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J. Cell. Mol. Med. Vol 10, No 3, 2006 pp. 796-805

Synaptic dysfunction and oxidative stress in Alzheimer's disease: Emerging mechanisms

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Received: January 20, 2006; Accepted: March 31, 2006

Abstract

In this paper, we review experimental advances in molecular neurobiology of Alzheimer's disease (AD), with special emphasis on analysis of neural function of proteins involved in AD pathogenesis, their relation with several signaling pathways and with oxidative stress in neurons. Molecular genetic studies have found that mutations in APP, PS1 and PS2 genes and polymorphisms in APOE gene are implicated in AD pathogenesis. Recent studies show that these proteins, in addition to its role in beta-amyloid processing, are involved in several neuroplasticity-signaling pathways (NMDA-PKA-CREB-BDNF, *reelin*, *wingless*, *notch*, among others). Genomic and proteomic studies show early synaptic protein alterations in AD brains and animal models. DNA damage caused by oxidative stress is not completely repaired in neurons and is accumulated in the genes of synaptic proteins. Several functional SNPs in synaptic genes may be interesting candidates to explore in AD as genetic correlates of this synaptopathy in a "synaptogenomics" approach. Thus, experimental evidence shows that proteins implicated in AD pathogenesis have differential roles in several signaling pathways related to neuromodulation and neurotransmission in adult and developing brain. Genomic and proteomic studies support these results. We suggest that oxidative stress effects on DNA and inherited variations in synaptic genes may explain in part the synaptic dysfunction seen in AD.

Keywords: Alzheimer's disease • molecular genetics • neurobiology • oxidative stress • synaptic plasticity

Alzheimer's disease (AD) is one of the main causes of dementia in the world. Increases in life expectancy in the majority of populations around the world have been associated with a higher rate of AD incidence in different countries in past and future decades [1]

AD is clinically characterized by cognitive impairment; there is an initial alteration in recent

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Reprinted from:
Journal of Cellular and Molecular Medicine
doi:10.2755/jcmm010.003.15

memory with other variable neuropsychiatric changes [2]. Neuropathologically, in AD brains there are a diminution in neuron number and a significant increase in the number of amyloid plaques (extracellular structures composed mainly of beta-amyloid peptide) and neurofibrillary tangles (intracellular structures composed mainly by tau protein). Interestingly, in AD brains there is a significant synaptic loss, which is the structural neuropathological variable with the greatest correlation with dementia degree [3].

Current treatment strategies are limited in relation to the detention of the underlying pathogenic process; on other hand, results in relation to search for specific prevention factors for AD are controversial [4]. It highlights the need for a detailed and objective study of AD pathogenesis. In recent years, a great part of AD research has been focused on amyloid hypothesis, which initially proposed that the main AD cause could be the extracellular aggregation of beta-amyloid protein [5]. Many recent articles, carried out by different research groups in the world, have demonstrated that AD-related proteins are participating as key factors in multiple neurosignaling pathways, and that it is possible that pathogenic molecular mechanisms underlying AD may be different from beta-amyloid aggregation [4].

In this article, we review new approaches toward the understanding of AD molecular pathophysiology, which may give a deeper understanding and be the basis for future better therapeutic and preventive strategies. It is done a special emphasis on the description of neural signalling pathways in which AD-related proteins are participating, on advances carried out in AD neurogenomics and neuroproteomics and on interrelation of synaptic dysfunction and oxidative stress in AD.

Molecular approaches

Current knowledge of molecular basis of AD comes from 3 main areas: Analysis of genetic changes in affected subjects and families, molecular neuropathological studies of AD patient brains and animal and cellular models in which genetic or environmental variables, previously associated with the disease in epidemiological studies, are modulated in an experimental approach [4].

From a molecular genetics viewpoint, it has been found mutations in three genes that, although they account by a small part of the overall number of patients in the world, they have been very useful for the development of animal and cellular models of AD [5].

Amyloid precursor protein and presenilins

To date, 25 mutations in Amyloid Precursor Protein (APP) (Chromosome 21), 155 mutations in Presenilin 1 (PS1) (Chromosome 14), and 10 mutations in Presenilin 2 (PS2) (Chromosome 1) genes have been found in AD families with early onset and autosomal dominant inheritance form different parts of the world (*Alzheimer Disease & Frontotemporal Dementia Mutation Database,* available at *http://www.molgen.ua.ac.be/AD Mutations/default.cfm*). Based in these findings, it has been developed in last years animal models in which mutations found in families are over expressed in a homozygote or heterozygote way or in which the homologs of these genes are turned off. As a result of these experimental analyses, it has been proposed that an altered function of these genes converges on the beta-amyloid generation and aggregation. However, many articles show that normal and pathological function of these genes are beyond from beta-amyloid aggregation and can involve other neural mechanisms. [6].

Although some of the first models showed cerebral deposition of beta-amyloid and learning alterations in animals over expressing mutations in APP or PS1, simple or double transgenics [7], systematic studies later showed that behavioural alterations are present previous to the appearance of beta-amyloid aggregates and are correlated in a greater level with functional alterations (hippocampal long-term potentiation –LTP-) or with altered patterns of synapse-related gene expression [8–10].

It was believed that the only function of PS1 could be its participation in APP cleavage to produce beta-amyloid [5], however, animal and cellular studies show that it has a role in the modulation of activity of other important neuronal proteins, mainly in the cleavage of notch protein, the neural adhesion molecule N-cadherin and the neurotrophin receptor p75NTR and through its relation with the b-catenin/GSK3b/wnt and CREB signalling path-

ways [11–13]. These molecular pathways are fundamental to the consolidation of connection patterns in developing brain and on adult brain function. For example, Notch protein, identified some years ago, is cleaved by PS1 after it binds its ligands (Delta-1 and Jagged-1) and translocates to the nucleus to modulate the expression of several downstream genes important for neuronal differentiation [14]. In this context, adult heterozygote Notch knock-out mice have memory alterations [15]. Some PS1 mutations, including several ones found in patients with frontotemporal dementia, have differential effects on these pathways, which are important for neuronal plasticity, as well as for intracellular calcium dynamics, axonal transport, and neurogenesis in adult brain [16–18]. These findings support theories relating common mechanisms between neurodevelopment and neurodegeneration at molecular level [19].

