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ASSAY AND ANIMAL MODEL DEVELOPMENT

Cell Cultures From Animal Models of Alzheimer's Disease as a Tool for Faster Screening and Testing of Drug Efficacy

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Abstract

Approximately 2 million people in the United States suffer from Alzheimer's disease (AD), which is the most common cause of chronic dementia among the aging population. During the last 7 yr, excellent opportunities to screen drugs against AD have been provided by animal models of the disease. Because even in the fastest model, AD pathology does not start before the end of the second month, it has been necessary to wait at least until that age to inject drugs into the animal to assess whether they prevent, reduce, or revert synaptic impairment, plaque formation, and increase of β-amyloid (Aβ) levels, the main features of the disease. A solution to the
problems mentioned above is achieved by the present fast, efficient, and reproducible cultured cell system from animal models of AD or Aβ-associated diseases, for the screening and testing of compounds for the treatment and therapy of AD or Aβ-associated diseases.

Index Entries: Alzheimer's disease; cell cultures; hippocampus; synaptic plasticity; vesicle cycling.

Introduction

Alzheimer's disease (AD) is the most common cause of chronic dementia among the aging population. Neuritic amyloid plaques, neurofibrillary degeneration, and granulovascular neuronal degeneration are major hallmarks of AD and are found in the brains of elderly people with Alzheimer's dementia. Some of these features, as well as learning and memory disruption, have already been reproduced in transgenic mouse models of AD. For example, transgenic mice in which mutant forms of amyloid precursor protein (APP) and presenilin I (PS1), a peptide that alters APP processing, are overexpressed (Sant'Angelo et al., 2003).

Tissue slices and live adult animals overexpressing APP and PS1 represent a powerful tool for screening drugs against AD. However, although transgenic mice constitute a step forward in AD research, it still takes months for these animals to develop AD pathology. The dissociated hippocampal cell cultures constitute another preparation that might be exploited for examining drugs against the disease. The cell culture system permits the investigation of the cellular mechanisms of learning in hippocampus, a structure within the brain temporal lobe

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that is particularly critical for memory storage (Milner, 1996). Synaptic connections among hippocampal cells in cultures can undergo long-lasting changes in synaptic strength that might underlie learning and memory (Bliss and Collingridge, 1993). Cell culture systems present numerous advantages over other preparations, for example, tissue slices or live adult animals, for evaluating the efficacy and use of compounds in the amelioration, treatment, or prevention of AD or β-amyloid (Aβ)-associated diseases. Illustrative examples of these advantages include (1) easy access for extracellularly applied drugs and compounds undergoing evaluation or testing, as well as the ability to deliver drugs intracellularly to either side of the synapse; (2) the ability to test drug efficacy after a shorter period of time from the birth of an animal that represents an animal model of disease; (3) visibility of synapses and cells for electrical and optical measurements; (4) the opportunity to identify pre- and postsynaptic neurons and to examine the monosynaptic response between them; (5) accessibility to the presynaptic terminal; (6) the possibility of having and maintaining long-term access to cells under a controlled environment for biochemical and genetic manipulation; and (7) simplification of a testing, diagnostic, or evaluation system, because of the exclusion of different types of neuronal and nonneuronal cells. To exploit these advantages, electrophysiological, optical, immunocytochemical, and biochemical methods have been combined with cell cultures derived from transgenic models of AD. We have utilized cultures from double transgenic mice expressing both the human APP mutation (K670N:M671L) (APP), and the human PS1 mutation (M146L) (PS1). Our data show the relevance of transgenic mouse cell cultures as a rapid system in which to test drugs for AD treatment or prevention.

Materials and Methods

Animals

The animals utilized for generating cell cultures were double transgenic mice, expressing both the human APP and the human PS1 mutation, as well as wild-type (WT) littermates. They were obtained by crossing APP (Hsiao et al., 1996) with PS1 (Duff et al., 1996) animals. To identify the genotype of the animals, we used the polymerase chain reaction on samples of the tail taken after dissection of the hippocampus (Duff et al., 1996; Hsiao et al., 1996).

