Complex environment experience rescues impaired neurogenesis, enhances synaptic plasticity, and attenuates neuropathology in familial Alzheimer’s disease-linked APPswe/PS1ΔE9 mice

Yuan-Shih Hu,* Peng Xu,† Gustavo Pigino,* Scott T. Brady,* John Larson,† and Orly Lazarov*1†

*Department of Anatomy and Cell Biology and †Psychiatric Institute, Department of Psychiatry, College of Medicine, University of Illinois, Chicago, Illinois, USA

ABSTRACT Experience in complex environments induces numerous forms of brain plasticity, improving structure and function. It has been long debated whether brain plasticity can be induced under neuropathological conditions, such as Alzheimer’s disease (AD), to an extent that would reduce neuropathology, rescue brain structure, and restore its function. Here we show that experience in a complex environment rescues a significant impairment of hippocampal neurogenesis in transgenic mice harboring familial AD-linked mutant APPswe/PS1ΔE9. Proliferation of hippocampal cells is enhanced significantly after enrichment, and these proliferating cells mature to become new neurons and glia. Enhanced neurogenesis was accompanied by a significant reduction in levels of hyperphosphorylated tau and oligomeric Aβ, the precursors of AD hallmarks, in the hippocampus and cortex of enriched mice. Interestingly, enhanced expression of the neuronal anterograde motor kinesin-1 was observed, suggesting enhanced axonal transport in hippocampal and cortical neurons after enrichment. Examination of synaptic physiology revealed that environmental experience significantly enhanced hippocampal long-term potentiation, without notable alterations in basal synaptic transmission. This study suggests that environmental modulation can rescue the impaired phenotype of the Alzheimer’s brain and that induction of brain plasticity may represent therapeutic and preventive avenues in AD.—Y.-S. Hu, P. Xu, G. Pigino, S. T. Brady, J. Larson, O. Lazarov. Complex environment experience rescues impaired neurogenesis, enhances synaptic plasticity, and attenuates neuropathology in familial Alzheimer’s disease-linked APPswe/PS1ΔE9 mice. *FASEB J. 24, 1667–1681 (2010). www.fasebj.org

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Alzheimer’s disease (AD) is a progressive neurodegenerative disease that is the most prevalent cause of dementia in adults. Affected individuals experience progressive memory loss, difficulties in learning, diminished recall accuracy, and impaired problem solving and cognition (1). Rare familial cases of AD (FAD) are caused by mutations in presenilin 1 (PS1), presenilin 2 (PS2), and amyloid precursor protein (APP) (for review, see ref. 2). The vast majority of AD cases are the sporadic, late-onset form of the disease, suggesting that environmental factors may play a role in disease development. The hallmarks of the disease are amyloid deposits of aggregated β-amyloid (Aβ) peptides and neurofibrillary tangles, which are intracellular aggregates of hyperphosphorylated tau. Aβ is a 4-kDa peptide that is a proteolytic cleavage product of APP. Soluble oligomeric forms of Aβ peptides accumulating in the disease are precursors of amyloid deposits. Oligomeric Aβ is thought to be neurotoxic and to impair learning and memory (3), synaptic plasticity, and hippocampal long-term potentiation (LTP) (4, 5) (for reviews, see refs. 6, 7). Hyperphosphorylation of the microtubule-associated protein tau reflects the misregulation of several kinases in AD that can compromise axonal transport, including GSK3 and CK2 (8–11). These changes lead to a pattern of dying-back neuropathy of mature neurons in AD. Progressive neuronal loss takes place in specific brain areas, such as the hippocampal formation and the entorhinal cortex (12–15). In addition, increasing evidence suggests that altered or compromised neurogenesis may contribute to the cognitive impairments and neuronal vulnerability that characterize the disease. Indeed, numerous studies report impaired hippocampal neurogenesis in mouse models exhibiting high levels of Aβ, amyloid deposition (16–21), and neurofibrillary tangles (22). In support of that, recent evidence suggests a crosstalk between neurogenic signaling and molecular players in FAD, such as APP and PS1 (for review, see ref. 23). Because the hippocampus is one of the earliest areas in the brain to be affected in AD, it is reasonable to assume that AD neuropathology may affect impaired recall accuracy, and impaired problem solving and cognition (1). Rare familial cases of AD (FAD) are caused by mutations in presenilin 1 (PS1), presenilin 2 (PS2), and amyloid precursor protein (APP) (for review, see ref. 2). The vast majority of AD cases are the sporadic, late-onset form of the disease, suggesting that environmental factors may play a role in disease development. The hallmarks of the disease are amyloid deposits of aggregated β-amyloid (Aβ) peptides and neurofibrillary tangles, which are intracellular aggregates of hyperphosphorylated tau. Aβ is a 4-kDa peptide that is a proteolytic cleavage product of APP. Soluble oligomeric forms of Aβ peptides accumulating in the disease are precursors of amyloid deposits. Oligomeric Aβ is thought to be neurotoxic and to impair learning and memory (3), synaptic plasticity, and hippocampal long-term potentiation (LTP) (4, 5) (for reviews, see refs. 6, 7). Hyperphosphorylation of the microtubule-associated protein tau reflects the misregulation of several kinases in AD that can compromise axonal transport, including GSK3 and CK2 (8–11). These changes lead to a pattern of dying-back neuropathy of mature neurons in AD. Progressive neuronal loss takes place in specific brain areas, such as the hippocampal formation and the entorhinal cortex (12–15). In addition, increasing evidence suggests that altered or compromised neurogenesis may contribute to the cognitive impairments and neuronal vulnerability that characterize the disease. Indeed, numerous studies report impaired hippocampal neurogenesis in mouse models exhibiting high levels of Aβ, amyloid deposition (16–21), and neurofibrillary tangles (22). In support of that, recent evidence suggests a crosstalk between neurogenic signaling and molecular players in FAD, such as APP and PS1 (for review, see ref. 23). Because the hippocampus is one of the earliest areas in the brain to be affected in AD, it is reasonable to assume that AD neuropathology may affect
and alter neurogenesis early in the disease. In this report, we show that transgenic mice harboring FAD-linked APPswe/PS1ΔE9 mutants exhibit impairments in hippocampal neurogenesis early in life, at the age of 2 mo. This observation is in agreement with other studies, suggesting a significant impairment of hippocampal neurogenesis in various models of AD in transgenic mice as young as 3 mo old, as well as older (24–27).