Approaches based in the classical amyloid theory proposed that the absence of PSs may be not deleterious for neurons; however a KO mouse for PS1 had a phenotype similar to that found in Notch mutants with massive brain alterations [20]. After it, in several works using conditional mice for PS1 (or for both PS1 and PS2), in which the expression of the protein is selectively diminished in adult brain, it is demonstrated the key role of PSs and associated pathways in neuronal function [21–24].

Studies focused in APP, have described new functions for this protein; it has been studied its role as a modulator of the activation of a protein that binds GTP, its possible function as a transmembrane receptor, its location at synapse and the modulation of its levels by neuronal activity [25]. For beta-amyloid generation it is necessary a cleavage by beta-secretase and after it by gamma-secretase, an alternate cleavage (by alpha-and gamma-secretases) generates some soluble fragments, which have neurotrophic activities *in vivo* and *in vitro* [26].

Intracytoplasmic portion of APP, which is generated in the production of beta-amyloid, has a behaviour similar to Notch, it translocates to the nucleus and modulates the expression of several genes, some of them related to the regulation of intracellular calcium [27–28]. These findings are correlated with several works that shows that the dynamics of APP processing has a clear effect on synaptic function [25, 29].

In relation to beta-amyloid peptide, several studies showed that this molecule has neuroprotective function in some functional contexts [30]. In this way, the new amyloid theory gives more importance to the presence of oligomers than to amyloid plaques (see below), and although it has been not described what is the molecular pathway by which beta-amyloid can achieve its supposed toxic function [31], a recent description of an effect of oligomeric beta-amyloid on modulation of ionic channels and regulation of the function of Protein Kinase A (PKA), calcineurin and CREB [32–34] may be a point of convergence with its description as a possible endogenous modulator of synaptic function [25].

Apolipoprotein E

In the field of molecular genetics of susceptibility to non-mendelian AD, the main consistent finding has been the association with the so-called E4 allele of apolipoprotein E (APOE) [35]. This gene has a 4 kb size, is located in long arm of chromosome 19, it encodes a protein of 299 amino acids expressed in glial and neural cells in the nervous system. Initially, it was identified three alleles which corresponds to the isoforms of this protein, these 3 classic alleles (E2, E3 and E4) are produced by the change of an cysteine to an arginine in positions 112 and 158 of the protein (a C-T change in positions 3937 and 4075 in the third exon in the gene sequence) [36]. These classical alleles are 3 haplotypes that may be extended through several SNPs (including promoter and intronic regions) through the length of the gene [37].

In 1994 it was identified an important association of APOE4 and AD in an US population, which has been confirmed in different populations around the world [35, 37]. It is to highlight that recent studies showed that preclinical alterations in brain activation patterns in APOE4 carriers many decades before of the possible dementia onset (at an age of 20–30 years) [38]. In addition, it has been found differences in presence of oxidative stress and expression of synaptic proteins between AD patients with different APOE genotypes [39–40].

Animal and cellular studies have demonstrated a possible negative dominant effect of APOE4 isoform on neural plasticity, learning and memory,

neuronal differentiation and activation of signalling pathways, such as CREB, because the activation of these mechanisms by APOE4 is significantly greater than the found in APOE KO mice [41–45]. ApoE receptors may be also activated by reelin, a important molecule for developing and adult brain organization, through the intracellular adaptor protein disabled and activation of CDK5 [46]. It is to highlight that a proteomics study showed that apoE is a key molecule in glia-promoted synaptogenesis [47]. Although some early structural studies tried to demonstrate a protective role of apoE absence on beta-amyloid accumulation produced by APP over expression *in vivo* [48], systematic studies showed, however, that the negative effect on neural function is enhanced [49].

Genomics and proteomics

In a disease as heterogeneous as AD, information provided by non-biased global molecular strategies is of the greatest relevance. Several studies have been carried out using DNA microarrays with AD brain tissues from different stages of the disease, they showed in a consistent fashion a diminution in levels of transcripts related to synaptic function and an overexpression of RNA messengers implicated in cellular stress response [50–52].

In addition, several proteomics studies, using bidimensional electrophoresis of proteins and mass spectrometry (MALDI) showed changes in the state of oxidation and levels of proteins implicated in neuromodulation and neurotransmission [53–54]. These findings are consistent with findings from animal models of AD and from molecular neuropathology studies carried out in AD at a lower scale [55].

In recent years, it has been carried out a great number of genetic association studies in AD trying to find the combination of polymorphisms that explain a greater part of the genetic risk for the development of non-mendelian AD, examples of these markers are polymorphisms in LRP1, MAPT, BDNF, IDE, A2M and ACE genes, among others [4, 5, 56]. It is possible that the synaptic dysfunction in AD may be based, at least in part, to inherited variations in the genes that encode synaptic proteins. It is important to highlight that in recent years it has been described functional SNPs in these genes [57–63]. Preliminary work, based in other formal hypothesis, shows that this association between polymorphisms in synaptic genes and AD may be possible [64], as it has been demonstrated in other neuropsychiatric diseases [65–68]. A ”synaptogenomics” approach may be important to discover genetic correlates of this neurodegenerative synaptopathy.

Etiology

Although synaptic dysfunction appears as a one of the main pathophysiological theories of AD [69, 70], it is important to find an etiological explanation for the alterations in synaptic signalling. Oxidative stress is one of the strongest explanations for neuronal dysfunction related to alterations in intra and interneuronal signalling mechanisms [71]. Some studies have found changes produced by oxidative stress on proteins, lipids, carbohydrates, RNA and DNA in AD brains and animal models [71]. This increase in free radicals comes mainly from cellular energetic metabolism [72] and generates activation of intracellular signalling mechanisms of stress response [73]. These changes appears very early in the disease process and represent an interesting target for prevention, because several works showed a protective use of antioxidants in patients and animal and cellular models [71, 74]. In this context, effect of oxidative stress on DNA in neurons appears as one of the most interesting mechanisms related to neurodegeneration, because neural cells are terminally differentiated and have less effective DNA repair mechanisms that only function in those genomic regions that are transcribed [75, 76]. A recent work showed that in cultured neurons the promoters of genes related to synaptic function, in contrast to other genes, have an increase in the susceptibility to accumulate oxidative stress damage, leading to a down regulation of the synaptic genes [77]. Also, it is possible that interindividual differences in cellular response to oxidative stress may be correlated with variations in the genes that encode proteins implicated in this type of compensatory response [78, 79].

