):18-29; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2663406/>.

\* **Regulation of angiogenesis** ( $A\beta_{1-42}$ )

$A\beta$  monomers stimulate angiogenesis, regulating blood vessel branching in tissues as varied as human umbilical vein, human endothelial cells, and zebrafish hindbrain. High physiological concentration of  $A\beta$  monomer induces angiogenesis by a conserved mechanism, establishing that the underlying mechanism has remained largely unchanged since amphibians diverged from bony fishes approximately 350 million years ago.<sup>1869</sup>

\* **Regulation of platelet activation during hemostasis** ( $A\beta_{1-40}$ ,  $A\beta_{1-42}$ ,  $A\beta_{25-35}$ )

Platelets express amyloid precursor protein APP751 and APP770, as well as all the enzymes for APP proteolysis through amyloidogenic and non-amyloidogenic pathways.<sup>1870</sup> In addition, a considerable amount of amyloidogenic  $A\beta_{1-40}$  and  $A\beta_{1-42}$  is stored in platelet  $\alpha$ -granules and is secreted upon platelet stimulation.<sup>1871</sup> Once released in the blood plasma,  $A\beta$  peptides are able to induce platelet activation, establishing a positive feedback to potentiate cell activation.<sup>1872</sup> Amyloid  $A\beta$  peptides present in the bloodstream mainly derive from circulating platelets.<sup>1873</sup>

---

<sup>1869</sup> Cameron DJ, Galvin C, Alkam T, Sidhu H, Ellison J, Luna S, Ethell DW. Alzheimer's-related peptide amyloid- $\beta$  plays a conserved role in angiogenesis. *PLoS One*. 2012;7(7):e39598; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3392248/>.

<sup>1870</sup> Catricala S, Torti M, Ricevuti G. Alzheimer disease and platelets: how's that relevant. *Immun Ageing*. 2012 Sep 17;9(1):20; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3545835/>.

<sup>1871</sup> Bush AI, Martins RN, Rumble B, Moir R, Fuller S, Milward E, Currie J, Ames D, Weidemann A, Fischer P, Multhaup G, Beyreuther K, Masters CL. The amyloid precursor protein of Alzheimer's disease is released by human platelets. *J Biol Chem*. 1990 Sep 15;265(26):15977-83; <http://www.jbc.org/content/265/26/15977.long>. Li QX, Whyte S, Tanner JE, Evin G, Beyreuther K, Masters CL. Secretion of Alzheimer's disease Abeta amyloid peptide by activated human platelets. *Lab Invest*. 1998 Apr;78(4):461-9; <http://www.ncbi.nlm.nih.gov/pubmed/9564890>. Kokjohn TA, Van Vickle GD, Maarouf CL, Kalback WM, Hunter JM, Dausgs ID, Luehrs DC, Lopez J, Brune D, Sue LI, Beach TG, Castaño EM, Roher AE. Chemical characterization of pro-inflammatory amyloid-beta peptides in human atherosclerotic lesions and platelets. *Biochim Biophys Acta*. 2011 Nov;1812(11):1508-14; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3185199/>.

<sup>1872</sup> Canobbio I, Guidetti GF, Oliviero B, Manganaro D, Vara D, Torti M, Pula G. Amyloid  $\beta$ -peptide-dependent activation of human platelets: essential role for  $Ca^{2+}$  and ADP in aggregation and thrombus formation. *Biochem J*. 2014 Sep 15;462(3):513-23; <http://www.ncbi.nlm.nih.gov/pubmed/24984073>.

<sup>1873</sup> Canobbio I, Catricalà S, Di Pasqua LG, Guidetti G, Consonni A, Manganaro D, Torti M. Immobilized amyloid  $A\beta$  peptides support platelet adhesion and activation. *FEBS Lett*. 2013 Aug 19;587(16):2606-11; <http://www.sciencedirect.com/science/article/pii/S0014579313004997>.

\* **Anti-microbial activity** ( $A\beta_{1-40}$ ,  $A\beta_{1-42}$ )

Synthetic  $A\beta$  peptides have demonstrated antibiotic activity against Gram-negative and Gram-positive bacteria and the yeast *C. albicans*,<sup>1874</sup> suggesting that  $A\beta$  may function *in vivo* as an antimicrobial peptide,<sup>1875</sup> and thus may play a role as an effector molecule of innate immunity.

\* **Binding to DNA in chromatin** ( $A\beta_{1-40}$ ,  $A\beta_{1-42}$ )

Structural studies have indicated that  $A\beta_{1-42}$  binds DNA *in vitro*.<sup>1876</sup> Binding of  $A\beta$  to DNA *in vitro* would be moot if the peptide did not exist intracellularly and interact with chromatin. While  $A\beta$  is normally a secreted peptide, oxidative stress can induce intracellular localization of the peptide. Investigation of chromatin-protein interaction within live cells suggests that the  $A\beta$  peptide interacts with the chromatin of SK-N-SH human neuroblastoma cells, strongly supporting the intranuclear localization of  $A\beta_{1-40}$  as suggested by live-cell imaging data.<sup>1877</sup> It was later reported that a novel chaperone protein,  $A\beta$ -related death-inducing protein ( $A\beta$ -DIP), regulates nuclear localization of intracellular  $A\beta_{1-42}$ .<sup>1878</sup>

---

<sup>1874</sup> Soscia SJ, Kirby JE, Washicosky KJ, Tucker SM, Ingelsson M, Hyman B, Burton MA, Goldstein LE, Duong S, Tanzi RE, Moir RD. The Alzheimer's disease-associated amyloid beta-protein is an antimicrobial peptide. *PLoS One*. 2010 Mar 3;5(3):e9505; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2831066/>.

<sup>1875</sup> Zaiou M. Multifunctional antimicrobial peptides: therapeutic targets in several human diseases. *J Mol Med (Berl)*. 2007 Apr;85(4):317-29; <http://www.ncbi.nlm.nih.gov/pubmed/17216206>.

<sup>1876</sup> Barrantes A, Rejas MT, Benítez MJ, Jiménez JS. Interaction between Alzheimer's Abeta1-42 peptide and DNA detected by surface plasmon resonance. *J Alzheimers Dis*. 2007 Dec;12(4):345-55; <http://www.ncbi.nlm.nih.gov/pubmed/18198421/>.

<sup>1877</sup> Ohyagi Y, Asahara H, Chui DH, Tsuruta Y, Sakae N, Miyoshi K, Yamada T, Kikuchi H, Taniwaki T, Murai H, Ikezoe K, Furuya H, Kawarabayashi T, Shoji M, Checler F, Iwaki T, Makifuchi T, Takeda K, Kira J, Tabira T. Intracellular Abeta42 activates p53 promoter: a pathway to neurodegeneration in Alzheimer's disease. *FASEB J*. 2005 Feb;19(2):255-7; <http://www.fasebj.org/content/early/2005/01/27/fj.04-2637fje.long>. Ohyagi Y, Tabira T. Intracellular amyloid beta-protein and its associated molecules in the pathogenesis of Alzheimer's disease. *Mini Rev Med Chem*. 2006 Oct;6(10):1075-80; <http://www.ncbi.nlm.nih.gov/pubmed/17073707/>.

<sup>1878</sup> Ohyagi Y. Intracellular amyloid beta-protein as a therapeutic target for treating Alzheimer's disease. *Curr Alzheimer Res*. 2008 Dec;5(6):555-61; <http://www.ncbi.nlm.nih.gov/pubmed/19075582/>.

\* **Transcriptional activation** ( $A\beta_{1-28}$ ,  $A\beta_{25-35}$ ,  $A\beta_{1-40}$ ,  $A\beta_{1-42}$ )

Intracellular  $A\beta_{1-42}$  can act as a heat-shock transcription factor in directing p53-activated neuron apoptosis.<sup>1879</sup> In cell culture,  $A\beta_{1-42}$  regulates the transcription factors Mash1 and OLIG2 – two basic helix-loop-helix transcription factors involved in initiating neurogenesis and oligodendrogenesis in the central nervous system.<sup>1880</sup> Low concentrations of  $A\beta_{1-40}$  play a role in translationally regulating the maturation of rat cerebellar granule neurons.<sup>1881</sup>

$A\beta$  may also regulate its own production through feedback on expression of its precursor protein and the  $\beta$ -secretase enzyme,<sup>1882</sup> in particular, the upregulation of native BACE1 (beta-secretase) gene transcription as observed in cell culture by the addition of  $A\beta_{1-42}$ .<sup>1883</sup> Such activity has also been demonstrated specifically with the  $A\beta_{25-35}$  peptide for the SLC38A1 gene.<sup>1884</sup>  $A\beta_{1-42}$ <sup>1885</sup> also mediates the overexpression of BACE1 with stimulation of BACE1 RNA transcription.

<sup>1879</sup> Ohyagi Y, Asahara H, Chui DH, Tsuruta Y, Sakae N, Miyoshi K, Yamada T, Kikuchi H, Taniwaki T, Murai H, Ikezoe K, Furuya H, Kawarabayashi T, Shoji M, Checler F, Iwaki T, Makifuchi T, Takeda K, Kira J, Tabira T. Intracellular Abeta42 activates p53 promoter: a pathway to neurodegeneration in Alzheimer's disease. *FASEB J.* 2005 Feb;19(2):255-7; <http://www.fasebj.org/content/early/2005/01/27/fj.04-2637fje.long>.

<sup>1880</sup> Uchida Y, Nakano S, Gomi F, Takahashi H. Differential regulation of basic helix-loop-helix factors Mash1 and Olig2 by beta-amyloid accelerates both differentiation and death of cultured neural stem/progenitor cells. *J Biol Chem.* 2007 Jul 6;282(27):19700-9; <http://www.jbc.org/content/282/27/19700.long>.

<sup>1881</sup> Zhan XQ, Yao JJ, Liu DD, Ma Q, Mei YA.  $A\beta_{40}$  modulates GABA(A) receptor  $\alpha 6$  subunit expression and rat cerebellar granule neuron maturation through the ERK/mTOR pathway. *J Neurochem.* 2014 Feb;128(3):350-62; <http://www.ncbi.nlm.nih.gov/pubmed/24118019>.

<sup>1882</sup> Bailey JA, Maloney B, Ge YW, Lahiri DK. Functional activity of the novel Alzheimer's amyloid  $\beta$ -peptide interacting domain ( $A\beta$ ID) in the APP and BACE1 promoter sequences and implications in activating apoptotic genes and in amyloidogenesis. *Gene.* 2011 Nov 15;488(1-2):13-22; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3372404/>. Marsden IT, Minamide LS, Bamburg JR. Amyloid- $\beta$ -induced amyloid- $\beta$  secretion: a possible feed-forward mechanism in Alzheimer's Disease. *J Alzheimers Dis.* 2011;24(4):681-91; <http://www.ncbi.nlm.nih.gov/pubmed/21297255>.

<sup>1883</sup> Tamagno E, Guglielmotto M, Giliberto L, Vitali A, Borghi R, Autelli R, Danni O, Tabaton M. JNK and ERK1/2 pathways have a dual opposite effect on the expression of BACE1. *Neurobiol Aging.* 2009 Oct;30(10):1563-73; <http://www.ncbi.nlm.nih.gov/pubmed/18255190>.

<sup>1884</sup> Buntup D, Skare O, Solbu TT, Chaudhry FA, Storm-Mathisen J, Thangnipon W. Beta-amyloid 25-35 peptide reduces the expression of glutamine transporter SAT1 in cultured cortical neurons. *Neurochem Res.* 2008 Feb;33(2):248-56; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2226019/>.

