28. N. Kohmura *et al.*, *Neuron* **20**, 1137 (1998).
29. D. L. Benson, H. Tanaka, J. *Neurosci.* **18**, 6892 (1998).
30. U. Staubli, P. Vanderklish, G. Lynch, *Behav. Neural Biol.* **53**, 1 (1990).
31. K. Obst-Pernberg, C. Redies, *J. Neurosci. Res.* **58**, 130 (1999).
32. A. M. Fannon, D. R. Colman, *Neuron* **17**, 423 (1996).
33. Q. Wu, T. Maniatis, *Cell* **97**, 779 (1999).
34. D. J. Hagler Jr., Y. Goda, *Neuron* **20**, 1059 (1998).
35. K. Arndt, S. Nakagawa, M. Takeichi, C. Redies, *Mol. Cell. Neurosci.* **10**, 211 (1998).
36. M. A. Norcross, *Ann. Immunol. (Paris)* **135D**, 113 (1984).
37. T. A. Springer, *Nature* **346**, 425 (1990).
38. M. L. Dustin, S. K. Bromely, Z. Kan, D. A. Peterson, E. R. Unanue, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 3909 (1997).
39. W. J. Poo, L. Conrad, C. A. Janeway Jr., *Nature* **332**, 378 (1988).
40. H. Kupfer, C. R. Monks, A. Kupfer, *J. Exp. Med.* **179**, 1507 (1994).
41. W. E. Paul, R. A. Seder, *Cell* **76**, 241 (1994).
42. C. R. Monks, B. A. Freiberg, H. Kupfer, N. Sciaky, A. Kupfer, *Nature* **395**, 82 (1998).
43. M. L. Dustin *et al.*, *Cell* **94**, 667 (1998).
44. A. Grakoui *et al.*, *Science* **285**, 221 (1999).
45. P. Anton van der Merwe, S. J. Davis, A. S. Shaw, M. L. Dustin, *Semin. Immunol.* **12**, 5 (2000).
46. J. R. Kuhn, M. Poenie, *Immunity* **16**, 111 (2002).
47. M. F. Krummel, M. D. Sjaastad, C. Wulfing, M. M. Davis, *Science* **289**, 1349 (2000).
48. K.-H. Lee *et al.*, *Science* **295**, 1539 (2002).
49. S. Stoll, J. Delon, T. M. Brotz, R. N. Germain, *Science* **296**, 1873 (2002).
50. P. Reichert, R. L. Reinhardt, E. Ingulli, M. K. Jenkins, *J. Immunol.* **166**, 4278 (2001).
51. S. J. Davis, P. A. van der Merwe, *Immunol. Today* **17**, 177 (1996).
52. A. S. Shaw, M. L. Dustin, *Immunity* **6**, 361 (1997).
53. M. K. Wild *et al.*, *J. Exp. Med.* **190**, 31 (1999).
54. M. L. Dustin *et al.*, *J. Biol. Chem.* **272**, 30889 (1997).
55. J. Braun, K. Fujiwara, T. D. Pollard, E. R. Unanue, *J. Cell Biol.* **79**, 409 (1978).
56. M. L. Dustin, J. A. Cooper, *Nature Immunol.* **1**, 23 (2000).
57. J. M. Penninger, G. R. Crabtree, *Cell* **96**, 9 (1999).
58. C. Wülfing, M. M. Davis, *Science* **282**, 2266 (1998).
59. S. Y. Qi, J. T. Groves, A. K. Chakraborty, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 6548 (2001).
60. S. J. Lee, Y. Hori, J. T. Groves, M. L. Dustin, A. K. Chakraborty, *Trends Immunol.*, in press.
61. C. Wülfing *et al.*, *Nature Immunol.* **3**, 42 (2002).
62. D. R. Klopfenstein, M. Tomishige, N. Stuurman, R. D. Vale, *Cell* **109**, 347 (2002).
63. S. K. Bromley *et al.*, *Annu. Rev. Immunol.* **19**, 375 (2001).
64. G. Iezzi, K. Karjalainen, A. Lanzavecchia, *Immunity* **8**, 89 (1998).
65. J. C. Stinchcombe, G. Bossi, S. Booth, G. M. Griffiths, *Immunity* **15**, 751 (2001).
66. M. Boes *et al.*, *Nature* **48**, 6901 (2002).
67. A. Chow, D. Toomre, W. Garrett, I. Mellman, *Nature* **418**, 988 (2002).
68. S. J. Turley *et al.*, *Science* **288**, 522 (2000).
69. S. K. Bromley *et al.*, *Nature Immunol.* **2**, 1159 (2001).
70. M. L. Dustin, A. C. Chan, *Cell* **103**, 283 (2000).
71. J. G. Egen, M. S. Kuhns, J. P. Allison, *Nature Immunol.* **3**, 611 (2002).
72. H. M. McConnell, T. H. Watts, R. M. Weis, A. A. Brian, *Biochim. Biophys. Acta* **864**, 95 (1986).
73. C. Wülfing, M. D. Sjaastad, M. M. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 6302 (1998).
74. P. Y. Chan *et al.*, *J. Cell Biol.* **115**, 245 (1991).
75. M. M. Al-Alwan, G. Rowden, T. D. Lee, K. A. West, *J. Immunol.* **166**, 1452 (2001).
76. H. A. Anderson, E. M. Hiltbold, P. A. Roche, *Nature Immunol.* **2**, 156 (2000).
77. R. W. Burry, *Neurochem. Pathol.* **5**, 345 (1986).
78. E. M. Ullian, S. K. Sapperstein, K. S. Christopherson, B. A. Barres, *Science* **291**, 657 (2001).
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VIEWPOINT