In relation to possible preventive therapeutic strategies based in these functional approaches to AD, it is important to highlight preclinical advances in the development of drugs that modulate the func-

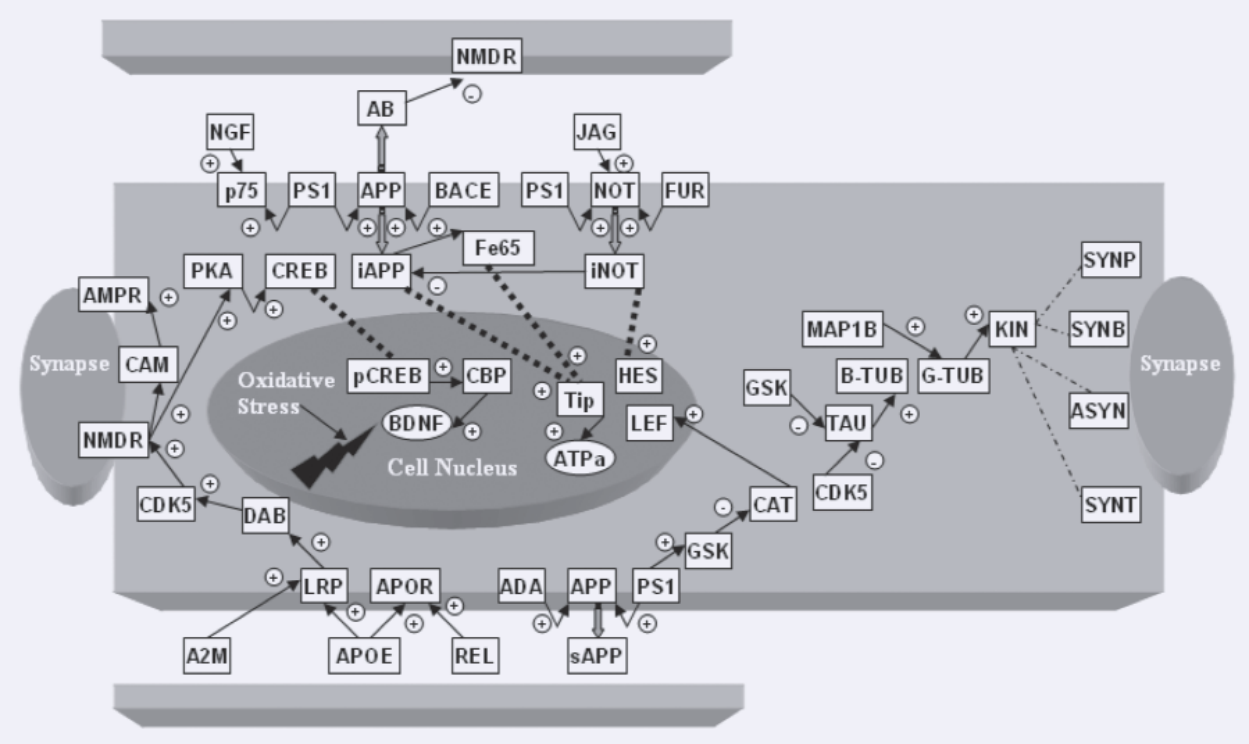

Fig. 1 Overview of molecular interactions described for AD related proteins and neuroplasticity in developing and adult brain. (Signs close to arrows indicate the type and direction of interactions. Squares and circles represent proteins and genes, respectively).

tion of proteins crucial for synaptic plasticity [80, 81] and the analysis of the effects of some environmental variables, such as diet and physical exercise, on molecular mechanisms of neuroplasticity [82].

An overview of interactions between AD-related proteins and molecules involved in neural plasticity pathways is showed in Fig. 1. More information about components of ***NMDA/PKA/CREB/BDNF*** (NMDAR, AMPAR, CAMKIV, PKA, CREB, CBP, BDNF), reelin (reelin, APOER2, DAB, CDK5), **wnt** (GSK3B, β-catenin, LEF) and **notch** (*Notch, HES*) pathways and mechanisms of presynaptic dynamics (*KINESIN, SYNT, SYNP and others*) may be found elsewhere [6].

Additional considerations

Some authors have proposed and demonstrated that the supposed causal structures for AD (plaques and tangles) may be physiological responses that protect the cells form several cell injuries [83], as showed in other neurodegenerative diseases [84]. An interesting example is the functional role of a increase in tau phosphorylation on instability of microtubule cytoskeleton, which is beneficial for a greater plasticity of the cell in response to external stimuli [85].

Another proposed protective mechanism is based in that the increase in beta-amyloid and tau, across aging, is able to diminish oxidative stress levels [86]. In this way, these two changes may have an antioxidant function that may limit the age-related neuronal dysfunction. However, in AD, oxidative damage associated to aging is accompanied by oxidative stress of metabolic and metallic origin [87, 88], which may enhance the levels of beta-amyloid and tau phosphorylation and leads to neurodegeneration and consequent dementia.

Although AD has been of the neuropsychiatric diseases to many economical and human resources have destined in last years, the presence of a bias of many researchers toward the amyloid theory has made difficult that quantitative advances in other conceptual approaches may be realized and consol-

idated into the scientific community [83]. It leads to a constant call to different researchers to consider another advances and approaches that may explain AD in a more comprehensive way.

The new amyloid theory, modified in 2002 year, try to demonstrate that beta-amyloid is the main single factor that damages the synapse, without an possible mechanism proposed to date [31]. However, it did not include all the evidence presented above. One of the main tests of amyloid theory was a clinical trial in AD patients using a vaccine against beta-amyloid; as expected in an immunization against a key protein in the brain [89, 90], it was found several encephalitis cases among treated patients [91]. This generates an ethic preoccupation in relation to future use of similar strategies in AD patients [90].