<sup>1885</sup> Giliberto L, Borghi R, Piccini A, Mangerini R, Sorbi S, Cirmena G, Garuti A, Ghetti B, Tagliavini F, Mughal MR, Mattson MP, Zhu X, Wang X, Guglielmotto M, Tamagno E, Tabaton M. Mutant presenilin 1 increases the expression and activity of BACE1. *J Biol Chem.* 2009 Apr 3;284(14):9027-38; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2666551/>.

**\* Regulation of cholesterol transport and synthesis ( $A\beta_{1-40}$ ,  $A\beta_{1-42}$ ,  $A\beta_{12-28}$ )**

One of the physiological functions of  $A\beta$  and APP may be to control cholesterol transport and homeostasis.<sup>1886</sup> Cholesterol binds to the  $\alpha$ -secretase cleavage site of APP and blocks the action of the enzyme, which results in the generation of  $A\beta_{1-40}$  rather than  $A\beta_{17-40}$ .  $A\beta_{1-40}$  affects neither the ApoE-mediated cholesterol efflux, which is elevated because of increased ApoE levels, nor the binding of cholesterol to ApoE. However,  $A\beta_{1-40}$  blocks the amount of cholesterol bound and transported by LDL into the cells, leading to decreased intracellular cholesterol levels. Accumulation of extracellular or membrane free cholesterol will result in neuronal dysfunction. Thus, the presence of excessive levels of cholesterol lead to increased production of  $A\beta$ , which binds cholesterol and in turn competes against and blocks the LDL-mediated cholesterol influx.

It has been shown that  $A\beta$  binds to ApoE (a protein that transports lipoproteins, vitamins, and cholesterol in the blood).<sup>1887</sup> The binding of cholesterol to ApoE or LDL (low-density lipoproteins) is abolished completely in the presence of  $A\beta_{1-42}$ . Increased extracellular free cholesterol levels are toxic to neurons. This toxicity is prevented by specific lipoproteins, such as high-density lipoproteins, which maintain their ability to bind cholesterol in the presence of  $A\beta$ .<sup>1888</sup>

$A\beta_{1-42}$  directly activates neutral SMase (sphingomyelinases) and downregulates SM (sphingomyelin) levels, whereas  $A\beta_{1-40}$  reduces cholesterol *de novo* synthesis by inhibition of HMGR (hydroxymethylglutaryl-CoA reductase) activity.<sup>1889</sup>  $A\beta_{1-42}$  increases ApoE protein levels in astrocytes which could affect cholesterol trafficking.<sup>1890</sup>  $A\beta_{1-42}$  also reduces caveolin-1

<sup>1886</sup> Yao ZX, Papadopoulos V. Function of beta-amyloid in cholesterol transport: a lead to neurotoxicity. *FASEB J.* 2002 Oct;16(12):1677-9; <http://www.fasebj.org/content/early/2002/10/02/fj.02-0285fje.long>.

<sup>1887</sup> Strittmatter WJ, Weisgraber KH, Huang DY, Dong LM, Salvesen GS, Pericak-Vance M, Schmechel D, Saunders AM, Goldgaber D, Roses AD. Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease. *Proc Natl Acad Sci U S A.* 1993 Sep 1;90(17):8098-102; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC47295/>. Näslund J, Thyberg J, Tjernberg LO, Wernstedt C, Karlström AR, Bogdanovic N, Gandy SE, Lannfelt L, Terenius L, Nordstedt C. Characterization of stable complexes involving apolipoprotein E and the amyloid beta peptide in Alzheimer's disease brain. *Neuron.* 1995 Jul;15(1):219-28; <http://www.sciencedirect.com/science/article/pii/S0896627395900799/pdf?md5=f38a15a8690c849767c578616aa11754&pid=1-s2.0-0896627395900799-main.pdf>. LaDu MJ, Lukens JR, Reardon CA, Getz GS. Association of human, rat, and rabbit apolipoprotein E with beta-amyloid. *J Neurosci Res.* 1997 Jul 1;49(1):9-18; <http://www.ncbi.nlm.nih.gov/pubmed/9211985>. Tokuda T, Calero M, Matsubara E, Vidal R, Kumar A, Permanne B, Zlokovic B, Smith JD, Ladu MJ, Rostagno A, Frangione B, Ghiso J. Lipidation of apolipoprotein E influences its isoform-specific interaction with Alzheimer's amyloid beta peptides. *Biochem J.* 2000 Jun 1;348 Pt 2:359-65; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1221074/>.

<sup>1888</sup> Yao ZX, Papadopoulos V. Function of beta-amyloid in cholesterol transport: a lead to neurotoxicity. *FASEB J.* 2002 Oct;16(12):1677-9; <http://www.fasebj.org/content/early/2002/10/02/fj.02-0285fje.long>.

<sup>1889</sup> Grimm MO, Grimm HS, Pätzold AJ, Zinser EG, Halonen R, Duering M, Tschäpe JA, De Strooper B, Müller U, Shen J, Hartmann T. Regulation of cholesterol and sphingomyelin metabolism by amyloid-beta and presenilin. *Nat Cell Biol.* 2005 Nov;7(11):1118-23; <http://www.ncbi.nlm.nih.gov/pubmed/16227967>.

<sup>1890</sup> LaDu MJ, Shah JA, Reardon CA, Getz GS, Bu G, Hu J, Guo L, van Eldik LJ. Apolipoprotein E receptors mediate the effects of beta-amyloid on astrocyte cultures. *J Biol Chem.* 2000 Oct 27;275(43):33974-80; <http://www.jbc.org/content/275/43/33974.long>. Igbavboa U, Pidcock JM, Johnson LN, Malo TM, Studniski AE, Yu S, Sun GY, Wood WG. Cholesterol distribution in the Golgi complex of

levels in the plasma membrane and increases its abundance in the Golgi complex.<sup>1891</sup> These results add to the growing body of data demonstrating that cholesterol carrier proteins are targets of A $\beta$ .

**\* Altered expression and activity of neuron K<sup>+</sup> channels (A $\beta$ <sub>1-40</sub>, A $\beta$ <sub>1-42</sub>)**

Voltage-gated potassium (Kv) channels (transmembrane proteins composed of multiple subunits) are prominent in the repolarization phase of the neuroelectric action potential, and the expression and activity of these channels can be modulated by endogenous A $\beta$  in primary cultures of central neurons. A-type K<sup>+</sup> ion currents, along with Kv4.2 and Kv4.3 subunit expression, are transiently increased in cerebellar granule neurons by the extracellular administration of 100 nM concentrations of A $\beta$ <sub>40</sub> and A $\beta$ <sub>42</sub> over a 2-24 hour period.<sup>1892</sup> Both protein expression and trafficking processes were altered by A $\beta$ , which is apparently an important physiological regulator of ion channel expression and hence neuronal excitability.

Inhibition of endogenous A $\beta$  production (by exposure to inhibitors either of  $\beta$ - or  $\gamma$ -secretases) or immunodepletion of A $\beta$  causes neuronal cell death, but neurons can be restored by addition of physiological (picomolar) levels of A $\beta$ . A $\beta$ <sub>1-40</sub> is the most effective in this regard, with significant effects at concentrations as low as 10 pM; A $\beta$ <sub>1-42</sub> affords only limited protection, and the A $\beta$ <sub>25-35</sub> fragment (which retains many of the toxic properties of A $\beta$ ) has very little protective effect.<sup>1893</sup> The underlying mechanism was believed to involve altered expression of K<sup>+</sup> channels, in part because they govern excitability and hence the excitotoxicity of released glutamate,<sup>1894</sup> but also because intracellular K<sup>+</sup> is a key determinant of apoptosis.<sup>1895</sup>

DITNC1 astrocytes is differentially altered by fresh and aged amyloid beta-peptide-(1-42). *J Biol Chem.* 2003 May 9;278(19):17150-7; <http://www.jbc.org/content/278/19/17150.long>. Igbavboa U, Johnson-Anuna LN, Rossello X, Butterick TA, Sun GY, Wood WG. Amyloid beta-protein1-42 increases cAMP and apolipoprotein E levels which are inhibited by beta1 and beta2-adrenergic receptor antagonists in mouse primary astrocytes. *Neuroscience.* 2006 Oct 27;142(3):655-60; <http://www.ncbi.nlm.nih.gov/pubmed/16904834>.

<sup>1891</sup> Igbavboa U, Sun GY, Weisman GA, He Y, Wood WG. Amyloid beta-protein stimulates trafficking of cholesterol and caveolin-1 from the plasma membrane to the Golgi complex in mouse primary astrocytes. *Neuroscience.* 2009 Aug 18;162(2):328-38; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3083247/>.

<sup>1892</sup> Plant LD, Webster NJ, Boyle JP, Ramsden M, Freir DB, Peers C, Pearson HA. Amyloid beta peptide as a physiological modulator of neuronal 'A'-type K<sup>+</sup> current. *Neurobiol Aging.* 2006 Nov;27(11):1673-83; <http://www.ncbi.nlm.nih.gov/pubmed/16271805>.

<sup>1893</sup> Plant LD, Boyle JP, Smith IF, Peers C, Pearson HA. The production of amyloid beta peptide is a critical requirement for the viability of central neurons. *J Neurosci.* 2003 Jul 2;23(13):5531-5; <http://www.jneurosci.org/content/23/13/5531.long>.

<sup>1894</sup> Pearson HA, Peers C. Physiological roles for amyloid beta peptides. *J Physiol.* 2006 Aug 15;575(Pt 1):5-10; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1819417/>.

<sup>1895</sup> Yu SP. Regulation and critical role of potassium homeostasis in apoptosis. *Prog Neurobiol.* 2003 Jul;70(4):363-86; <http://www.ncbi.nlm.nih.gov/pubmed/12963093>.

**\* Altered expression and activity of neuron Ca<sup>2+</sup> channels (A $\beta$ <sub>1-40</sub>, A $\beta$ <sub>9-42</sub>, A $\beta$ <sub>17-42</sub>)**

A 24-hour pretreatment with 1  $\mu$ M of unaggregated A $\beta$ <sub>1-40</sub> stimulates an increase in voltage-dependent Ca<sup>2+</sup> channel current activity (both N- and P-type current), whereas the same pretreatment with aggregated A $\beta$ <sub>1-40</sub> reduces Ca<sup>2+</sup> channel current density in cortical neurons via action on N-type Ca<sup>2+</sup> current, revealing another normal functional role for amyloid peptides in the central nervous system.<sup>1896</sup>

Interestingly, non-amyloidogenic A $\beta$ <sub>9-42</sub> and A $\beta$ <sub>17-42</sub> fragments self-assemble into ion channels with loosely attached subunits that elicit single-channel conductances, allowing calcium uptake in amyloid precursor protein-deficient cells.<sup>1897</sup> Atomic force microscopy (AFM) images of A $\beta$  peptides reconstituted in lipid bilayers show heteromeric (rectangular to hexagonal) ion channel-like structures with a  $\sim$ 2.0-nm central pore and 8- to 12-nm outer diameters.