Cell Culture Preparation

Primary cell cultures were prepared from 1-d-old mouse pups. The hippocampus was surgically dissected from the remaining part of the brain under a stereomicroscope. Cells were dissociated using enzymatic treatment with 0.25% trypsin for 30 min and subsequent trituration. The cells were plated on glass coverslips previously coated with 10 μg/mL poly(D-lysine) for at least 3 h at 4°C, followed by 1 mg/50 mL laminin for at least 1 h in an incubator containing 5% CO_2. A ring was placed at the center of the glass coverslip. Each glass coverslip was contained in a 35 × 10 mm cell culture dish. Approximately 150 μL of solution containing approx. 100,000 cells was placed inside the ring. Hippocampal cells were grown in medium containing 84% Eagle's minimum essential medium (MEM), 10% heat-inactivated fetal calf serum (FCS), 45 m*M* glucose, 1% MEM vitamin solution, and 2 m*M* glutamine. Cells were kept inside a 5% CO_2 incubator at 37°C. After 24 h, this medium was replaced by one containing 96.5% neurobasal A, 2% B27 nutrient, 1% heat-inactivated FCS, 0.4 m*M* glutamine, 0.5 mM kynurenic acid, 6.6 ng/mL 5-fluoro-2-deoxyuridine, and 16.4 ng/mL uridine. This medium was no longer replaced in the following days.

Biochemistry

β-Amyloid (Aβ) levels were assayed from supernatant derived from the medium collected from culture dishes and spun at 5000 rpm for 5 min at 4°C, using an ELISA method in which Aβ was trapped with either monoclonal antibody to Aβ 40 (JRF/cAβ40/10) or Aβ 42 (JRF/cAβ42/26) and then detected with horseradish peroxidase-conjugated JRF/Aβtot/17 (Janus et al., 2000). The dilution of JRF/Aβtot/17 and samples was optimized to detect Aβ in the range of 25–400 fmol/mL. ELISA signals were reported as the mean ± S.E.M. of two replica wells in fmoles of Aβ per mg of protein (determined with the BCA Protein Assay Reagent Kit, Pierce), based on standard curves using synthetic Aβ 40 and Aβ 42 peptide standards (American Peptide Co., Sunnyvale, CA).

Electrophysiological Analysis

Cultured neurons were voltage clamped with the whole cell ruptured patch technique throughout the experiment as described previously (Arancio et al., 1995, 2001). The bath solution contained 119 m*M* NaCl, 5 m*M* KCl, 20 m*M* HEPES, 2 m*M* $CaCl_2$, 2 m*M*

$MgCl_2$, 30 m*M* glucose, 0.001 m*M* glycine, and 0.1 m*M* picrotoxin (pH 7.3), with osmolarity adjusted to 330 osmol with sucrose. The solution in the whole cell patch electrode contained 130 m*M* K-gluconate, 10 m*M* KCl, 5 m*M* $MgCl_2$, 0.6 m*M* EGTA, 5 m*M* HEPES, 0.06 m*M* $CaCl_2$, 2 m*M* Mg-ATP, 0.2 m*M* GTP, 0.2 m*M* leupeptin, 20 m*M* phosphocreatine, and 50 U/mL creatine-phosphokinase. Currents were recorded with a Warner amplifier (model PC-501A) (CT), and filtered at 1 kHz. To eliminate artifacts due to variation of the seal properties, access resistance was monitored for constancy throughout all experiments. To suppress action potentials, 1 µ*M* tetrodotoxin was added to the bath when recording miniature excitatory postsynaptic currents (mEPSCs). They were digitized and analyzed with the mini-analysis program (v. 4.0) from Synaptosoft (GA). A baseline of 10-min duration was acquired before inducing potentiation through 200 µ*M* glutamate application in Mg^{2+}-free solution.