Alzheimer's Disease Is a Synaptic Failure

Dennis J. Selkoe

In its earliest clinical phase, Alzheimer's disease characteristically produces a remarkably pure impairment of memory. Mounting evidence suggests that this syndrome begins with subtle alterations of hippocampal synaptic efficacy prior to frank neuronal degeneration, and that the synaptic dysfunction is caused by diffusible oligomeric assemblies of the amyloid β protein.

Among the remarkable opportunities emerging from recent progress in molecular neuroscience, the prospect of understanding and preventing neurodegenerative diseases looms large. Disorders like Alzheimer's, Huntington's, and Parkinson's diseases once epitomized the mechanistic ignorance and therapeutic nihilism surrounding human neurodegeneration. But in the past decade, genes causing familial forms of such disorders have been identified, protein pathways involving the gene products have been delineated, and specific treatments directed at these pathways have begun to enter human trials.

The example of Alzheimer's disease (AD) is of special interest to neuroscientists, not only because it is the most common of the brain degenerations, but also because it usually begins with a remarkably pure impairment of cognitive function. Patients with this devastating disorder of the limbic and association cortices lose their ability to encode new memories, first of trivial and then of important details of life. The insidious dissolution of the ability to learn new information evolves in an individual whose motor and sensory functions are very well preserved and who is otherwise neurologically intact. Over time, both declarative and nondeclarative memory become profoundly impaired, and the capacities for reasoning, abstraction, and language slip away. But the subtlety and variability of the earliest amnestic symptoms, occurring in the absence of any other clinical signs of brain injury, suggest that something is discretely, perhaps intermittently, interrupting the function of synapses that help encode new declarative memories. A wealth of evidence now suggests that this "something" is the amyloid β protein (Aβ), a 42-residue hydrophobic peptide with an ominous tendency to assemble into long-lived oligomers and polymers.

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New Ways to Approach the Problem

As scientists proceed to decipher ever more precisely the basis of memory and cognitive impairments in AD, new rules for how this should be accomplished are emerging. First, it has become clear that we must focus our clinicopathological analyses on the earliest stages in the disorder. Studying the brains of individuals dying (for other reasons) with minimal cognitive impairment (MCI) (*1*, *2*), a very subtle memory syndrome that is often the harbinger of AD, is far more likely to yield compelling mechanistic and therapeutic insights than are further studies of late-stage AD brains. The enormous number of structural and biochemical changes already documented in the latter precludes their utility in identifying events that initiate AD-type neuronal dysfunction. The same is true for rodent models in which the process can be examined dynamically: The earlier one looks, the better. Synapse loss matters; loss of whole neurons comes later and matters less. Second, we must use methods that can reveal functional rather than just structural changes in the brain. The latest mouse models that coexpress transgenes encoding mutant human tau and amyloid β protein precursor (APP) (*3*) are particularly compelling and can be used to perform in vivo electrophysiological analyses and correlate the results with both behavioral and biochemical measures. Third, we must emphasize studies of natural assemblies of human Aβ arising under physiological conditions. Synthetic Aβ peptides have generally been applied at micromolar concentrations (in contrast to the low nanomolar levels of natural Aβ found in the brain and cerebrospinal fluid), and they can aggregate

into an array of assembly forms, some of which may have biophysical properties unlike those found in vivo. Moreover, assessing the effects of such synthetic aggregates is tricky, because they occur as complex mixtures and may undergo rapid transitions to more or less neurotoxic forms in cell culture models or after injection into the brain.