**\* Modulation of synaptic activity (A $\beta$ <sub>1-40</sub>, A $\beta$ <sub>1-42</sub>, A $\beta$ <sub>25-35</sub>)**

A $\beta$ <sub>1-42</sub> has high affinity for neuronal nicotinic acetylcholine receptors.<sup>1898</sup> An extracellularly-applied 100 nM concentration of A $\beta$ <sub>1-42</sub> alters glutamatergic (glutamate-mediated) neurotransmission in the rat basal forebrain.<sup>1899</sup> It has also been shown that neuronal activity modulates the production and secretion of A $\beta$ .<sup>1900</sup> In turn, A $\beta$  can depress this neuronal activity, via glutamatergic receptors, creating a negative feedback loop<sup>1901</sup> that may act as a sort of

<sup>1896</sup> Ramsden M, Henderson Z, Pearson HA. Modulation of Ca<sup>2+</sup> channel currents in primary cultures of rat cortical neurones by amyloid beta protein (1-40) is dependent on solubility status. *Brain Res.* 2002 Nov 29;956(2):254-61; <http://www.ncbi.nlm.nih.gov/pubmed/12445693>.

<sup>1897</sup> Jang H, Arce FT, Ramachandran S, Capone R, Azimova R, Kagan BL, Nussinov R, Lal R. Truncated beta-amyloid peptide channels provide an alternative mechanism for Alzheimer's Disease and Down syndrome. *Proc Natl Acad Sci U S A.* 2010 Apr 6;107(14):6538-43; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2851998/>.

<sup>1898</sup> Wang HY, Lee DH, D'Andrea MR, Peterson PA, Shank RP, Reitz AB. beta-Amyloid(1-42) binds to alpha7 nicotinic acetylcholine receptor with high affinity. Implications for Alzheimer's disease pathology. *J Biol Chem.* 2000 Feb 25;275(8):5626-32; <http://www.jbc.org/content/275/8/5626.long>.

<sup>1899</sup> Chin JH, Ma L, MacTavish D, Jhamandas JH. Amyloid beta protein modulates glutamate-mediated neurotransmission in the rat basal forebrain: involvement of presynaptic neuronal nicotinic acetylcholine and metabotropic glutamate receptors. *J Neurosci.* 2007 Aug 29;27(35):9262-9; <http://www.jneurosci.org/content/27/35/9262.long>.

<sup>1900</sup> Nitsch RM, Farber SA, Growdon JH, Wurtman RJ. Release of amyloid beta-protein precursor derivatives by electrical depolarization of rat hippocampal slices. *Proc Natl Acad Sci U S A.* 1993 Jun 1;90(11):5191-3; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC46681/>.

<sup>1901</sup> Kamenetz F, Tomita T, Hsieh H, Seabrook G, Borchelt D, Iwatsubo T, Sisodia S, Malinow R. APP processing and synaptic function. *Neuron.* 2003 Mar 27;37(6):925-37; [http://www.cell.com/neuron/pdf/S0896-6273\(03\)00124-7.pdf](http://www.cell.com/neuron/pdf/S0896-6273(03)00124-7.pdf). Shemer I, Holmgren C, Min R, Fülöp L, Zilberter M, Sousa KM, Farkas T, Härtig W, Penke B, Burnashev N, Tanila H, Zilberter Y, Harkany T. Non-fibrillar beta-amyloid abates spike-timing-dependent synaptic potentiation at excitatory synapses in layer 2/3 of the neocortex by targeting postsynaptic AMPA receptors. *Eur J Neurosci.* 2006 Apr;23(8):2035-47; <http://www.ncbi.nlm.nih.gov/pubmed/16630051/>.

synaptic homeostasis mechanism, preventing excitotoxicity. Thus A $\beta$  may have a normal negative feedback function: increased neuronal activity produces more A $\beta$ , the enhanced A $\beta$  production then depresses synaptic function, and the depressed synaptic function then decreases neuronal activity.

**\* Alteration of glucose metabolism to reduce cellular Reactive Oxygen Species (A $\beta_{1-42}$ )**

A $\beta$  may provide direct neuroprotection in aging neurons.<sup>1902</sup> The ability of A $\beta$  to increase glycolysis, increase HMS (hexose monophosphate shunt) activity to produce the natural antioxidant NADPH, and increase antioxidant defenses may explain the observation that low levels of A $\beta$  are neuroprotective against the pro-oxidant conditions of tissue culture, while higher concentrations are toxic.<sup>1903</sup>

**\* Neuroprotection against metal-induced oxidative damage (A $\beta_{1-40}$ , A $\beta_{1-42}$ )**

Monomeric A $\beta_{1-40}$  and A $\beta_{1-42}$  function as natural antioxidant molecules that prevent neuronal death caused by transition metal-induced oxidative damage, probably by quenching metal-inducible oxygen radical generation and thereby inhibiting neurotoxicity. Oligomeric and aggregated A $\beta_{1-40}$  and A $\beta_{1-42}$  were thought to lose their neuroprotective activity because they were believed to be oxygen radical generators.<sup>1904</sup> However, a later study found that A $\beta_{1-40}$ , either monomeric or aggregated and at either nanomolar or micromolar concentrations, is a highly potent antioxidant in cell-free oxidative systems, acting mainly as a radical scavenger.<sup>1905</sup>

---

<sup>1902</sup> Soucek T, Cumming R, Dargusch R, Maher P, Schubert D. The regulation of glucose metabolism by HIF-1 mediates a neuroprotective response to amyloid beta peptide. *Neuron*. 2003 Jul 3;39(1):43-56; [http://www.cell.com/neuron/pdf/S0896-6273\(03\)00367-2.pdf](http://www.cell.com/neuron/pdf/S0896-6273(03)00367-2.pdf).

<sup>1903</sup> Yankner BA, Duffy LK, Kirschner DA. Neurotrophic and neurotoxic effects of amyloid beta protein: reversal by tachykinin neuropeptides. *Science*. 1990 Oct 12;250(4978):279-82; <http://www.sciencemag.org/content/250/4978/279.long>.

<sup>1904</sup> Zou K, Gong JS, Yanagisawa K, Michikawa M. A novel function of monomeric amyloid beta-protein serving as an antioxidant molecule against metal-induced oxidative damage. *J Neurosci*. 2002 Jun 15;22(12):4833-41; <http://www.jneurosci.org/content/22/12/4833.long>.

<sup>1905</sup> Baruch-Suchodolsky R, Fischer B. Abeta40, either soluble or aggregated, is a remarkably potent antioxidant in cell-free oxidative systems. *Biochemistry*. 2009 May 26;48(20):4354-70; <http://www.ncbi.nlm.nih.gov/pubmed/19320465>.

\* **A $\beta$ <sub>40</sub> may provide neuroprotection from A $\beta$ <sub>42</sub> (A $\beta$ <sub>1-40</sub>)**

A $\beta$ <sub>1-40</sub> may protect neurons from the deleterious effects of A $\beta$ <sub>1-42</sub>.<sup>1906</sup> The reduction in the A $\beta$ <sub>1-40</sub> / A $\beta$ <sub>1-42</sub> ratio in the AD brain correlates with the severity of neurodegeneration.<sup>1907</sup>

\* **Possible blood-brain barrier vascular sealant (A $\beta$ <sub>1-40</sub>, A $\beta$ <sub>1-42</sub>)**

Unlike the transgenic mouse brain and vasculature, where amyloid- $\beta$  deposition is simply a result of the overexpression of amyloid- $\beta$  protein precursor, amyloid- $\beta$  deposition during adult life is normally associated with injury. It has been hypothesized that this deposition of amyloid- $\beta$  could be a neuroprotective response to injury.<sup>1908</sup> Given the extensive vasculature of the brain and that peripheral mechanisms of preventing hemorrhage (i.e., platelet closure) would prevent nourishment of neurons, other mechanisms may have evolved to rapidly seal vascular ruptures without activating the coagulation cascade. One proposal is that amyloid- $\beta$  is just such a sealant, given its physiochemical properties that allow it to aggregate under inflammatory conditions<sup>1909</sup> and form an intracranial “scab,” thereby maintaining structural integrity of the blood-brain barrier. Such a notion would explain the acute phase generation and rapid cortical deposition of amyloid- $\beta$  in stroke and after head trauma<sup>1910</sup> and its resolution after recovery,<sup>1911</sup> important

<sup>1906</sup> Zou K, Kim D, Kakio A, Byun K, Gong J-S, Kim J, Kim M, Sawamura N, Nishimoto S-i, Matsuzaki K, Lee B, Yanagisawa K, Michikawa M. Amyloid  $\beta$ -protein (A $\beta$ )1-40 protects neurons from damage induced by A $\beta$ 1-42 in culture and in rat brain. *J Neurochem.* 2003;87(3):609-619; <http://www.ncbi.nlm.nih.gov/pubmed/14535944>. Yan Y, Wang C. A[beta]40 Protects Non-toxic A[beta]42 Monomer from Aggregation. *J Mol Biol.* 2007;369(4):909-916; <http://www.ncbi.nlm.nih.gov/pubmed/17481654>. Kim J, Onstead L, Randle S, Price R, Smithson L, Zwizinski C, Dickson DW, Golde T, McGowan E. A{beta}40 Inhibits Amyloid Deposition *In Vivo*. *J Neurosci.* 2007;27(3):627-633; <http://www.jneurosci.org/content/27/3/627.long>. Murray MM, Bernstein SL, Nyugen V, Condron MM, Teplow DB, *et al.* Amyloid beta protein: Abeta40 inhibits Abeta42 oligomerization. *J Am Chem Soc* 131(2009):6316-6317; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2697393/>.

<sup>1907</sup> Kumar-Singh S, Theuns J, Van Broeck B, Pirici D, Vennekens KI, Corsmit E, Cruts M, Dermaut B, Wang R, Van Broeckhoven C. Mean age-of-onset of familial Alzheimer disease caused by presenilin mutations correlates with both increased A $\beta$ 42 and decreased A $\beta$ 40. *Hum Mutat.* 2006;27(7):686-695; <http://www.ncbi.nlm.nih.gov/pubmed/16752394>. Omtri RS, Davidson MW, Arumugam B, Poduslo JF, Kandimalla KK. Differences in the cellular uptake and intracellular itineraries of amyloid beta proteins 40 and 42: ramifications for the Alzheimer’s drug discovery. *Mol Pharm.* 2012 Jul 2;9(7):1887-97; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3858471/>.

<sup>1908</sup> Atwood CS, Moir RD, Huang X, Scarpa RC, Bacarra NM, Romano DM, Hartshorn MA, Tanzi RE, Bush AI. Dramatic aggregation of Alzheimer abeta by Cu(II) is induced by conditions representing physiological acidosis. *J Biol Chem.* 1998 May 22;273(21):12817-26; <http://www.jbc.org/content/273/21/12817.long>.

<sup>1909</sup> Atwood CS, Bishop GM, Perry G, Smith MA. Amyloid-beta: a vascular sealant that protects against hemorrhage? *J Neurosci Res.* 2002 Nov 1;70(3):356; <http://www.ncbi.nlm.nih.gov/pubmed/12391596>.