Vesicle Cycling

Cationic styrylpyridinium dye FM 1-43 has become an established tool for identifying actively firing neurons and for investigating the mechanisms of activity-dependent vesicle cycling in widely different species (Ryan et al., 1993). These water-soluble dyes, which are nontoxic to cells virtually nonfluorescent in aqueous medium, become internalized within recycled synaptic vesicles and the nerve terminals become brightly stained (Ryan et al., 1993). The amount of FM 1-43 taken up per vesicle by endocytosis equals the amount of dye released upon exocytosis. One of the methods to induce exocytosis/endocytosis in the neuronal culture is by perfusion of hyperkalemic solution (Ryan et al., 1993). Loading of FM 1-43 was induced by changing the perfusion medium from normal saline bath solution (119 m*M* NaCl, 2.5 m*M* KCl, 2 m*M* $CaCl_2$, 2 m*M* $MgCl_2$, 25 m*M* HEPES, and 30 m*M* glucose) to hyeprkalemic bath solution (31.5 m*M* NaCl, 90 m*M* KCl, 2 m*M* $CaCl_2$, 2 m*M* $MgCl_2$, 25 m*M* HEPES, and 30 m*M* glucose) with 5 µ*M* FM 1-43 for 45 s. The perfusion solution was then changed back to normal bath solution for 10 min to wash off the dye from the external medium. ADVASEP-7 (1 mM, CyDex, Inc., Overland Park, KS), an anionic cyclodextrin complexing agent, was introduced for 60 s in the washing bath solution at 1 and 6 min of washing for enhanced removal of dye from the external medium. After a 10-min wash period, which was sufficient for the complete recycling and repriming of the dye-stained population of synaptic vesicles, an image was taken to record the loading of FM 1-43 in the synaptic boutons. The culture was then exposed to multiple 15-s applications of hyperkalemic bath solution (without FM 1-43) to evoke repeated cycles of exocytosis, which facilitated release of the dye from the vesicles. An image was taken after 30 min of repeated cycles of exocytosis and washing with normal bath solution. The difference between the images before and after multiple exposures to hyperkalemic solution gave the measure of FM 1-43-stained vesicles. The number of active boutons per uniform length of randomly selected neurites (15 × 6.85-µm field) at 12 µ*M* from the cell body was measured by an investigator blinded to experimental conditions. To study glutamate-induced presynaptic plasticity changes, the culture was exposed to 200 µ*M* glutamate in Mg^{2+}-free bath solution for 30 s and washed out in approx 1 min. After 30 min of glutamate exposure, the staining and destaining procedure was repeated. NMDA receptor antagonist, D-AP5 (40 µ*M*), and non-NMDA receptor antagonist, CNQX (20 µ*M*), were included in the hyperkalemic solution to block possible recurrent excitation and induction of activity-dependent plasticity. All images were acquired using the Nikon D-Eclipse C1 confocal microscope. Cultures were viewed with 20 × 0.50 nA water immersion objective. An investigator blinded to experimental conditions obtained quantitative data using NIH Image software (v. 1.61). The total number of boutons was assessed from randomly selected fields (30.8 × 30.8 µm).

Immunocytochemistry

Glutamate in Mg^{2+}-free bath solution, or normal bath solution (control), was added directly to the culture dish and washed out after approx 1 min by exchanging the solution with phosphate-buffered saline (PBS), at pH 7.4, twice, as described previously (Antonova et al., 2001). The cultures were fixed starting approx 30 min after the application of glutamate or control solution. Immunocytochemical methods were described previously (Antonova et al., 2001). The cultures were incubated overnight with affinity-purified rabbit anti-synapsin I (Molecular Probes), diluted 1:200. The secondary antibodies were goat anti-rabbit labeled with Rhodamine Red, diluted 1:500 in 4% goat serum in PBS. Cells were mounted in Vectashield

(Vector Labs) and examined by confocal microscopy (Nikon D-Eclipse C1). Kalman averages of four scans were collected for each image. Ten neurons in each culture dish were selected at random and analyzed by an observer who was blind to the experimental treatment. Synapsin I puncta in a representative field (94 × 142 mm) around the neuron were quantified using NIH Image software (v. 1.61). The fields were chosen to have equal numbers and lengths of neurites. Individual puncta were identified based on having a fluorescence intensity that exceeded a threshold set above background and a diameter between 0.5 and 5 μm. For all measures, the mean result from the 10 neurons in the dish was normalized to the mean from control dishes in the same culture batch because variability between different culture batches was greater than that between dishes in the same batch. These values were used for statistical comparison of dishes that received different experimental treatments.

Statistical Analysis

Statistical analysis was performed with ANOVA with post hoc correction, and Student's *t*-test was used for comparison between two groups. Results were expressed as mean ± S.E.M.

Results

Production of elevated amounts of Aβ peptides is one of the key features of AD. Therefore, in a first series of experiments, we examined Aβ levels and found that they were high in the medium from hippocampal cell cultures derived from APP/PS1 mice compared with WT animals. More specifically, at 12 d after plating the cells, high levels of human Aβ 40 and 42, two major types of Aβ peptides, were detected in double transgenic cultures (average values of Aβ40 = 578.53 ± 79.55 fmol/mg protein, and Aβ42 = 175.78 ± 28.34 fmol/mg protein, $n = 7$ dishes), whereas they were undetectable in control cultures.