Synapses as the Initial Target in Alzheimer's Disease

Early neurochemical analyses of AD brain tissue revealed that the enzymes that generate and metabolize acetylcholine are substantially depleted (*4*). This finding fits well with the facts that the defining lesions of AD—the Aβ-containing neuritic plaques and the tau-containing neurofibrillary tangles—are present in septal-hippocampal and basal forebrain-neocortical pathways that are cholinergic, and that frank cell loss is observed in the projection neurons of these pathways (*5*). Although deficits in numerous neurotransmitters (including corticotropin-releasing factor, somatostatin, GABA, and serotonin) accrue as the disease progresses, the early symptoms appear to correlate with dysfunction of cholinergic and glutamatergic synapses. In addition to the transmitter alterations, many other biochemical and morphological indicators suggest that AD represents, at least initially, an attack on synapses [reviewed in (*6*)]. Of special relevance is a quantitative morphometric study of temporal and frontal cortical biopsies performed within an average of 2 to 4 years of the onset of clinical AD (*7*). This revealed a ~25 to 35% decrease in the numerical density of synapses (painstakingly counted in electron micrographs) in biopsied AD cortex, and a ~15 to 35% decrease in the number of synapses per cortical neuron. Even at the end of the disease, quantitative correlations of postmortem cytopathology with premortem cognitive deficits indicate that synapse loss is more robustly correlated than are numbers of plaques or tangles, degree of neuronal perikaryal loss, or extent of cortical gliosis (*8*).

The degree of cognitive decline in patients with AD has been correlated with changes in the presynaptic vesicle protein synaptophysin in the hippocampus and association cortices (*8–10*). Indeed, synaptophysin immunoreactivity has been reported to be decreased ~25% in the cortex of patients with MCI or very mild AD, relative to age-matched subjects with normal memory function (*11*). Interestingly, in some APP transgenic mouse lines, the numbers of synaptophysin-positive presynaptic terminals and microtubule-associated protein (MAP2)–positive neurons are ~30% less than in non-transgenic controls at age 2 to 3 months, well before any Aβ plaque formation (*12*). Comparisons of transgenic lines having varying APP expression suggest that decreases in presynaptic terminals are critically dependent on cortical Aβ levels, not on Aβ plaque burden or APP levels (*13*). In accord with this finding, presynaptic terminals are already significantly depleted in 2- to 4-month-old APP transgenic mice as their soluble Aβ levels rise, but before Aβ deposition (i.e., plaque formation) begins. This animal work fits nicely with growing evidence that memory and cognitive deficits in MCI and AD patients correlate far better with cortical Aβ levels than with plaque numbers (*14*) and correlate best with the soluble pool of cortical Aβ, which includes soluble oligomers (*15–17*). Even in very mildly impaired patients, soluble Aβ levels in the cortex show a significant correlation with degree of synaptic loss (*17*).

Advances in brain imaging allow humans with very early or no amnestic symptoms to be evaluated in vivo for subtle alterations of neuronal function by positron emission tomography (PET) or functional magnetic resonance imaging (fMRI). For example, cognitively normal middle-aged and elderly subjects carrying an apolipoprotein E4 (ApoE4) allele—a strong genetic risk factor for AD—showed substantially increased hippocampal and neocortical activation by fMRI, relative to age- and education-matched non-E4 carriers, during memory tasks (*18*). When some of these subjects were reassessed 2 years later, the degree of baseline functional activation correlated with the degree of decline in memory. In addition, PET studies have revealed deficits in cortical glucose metabolism already in middle-aged, cognitively normal subjects with the ApoE4 allele (*19*). The important link here is that ApoE4 carriers undergo substantially greater accumulation of cortical Aβ than do non-E4 carriers, and this occurs well before the development of any symptoms of AD (*20*) (Fig. 1).