It may be possible that new variants of high-penetrance can be found for AD in the near future [56]. Recent gene mapping efforts trying to use subphenotypes of AD (AD + psychosis, for example) are very interesting approaches for the identification of new genetic variants [92–93]. Animal (knockout and transgenic mice for specific mutations) and cellular models of those possible new genetic changes would be important for the understanding of pathobiology of AD, in addition to those ones already available for APP, APOE and PS1/PS2 variants found in human patients (see references for key papers) [94–101].

It is important to highlight recent advances in research on other neuropsychiatric diseases, such as schizophrenia, bipolar disorder, major depression and hereditary mental retardation, in which it has been possible to advance on the knowledge of underlying pathological mechanisms from a functional molecular perspective [102–104]. One interesting point to consider may be the study of variants in genes related to other mechanisms of synaptic plasticity and response to oxidative stress.

Conclusions

Although amyloid hypothesis remains as one of the main theories in which the research on AD is based, many multidisciplinary experimental studies demonstrate that AD related proteins are involved in multiple signalling pathways crucial for neuronal plasticity in developing and adult brain, such as the NMDA/PKA/CREB/BDNF, reelin, wnt and notch pathways, among others. Genomics and proteomics studies show early and specific alterations in proteins related to neural and synaptic plasticity in AD brains. These changes in the expression of synaptic proteins may be related to a enhanced susceptibility of synaptic protein-encoded genes to oxidative stress in neurons or to inherited genetic variations. A continuation of basic and clinical research based in other functional conceptions of AD pathophysiology would allow a development of preventive, diagnostic and therapeutic more effective that existent ones and those that could be developed based in amyloid hypothesis.

Acknowledgements

Work in the labs of the authors is supported by grants from COLCIENCIAS and DIB-UNAL (DAF y HA) and by National Institutes of Health and Alzheimer's Association (GC y GP).

Reference

1. **Wimo A, Winblad B, Aguero-Torres H, von Strauss E**. The magnitude of dementia occurrence in the world. *Alzheimer Dis Assoc Disord.* 2003; 17: 63–7.
2. **Cummings JL**. Alzheimer's disease. *N Engl J Med*. 2004; 351: 56–67.
3. **Scheff SW, Price DA**. Synaptic pathology in Alzheimer's disease: a review of ultrastructural studies. *Neurobiol Aging* 2003; 24: 1029–46.
4. **Mattson MP**. Pathways towards and away from Alzheimer's disease. *Nature* 2004; 430: 631–9.
5. **Hardy J, Selkoe DJ**. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 2002; 297: 353–6.
6. **Arendt T**. Synaptic plasticity and cell cycle activation in neurons are alternative effector pathways: the 'Dr. Jekyll and Mr. Hyde concept' of Alzheimer's disease or the yin and yang of neuroplasticity. *Prog Neurobiol*. 2003; 71: 83–248.
7. **Duff K, Eckman C, Zehr C, Yu X, Prada CM, Perez-Tur J, Hutton M, Buee L, Harigaya Y, Yager D, Morgan D, Gordon MN, Holcomb L, Refolo L, Zenk B, Hardy J, Younkin S**. Increased amyloid-beta42(43) in brains of mice expressing mutant presenilin 1. *Nature* 1996; 383: 710–3.
8. **Moechars D, Dewachter I, Lorent K, Reverse D, Baekelandt V, Naidu A, Tesseur I, Spittaels K, Haute CV, Checler F, Godaux E, Cordell B, Van Leuven F**.

Early phenotypic changes in transgenic mice that overexpress different mutants of amyloid precursor protein in brain. *J Biol Chem*. 1999; 274: 6483–92.