<sup>1910</sup> Roberts GW, Gentleman SM, Lynch A, Murray L, Landon M, Graham DI. Beta amyloid protein deposition in the brain after severe head injury: implications for the pathogenesis of Alzheimer’s disease. *J Neurol Neurosurg Psychiatry.* 1994 Apr;57(4):419-25; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1072869/>.

physiological responses that would limit the loss of terminally differentiated neurons. Removal of such a seal would lead to hemorrhage and an inflammatory immune response.<sup>1912</sup>

There are also several well-known pathological functions of A $\beta$ :

**\* Cellular damage (A $\beta$ <sub>1-42</sub>)**

Excessive intracellular A $\beta$  accumulation, which may commence long before extracellular accumulation, seems to be involved in various types of cellular damage such as mitochondrial toxicity, proteasome impairment, and synaptic damage.<sup>1913</sup> For example, A $\beta$ <sub>1-42</sub> binds to the neural cell adhesion molecule NCAM2, inducing its removal from synapses in AD hippocampus and thus may contribute to synapse loss.<sup>1914</sup> A $\beta$ <sub>1-42</sub> also induces activation of primary rat astrocyte cultures, as measured by changes in morphology and an increase in IL-1 $\beta$  mRNA, in a time- and dose-dependent manner.<sup>1915</sup>

**\* Neurotoxic A $\beta$ <sub>43</sub> oligomers and plaques (A $\beta$ <sub>1-43</sub>)**

Longer A $\beta$  isoforms like A $\beta$ <sub>1-43</sub> are gaining attention for their higher propensity to aggregate into neurotoxic oligomers. Although the total levels of A $\beta$ <sub>1-43</sub> in human brain are low compared to A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub>, it is possible that A $\beta$ <sub>1-43</sub> could initiate the formation of oligomers and amyloid plaques and thereby be crucial to AD pathogenesis.<sup>1916</sup>

<sup>1911</sup> Pluta R, Barcikowska M, Misicka A, Lipkowski AW, Spisacka S, Januszewski S. Ischemic rats as a model in the study of the neurobiological role of human beta-amyloid peptide. Time-dependent disappearing diffuse amyloid plaques in brain. *Neuroreport*. 1999 Nov 26;10(17):3615-9; <http://www.ncbi.nlm.nih.gov/pubmed/10619654>.

<sup>1912</sup> Atwood CS, Perry G, Smith MA. Cerebral hemorrhage and amyloid-beta. *Science*. 2003 Feb 14;299(5609):1014; <http://www.sciencemag.org/content/299/5609/1014.1.long>.

<sup>1913</sup> Tabaton M, Zhu X, Perry G, Smith MA, Giliberto L. Signaling effect of amyloid-beta(42) on the processing of AbetaPP. *Exp Neurol*. 2010 Jan;221(1):18-25; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2812589/>.

<sup>1914</sup> Leshchyn'ska I, Liew HT, Shepherd C, Halliday GM, Stevens CH, Ke YD, Ittner LM, Sytnyk V. A $\beta$ -dependent reduction of NCAM2-mediated synaptic adhesion contributes to synapse loss in Alzheimer's disease. *Nat Commun*. 2015 Nov 27;6:8836; <http://www.nature.com/ncomms/2015/151127/ncomms9836/full/ncomms9836.html>.

<sup>1915</sup> Hu J, Akama KT, Krafft GA, Chromy BA, Van Eldik LJ. Amyloid-beta peptide activates cultured astrocytes: morphological alterations, cytokine induction and nitric oxide release. *Brain Res*. 1998 Mar 2;785(2):195-206; <http://www.ncbi.nlm.nih.gov/pubmed/9518610>.

<sup>1916</sup> Sandebring A, Welander H, Winblad B, Graff C, Tjernberg LO. The pathogenic a $\beta$ 43 is enriched in familial and sporadic Alzheimer disease. *PLoS One*. 2013;8(2):e55847; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3569467/>.

Recent data have raised the possibility that A $\beta$ <sub>1-43</sub> peptides might be instrumental in AD pathogenesis because they are frequently observed in both dense and diffuse amyloid plaques from human AD brains and are highly amyloidogenic *in vitro*. A $\beta$ <sub>1-43</sub> peptides are mainly insoluble, highly toxic *in vivo*, and can trigger the aggregation of the typically soluble A $\beta$ <sub>1-40</sub> leading to synergistic toxic effects – supporting the idea that A $\beta$ <sub>1-43</sub> contributes to the pathological events leading to neurodegeneration in AD.<sup>1917</sup>

\* **Vascular damage** (A $\beta$ <sub>1-40</sub>, A $\beta$ <sub>1-42</sub>)

A $\beta$  peptides are also present in small arteries and capillaries of leptomeninges and cerebral cortex, causing cerebral amyloid angiopathy (CAA),<sup>1918</sup> which is the deposition of A $\beta$  in the blood vessels of the brain. CAA may be the basis of the inflammatory response that halted a Phase IIa trial of active A $\beta$ <sub>1-42</sub> immunization in AD.<sup>1919</sup> Differences in A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub> levels between healthy elderly subjects and patients with either AD or CAA have been investigated.<sup>1920</sup> Whereas concentrations of A $\beta$ <sub>1-42</sub> were reduced in both the AD and the CAA groups, A $\beta$ <sub>1-40</sub> levels were also lower in CAA compared to both the healthy controls and the AD subjects. These results are consistent with the hypothesis that the large component of A $\beta$ <sub>1-40</sub> deposited in vessels in advanced CAA would deplete this peptide from CSF in an analogous manner to AD-associated reductions in CSF A $\beta$ <sub>1-42</sub>.

A $\beta$  peptides are also involved in the inflammatory pathology of atherosclerotic vascular disease.<sup>1921</sup>

---

<sup>1917</sup> Burnouf S, Gorsky MK, Dols J, Grönke S, Partridge L. A $\beta$ 43 is neurotoxic and primes aggregation of A $\beta$ 40 *in vivo*. *Acta Neuropathol.* 2015 Apr 11; <http://www.ncbi.nlm.nih.gov/pubmed/25862636>.

<sup>1918</sup> Viswanathan A, Greenberg SM. Cerebral amyloid angiopathy in the elderly. *Ann Neurol.* 2011 Dec;70(6):871-80; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4004372/>.

<sup>1919</sup> Orgogozo JM, Gilman S, Dartigues JF, Laurent B, Puel M, Kirby LC, Jouanny P, Dubois B, Eisner L, Flitman S, Michel BF, Boada M, Frank A, Hock C. Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization. *Neurology.* 2003 Jul 8;61(1):46-54; <http://www.ncbi.nlm.nih.gov/pubmed/12847155/>.

<sup>1920</sup> Verbeek MM, Kremer BP, Rikkert MO, Van Domburg PH, Skehan ME, Greenberg SM. Cerebrospinal fluid amyloid beta(40) is decreased in cerebral amyloid angiopathy. *Ann Neurol.* 2009 Aug;66(2):245-9; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3697750/>.

<sup>1921</sup> Kokjohn TA, Van Vickle GD, Maarouf CL, Kalback WM, Hunter JM, Dausgs ID, Luehrs DC, Lopez J, Brune D, Sue LI, Beach TG, Castaño EM, Roher AE. Chemical characterization of pro-inflammatory amyloid-beta peptides in human atherosclerotic lesions and platelets. *Biochim Biophys Acta.* 2011 Nov;1812(11):1508-14; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3185199/>.

\* **Skeletal muscle inflammation** ( $A\beta_{1-42}$ ,  $A\beta_{1-44}$ ,  $A\beta_{1-45}$ )

The cholinergic neuromuscular junctions of skeletal muscle are a rich source of  $A\beta$  peptides,<sup>1922</sup> the production of which is exacerbated in inclusion body myositis, the most common skeletal muscle inflammatory disease among the elderly.<sup>1923</sup> The significant differences in  $A\beta_{42}$  between normal and AD populations suggests that AD might have systemic manifestations. Skeletal muscle also generates even longer  $A\beta$  peptides ending at residues 44 and 45. The potential contribution of skeletal muscle  $A\beta$  to the circulating pool should be considered since the total muscle mass represents about one third of body weight.

\*  **$A\beta$  fragment activity** ( $A\beta_{17-20}$ ,  $A\beta_{17-21}$ ,  $A\beta_{25-35}$ ,  $A\beta_{25-40}$ ,  $A\beta_{26-35}$ ,  $A\beta_{30-35}$ ,  $A\beta_{41-42}$ )

Shen,<sup>1924</sup> Canobbio,<sup>1925</sup> and others have demonstrated that the ability of  $A\beta_{1-40}$  to promote platelet activation and to potentiate the effect of weak agonists is completely conserved by the smaller  $A\beta_{25-35}$  peptide, a core fragment of 11 amino acids corresponding to the intermembrane segment of the entire  $A\beta$  peptide. Apparently  $A\beta_{25-35}$  reproduces all the biological functions of  $A\beta$  peptides, and it retains the cytotoxicity and neurodegenerative properties of the entire peptides

---

<sup>1922</sup> Kuo YM, Kokjohn TA, Watson MD, Woods AS, Cotter RJ, Sue LI, Kalback WM, Emmerling MR, Beach TG, Roher AE. Elevated abeta42 in skeletal muscle of Alzheimer disease patients suggests peripheral alterations of AbetaPP metabolism. *Am J Pathol*. 2000 Mar;156(3):797-805; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1876838/>.

<sup>1923</sup> Querfurth HW, Suhara T, Rosen KM, McPhie DL, Fujio Y, Tejada G, Neve RL, Adelman LS, Walsh K. Beta-amyloid peptide expression is sufficient for myotube death: implications for human inclusion body myopathy. *Mol Cell Neurosci*. 2001 May;17(5):793-810; <http://www.ncbi.nlm.nih.gov/pubmed/11358479>. Askanas V, Engel WK. Molecular pathology and pathogenesis of inclusion-body myositis. *Microsc Res Tech*. 2005 Jul;67(3-4):114-20; <http://www.ncbi.nlm.nih.gov/pubmed/16104000/>. Finch CE. A perspective on sporadic inclusion-body myositis: the role of aging and inflammatory processes. *Neurology*. 2006 Jan 24;66(2 Suppl 1):S1-6; <http://www.ncbi.nlm.nih.gov/pubmed/16432135>.

<sup>1924</sup> Shen MY, Hsiao G, Fong TH, Chou DS, Sheu JR. Expression of amyloid beta peptide in human platelets: pivotal role of the phospholipase Cgamma2-protein kinase C pathway in platelet activation. *Pharmacol Res*. 2008 Feb;57(2):151-8; <http://www.ncbi.nlm.nih.gov/pubmed/18313326>.

<sup>1925</sup> Canobbio I, Catricalà S, Di Pasqua LG, Guidetti G, Consonni A, Manganaro D, Torti M. Immobilized amyloid  $A\beta$  peptides support platelet adhesion and activation. *FEBS Lett*. 2013 Aug 19;587(16):2606-11; <http://www.sciencedirect.com/science/article/pii/S0014579313004997>. Canobbio I, Guidetti GF, Oliviero B, Manganaro D, Vara D, Torti M, Pula G. Amyloid  $\beta$ -peptide-dependent activation of human platelets: essential role for  $Ca^{2+}$  and ADP in aggregation and thrombus formation. *Biochem J*. 2014 Sep 15;462(3):513-23; <http://www.ncbi.nlm.nih.gov/pubmed/24984073>.

and is able to aggregate innately in fibrils with  $\beta$ -structure.<sup>1926</sup>  $A\beta_{25-35}$  is also physiologically present in elderly people, suggesting its possible importance in the pathogenesis of AD.<sup>1927</sup>

Using different  $A\beta$  fragments or truncated  $A\beta$  peptides, several sequence regions including residues  $A\beta_{25-40}$  and  $A\beta_{26-35}$ ,<sup>1928</sup>  $A\beta_{17-21}$  and  $A\beta_{41-42}$ ,<sup>1929</sup> and  $A\beta_{17-20}$  and  $A\beta_{30-35}$ ,<sup>1930</sup> have been shown to play roles in protein conformation and toxicity with possible relevance to AD.