In an attempt to find reliable parameters that could be used as a tool for testing drugs for AD in our cell culture system, we first examined the basal mEPSC frequency of neurotransmitter release. Spontaneous neurotransmitter release, also known as miniature excitatory postsynaptic currents (mEPSCs), corresponding to the spontaneous and random release of neurotransmitter from the presynaptic terminal were examined to determine whether overexpression of APP and PS1 altered synaptic transmission. We found an approximate twofold increase in basal mEPSC frequency of cultures from double transgenic APP/PS1 animals compared with cultures from WT mice. These novel results suggest that the probability of transmitter release increased in cultures from double transgenic mice. We then studied the capability of cells to undergo plastic changes. Specifically, we found that glutamate-induced long-term enhancement of mEPSC frequency was altered following overexpression of APP and PS1 transgenes. A brief (approx 1 min) application of glutamate (200 μ*M*) did not produce enhancement of mEPSC frequency in cell cultures prepared from double transgenic mice compared with cell cultures prepared from WT mice. These results indicate that overexpression of the two transgenes causing elevated levels of Aβ peptides blocks the capabilities of cells to undergo plastic changes.

In another series of experiments, we examined another synaptic parameter, the number of functional synaptic boutons before and after glutamate application. We intended to determine whether overexpression of APP and PS1 transgenes altered both the basal number of active synaptic boutons and the glutamate-induced increase in the number of active boutons. We found an almost twofold increase in the basal number of active synaptic boutons in cultures from double transgenic animals compared with those in WT cultures. We also found that brief application (approx 30 s) of 200 μ*M* glutamate in Mg^{2+}-free bath solution produced a two- to threefold increase in the number of active synaptic boutons in cultures from WT mice. However, similar application of glutamate failed to increase the number of active synaptic boutons in APP/PS1 cultures. These results are entirely consistent with the electrophysiological experiments, which demonstrate that synaptic plasticity is impaired in APP/PS1 mice primarily because of presynaptic dysfunction.

Next, we examined the number of immunoreactive clusters for the presynaptic protein synapsin I, before and after glutamate application, to determine whether overexpression of APP and PS1 transgenes altered the basal number of clusters or the glutamate-induced long-lasting increase in the number of clusters. We found a 0.5-fold increase in the basal number of synapsin I immunoreactive clusters in APP/PS1 cultures compared with that in WT cultures. We also found an approximate twofold increase in the number of synapsin I-

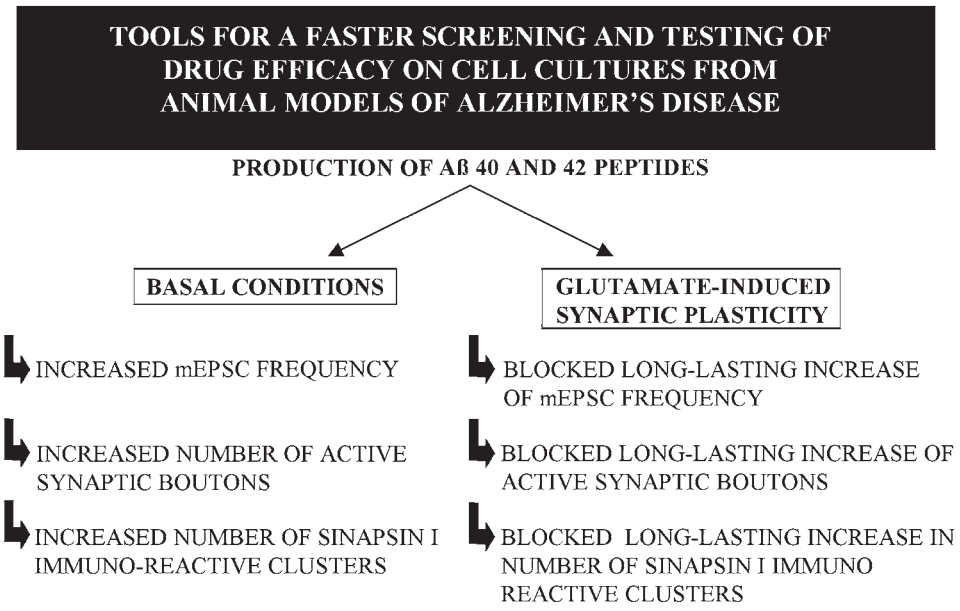

Fig. 1. Tools for faster screening and testing of drug efficacy.

immunoreactive clusters after glutamate application in WT cultures, whereas such increase was not induced in APP/PS1 cultures. These results provide additional evidence that presynaptic release machinery is altered in cultures from transgenic animals producing elevated levels of Aβ peptides. Moreover, these findings are consistent with the block of glutamate-induced plasticity observed with electrophysiological and video-imaging experiments.