The “enriched environment” experimental paradigm (28), was instrumental in demonstrating that brain structure is dynamically responsive to experience, even in adults (29–31). In an enriched environment, the animals are exposed to a complex array of stimuli (e.g., toys, obstacles, and tunnels), allowed freedom to move and exercise, and provided with more social stimulation than animals housed in standard laboratory conditions. Numerous studies indicate that environmental enrichment induces dendritic growth, stimulates dendritic branching, promotes the formation of new dendritic spines, enhances hippocampal neurogenesis, and results in increased numbers of synapses per neuron in many forebrain structures (32–35). Enrichment also enhances memory function in various learning tasks (31, 36–39).

These attributes raised the question of whether enrichment-induced brain plasticity would prevent or attenuate neuropathology in neurodegenerative diseases, particularly in AD. Using the enriched environment paradigm, we and others have shown that modulation of environmental factors significantly attenuates steady-state levels of Aβ and reduces extent of amyloid deposits in the brains of transgenic mice harboring FAD-linked APP and PS1 mutations (40–43). However, using different experimental conditions that affect variables such as duration of treatment related to the onset of deposition, some studies found no change in amyloid levels (44, 45) or even an increase (46).

Although the effect of environmental enrichment on amyloid metabolism is controversial, the consensus seems to be that the experience of FAD-linked transgenic mice in an enriched environment enhances learning and memory (43, 45, 47). However, the mechanism by which enrichment enhances learning and memory is largely unknown. The effect of an experience in a complex environment on tau pathology, another neuropathological hallmark of the disease, is also unclear. Finally, while enriched environment unequivocally induces hippocampal neurogenesis (48), it is not clear whether it would attenuate impaired neurogenesis in the APPswe/PS1ΔE9 transgenic mice, because neurogenesis could not be enhanced after enrichment in transgenic mice harboring FAD-linked PS1 mutant variants (49).

In an attempt to reconcile these findings, we examined the effect of enrichment experience on neuropathology, neurophysiology, and neurogenesis in young transgenic mice harboring FAD-linked APPswe/PS1ΔE9 before the onset of deposition. We reasoned that because these are the soluble oligomeric forms of Aβ that are highly neurotoxic (7), it is critical to determine whether experience in a complex environment would modulate these forms specifically. In addition, we explored the possibility that enrichment-induced facilitation of learning and memory in these mice may be the result, at least in part, of enhanced hippocampal neurogenesis, LTP, or both (48, 50, 51). Transgenic mice harboring FAD-linked APPswe/PS1ΔE9 were used because these mice exhibit high levels of oligomeric Aβ, leading to amyloid deposition at 4–5 mo of age. In addition, these mice exhibit deficits in learning and memory (47) and impaired neurogenesis in the subgranule layer (SGL) of the dentate gyrus (DG) and the subventricular zone (SVZ) of the lateral ventricles. Interestingly, high levels of hyperphosphorylated tau are also observed in neurogenic areas of these mice (19).