9. **Mucke L, Masliah E, Yu GQ, Mallory M, Rockenstein EM, Tatsuno G, Hu K, Kholodenko D, Johnson-Wood K, McConlogue L**. High-level neuronal expression of abeta 1-42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. *J Neurosci.* 2000; 20: 4050–8.
10. **Schneider I, Reverse D, Dewachter I, Ris L, Caluwaerts N, Kuiperi C, Gilis M, Geerts H, Kretzschmar H, Godaux E, Moechars D, Van Leuven F, Herms J.** Mutant presenilins disturb neuronal calcium homeostasis in the brain of transgenic mice, decreasing the threshold for excitotoxicity and facilitating long-term potentiation. *J Biol Chem.* 2001; 276: 11539–44.
11. **Marambaud P, Wen PH, Dutt A, Shioi J, Takashima A, Siman R, Robakis NK.** A CBP binding transcriptional repressor produced by the PS1/epsilon-cleavage of N-cadherin is inhibited by PS1 FAD mutations. *Cell* 2003; 114: 635–45.
12. **Song W, Nadeau P, Yuan M, Yang X, Shen J, Yankner BA**. Proteolytic release and nuclear translocation of Notch-1 are induced by presenilin-1 and impaired by pathogenic presenilin-1 mutations. *Proc Natl Acad Sci USA*. 1999; 96: 6959–63.
13. **Mitsuda N, Ohkubo N, Tamatani M, Lee YD, Taniguchi M, Namikawa K, Kiyama H, Yamaguchi A, Sato N, Sakata K, Ogihara T, Vitek MP, Tohyama M**. Activated cAMP-response element-binding protein regulates neuronal expression of presenilin-1. *J Biol Chem.* 2001; 276: 9688–98.
14. **Sestan N, Artavanis-Tsakonas S, Rakic P**. Contact-dependent inhibition of cortical neurite growth mediated by notch signaling. *Science* 1999; 286: 741–6.
15. **Costa RM, Honjo T, Silva AJ**. Learning and memory deficits in Notch mutant mice. *Curr Biol.* 2003; 13: 1348–54.
16. **Figueroa DJ, Morris JA, Ma L, Kandpal G, Chen E, Li YM, Austin CP**. Presenilin-dependent gamma-secretase activity modulates neurite outgrowth. *Neurobiol Dis.* 2002; 9: 49–60.
17. **Pigino G, Morfini G, Pelsman A, Mattson MP, Brady ST, Busciglio J.** Alzheimer's presenilin 1 mutations impair kinesin-based axonal transport. *J Neurosci.* 2003; 23: 4499–508.
18. **Amtul Z, Lewis PA, Piper S, Crook R, Baker M, Findlay K, Singleton A, Hogg M, Younkin L, Younkin SG, Hardy J, Hutton M, Boeve BF, Tang-Wai D, Golde TE**. A presenilin 1 mutation associated with familial frontotemporal dementia inhibits gamma-secretase cleavage of APP and notch. *Neurobiol Dis.* 2002; 9: 269–73.
19. **Bothwell M, Giniger E**. Alzheimer's disease: neurodevelopment converges with neurodegeneration. *Cell* 2000; 102: 271–3.
20. **Shen J, Bronson RT, Chen DF, Xia W, Selkoe DJ, Tonegawa S**. Skeletal and CNS defects in Presenilin-1-deficient mice. *Cell* 1997; 89: 629–69.
21. **Dewachter I, Reverse D, Caluwaerts N, Ris L, Kuiperi C, Van den HC, Spittaels K, Umans L, Serneels L, Thiry E, Moechars D, Mercken M, Godaux E, Van Leuven F**. Neuronal deficiency of presenilin 1 inhibits amyloid plaque formation and corrects hippocampal long-term potentiation but not a cognitive defect of amyloid precursor protein [V717I] transgenic mice. *J Neurosci.* 2002; 22: 3445–53.
22. **Feng R, Rampon C, Tang YP, Shrom D, Jin J, Kyin M, Sopher B, Miller MW, Ware CB, Martin GM, Kim SH, Langdon RB, Sisodia SS, Tsien JZ**. Deficient neurogenesis in forebrain-specific presenilin-1 knockout mice is associated with reduced clearance of hippocampal memory traces. *Neuron* 2001; 32: 911–26.
23. **Saura CA, Choi SY, Beglopoulos V, Malkani S, Zhang D, Shankaranarayana Rao BS, Chattarji S, Kelleher RJ, III, Kandel ER, Duff K, Kirkwood A, Shen J**. Loss of presenilin function causes impairments of memory and synaptic plasticity followed by age-dependent neurodegeneration. *Neuron* 2004; 42: 23–36.
24. **Yu H, Saura CA, Choi SY, Sun LD, Yang X, Handler M, Kawarabayashi T, Younkin L, Fedeles B, Wilson MA, Younkin S, Kandel ER, Kirkwood A, Shen J**. APP processing and synaptic plasticity in presenilin-1 conditional knockout mice. *Neuron* 2001; 31: 713–26.
25. **Kamenetz F, Tomita T, Hsieh H, Seabrook G, Borchelt D, Iwatsubo T, Sisodia S, Malinow R.** APP processing and synaptic function. *Neuron* 2003; 37: 925–37.
26. **Meziane H, Dodart JC, Mathis C, Little S, Clemens J, Paul SM, Ungerer A**. Memory-enhancing effects of secreted forms of the beta-amyloid precursor protein in normal and amnestic mice. *Proc Natl Acad Sci USA*. 1998; 95: 12683–8.
27. **Cao X, Sudhof TC**. A transcriptionally active complex of APP with Fe65 and histone acetyltransferase Tip60. *Science* 2001; 293: 115–20.
28. **Leissring MA, Murphy MP, Mead TR, Akbari Y, Sugarman MC, Jannatipour M, Anliker B, Muller U, Saftig P, De SB, Wolfe MS, Golde TE, LaFerla FM**. A physiologic signaling role for the gamma -secretase-derived intracellular fragment of APP. *Proc Natl Acad Sci USA*. 2002; 99: 4697–702.
29. **Yang G, Gong YD, Gong K, Jiang WL, Kwon E, Wang P, Zheng H, Zhang XF, Gan WB, Zhao NM**. Reduced synaptic vesicle density and active zone size in mice lacking amyloid precursor protein (APP) and APP-like protein 2. *Neurosci Lett.* 2005; 384: 66–71.
30. **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; 23: 5531–5.
31. **Selkoe DJ**. Alzheimer's disease is a synaptic failure. *Science* 2002; 298: 789–91.
32. **Tong L, Thornton PL, Balazs R, Cotman CW**. Beta -amyloid-(1-42) impairs activity-dependent cAMP-response element-binding protein signaling in neurons at concentrations in which cell survival is not compromised. *J Biol Chem.* 2001; 276: 17301–6.
33. **Tong L, Balazs R, Thornton PL, Cotman CW.** Beta-amyloid peptide at sublethal concentrations downregulates brain-derived neurotrophic factor functions in cultured cortical neurons. *J Neurosci.* 2004; 24: 6799–809.