Discussion

Neurotransmission in hippocampal cultures exhibits fundamental characteristics that are highly similar to those in vivo and/or in tissue slices. As a result, cell cultures can be used in lieu of other preparations to examine neurotransmission. Here, we report that cultures derived from APP/PS1 mice producing elevated levels of Aβ 40 and 42 peptides present important synaptic changes, including an increase in the basal frequency of spontaneous release of neurotransmitter, in the basal number of active boutons, and in the basal number of immunoreactive clusters for synapsin I. These changes are associated with lack of synaptic plasticity consisting of glutamate-induced increase in mEPSC frequency, active bouton number, and number of synapsin I immunoreactive clusters (Fig. 1).

Much evidence has been provided to suggest that, in AD, memory starts to fail before brain cells die and that the disease, with its memory loss, begins as an interruption or dysfunction of the signaling between living and healthy brain cells (Masliah, 1995). Frequency of spontaneous neurotransmitter release from the presynaptic terminal, a type of activity occurring at the synaptic level, was increased in cultures from double transgenic APP/PS1 animals compared with cultures from WT mice. Interestingly, this increase precedes any morphological and behavioral change occurring in live adult animals (Sant'Angelo et al., 2003; Trinchese et al., 2004). The Tg2576 transgenic mouse, for instance, expressing the Swedish mutation (K670N:M671L) in APP, shows increased brain Aβ levels by 6–8 mo of age, followed by development of neuritic plaques in the neocortex and hippocampus (Hsiao et al., 1996; Irizarry et al., 1997; Frautschy et al., 1998), and impairment of the cognitive function by 9–10 mo (Holcomb et al., 1998; Chapman et al., 1999; King et al., 1999). Coexpression of mutant PS1, which increases Aβ 42 generation, potentiates amyloid deposition in Tg2576 mice to the extent that some lines begin to develop plaques (McGowan et al., 1999), together with abnormal spatial working memory at about 2 mo of age; Trinchese et al., 2004). Thus, basal mEPSC frequency measured in cell cultures about 7–14 d after birth is one parameter that can be used as a tool in an in vitro screening or testing system to

assess and evaluate drug efficacy for use in therapy for AD. In this manner, cultured cells from double transgenic APP/PS1 animals, or from other transgenic animal models of AD, are useful for testing a compound and determining whether the compound can return the basal frequency of mEPSC to normal levels.

An additional parameter, synaptic plasticity, can also be addressed in cell cultures. Glutamate-induced, long-term enhancement of mEPSC frequency, a form of synaptic plasticity (Antonova et al., 2001; Malgaroli and Tsien, 1992), is also altered in hippocampal cell cultures prepared from double transgenic mice compared with that from WT littermates. In this manner, cultured cells from double transgenic APP/PS1 animals, or from other transgenic animal models of AD, are useful for testing a compound and to determine whether the compound can restore the capability of the cultured transgenic cells to regain the ability to undergo plastic changes.

Synaptic transmission is attributable to the release of neurotransmitter that is stored in synaptic vesicles and secreted by exocytosis from the presynaptic terminal. This process is triggered by membrane depolarization due to an action potential, which causes Ca^{2+} influx into the nerve terminal through voltage-gated calcium channels. The next steps include the participation of a series of vesicle-associated proteins, called v-SNARE, together with presynaptic plasma membrane proteins, called t-SNARE, to ensure processes of docking and fusion of synaptic vesicles to the cell membrane of the bouton. After exocytosis, empty synaptic vesicles are rapidly recovered and recycled (Sollner et al., 1993a; 1993b; Sollner and Rothman, 1994; Hay and Scheller, 1997). We have found that the number of active synaptic boutons, both in basal conditions and after glutamate application in APP/PS1 cultures, is an additional test that can be used as a tool for testing a compound, as well as for determining whether that compound can restore the capability of the cultured transgenic cells to undergo normal changes in the number of functional boutons. Most important, findings with these optical techniques can be adapted in a high-throughput type of screening.