---

<sup>1926</sup> Kaminsky YG, Marlatt MW, Smith MA, Kosenko EA. Subcellular and metabolic examination of amyloid-beta peptides in Alzheimer disease pathogenesis: evidence for Abeta(25-35). *Exp Neurol*. 2010 Jan;221(1):26-37; <http://www.ncbi.nlm.nih.gov/pubmed/19751725>.

<sup>1927</sup> Millucci L, Ghezzi L, Bernardini G, Santucci A. Conformations and biological activities of amyloid beta peptide 25-35. *Curr Protein Pept Sci*. 2010 Feb;11(1):54-67; <http://www.ncbi.nlm.nih.gov/pubmed/20201807>.

<sup>1928</sup> Kubo T, Kumagae Y, Miller CA, Kaneko I. Beta-amyloid racemized at the Ser26 residue in the brains of patients with Alzheimer disease: implications in the pathogenesis of Alzheimer disease. *J Neuropathol Exp Neurol*. 2003 Mar;62(3):248-59; <http://www.ncbi.nlm.nih.gov/pubmed/12638729>.

<sup>1929</sup> Liao MQ, Tzeng YJ, Chang LY, Huang HB, Lin TH, Chyan CL, Chen YC. The correlation between neurotoxicity, aggregative ability and secondary structure studied by sequence truncated Abeta peptides. *FEBS Lett*. 2007 Mar 20;581(6):1161-5; <http://www.sciencedirect.com/science/article/pii/S0014579307001822>.

<sup>1930</sup> Liu R, McAllister C, Lyubchenko Y, Sierks MR. Residues 17-20 and 30-35 of beta-amyloid play critical roles in aggregation. *J Neurosci Res*. 2004 Jan 15;75(2):162-71; <http://www.ncbi.nlm.nih.gov/pubmed/14705137>.

## Appendix B. Alzheimer's Drugs in Development as of 2013<sup>1931</sup>

Alzheimer's Disease, Therapeutics			
Product Name	Sponsor	Indication	Development Phase*
AAB-003/PF-05236812 (beta-amyloid protein inhibitor mAb)	Janssen Alzheimer Immunotherapy South San Francisco, CA Pfizer New York, NY	Alzheimer's disease	Phase I www.janssenrnd.com www.pfizer.com
ABT-126 (alpha-7-NNR antagonist)	AbbVie North Chicago, IL	Alzheimer's disease	Phase II www.abbvie.com
ABT-354 (serotonin 5-HT6 receptor antagonist)	AbbVie North Chicago, IL	Alzheimer's disease	Phase I www.abbvie.com
ABT-957 (calpain inhibitor)	AbbVie North Chicago, IL	Alzheimer's disease	Phase I www.abbvie.com
AC-1204 (glucose stimulant)	Accera Broomfield, CO	mild to moderate Alzheimer's disease	Phase II/III www.accelpharma.com
AD02 vaccine (amyloid-beta protein inhibitor)	Affiris Vienna, Austria GlaxoSmithKline Rsch. Triangle Park, NC	Alzheimer's disease	Phase II www.affiris.com www.gsk.com
AD03 vaccine (amyloid-beta protein inhibitor)	Affiris Vienna, Austria GlaxoSmithKline Rsch. Triangle Park, NC	Alzheimer's disease	Phase I www.affiris.com www.gsk.com
APH-0703 (protein kinase C stimulant)	Aphios Woburn, MA	Alzheimer's disease	Phase II www.aphios.com
ARC029 (nilvadipine)	Archer Pharmaceuticals Sarasota, FL	Alzheimer's disease	Phase I/II www.archerpharma.com
ARC031 (soluble amyloid reducing/clearing agent)	Archer Pharmaceuticals Sarasota, FL	Alzheimer's disease	Phase I www.archerpharma.com
AVN 101 (serotonin 5-HT6 receptor antagonist)	Avineuro Pharmaceuticals San Diego, CA	Alzheimer's disease	Phase II www.avineuro.com
AVN 322 (serotonin 6 receptor antagonist)	Avineuro Pharmaceuticals San Diego, CA	Alzheimer's disease	Phase I www.avineuro.com

<sup>1931</sup> “Medicines in Development: Alzheimer's Disease,” Pharmaceutical Research and Manufacturers of America, Washington DC, 2013; <http://www.phrma.org/sites/default/files/Alzheimer%27s%202013.pdf>.

## Alzheimer's Disease, Therapeutics

Product Name	Sponsor	Indication	Development Phase
AVP-923 (dextromethorphan/quinidine fixed-dose combination)	Avanir Pharmaceuticals <i>Aliso Viejo, CA</i>	agitation in Alzheimer's disease	Phase II <a href="http://www.avanir.com">www.avanir.com</a>
AZD3293 (beta secretase)	Astex Pharmaceuticals <i>Dublin, CA</i> AstraZeneca <i>Wilmington, DE</i>	Alzheimer's disease	Phase I <a href="http://www.astx.com">www.astx.com</a> <a href="http://www.astrazeneca.com">www.astrazeneca.com</a>
BACE inhibitor	Janssen Pharmaceuticals <i>Titusville, NJ</i> Shionogi <i>Florham Park, NJ</i>	Alzheimer's disease	Phase I <a href="http://www.janssenpharmaceuticalsinc.com">www.janssenpharmaceuticalsinc.com</a> <a href="http://www.shionogi.com">www.shionogi.com</a>
BAN2401 (amyloid beta-protein inhibitor)	Eisai <i>Woodcliff Lake, NJ</i>	early stage Alzheimer's disease	Phase II <a href="http://www.eisai.com">www.eisai.com</a>
BIB037 (human anti-amyloid beta mAb)	Biogen Idec <i>Weston, MA</i>	Alzheimer's disease	Phase I <a href="http://www.biogenidec.com">www.biogenidec.com</a>
bisnorcymserine (BNC)	QR Pharma <i>Berwyn, PA</i>	moderate to severe Alzheimer's disease	Phase I <a href="http://www.qrpharma.com">www.qrpharma.com</a>
BMS-241027 (microtubule stabilizer)	Bristol-Myers Squibb <i>Princeton, NJ</i>	Alzheimer's disease	Phase I <a href="http://www.bms.com">www.bms.com</a>
CAD106 (amyloid beta-protein inhibitor)	Novartis Pharmaceuticals <i>East Hanover, NJ</i>	Alzheimer's disease	Phase II <a href="http://www.novartis.com">www.novartis.com</a>
CERE-110 (AAV-NGF)	Ceregene <i>San Diego, CA</i> Sangamo BioSciences <i>Richmond, VA</i>	Alzheimer's disease	Phase II <a href="http://www.ceregene.com">www.ceregene.com</a> <a href="http://www.sangamo.com">www.sangamo.com</a>
crenezumab (anti-amyloid-beta mAb)	Genentech <i>South San Francisco, CA</i>	Alzheimer's disease	Phase II <a href="http://www.gene.com">www.gene.com</a>
donepezil/memantine extended release (fixed-dose combination)	Adamas Pharmaceuticals <i>Emeryville, CA</i> Forest Laboratories <i>New York, NY</i>	moderate to severe Alzheimer's disease	Phase III <a href="http://www.adamaspharma.com">www.adamaspharma.com</a> <a href="http://www.frx.com">www.frx.com</a>
DSP-8658 (PPAR alpha/gamma agonist)	Sunovion Pharmaceuticals <i>Marlborough, MA</i>	Alzheimer's disease	Phase I <a href="http://www.sunovion.com">www.sunovion.com</a>

## Alzheimer's Disease, Therapeutics

Product Name	Sponsor	Indication	Development Phase
E2212 (amyloid precursor protein secretase modulator)	Eisai <i>Woodcliff Lake, NJ</i>	Alzheimer's disease	Phase I completed <a href="http://www.eisai.com">www.eisai.com</a>
E2609 (BACE1 protein inhibitor)	Eisai <i>Woodcliff Lake, NJ</i>	Alzheimer's disease	Phase I completed <a href="http://www.eisai.com">www.eisai.com</a>
ELND005	Speranza Therapeutics <i>Dublin, Ireland</i>	neuropsychiatric symptoms in Alzheimer's disease (Fast Track)	Phase II
EVP-0962 (gamma secretase modulator)	EnVivo Pharmaceuticals <i>Watertown, MA</i>	Alzheimer's disease	Phase I <a href="http://www.envivopharma.com">www.envivopharma.com</a>
EVP-6124 (alpha7 nicotinic acetylcholine receptor agonist)	EnVivo Pharmaceuticals <i>Watertown, MA</i>	Alzheimer's disease	Phase II <a href="http://www.envivopharma.com">www.envivopharma.com</a>
<b>Exebryl-1<sup>®</sup></b> (amyloid-beta-protein/tau protein inhibitor)	ProteoTech <i>Kirkland, WA</i>	Alzheimer's disease	Phase I <a href="http://www.proteotech.com">www.proteotech.com</a>
gantenerumab (RG1450)	Roche <i>Nutley, NJ</i>	early stage Alzheimer's disease	Phase II/III <a href="http://www.roche.com">www.roche.com</a>
GM6 (peptide therapeutic)	Genervon Biopharmaceuticals <i>Pasadena, CA</i>	Alzheimer's disease	Phase I <a href="http://www.genervon.com">www.genervon.com</a>
GSK2647544 (Lp-PLA2 inhibitor)	GlaxoSmithKline <i>Rsch. Triangle Park, NC</i>	Alzheimer's disease	Phase I <a href="http://www.gsk.com">www.gsk.com</a>
HPP854 (BACE1 inhibitor)	Transtech Pharma <i>High Point, NC</i>	Alzheimer's disease	Phase I <a href="http://www.ttpharma.com">www.ttpharma.com</a>
JNJ-54861911	Janssen Research & Development <i>Raritan, NJ</i>	Alzheimer's disease	Phase I <a href="http://www.janssenrnd.com">www.janssenrnd.com</a>
KU-046 (amyloid beta-protein modulator)	Kareus Therapeutics <i>La Chaux-de-Fonds, Switzerland</i>	Alzheimer's disease	Phase I <a href="http://www.kareustherapeutics.com">www.kareustherapeutics.com</a>
<b>LMTX<sup>™</sup></b> tau aggregation inhibitor	TauRx Pharmaceuticals <i>Singapore</i>	Alzheimer's disease, frontotemporal dementia	Phase III <a href="http://www.taurx.com">www.taurx.com</a>
Lu AE58054 (5-HT6 receptor antagonist)	Lundbeck <i>Deerfield, IL</i> Otsuka America Pharmaceutical <i>Rockville, MD</i>	Alzheimer's disease (cognition)	Phase II <a href="http://www.lundbeck.com">www.lundbeck.com</a> <a href="http://www.otsuka.com">www.otsuka.com</a>