Aβ Induces Changes in Synaptic Efficacy in Vivo

The concept that progressive accumulation of Aβ in brain regions important for memory and cognition initiates AD is currently the leading theory of causation. However, the hypothesis remains controversial, in part because a specific neurotoxic species of Aβ and the nature of its effects on synaptic function in vivo have been ill-defined. Genetic mutations that cause aggressive, early-onset forms of AD elevate Aβ production [reviewed in (*21*)], and certain mouse models expressing these mutations reproduce some of the cardinal neuropathological and even behavioral features of the disease [e.g., (*3, 22*)]. Admittedly, current transgenic mice do not yet provide a model of full-blown AD, because they largely lack tangle formation and neuronal loss. The latter presumably represents a relatively advanced change in AD that follows synaptic dysfunction, although the long presymptomatic phase of the human disease means that both synaptic alteration and subsequent neuronal death could contribute to early symptoms. In any event, several electrophysiological studies of young mice transgenic for human APP with AD-causing mutations have revealed significant deficits in basal synaptic transmission and/or long-term potentiation

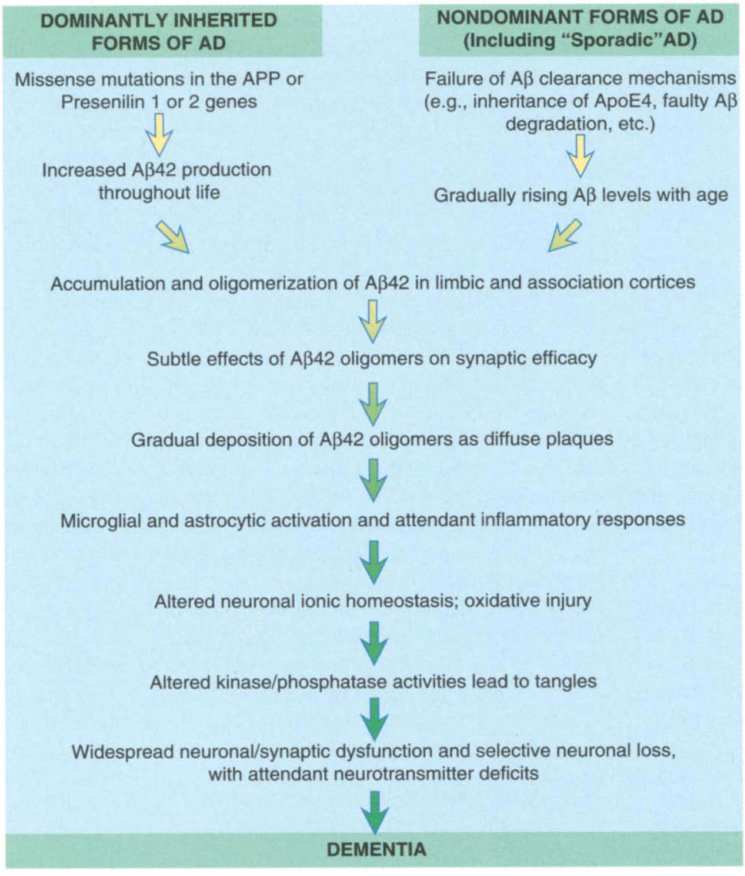

Fig. 1. A hypothetical sequence of the pathogenetic steps of AD, based on currently available evidence. Aβ-42, the 42-residue form of Aβ.

(LTP, an electrophysiological correlate of synaptic plasticity) in the hippocampus, well before the development of microscopically detectable Aβ deposits. For example, one study in mice bearing the $Val^{717} \rightarrow$ Phe (V717F) APP mutation reported smaller excitatory postsynaptic potentials and rapid decay of LTP, relative to nontransgenic mice of matched age and genetic background, at age 4 to 5 months (*23*). A study in another mutant APP mouse line ($Val^{642} \rightarrow$ Ile) found a similar failure to maintain LTP at age 5 to 7 months (*24*). In a study of a third line bearing the $Lys^{670} \rightarrow$ Asn, $Met^{671} \rightarrow$ Leu double mutation, no changes in basal synaptic transmission were observed at age 2 to 8 months (no Aβ deposits) or at age 15 to 17 months (many Aβ deposits), but hippocampal LTP measured in vivo became severely impaired by the latter age (*25*). The LTP deficit in these older mice was associated with impaired performance in a spatial working memory task but little or no loss of certain synaptic markers, suggesting that functional—not structural—synaptic changes were responsible for the cognitive deficits. A separate study in hippocampal slices taken from the same line found decreased basal synaptic transmission but no change in LTP at ages 12 and 18 months (*26*).