34. **Vitolo OV, Sant'Angelo A, Costanzo V, Battaglia F, Arancio O, Shelanski M**. Amyloid beta -peptide inhibition of the PKA/CREB pathway and long-term potentiation: reversibility by drugs that enhance cAMP signaling. *Proc Natl Acad Sci USA*. 2002; 99: 13217–21.
35. **Cedazo-Minguez A, Cowburn RF.** Apolipoprotein E: a major piece in the Alzheimer's disease puzzle. *J Cell Mol Med.* 2001; 5: 254-66.
36. **Stengard JH, Clark AG, Weiss KM, Kardia S, Nickerson DA, Salomaa V, Ehnholm C, Boerwinkle E, Sing CF**. Contributions of 18 additional DNA sequence variations in the gene encoding apolipoprotein E to explaining variation in quantitative measures of lipid metabolism. *Am J Hum Genet.* 2002; 71: 501–17.
37. **Arboleda GH, Yunis JJ, Pardo R, Gomez CM, Hedmont D, Arango G, Arboleda H**. Apolipoprotein E genotyping in a sample of Colombian patients with Alzheimer's disease. *Neurosci Lett.* 2001; 305: 135–8.
38. **Reiman EM, Chen K, Alexander GE, Caselli RJ, Bandy D, Osborne D, Saunders AM, Hardy J.** Functional brain abnormalities in young adults at genetic risk for late-onset Alzheimer's dementia. *Proc Natl Acad Sci USA*. 2004; 101: 284–9.
39. **Ramassamy C, Averill D, Beffert U, Theroux L, Lussier-Cacan S, Cohn JS, Christen Y, Schoofs A, Davignon J, Poirier** J. Oxidative insults are associated with apolipoprotein E genotype in Alzheimer's disease brain. *Neurobiol Dis.* 2000; 7: 23–37.
40. **Xu PT, Li YJ, Qin XJ, Scherzer CR, Xu H, Schmechel DE, Hulette CM, Ervin J, Gullans SR, Haines J, Pericak-Vance MA, Gilbert JR**. Differences in apolipoprotein E3/3 and E4/4 allele-specific gene expression in hippocampus in Alzheimer disease. *Neurobiol Dis.* 2006; 21: 256-75.
41. **Hartman RE, Wozniak DF, Nardi A, Olney JW, Sartorius L, Holtzman DM**. Behavioral phenotyping of GFAP-apoE3 and -apoE4 transgenic mice: apoE4 mice show profound working memory impairments in the absence of Alzheimer's-like neuropathology. *Exp Neurol.* 2001; 170: 326–44.
42. **Kitamura HW, Hamanaka H, Watanabe M, Wada K, Yamazaki C, Fujita SC, Manabe T, Nukina N**. Age-dependent enhancement of hippocampal long-term potentiation in knock-in mice expressing human apolipoprotein E4 instead of mouse apolipoprotein E. *Neurosci Lett*. 2004; 369: 173–8.
43. **Nathan BP, Jiang Y, Wong GK, Shen F, Brewer GJ, Struble RG**. Apolipoprotein E4 inhibits, and apolipoprotein E3 promotes neurite outgrowth in cultured adult mouse cortical neurons through the low-density lipoprotein receptor-related protein. *Brain Res*. 2002; 928: 96–105.
44. **Ohkubo N, Mitsuda N, Tamatani M, Yamaguchi A, Lee YD, Ogihara T, Vitek MP, Tohyama M**. Apolipoprotein E4 stimulates cAMP response element-binding protein transcriptional activity through the extracellular signal-regulated kinase pathway. *J Biol Chem*. 2001; 276: 3046–53.
45. **Wang C, Wilson WA, Moore SD, Mace BE, Maeda N, Schmechel DE, Sullivan PM**. Human apoE4-targeted replacement mice display synaptic deficits in the absence of neuropathology. *Neurobiol Dis.* 2005; 18: 390–8.
46. **Weeber EJ, Beffert U, Jones C, Christian JM, Forster E, Sweatt JD, Herz J**. Reelin and ApoE receptors cooperate to enhance hippocampal synaptic plasticity and learning. *J Biol Chem.* 2002; 277: 39944-52.
47. **Mauch DH, Nagler K, Schumacher S, Goritz C, Muller EC, Otto A, Pfrieger FW**. CNS synaptogenesis promoted by glia-derived cholesterol. *Science* 2001; 294: 1354–7.
48. **Holtzman DM, Fagan AM, Mackey B, Tenkova T, Sartorius L, Paul SM, Bales K, Ashe KH, Irizarry MC, Hyman BT**. Apolipoprotein E facilitates neuritic and cerebrovascular plaque formation in an Alzheimer's disease model. *Ann Neurol.* 2000; 47: 739–47.
49. **Buttini M, Yu GQ, Shockley K, Huang Y, Jones B, Masliah E, Mallory M, Yeo T, Longo FM, Mucke L**. Modulation of Alzheimer-like synaptic and cholinergic deficits in transgenic mice by human apolipoprotein E depends on isoform, aging, and overexpression of amyloid beta peptides but not on plaque formation. *J Neurosci.* 2002; 22: 10539–48.
50. **Colangelo V, Schurr J, Ball MJ, Pelaez RP, Bazan NG, Lukiw WJ**. Gene expression profiling of 12633 genes in Alzheimer hippocampal CA1: transcription and neurotrophic factor down-regulation and up-regulation of apoptotic and pro-inflammatory signaling. *J Neurosci Res.* 2002; 70: 462–73.
51. **Ho L, Guo Y, Spielman L, Petrescu O, Haroutunian V, Purohit D, Czernik A, Yemul S, Aisen PS, Mohs R, Pasinetti GM**. Altered expression of a-type but not b-type synapsin isoform in the brain of patients at high risk for Alzheimer's disease assessed by DNA microarray technique. *Neurosci Lett.* 2001; 298: 191–4.
52. **Yao PJ, Zhu M, Pyun EI, Brooks AI, Therianos S, Meyers VE, Coleman PD**. Defects in expression of genes related to synaptic vesicle trafficking in frontal cortex of Alzheimer's disease. *Neurobiol Dis.* 2003; 12: 97–109.
53. **Castegna A, Aksenov M, Aksenova M, Thongboonkerd V, Klein JB, Pierce WM, Booze R, Markesbery WR, Butterfield DA**. Proteomic identification of oxidatively modified proteins in Alzheimer's disease brain. Part I: creatine kinase BB, glutamine synthase, and ubiquitin carboxy-terminal hydrolase L-1. *Free Radic Biol Med.* 2002; 33: 562–71.
54. **Schonberger SJ, Edgar PF, Kydd R, Faull RL, Cooper GJ**. Proteomic analysis of the brain in Alzheimer's disease: molecular phenotype of a complex disease process. *Proteomics* 2001; 1: 1519–28.
55. **Honer WG**. Pathology of presynaptic proteins in Alzheimer's disease: more than simple loss of terminals. *Neurobiol Aging* 2003; 24: 1047–62.
56. **Bertram L, Tanzi RE**. Alzheimer's disease: one disorder, too many genes? *Hum Mol Genet.* 2004; 13: R135–41.
57. **Smith SK, Hoogendoorn B, Guy CA, Coleman SL, O'Donovan MC, Buckland PR**. Lack of functional promoter polymorphisms in genes involved in glutamate neurotransmission. *Psychiatr Genet.* 2003; 13: 193–19.
58. **Egan MF, Straub RE, Goldberg TE, Yakub I, Callicott JH, Hariri AR, Mattay VS, Bertolino A, Hyde TM, Shannon-Weickert C, Akil M, Crook J, Vakkalanka**