## Alzheimer's Disease, Therapeutics

Product Name	Sponsor	Indication	Development Phase
LY2886721 (beta secretase inhibitor)	Eli Lilly <i>Indianapolis, IN</i>	Alzheimer's disease (slow disease progression)	Phase II <a href="http://www.lilly.com">www.lilly.com</a>
LY3002813 (N3pG-AB mAb)	Eli Lilly <i>Indianapolis, IN</i>	Alzheimer's disease	Phase I <a href="http://www.lilly.com">www.lilly.com</a>
MK-8931 (BACE1 protein inhibitor)	Merck <i>Whitehouse Station, NJ</i>	Alzheimer's disease	Phase II/III <a href="http://www.merck.com">www.merck.com</a>
MSDC-0160 (mTOT modulator insulin sensitizer)	Metabolic Solutions Development Company <i>Kalamazoo, MI</i>	Alzheimer's disease	Phase II <a href="http://www.msdrx.com">www.msdrx.com</a>
NICS-15 (amyloid precursor protein secretase inhibitor)	Humanetics <i>Minneapolis, MN</i>	Alzheimer's disease	Phase II <a href="http://www.humaneticscorp.com">www.humaneticscorp.com</a>
PF-05212377 (SAM-760)	Pfizer <i>New York, NY</i>	Alzheimer's disease	Phase II <a href="http://www.pfizer.com">www.pfizer.com</a>
pioglitazone low-dose	Takeda Pharmaceuticals U.S.A. <i>Deerfield, IL</i> Zinfandel Pharmaceuticals <i>Chapel Hill, NC</i>	mild cognitive impairment due to Alzheimer's disease	Phase III <a href="http://www.takeda.com">www.takeda.com</a>
<b>Posiphen</b> <sup>®</sup> R-phenserine	QR Pharma <i>Berwyn, PA</i>	early Alzheimer's disease	Phase II <a href="http://www.qrpharma.com">www.qrpharma.com</a>
RG1577 (MAO-B inhibitor)	Roche <i>Nutley, NJ</i>	Alzheimer's disease	Phase II <a href="http://www.roche.com">www.roche.com</a>
RG7129 (BACE1 protein inhibitor)	Roche <i>Nutley, NJ</i>	Alzheimer's disease	Phase I <a href="http://www.roche.com">www.roche.com</a>
rilapladib (Lp-PLA2 inhibitor)	GlaxoSmithKline <i>Rsch. Triangle Park, NC</i>	Alzheimer's disease	Phase II <a href="http://www.gsk.com">www.gsk.com</a>
RVX-208 (BET protein inhibitor)	Resverlogix <i>Calgary, Canada</i>	Alzheimer's disease	Phase I <a href="http://www.resverlogix.com">www.resverlogix.com</a>
SAR110894 (H3 antagonist)	Sanofi US <i>Bridgewater, NJ</i>	Alzheimer's disease	Phase II <a href="http://www.sanofi.com">www.sanofi.com</a>
SAR228810 (anti-protofibrillar AB mAb)	Sanofi US <i>Bridgewater, NJ</i>	Alzheimer's disease	Phase I <a href="http://www.sanofi.com">www.sanofi.com</a>

## Alzheimer's Disease, Therapeutics

Product Name	Sponsor	Indication	Development Phase
sGC-1061 (nomethiazole)	sGC Pharma <i>Wellesley, MA</i>	Alzheimer's disease	Phase I <a href="http://www.sgcpharma.com">www.sgcpharma.com</a>
solanezumab (amyloid-beta protein inhibitor)	Eli Lilly <i>Indianapolis, IN</i>	mild Alzheimer's disease	Phase III <a href="http://www.lilly.com">www.lilly.com</a>
ST101	Sonexa Therapeutics <i>San Diego, CA</i>	Alzheimer's disease	Phase II completed <a href="http://www.sonexa.com">www.sonexa.com</a>
T3D-959 (dual PPAR agonist)	T3D Therapeutics <i>Rsch. Triangle Park, NC</i>	Alzheimer's disease	Phase I completed <a href="http://www.t3dtherapeutics.com">www.t3dtherapeutics.com</a>
T-817MA	Toyama Chemical <i>Tokyo, Japan</i>	Alzheimer's disease	Phase II <a href="http://www.toyama-chemical.co.jp">www.toyama-chemical.co.jp</a>
TC-1734 (ispronicline)	Targacept <i>Winston-Salem, NC</i>	Alzheimer's disease	Phase II <a href="http://www.targacept.com">www.targacept.com</a>
TC-5619 (alpha7nAChR)	Targacept <i>Winston-Salem, NC</i>	Alzheimer's disease	Phase I <a href="http://www.targacept.com">www.targacept.com</a>
TTP488 (RAGE inhibitor)	Transtech Pharma <i>High Point, NC</i>	Alzheimer's disease (Fast Track)	Phase II <a href="http://www.ttpharma.com">www.ttpharma.com</a>
TTP4000 (IgG and RAGE inhibitor)	Transtech Pharma <i>High Point, NC</i>	Alzheimer's disease	Phase I <a href="http://www.ttpharma.com">www.ttpharma.com</a>
UB-311 (liquid intramuscular amyloid beta protein inhibitor vaccine)	United Biomedical <i>Hauppauge, NY</i>	mild to moderate Alzheimer's disease	Phase II <a href="http://www.unitedbiomedical.com">www.unitedbiomedical.com</a>
V950 vaccine	Merck <i>Whitehouse Station, NJ</i>	Alzheimer's disease	Phase I completed <a href="http://www.merck.com">www.merck.com</a>
VI-1121	VIVUS <i>Mountain View, CA</i>	Alzheimer's disease	Phase II <a href="http://www.vivus.com">www.vivus.com</a>

## Alzheimer's Disease, Diagnostics

Product Name	Sponsor	Indication	Development Phase
AZD2184 (PET enhancer)	Navidea Pharmaceuticals <i>Dublin, OH</i>	Alzheimer's disease (diagnosis)	Phase I <a href="http://www.navidea.com">www.navidea.com</a>
AZD2995 (PET enhancer)	Navidea Pharmaceuticals <i>Dublin, OH</i>	Alzheimer's disease (diagnosis)	Phase I <a href="http://www.navidea.com">www.navidea.com</a>

## Alzheimer's Disease, Diagnostics

Product Name	Sponsor	Indication	Development Phase
F18-florbetaben (PET/SPECT imaging agent)	IBA Molecular Dulles, VA Piramal Healthcare Mumbai, India	Alzheimer's disease (diagnosis)	application submitted www.iba-molecular.com www.piramal.com
F18-flutemetamol (PET imaging agent)	GE Healthcare Waukesha, WI	Alzheimer's disease (diagnosis)	application submitted www.gehealthcare.com
LymPro® neurotrophic factor companion diagnostic	Amarantus BioSciences Sunnyvale, CA	Alzheimer's disease (diagnosis)	Phase II www.amarantus.com
NAV4694 (fluorine-18 labeled precision radiopharmaceutical)	Navidea Biopharmaceuticals Dublin, OH	Alzheimer's disease (diagnosis)	Phase III www.navidea.com
NAV5001 (123I-labelled imaging agent)	Navidea Biopharmaceuticals Dublin, OH	dementia with Lewy bodies (diagnosis)	Phase II www.navidea.com
pioglitazone companion diagnostic (AD4833/TOMM40)	Takeda Pharmaceuticals U.S.A. Deerfield, IL Zinfandel Pharmaceuticals Chapel Hill, NC	Alzheimer's disease (diagnosis)	Phase III www.takeda.com
tau imaging agent (PET imaging agent)	Avid Radiopharmaceuticals Philadelphia, PA Eli Lilly Indianapolis, IN	Alzheimer's disease (diagnosis)	Phase I www.lilly.com

The content of this report has been obtained through public, government and industry sources, and the Adis "R&D Insight" data-base based on the latest information. **Report current as of October 8, 2013.** The medicines in this report include medicines being developed by U.S. based companies conducting trials in the United States and abroad, PhRMA-member companies conducting trials in the United States and abroad, and foreign companies conducting clinical trials in the United States. The information in this report may not be comprehensive. For more specific information about a particular product, contact the individual company directly or go to [www.clinicaltrials.gov](http://www.clinicaltrials.gov). The entire series of Medicines in Development is available on PhRMA's website.

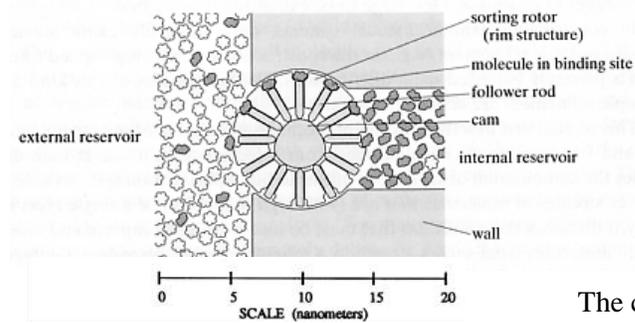
A publication of PhRMA's Communications & Public Affairs Department. (202) 835-3460

[www.phrma.org](http://www.phrma.org) | [www.innovation.org](http://www.innovation.org) | [www.pparx.org](http://www.pparx.org)

Provided as a Public Service by PhRMA. Founded in 1958 as the Pharmaceutical Manufacturers Association.

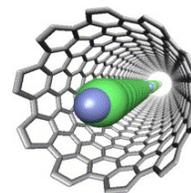
## Appendix C. Molecular Sorting Rotors

Molecular filters can consist of a barrier or wall penetrated by one or more nanomechanical devices that act as molecule-specific pumps, analogous to the transporter pumps found on the surfaces of living biological cells. One simple such pump is a nanomechanical device called a “molecular sorting rotor” that is capable of selectively binding molecules from solution and then



transporting these bound molecules against concentration gradients (image, left), moving only the molecules of a specific type (such as  $\text{CO}_2$ ) from one side of the wall to the other. Each pump mechanically transports individual molecules, one by one, through the barrier.

The classical sorting rotor illustrated above<sup>1932</sup> is a disk about 10 nm in diameter and about 3 nm thick having 12 binding site “pockets” along the rim that are exposed alternately to the source fluid at left and the receiving chamber at right by the clockwise axial rotation of the disk. (Other designs may have more, or fewer, pockets.) Each pocket selectively binds a specific molecule when exposed to the source fluid at left. The rotor turns clockwise, moving the pocket containing the bound molecule through the wall from left to right. Once the binding site has rotated far enough to expose it to the receiving chamber at right, the bound molecules are forcibly ejected by rods (e.g., polyynes) thrust outward by the cam surface. Note that short polyyne sections ( $\text{C}_{10}\text{H}_2$ ) have been synthesized experimentally inside carbon nanotubes (image, right),<sup>1933</sup> longer polyyne chains have been assembled inside double-walled carbon nanotubes (image, left),<sup>1934</sup> and isolated 44-carbon-atom polyyne chains (stabilized with end caps) also appear to be stable.<sup>1935</sup> Of course, other means, whether mechanical or electronic, could also be used to reversibly alter the binding site affinity for the transported molecule during the transport process.