In work on another mouse line bearing the V717F APP mutation, young animals (age 1 to 4 months) had a ~40% loss in basal synaptic transmission in hippocampal slices but no change in LTP (*12*). By age 8 to 10 months, these mice showed an ~80% deficit in synaptic transmission. Electrophysiologically, the impairment appeared to be due to a significant reduction in synaptic number, not synaptic strength, occurring between 2 and 10 months. The authors analyzed a second mouse line having lower APP transgene expression but higher Aβ production and observed even worse synaptic transmission deficits at 2 to 4 months, again attributable to the accumulation of diffusible forms of Aβ before plaque formation (*12*). [Note that cortical Aβ42 levels rise steadily in APP transgenic mice from ≤0.1 nmol/g cortex at 6 months (before visible deposits) to ≥5 nmol/g at 18 months (abundant deposits) (*27*). By comparison, cortical Aβ42 levels in AD patients vary widely from <3 to >10 nmol/g at the time of death (*28*).]

Although the use of different electrophysiological protocols and mouse lines has led to some variability, all of the above studies support the concept that mutant APP transgenic mice undergo synaptic dysfunction before plaque formation. However, the nature of the synaptotoxic Aβ species in the brain is very difficult to define, because the animals accumulate a mixture of Aβ forms (monomers, soluble oligomers, insoluble oligomers, and some insoluble amyloid fibrils) that are likely to exist in dynamic equilibrium. In certain cultured cell lines expressing mutant human APP, natural oligomers of human Aβ are formed soon after generation of the peptide within intracellular vesicles and are later secreted from the cell at low nanomolar levels (*29*). Intracerebroventricular microinjection of cell medium containing these oligomers and abundant monomers (but no amyloid fibrils) potently inhibited hippocampal LTP in adult rats. Immunodepletion from the medium of all Aβ species abrogated the LTP block. Pretreatment of the medium with a protease that selectively degrades Aβ monomers but not oligomers failed to prevent the LTP inhibition. Conversely, treatment of the cells with an inhibitor of γ-secretase (one of the two proteases that generate Aβ from APP) markedly decreased oligomer formation at doses that still allowed appreciable monomer production, and such medium no longer disrupted LTP (*29*). These experiments allow inhibition of hippocampal LTP in vivo to be attributed specifically to soluble oligomers, not monomers or fibrils, of secreted human Aβ.

Synthetic Aβ peptides can induce similar changes but generally require low micromolar doses. For example, metastable oligomers of synthetic Aβ can cause acute electrophysiological changes in cultured neurons or hippocampal slices (*30, 31*). Also, microinjection of Aβ43 plus Aβ40 synthetic peptides into rat hippocampus led to in vivo aggregation of the peptides and the development of focal amyloid deposits that were associated with deficits in basal synaptic transmission and maintenance of LTP (*32*). These electrophysiological changes were accompanied by small but significant deficits in short-term ("working") memory; long-term ("reference") memory was unchanged (*32*).

Therapeutic Strategies to Prevent Aβ-induced Synaptic Dysfunction

The above experimental findings have recently been complemented by manipulations intended to reduce the levels of synaptotoxic forms of Aβ in the brains of mouse models. For example, neuron-specific postnatal inactivation of the presenilin 1 gene [which encodes the probable active-site component of γ-secretase (*21*)] resulted in mice with normal brain morphology in which levels of endogenous Aβ were sharply lowered (*33*). Crossing such mice with mutant human APP transgenic mice yielded progeny in which no Aβ accumulation occurred, and the LTP deficits of the latter line were rescued. This genetic lowering of γ-secretase activity thus mirrors the beneficial electrophysiological effects of γ-secretase inhibitors seen in the culture medium injection paradigm discussed above.