Presynaptic proteins have been demonstrated to be altered in AD. Analyses in postmortem AD patients brains revealed a significant decrease in presynaptic proteins (Sze et al., 2000), whereas studies on transgenic animals overexpressing APP revealed an increase caused by Aβ peptides (Mucke et al., 1994). The basal number of immunoreactive clusters for synapsin I is increased in APP/PS1 cultures compared with the number in WT cultures. Also the capability to undergo increase in the number of immunoreactive clusters for synapsin I following glutamate application is impaired in hippocampal cultures of double transgenic APP/PS1 mice. Therefore, the increase in the number of presynaptic protein clusters following glutamate application might be used as a parameter to test potential pharmaceutical therapies on this cell culture system.

It is likely that Aβ peptides play a role in neuronal dysfunction and AD pathological changes (Hardy, 1997; Calhoun et al., 1998; Price and Sisodia, 1998; Hsia et al., 1999; Selkoe, 1999; Mucke et al., 2000). In this context, attention should be directed to the fact that cell cultures from AD models offer the advantage of producing Aβ, which can be exploited in at least two ways: (1) for screening drugs that interfere with the production of Aβ, and (2) as a source for production of Aβ that can be isolated and used for behavioral, electrophysiological, and toxicological testing.

According to the method described in this paper, cell cultures constitute an easy, fast, and inexpensive way of screening drugs for use in the treatment or prevention of AD. The example of APP/PS1 cultures should be of special interest to the neuroscience community, provided that cell cultures can be established from a variety of different types of available animal models of AD and, therefore, provide researchers not only with different systems to test drugs for AD but also with an effective approach to further their knowledge of the pathologic processes of the disease itself.

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References

Antonova I., Arancio O., Trillat A. C., Wang H. G., Zablow L., Udo H., et al. (2001) Rapid increase in immunoreactive presynaptic terminals during long-lasting potentiation. *Science* **294,** 1547–1550.

Arancio O., Antonova I., Gambaryan S., Lohmann S. M., Wood J. S., Lawrence D. S., and Hawkins R. D. (2001) Presynaptic role of cGMP-dependent protein kinase during long-lasting potentiation. *J. Neurosci.* **21**, 143–149.

Arancio O., Kandel E. R., and Hawkins R. D. (1995) Activity-dependent long-term enhancement of transmitter release by presynaptic 3′,5′-cyclic GMP in cultured hippocampal neurons. *Nature* **376**, 74–80.

Bliss T. V. and Collingridge G. L. (1993) A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361**, 31–39.

Calhoun M., Wiederhold K., Abramowski D., Phinney A., Probst A., Sturcheler-Pierrat C., et al. (1998) Neuronal loss in APP transgenic mice. *Nature* **395**, 755–756.

Chapman P. F., White G. L., Jones M. W., Cooper-Blacketer D., Marshal V. J., Irizarry, M., et al. (1999) Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. *Nat. Neurosci.* **2**, 271–276.

Duff K., Eckman C., Zehr C., Yu X., Prada C. M., Perez-tur J., et al. (1996) Increased amyloid-beta42(43) in brains of mice expressing mutant presenilin 1. *Nature* **383**, 710–713.

Frautschy S. A., Yang F., Irrizarry M., Hyman B., Saido T. C., Hsiao K., and Cole G. M. (1998) Microglial response to amyloid plaques in APPsw transgenic mice *Am. J. Pathol.* **152**, 307–317.

Hardy J. (1997) Amyloid, the presenilins and Alzheimer's disease. *Trends Neurosci.* **29556**, 28570–26998.

Hay J.C. and Scheller R.H. (1997) SNAREs and NSF in tar-geted membrane fusion. *Curr. Opin. Cell Biol.* **9**, 505–512.

Holcomb L., Gordon M. N., McGowan E., Yu X., Benkovic S. Jantzen P., et al. (1998) Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat. Med.* **4**, 97–100.

Hsia A.Y., Masliah E., McConlogue L., Yu G.- Q., Tatsuno G., Hu K., et al. (1999) Plaque-independent disruption of neural circuits in Alzheimer's disease mouse models. *Proc. Natl. Acad. Sci. U. S. A.* **96**, 3228–3233.