RK, Balkissoon R, Gibbs RA, Kleinman JE, Weinberger DR. Variation in GRM3 affects cognition, prefrontal glutamate, and risk for schizophrenia. *Proc Natl Acad Sci USA*. 2004; 101: 12604–9.
59. **Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR**. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* 2003; 112: 257–69.
60. **Hariri AR, Mattay VS, Tessitore A, Kolachana B, Fera F, Goldman D, Egan MF, Weinberger DR**. Serotonin transporter genetic variation and the response of the human amygdala. *Science* 2002; 297: 400–3.
61. **Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, Goldman D, Weinberger DR**. Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci USA*. 2001; 98: 6917–22.
62. **Canli T, Omura K, Haas BW, Fallgatter A, Constable RT, Lesch KP**. Beyond affect: a role for genetic variation of the serotonin transporter in neural activation during a cognitive attention task. *Proc Natl Acad Sci USA*. 2005; 102: 12224–9.
63. **Iwayama-Shigeno Y, Yamada K, Itokawa M, Toyota T, Meerabux JM, Minabe Y, Mori N, Inada T, Yoshikawa T**. Extended analyses support the association of a functional (GT)n polymorphism in the GRIN2A promoter with Japanese schizophrenia. *Neurosci Lett.* 2005; 378: 102–5.
64. **Ventriglia M, Bocchio CL, Benussi L, Binetti G, Zanetti O, Riva MA, Gennarelli M**. Association between the BDNF 196 A/G polymorphism and sporadic Alzheimer's disease. *Mol Psychiatry* 2002; 7: 136–7.
65. **Garcia CC, Blair HJ, Seager M, Coulthard A, Tennant S, Buddles M, Curtis A, Goodship JA**. Identification of a mutation in synapsin I, a synaptic vesicle protein, in a family with epilepsy. *J Med Genet.* 2004; 41: 183–6.
66. **Arai M, Itokawa M, Yamada K, Toyota T, Arai M, Haga S, Ujike H, Sora I, Ikeda K, Yoshikawa T**. Association of neural cell adhesion molecule 1 gene polymorphisms with bipolar affective disorder in Japanese individuals. *Biol Psychiatry* 2004; 55: 804-10.
67. **Goldberger C, Gourion D, Leroy S, Schurhoff F, Bourdel MC, Leboyer M, Krebs MO.** Population-based and family-based association study of 5'UTR polymorphism of the reelin gene and schizophrenia. *Am J Med Genet B Neuropsychiatr Genet.* 2005; 137: 51–5.
68. **Mill J, Richards S, Knight J, Curran S, Taylor E, Asherson P**. Haplotype analysis of SNAP-25 suggests a role in the aetiology of ADHD. *Mol Psychiatry* 2004; 9: 801–10.
69. **Arendt T.** Neurodegeneration and plasticity. *Int J Dev Neurosci.* 2004; 22: 507–14.
70. **Mesulam MM**. A plasticity-based theory of the pathogenesis of Alzheimer's disease. *Ann N Y Acad Sci. 2000*; 924: 42–52.
71. **Zhu X, Raina AK, Lee HG, Casadesus G, Smith MA, Perry G**. Oxidative stress signalling in Alzheimer's disease. *Brain Res.* 2004; 1000: 32–9.
72. **Mattson MP, Liu D**. Energetics and oxidative stress in synaptic plasticity and neurodegenerative disorders. *Neuromolecular Med.* 2002; 2: 215–31.
73. **Zhu X, Raina AK, Perry G, Smith MA.** Alzheimer's disease: the two-hit hypothesis. *Lancet Neurol.* 2004; 3: 219–26.
74. **Nunomura A, Perry G, Aliev G, Hirai K, Takeda A, Balraj EK, Jones PK, Ghanbari H, Wataya T, Shimohama S, Chiba S, Atwood CS, Petersen RB, Smith MA.** Oxidative damage is the earliest event in Alzheimer disease. *J Neuropathol Exp Neurol.* 2001; 60: 759–67.
75. **Brooks PJ.** DNA repair in neural cells: basic science and clinical implications. *Mutat Res.* 2002; 509: 93–108.
76. **Nouspikel T, Hanawalt PC.** Terminally differentiated human neurons repair transcribed genes but display attenuated global DNA repair and modulation of repair gene expression. *Mol Cell Biol.* 2000; 20: 1562–70.
77. **Lu T, Pan Y, Kao SY, Li C, Kohane I, Chan J, Yankner BA.** Gene regulation and DNA damage in the ageing human brain. *Nature* 2004; 429: 883–91.
78. **Zhang ZJ, Zhang XB, Hou G, Yao H, Reynolds GP.** Interaction between polymorphisms of the dopamine D3 receptor and manganese superoxide dismutase genes in susceptibility to tardive dyskinesia. *Psychiatr Genet.* 2003; 13: 187–92.
79. **Christiansen L, Petersen HC, Bathum L, Frederiksen H, McGue M, Christensen K.** The catalase -262C/T promoter polymorphism and aging phenotypes. *J Gerontol A Biol Sci Med Sci.* 2004; 59: B886–9.
80. **Gong B, Vitolo OV, Trinchese F, Liu S, Shelanski M, Arancio O.** Persistent improvement in synaptic and cognitive functions in an Alzheimer mouse model after rolipram treatment. *J Clin Invest.* 2004; 114: 1624–34.
81. **Tully T, Bourtchouladze R, Scott R, Tallman J.** Targeting the CREB pathway for memory enhancers. *Nat Rev Drug Discov.* 2003; 2: 267–77.
82. **Mattson MP.** Gene-diet interactions in brain aging and neurodegenerative disorders. *Ann Intern Med.* 2003; 139: 441–4.
83. **Joseph J, Shukitt-Hale B, Denisova NA, Martin A, Perry G, Smith MA.** Copernicus revisited: amyloid beta in Alzheimer's disease. *Neurobiol Aging* 2001; 22: 131–46.
84. **Arrasate M, Mitra S, Schweitzer ES, Segal MR, Finkbeiner S.** Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature* 2004; 431: 805–10.
85. **Arendt T, Stieler J, Strijkstra AM, Hut RA, Rudiger J, Van der Zee EA, Harkany T, Holzer M, Hartig W.** Reversible paired helical filament-like phosphorylation of tau is an adaptive process associated with neuronal plasticity in hibernating animals. *J Neurosci.* 2003; 23: 6972–81.
86. **Smith MA, Casadesus G, Joseph JA, Perry G.** Amyloid-beta and tau serve antioxidant functions in the aging and Alzheimer brain. *Free Radic Biol Med.* 2002; 33: 1194–9.
87. **Sayre LM, Zelasko DA, Harris PL, Perry G, Salomon RG, Smith MA.** 4-Hydroxynonenal-derived advanced lipid peroxidation end products are increased in Alzheimer's disease. *J Neurochem.* 1997; 68: 2092–7.