An even more striking reversal of the synaptic dysfunction associated with soluble Aβ assemblies was effected by single systemic injections of an antibody to Aβ into 22-month-old APP transgenic mice (*34*). The impaired memory these mice showed in an object recognition task was essentially eliminated overnight, in the absence of any change in overall levels of Aβ deposits (electrophysiological studies were not performed). If confirmed, this result strongly suggests that antibodies to Aβ can interfere acutely with the synaptic dysfunction caused by a diffusible Aβ species without necessarily decreasing mature, insoluble deposits.

Conclusions

Attempts to decipher the subtle alterations in synaptic function that underlie the earliest cognitive features of AD are perhaps the most advanced example of rapid progress in understanding the mechanisms of human neurodegenerative diseases. Analogous efforts in the glutamine repeat disorders (e.g., Huntington's disease, spinocerebellar ataxia), Parkinson's disease, and amyotrophic lateral sclerosis will likely yield powerful clues to the genotype-to-phenotype conversions underlying the development of these syndromes. The inextricably linked goals of such work are to contribute to the elucidation of neuronal function and to prevent the earliest symptoms of dysfunction before they occur.

References and Notes

1. J. C. Morris *et al.*, *Neurology* **46**, 707 (1996).
2. R. C. Petersen *et al.*, *Arch. Neurol.* **56**, 303 (1999).
3. J. Lewis *et al.*, *Science* **293**, 1487 (2001).
4. P. Davies, A. J. Maloney, *Lancet* **ii**, 1403 (1976).
5. P. J. Whitehouse *et al.*, *Science* **215**, 1237 (1982).
6. D. H. Small, S. S. Mok, J. C. Bornstein, *Nature Rev. Neurosci.* **2**, 595 (2001).
7. C. A. Davies, D. M. Mann, P. Q. Sumpter, P. O. Yates, *J. Neurol. Sci.* **78**, 151 (1987).
8. R. D. Terry *et al.*, *Ann. Neurol.* **30**, 572 (1991).
9. D. W. Dickson *et al.*, *Neurobiol. Aging* **16**, 285 (1995).
10. C. I. Sze *et al.*, *J. Neuropathol. Exp. Neurol.* **56**, 933 (1997).
11. E. Masliah *et al.*, *Neurology* **56**, 127 (2001).
12. A. Y. Hsia *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 3228 (1999).
13. L. Mucke *et al.*, *J. Neurosci.* **20**, 4050 (2000).
14. J. Naslund *et al.*, *JAMA* **283**, 1571 (2000).
15. Y.-M. Kuo *et al.*, *J. Biol. Chem.* **271**, 4077 (1996).
16. C. A. McLean *et al.*, *Ann. Neurol.* **46**, 860 (1999).
17. L. F. Lue *et al.*, *Am. J. Pathol.* **155**, 853 (1999).
18. S. Y. Bookheimer *et al.*, *N. Engl. J. Med.* **343**, 450 (2000).
19. E. M. Reiman *et al.*, *N. Engl. J. Med.* **334**, 752 (1996).
20. T. Polvikoski *et al.*, *N. Engl. J. Med.* **333**, 1242 (1995).
21. D. J. Selkoe, *Physiol. Rev.* **81**, 742 (2001).
22. G. Chen *et al.*, *Nature* **408**, 975 (2000).
23. J. Larson, G. Lynch, D. Games, P. Seubert, *Brain Res.* **840**, 23 (1999).
24. D. Moechars *et al.*, *J. Biol. Chem.* **274**, 6483 (1999).
25. P. F. Chapman *et al.*, *Nature Neurosci.* **2**, 271 (1999).
26. S. M. Fitzjohn *et al.*, *J. Neurosci.* **21**, 4691 (2001).
27. K. Johnson-Wood *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 1550 (1997).
28. S. A. Gravina *et al.*, *J. Biol. Chem.* **270**, 7013 (1995).
29. D. Walsh *et al.*, *Nature* **416**, 535 (2002).
30. M. P. Lambert *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 6448 (1998).
31. D. Hartley *et al.*, *J. Neurosci.* **19**, 8876 (1999).
32. A. Stephan, S. Laroche, S. Davis, *J. Neurosci.* **21**, 5703 (2001).
33. I. Dewachter *et al.*, *J. Neurosci.* **22**, 3445 (2002).
34. J. C. Dodart *et al.*, *Nature Neurosci.* **5**, 452 (2002).

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