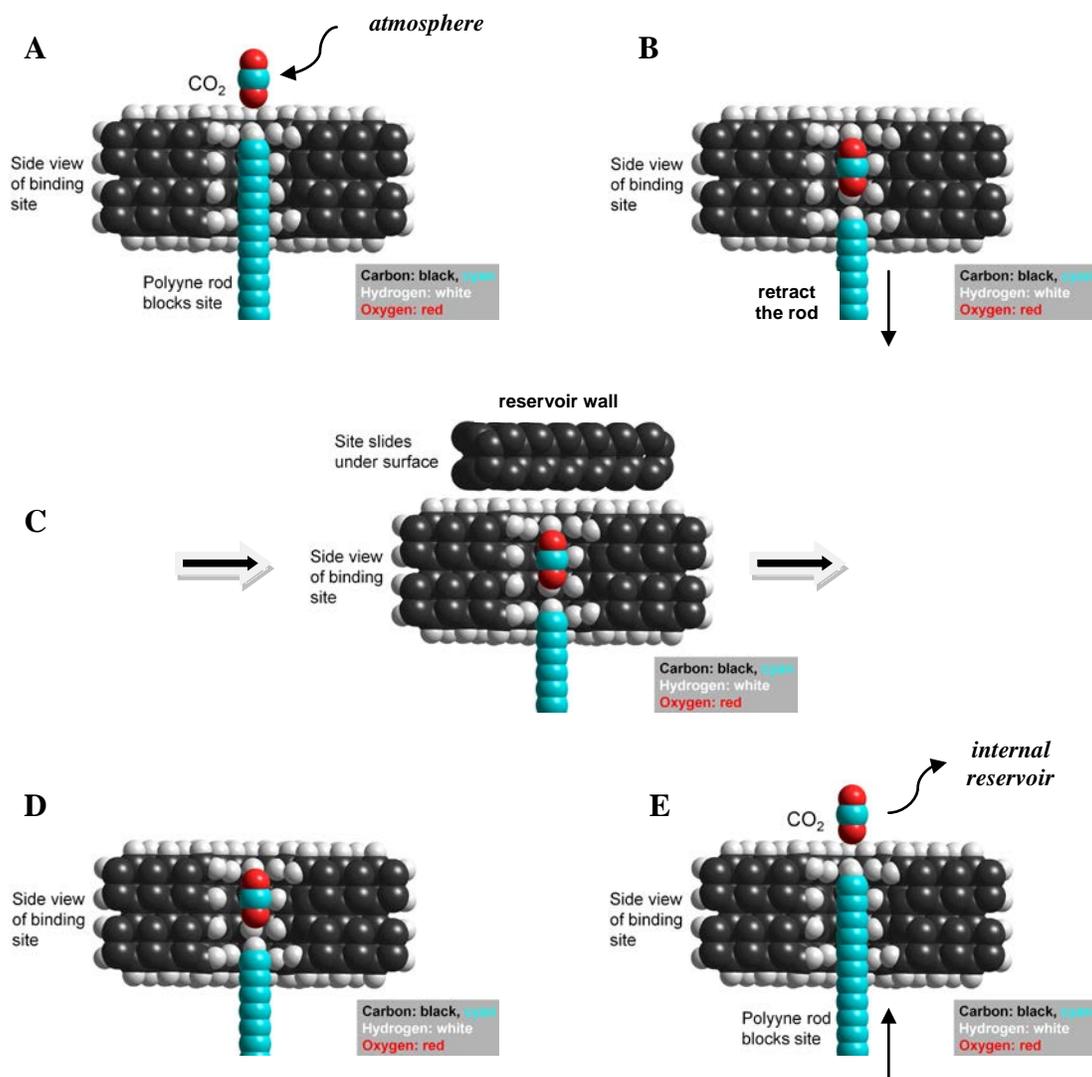


<sup>1932</sup> Drexler KE. *Nanosystems: Molecular Machinery, Manufacturing, and Computation*. John Wiley & Sons, New York, 1992, Section 13.2.1(a).

<sup>1933</sup> Nishide D, Dohi H, Wakabayashi T, Nishibori E, Aoyagi S, Ishida M, Kikuchi S, Kitaura R, Sugai T, Sakata M, Shinohara H. Single-wall carbon nanotubes encaging linear chain  $\text{C}_{10}\text{H}_2$  polyyne molecules inside. *Chem Phys Lett*. 2006 Sep 20;428(4-6):356-360; <http://www.sciencedirect.com/science/article/pii/S0009261406010256>.

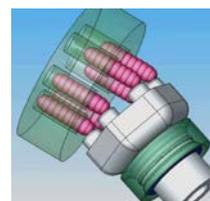
<sup>1934</sup> Zhao C, Kitaura R, Hara H, Irle S, Shinohara H. Growth of linear carbon chains inside thin double-wall carbon nanotubes. *J Phys Chem*. 2011 Jun 7;115(27):13166-13170; <http://pubs.acs.org/doi/abs/10.1021/jp201647m>.

<sup>1935</sup> Chalifoux WA, Tykwinski RR. Synthesis of polyynes to model the sp-carbon allotrope carbyne. *Nat Chem*. 2010;2:967-971; [www.nature.com/nchem/journal/v2/n11/full/nchem.828.html](http://www.nature.com/nchem/journal/v2/n11/full/nchem.828.html).



A specific sequence, focusing on a single binding pocket, is illustrated above. Such molecular pumps generally operate in a four-phase sequence: (1) recognition (and binding) by the transporter of the target molecule from a variety of molecules presented to the pump in the source fluid; (2) translocation of the target molecule through a wall, into the interior of the transporter mechanism; (3) release of the molecule by the transporter mechanism; and (4) return of the transporter to its original condition, outside the wall, so that it is ready to accept another target molecule. Molecular transporters that rely on protein conformational changes are ubiquitous in biological systems.

Mechanical molecular sorting rotors (including the newer “revolver” motif; image, right) can be designed from about 100,000 atoms, comprising ~20,000 atoms for the rotor mechanism with binding sites plus another ~80,000 atoms for the rotor housing and pro rata share of the mechanical drive system. Each complete rotor mechanism might measure roughly 7 nm (wide) x 14 nm (tall) x 14 nm (deep) in size with a mass of about  $2 \times 10^{-21}$  kg if composed mostly of diamondoid structure. The classic sorting rotor can turn at up to ~86,000



rev/sec which exposes 1 million binding sites per second to the source fluid, giving a conservative rim speed of 2.7 mm/sec, sorting and transporting small molecules at a rate of up to  $10^6$  molecules/sec assuming laminar flow as in the case of an aqueous source fluid.

As noted elsewhere,<sup>1936</sup> the minimum energy required to pump<sup>1937</sup> uncharged molecules is the change in free energy  $\Delta G_c$  (joules) in transporting the species from one environment having concentration  $c_1$  to a second environment having concentration  $c_2$ , given by:<sup>1938</sup>

$$\Delta G_c = k_B T \ln(c_2/c_1) \quad (1)$$

where  $k_B = 0.01381$  zJ/K (Boltzmann constant) and  $T =$  temperature in kelvins.<sup>1939</sup> For example, transport of one uncharged molecule from a low concentration to a high concentration environment across a  $c_2/c_1 = 1000$  gradient (typical in biology) costs  $\Delta G_c \sim 30$  zJ/molecule at 300 K. Pumping against a more aggressive  $c_2/c_1 = 10^6$  concentration gradient costs  $\Delta G_c \sim 60$  zJ/molecule.

Besides the compression energy  $\Delta G_c$ , there is also less disorder (lower entropy) in the world when two gases are separated than when they share a common volume, and the entropy of the world can only be decreased by the expenditure of energy. Thermodynamics predicts the minimum amount of energy  $\Delta G_S$  needed to achieve such a separation, a value that depends on the absolute temperature and the initial and final concentrations and pressures. If  $X_{CO_2}$  is the mole fraction of carbon dioxide, and if all of the captured  $CO_2$  is pure and all of the  $CO_2$  in the original mixture is removed, then the minimum energy per unit mass of  $CO_2$  removed is given by:<sup>1940</sup>

$$\Delta G_S = -k_B T [X_{CO_2} \ln(X_{CO_2}) + (1-X_{CO_2}) \ln(1-X_{CO_2})] / X_{CO_2} \quad (2)$$

For example, in the simple case of ambient air capture of  $CO_2$  at  $T = 300$  K,  $X_{CO_2} = 0.0004$  (400 ppm) and  $\Delta G_S = 36.6$  zJ/molecule  $CO_2$ . The total energy of  $CO_2$  compression and separation would then be:

$$\Delta G_{CO_2} = \Delta G_c + \Delta G_S. \quad (3)$$

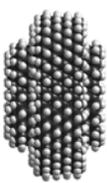
<sup>1936</sup> Freitas RA Jr. The Nanofactory Solution to Global Climate Change: Atmospheric Carbon Capture. IMM Report No. 45, Dec 2015; <http://www.imm.org/Reports/rep045.pdf>.

<sup>1937</sup> If molecules are allowed to flow in the reverse direction from higher concentration to lower concentration, then the sorting rotor becomes a pressure-driven motor, selectively transporting just the target molecular species from right to left while generating power rather than consuming it.

<sup>1938</sup> Lackner KS. Capture of carbon dioxide from ambient air. Eur. Phys. J. Special Topics 176(2009):93-106, Eqn. (1), p. 95; [http://death.ocf.berkeley.edu/~step/Journal\\_Club/paper4\\_02092010.pdf](http://death.ocf.berkeley.edu/~step/Journal_Club/paper4_02092010.pdf).

<sup>1939</sup> The energy unit “zJ” refers to the zeptojoule or  $10^{-21}$  joules.

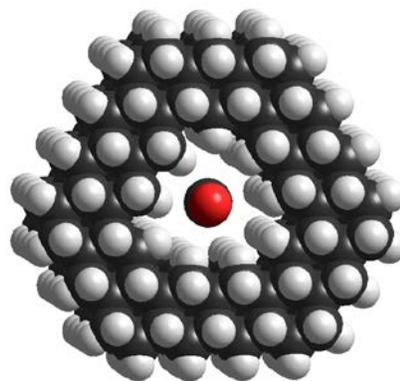
<sup>1940</sup> “Direct Air Capture of  $CO_2$  with Chemicals,” American Physical Society, 1 Jun 2011; <http://www.aps.org/policy/reports/assessments/upload/dac2011.pdf>.



If we plausibly assume the use of incommensurate-surfaced molecular bearings (image, left) exhibiting superlubricity<sup>1941</sup> inside the rotor mechanism, then the primary source of mechanical energy loss is speed-dependent viscous drag of the rotor surface as it moves through the fluid environment on either side of the barrier wall. For a fluid environment having the approximate viscosity of water ( $\sim 10^{-3}$  kg/m-sec at 20 °C) on both sides of the wall, the sorting rotor described and operated as above has an estimated continuous drag power loss of  $10^{-16}$  W while transporting  $10^6$  molecules/sec,<sup>1942</sup> or  $\sim 0.1$  zJ/molecule transported. At low speeds, drag power scales linearly with viscosity, so rotors turning in air at STP (viscosity  $\sim 0.02 \times 10^{-3}$  kg/m-sec) for the purpose of O<sub>2</sub> or CO<sub>2</sub> extraction from the atmosphere would dissipate  $\sim 0.002$  zJ/molecule transported. This is  $10^{-2}$  to  $10^{-4}$  of the required  $\Delta G_{\text{CO}_2}$  transport energy, hence this source of energy loss may be regarded as negligible for the *in vivo* medical device application.

The strength of the binding of the target molecule to the artificial receptor site can be designed to be sufficient to achieve high occupancy of all pockets (e.g., 99%) at the given relatively low speeds of rotor rotation. The mechanical energy consumed to force the target molecule out of its binding site into the receiving chamber is delivered from the cam to the rods, but this energy is largely returned with minimal losses to the cam on the source side by the compression of the rods during the binding of the target molecule to the receptor, a process that regenerates mechanical energy. The artificial receptors are best designed for high affinity binding in the presence of a dominant background of quite different molecules. Analogies with antibodies suggest that a rotor with binding pockets of this type could deliver a product stream with impurity fractions on the order of  $10^{-4}$  to  $10^{-9}$  (i.e., 99.99% purity or better) depending on affinities, specificities, and the concentrations of the effectively competing ligands.<sup>1943</sup> A detailed computational modeling and simulation effort will be required to create ideal selective binding site designs for CO<sub>2</sub> and also for other specific molecules of medical interest including O<sub>2</sub>, H<sub>2</sub>O, glucose, and so forth.