88. **Takeda A, Smith MA, Avila J, Nunomura A, Siedlak SL, Zhu X, Perry G, Sayre LM.** In Alzheimer's disease, heme oxygenase is coincident with Alz50, an epitope of tau induced by 4-hydroxy-2-nonenal modification. *J Neurochem.* 2000; 75: 1234–41.
89. **Furlan R, Brambilla E, Sanvito F, Roccatagliata L, Olivieri S, Bergami A, Pluchino S, Uccelli A, Comi G, Martino G.** Vaccination with amyloid-beta peptide induces autoimmune encephalomyelitis in C57/BL6 mice. *Brain* 2003; 126: 285–91.
90. **Smith MA, Atwood CS, Joseph JA, Perry G.** Predicting the failure of amyloid-beta vaccine. *Lancet* 2002; 359: 1864–5.
91. **Nicoll JA, Wilkinson D, Holmes C, Steart P, Markham H, Weller RO.** Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. *Nat Med.* 2003; 9: 448–52.
92. **Holmans P, Hamshere M, Hollingworth P, Rice F, Tunstall N, Jones S, Moore P, Wavrant DeVrieze F, Myers A, Crook R, Compton D, Marshall H, Meyer D, Shears S, Booth J, Ramic D, Williams N, Norton N, Abraham R, Kehoe P, Williams H, Rudrasingham V, O'Donovan M, Jones L, Hardy J, Goate A, Lovestone S, Owen M, Williams J.** Genome screen for loci influencing age at onset and rate of decline in late onset Alzheimer's disease. *Am J Med Genet B Neuropsychiatr Genet*. 2005; 135: 24–32.
93. **Bacanu SA, Devlin B, Chowdari KV, DeKosky ST, Nimgaonkar VL, Sweet RA.** Linkage analysis of Alzheimer disease with psychosis. *Neurology* 2002; 59: 118–20.
94. **Goate A, Chartier-Harlin MC, Mullan M, Brown J, Crawford F, Fidani L, Giuffra L, Haynes A, Irving N, James L, Mant R, Newton P, Rooke K, Roques P, Talbot C, Pericak-Vance M, Roses A, Williamson R, Rossor M, Owen M, Hardy J.** Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* 1991; 349: 704–6.
95. **Sherrington R, Rogaev EI, Liang Y, Rogaeva EA, Levesque G, Ikeda M, Chi H, Lin C, Li G, Holman K, Tsuda T, Mar L, Foncin JF, Bruni AC, Montesi MP, Sorbi S, Rainero I, Pinessi I, Nee L, Chumakov I, Pollen D, Brookes A, Sanseau P, Polinsky RJ, Wasco W, Da Silva HAR, Haines JL, Pericak-Vance MA, Tanzi RE, Roses AD, Fraser PE, Rommens JM, St George-Hyslop PH.** Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* 1995; 375: 754–60.
96. **Rogaev EI, Sherrington R, Rogaeva EA, Levesque G, Ikeda M, Liang Y, Chi H, Lin C, Holman K, Tsuda T, Mar L, Sorbi S, Nacmias B, Piacentini S, Amaducci L, Chumakov I, Cohen D, Lannfelt L, Fraser PE, Rommens JM, St George-Hyslop PH.** Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature* 1995; 376: 775–8.
97. **Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD.** Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci USA.* 1993; 90: 1977–81.
98. **Games D, Adams D, Alessandrini R, Barbour R, Berthelette P, Blackwell C, Carr T, Clemens J, Donaldson T, Gillespie F, Guido T, Hagopian S, Johnson-Wood K, Khan K, Lee M, Leibowitz P, Lieberburg I, Little S, Masliah E, McConlogue L, Montoya-Zavala M, Mucke L, Paganini L, Penniman E, Power M, Schenk D, Seubert P, Snyder B, Soriano F, Tan H, Vitale J, Wadsworth S, Wolozin B, Zhaoet J.** Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. *Nature* 1995; 373: 523–7.
99. **Masliah E, Mallory M, Ge N, Alford M, Veinbergs I, Roses AD.** Neurodegeneration in the central nervous system of apoE-deficient mice. *Exp Neurol*. 1995; 136: 107-22.
100. **Raber J, Wong D, Buttini M, Orth M, Bellosta S, Pitas RE, Mahley RW, Mucke L.** Isoform-specific effects of human apolipoprotein E on brain function revealed in ApoE knockout mice: increased susceptibility of females. *Proc Natl Acad Sci USA*. 1998; 95: 10914-9.
101. **Roberds SL, Anderson J, Basi G, Bienkowski MJ, Branstetter DG, Chen KS, Freedman SB, Frigon NL, Games D, Hu K, Johnson-Wood K, Kappenman KE, Kawabe TT, Kola I, Kuehn R, Lee M, Liu W, Motter R, Nichols NF, Power M, Robertson DW, Schenk D, Schoor M, Shopp GM, Shuck ME, Sinha S, Svensson KA, Tatsuno G, Tintrup H, Wijsman J, Wright S, McConlogue L.** BACE knockout mice are healthy despite lacking the primary beta-secretase activity in brain: implications for Alzheimer's disease therapeutics. *Hum Mol Genet.* 2001; 10: 1317–24**.**
102. **Blanpied TA, Ehlers MD.** Microanatomy of dendritic spines: emerging principles of synaptic pathology in psychiatric and neurological disease. *Biol Psychiatry* 2004; 55: 1121–7.
103. **Zoghbi HY.** Postnatal neurodevelopmental disorders: meeting at the synapse? *Science* 2003; 302: 826–30.
104. **Duman RS.** Pathophysiology of depression: the concept of synaptic plasticity. *Eur Psychiatry* 2002; 17: 306–10.