Hsiao K., Chapman P., Nilsen S., Heckman C., Harigaya Y., Younkin S., et al. (1996) Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science* **274**, 99–102.

Irizarry M. C., McNamara M., Fedorchak K., Hsiao K., and Hyman B. T. (1997) APPSw transgenic mice develop age-related A beta deposits and neuropil abnormalities, but no neuronal loss in CA1. *J. Neuropathol. Exp. Neurol.* **56**, 965–973.

Janus C., Pearson J., McLaurin J., Mathews P.M., Jiang Y., Schmidt S. D., et al. (2000) A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature* **408**, 979–982.

King D. L., Arendash G. W., Crawford F., Sterk T., Menendez J., and Mullan M. J. (1999) Progressive and gender-dependent cognitive impairment in the APP(SW) transgenic mouse model for Alzheimer's Disease. *Behav. Brain Res.* **103**, 145–162.

Malgaroli A.and Tsien R.W. (1992) Glutamate-induced long-term potentiation of the frequency of miniature synaptic currents in cultured hippocampal neurons. *Nature* **357,** 134-139.

Masliah E. (1995) Mechanisms of synaptic dysfunction in Alzheimer's disease. *Histol. Histopathol.* **10**, 509–519.

McGowan E., Sanders S., Iwatsubo T., Takeuchi A., Saido T., Zehr C., et al. (1999) Amyloid phenotype characterization of transgenic mice overexpressing both mutant amyloid precursor protein and mutant presenilin 1 transgenes. *Neurobiol. Dis.* **4**, 231–244.

Milner B. (1996) Amnesia following operation on the temporal lobes, in *Amnesia*, Whitty, C. W. M., and Zangwill, O. L., eds., Butterworths, London, UK, pp. 109–133.

Mucke L., Masliah E., Johnson W. B., Ruppe M. D., Alford M., Rockenstein E. M., et al. (1994) Synaptotrophic effects of human amyloid beta protein precursors in the cortex of transgenic mice. *Brain Res.* **666**, 151–167.

Mucke L., Masliah E., Yu G.- Q., Mallory M., Rockenstein E. M., Tatsuno G., et al. (2000) High-level neuronal expression of $A\beta_{1\text{-}42}$ in wild-type human amyloid protein precursor transgenic mice: Synaptotoxicity without plaque formation. *J. Neurosci.* **20,** 4050–4058.

Price D. L. and Sisodia S. S. (1998) Mutant genes in familial Alzheimer's disease and transgenic models. *Annu. Rev. Neurosci.* **21,** 479–505.

Ryan T. A., Reuter H., Wendland B., Schweizer F. E., Tsien R. W., and Smith S. J. (1993) The kinetics of synaptic vesicle recycling measured at single presynaptic boutons. *Neuron* **11,** 713–724.

Sant'Angelo A., Trinchese F., and Arancio O. (2003) Usefulness of behavioral and electrophysiological studies in transgenic models of Alzheimer's disease. *Neurochem. Res.* **28,** 1009–1015.

Selkoe D. J. (1999) Translating cell biology into therapeutic advances in Alzheimer's disease. *Nature* **399,** A23–A31.

Sollner T. and Rothman J. E. (1994) Neurotransmission: harnessing fusion machinery at the synapse. *Trends Neurosci.* **17,** 344–348.

Sollner T., Bennett M. K., Whiteheart S. W., Scheller R. H., and Rothman J. E. (1993a) A protein assembly-disassembly pathway in vitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. *Cell* **75,** 409–418.

Sollner T., Sollner T., Whiteheart S. W., Brunner M., Erdjument-Bromage H., Geromanos S., et al. (1993b) SNAP receptors implicated in vesicle targeting and fusion. *Nature* **362,** 318–324.

Sze C. I., Bi H., Kleinschmidt-DeMasters B. K., Filley C. M., and Martin L. J. (2000) Selective regional loss of exocytotic presynaptic vesicle proteins in Alzheimer's disease brains. *J. Neurol. Sci.* **175,** 81–90.

Trinchese F., Luis S., Baltaglia F., Walter S., Matthews P. M., and Arancio O. (2004) Progressive age-related development of Alzheimers-like pathology in APP/PSI mice. *Ann. Neurol.* **55,** 801–814.