However, even an extremely simple binding site may be surprisingly effective. The image at right<sup>1944</sup> shows an example of a very simple 420-atom binding site (C = black, H = white, O = red) that does not employ strong covalent



<sup>1941</sup> Dienwiebel M, Verhoeven GS, Pradeep N, Frenken JWM, Heimberg JA, Zandbergen HW. Superlubricity of graphite. *Phys. Rev. Lett.* 2004; 92:126101. Erdemir A, Martin J-M, eds., *Superlubricity*, Elsevier, 2007; <http://www.sciencedirect.com/science/book/9780444527721>. Liu Z, Yang J, Grey F, Liu JZ, Liu Y, Wang Y, Yang Y, Cheng Y, Zheng Q. Observation of superlubricity in microscale graphite. *Phys. Rev. Lett.* 2012; 108:205503.

<sup>1942</sup> Drexler KE. *Nanosystems: Molecular Machinery, Manufacturing, and Computation*. John Wiley & Sons, New York, 1992, Section 13.2.1(e).

<sup>1943</sup> Drexler KE. *Nanosystems: Molecular Machinery, Manufacturing, and Computation*. John Wiley & Sons, New York, 1992, Section 13.2.2(b).

<sup>1944</sup> Design by Ralph C. Merkle; personal communication, 2015.

bonds between the site and the target molecule ( $\text{CO}_2$ ). To design this binding site, we took a thin sheet of hydrogen-terminated diamond with the C(111) lattice on the top and bottom horizontal faces, cut out a hexagonal perimeter with the C(100) lattice on all six vertical sides, punched a small hole in the center, and hydrogenated the inner walls of the pore, making a 420-atom ( $\text{C}_{240}\text{H}_{180}$ ) all-hydrocarbon binding site. Such a structure could readily be nanofabricated as a solid block using a specific sequence of positionally-controlled tip-based mechanosynthetic reactions.<sup>1945</sup> The image above shows a single  $\text{CO}_2$  molecule nestled snugly inside the hydrogen-terminated pore, which employs only van der Waals attractions to provide the necessary binding interaction.

To assess the performance of our simple binding site for capture  $\text{CO}_2$  from normal sea-level atmosphere, we first list 38 major and trace atmospheric constituents typically found in non-urban air along with their fractional molar concentration,<sup>1946</sup> then employ molecular mechanics methods using the AMBER99 force field<sup>1947</sup> (known to be highly parameterized for nonbonded interactions, using the computational tools of quantum chemistry) to estimate the binding energy ( $E_b$ ) of the pore for each molecular species (**Table C1**). This is calculated as the difference between the total energy of the binding site with the molecule bound inside it and the sum of the total energies of the empty binding site and the isolated molecule. The fractional binding site occupancy of a molecular species, or  $\exp(-E_b/k_B T)$  where  $k_B = 8.625 \times 10^{-5}$  eV/molecule-K and ambient temperature  $T = 300$  K, is then multiplied by the fractional atmospheric molar concentration ( $f_{\text{air}}$ ) of that molecule, then renormalized to obtain the percentage of each molecular species likely to pass through the binding site filtration mechanism in the first pass. Our simple binding site pore has very high affinity for  $\text{CO}_2$  and even higher affinity for eight other molecules, but those other eight molecules are present in normal air at such low concentrations that the resulting gaseous filtrate is ~99% pure  $\text{CO}_2$ , with just ~1% of  $\text{O}_2$  and  $\text{N}_2$  and mere traces of everything else.

---

<sup>1945</sup> For mechanosynthesis theory work generally, see: Freitas RA Jr, Merkle RC. A Minimal Toolset for Positional Diamond Mechanosynthesis. *J Comput Theor Nanosci.* 2008 May;5:760-861; <http://www.molecularassembler.com/Papers/MinToolset.pdf>. For experimental mechanosynthesis work, see: Sugimoto Y, Pou P, Custance O, Jelinek P, Abe M, Perez R, Morita S. Complex Patterning by Vertical Interchange Atom Manipulation Using Atomic Force Microscopy. *Science.* 2008 Oct 17;322:413-417; <http://www.sciencemag.org/content/322/5900/413.full>.

<sup>1946</sup> Killinger DK, Churnside JH, Rothman LS. Chapter 44. Atmospheric Optics. Bass M, Van Stryland EW, Williams DR, Wolfe WL, eds., *Handbook of Optics, Volume I: Fundamentals, Techniques, and Design*, Second Edition, McGraw-Hill, Inc., New York, 1995, pp. 44.1-44.50. Weast RC, *Handbook of Chemistry and Physics*, 49th Edition, CRC, Cleveland OH, 1968.

<sup>1947</sup> Cornell WD, Cieplak P, Bayly CI, Gould IR, Merz KM Jr, Ferguson DM, Spellmeyer DC, Fox T, Caldwell JW, Kollman PA. A second generation force field for the simulation of proteins, nucleic acids, and organic molecules. *J Am Chem Soc.* 1995;117: 5179-5197; [http://homepage.univie.ac.at/mario.barbatti/papers/method/amber\\_1995.pdf](http://homepage.univie.ac.at/mario.barbatti/papers/method/amber_1995.pdf). Wang J, Cieplak P, Kollman PA. How well does a restrained electrostatic potential (RESP) model perform in calculating conformational energies of organic and biological molecules? *J Comput Chem.* 2000;21(12):1049-1074; [http://banana.cns.m.csulb.edu/ffamber/pdfs/wang\\_amber99\\_2000jcc.pdf](http://banana.cns.m.csulb.edu/ffamber/pdfs/wang_amber99_2000jcc.pdf).

Filtrate purity will vary slightly with ambient environmental temperature (e.g., the mechanism passes 99.26% CO<sub>2</sub> at 290 K, 98.49% CO<sub>2</sub> at 310 K).

<b>Table C1. Gas molecule concentration in ambient U.S. dry atmosphere, their binding energy in a simple pore of a molecular filter, and their concentration in the resulting filtrate.</b>			
<b>Atmospheric Molecular Component</b>	<b>Fractional Atmospheric Concentration (<math>f_{\text{air}}</math>)</b>	<b>Amber99 Binding Energy of Molecular Component in Simple Binding Site (<math>E_b</math>)</b>	<b>% Concentration of Air Component in Gas Filtrate</b>
Nitrogen (N <sub>2</sub> )	7.80840 x 10 <sup>-1</sup>	-0.2521 eV	0.1386%
Oxygen (O <sub>2</sub> )	2.09460 x 10 <sup>-1</sup>	-0.3336 eV	0.8665%
Water vapor (H <sub>2</sub> O)	~1.00 x 10 <sup>-2</sup>	-0.2936 eV	0.0088%
Argon (Ar)	9.340 x 10 <sup>-3</sup>	-0.0586 eV	< 1 ppm
Carbon Dioxide (CO <sub>2</sub> )	3.870 x 10 <sup>-4</sup>	-0.6190 eV	98.9341%
Neon (Ne)	1.818 x 10 <sup>-5</sup>	-0.1021 eV	< 1 ppm
Helium (He)	5.24 x 10 <sup>-6</sup>	-0.0535 eV	< 1 ppm
Methane (CH <sub>4</sub> )	1.79 x 10 <sup>-6</sup>	-0.3210 eV	< 1 ppm
Krypton (Kr)	1.14 x 10 <sup>-6</sup>	-0.0904 eV	< 1 ppm
Hydrogen (H <sub>2</sub> )	5.50 x 10 <sup>-7</sup>	-0.0461 eV	< 1 ppm
Nitrous Oxide (N <sub>2</sub> O)	3.20 x 10 <sup>-7</sup>	-0.4611 eV	0.0002%
Carbon Monoxide (CO)	1.50 x 10 <sup>-7</sup>	-0.2999 eV	< 1 ppm
Xenon (Xe)	8.70 x 10 <sup>-8</sup>	-0.1028 eV	< 1 ppm
Ozone (O <sub>3</sub> )	2.66 x 10 <sup>-8</sup>	-0.6287 eV	0.0099%
Formaldehyde (H <sub>2</sub> CO)	2.4 x 10 <sup>-9</sup>	-0.5542 eV	0.0001%
Ethane (C <sub>2</sub> H <sub>6</sub> )	2.0 x 10 <sup>-9</sup>	-0.6816 eV	0.0058%
Hydrogen Chloride (HCl)	1.0 x 10 <sup>-9</sup>	-0.3833 eV	< 1 ppm
Methyl Chloride (CH <sub>3</sub> Cl)	7.0 x 10 <sup>-10</sup>	-0.6842 eV	0.0022%
Carbonyl Sulfide (OCS)	6.0 x 10 <sup>-10</sup>	-0.7324 eV	0.0123%
Acetylene (C <sub>2</sub> H <sub>2</sub> )	3.0 x 10 <sup>-10</sup>	-0.5079 eV	< 1 ppm
Sulfur Dioxide (SO <sub>2</sub> )	3.0 x 10 <sup>-10</sup>	-0.7650 eV	0.0216%
Nitric Oxide (NO)	3.0 x 10 <sup>-10</sup>	-0.3052 eV	< 1 ppm
Hydrogen Peroxide (H <sub>2</sub> O <sub>2</sub> )	2.0 x 10 <sup>-10</sup>	-0.4974 eV	< 1 ppm
Hydrogen Cyanide (HCN)	1.7 x 10 <sup>-10</sup>	-0.5523 eV	< 1 ppm
Nitric Acid (HNO <sub>3</sub> )	5.0 x 10 <sup>-11</sup>	-0.4311 eV	< 1 ppm
Ammonia (NH <sub>3</sub> )	5.0 x 10 <sup>-11</sup>	-0.1887 eV	< 1 ppm
Nitrogen Dioxide (NO <sub>2</sub> )	2.3 x 10 <sup>-11</sup>	-0.5395 eV	< 1 ppm
Hypochlorous Acid (HOCl)	7.7 x 10 <sup>-12</sup>	-0.5970 eV	< 1 ppm
Hydrogen Iodide (HI)	3.0 x 10 <sup>-12</sup>	-0.4993 eV	< 1 ppm
Hydrogen Bromide (HBr)	1.7 x 10 <sup>-12</sup>	-0.4707 eV	< 1 ppm
Hydroxyl radical (OH <sup>•</sup> )	4.4 x 10 <sup>-14</sup>	-0.3443 eV	< 1 ppm
Hydrogen Fluoride (HF)	1.0 x 10 <sup>-14</sup>	-0.2439 eV	< 1 ppm
Chlorine Monoxide (ClO)	1.0 x 10 <sup>-14</sup>	-0.6515 eV	< 1 ppm
Formic Acid (HCOOH)	1.0 x 10 <sup>-14</sup>	-0.7281 eV	< 1 ppm
Carbonyl Fluoride (COF <sub>2</sub> )	1.0 x 10 <sup>-14</sup>	-0.3756 eV	< 1 ppm
Sulfur Hexafluoride (SF <sub>6</sub> )	1.0 x 10 <sup>-14</sup>	0.3578 eV	< 1 ppm
Hydrogen Sulfide (H <sub>2</sub> S)	1.0 x 10 <sup>-14</sup>	-0.3699 eV	< 1 ppm
Phosphine (PH <sub>3</sub> )	1.0 x 10 <sup>-20</sup>	-0.4062 eV	< 1 ppm