We demonstrate that the experience of APPswe/PS1ΔE9 mice in enriched, complex environmental conditions enhances neural progenitor cell (NPC) proliferation, differentiation, and maturation in the hippocampus. Enrichment-induced increases in the number of new neurons and astrocytes were comparable to the increase observed in nontransgenic (NonTg) wild-type mice, suggesting that experience in enriched environments can rescue the deficient neurogenic phenotype of these mice. Rescue of neurogenesis was accompanied by a significant reduction in levels of neurotoxic soluble oligomeric Aβ peptides and in levels of hyperphosphorylated tau in the hippocampus and cortex of these mice. Interestingly, the expression levels of the main anterograde motor, kinesin-1, were up-regulated in brains of transgenic mice that experienced an enriched environment, suggesting enhanced axonal transport. Finally, an enriched environment enhanced LTP in the CA1 field of hippocampal slices.

These results indicate that experience in an enriched environment attenuates neuropathology, enhances neurogenesis, and facilitates synaptic plasticity in APPswe/PS1ΔE9 mice. Further efforts should be made to elucidate the full mechanisms and translate this promising approach to a preventive or therapeutic regimen for the amelioration of cognitive decline and neuropathology in AD.

MATERIALS AND METHODS

Transgenic animals and environmental enrichment

The generation of mice coexpressing human PS1 encoding ΔE9 mutation and mouse APP containing humanized Aβ and Swedish mutations (K595N, M596L) was described previously (52). The animals were maintained in standard laboratory conditions (14/10 h light-dark cycle) and with full access to food and water ad libitum. Male FAD-linked APPswe/PS1ΔE9 transgenic mice (total n=26; n=11 for biochemical and immunohistochemical analysis, n=15 for LTP experiment) and NonTg littermate mice (total n=19; n=10 for biochemical and immunohistochemical analysis, n=9 for LTP experiment) were exposed to enriched environmental conditions right after weaning (P21) for a period of 1 mo (or 2 mo for long-term cell survival studies). Mice were maintained in groups of 5 or 4 males/cage. The enriched environment was
composed of running wheels, colored tunnels, visual stimulating toys, and free access to food pellets and water in the enlarged cages (~24×17×11 inches), where objects in the cage were repositioned for novel stimulation every day. Mice were exposed to the enriched environment for 3 h every day and returned to the standard housing cages (~11×6×8 inches) for the rest of the day. Control groups of transgenic mice (total n=22; n=12 for biochemical and immunohistochemical analysis, n=10 for LTP experiment) and NonTg (total n=21; n=12 for biochemical and immunohistochemical analysis, n=9 for LTP experiment) animals were singly housed in standard housing for 1 mo (or 2 mo for the long-term survival study).

5′-Bromo-2′-deoxyuridine (BrdU) administration

A solution of BrdU (Sigma-Aldrich, St. Louis, MO, USA) was prepared at 20 mg/ml in sterile saline and injected intraperitoneally at a dose of 100 mg/kg every 12 h for 3 consecutive days, on PS8 to PS9 of environmental enrichment or standard housing. The animals were sacrificed 3 h or 1 mo after the last BrdU injection.

Immunohistochemistry

Mice were anesthetized with ketamine (100 mg/kg) and xylazine (5 mg/kg) and transcardially perfused with ice-cold 1X PBS. The brains were then dissected into two hemispheres, one of which was frozen directly for biochemical analysis. The other hemisphere was placed in 4% paraformaldehyde for 3 d at 4°C and transferred to a 30% sucrose solution until used. Brains were sectioned sagittally into 50-μm-thick sections, and each section was stored separately in a 96-well plate immersed in cryoprotectant. Brain sections were pretreated with deionized formamide/SSC solution for 2 h at 65°C, incubated in 2N HCl for 30 min at room temperature, and rinsed in 0.1 M borate buffer for 10 min at room temperature. Sections were subsequently incubated in primary antibody cocktails that consisted of monoclonal rat anti-BrdU (1:500; Accurate Chemical & Scientific Corporation, Westbury, NY, USA), biotinylated polyclonal rabbit anti-glia fibrillary acidic protein (GFAP; 1:500; DakoCytomation, Glostrup, Denmark), polyclonal goat anti-dou- blecortin (DCX; 1:400; Santa Cruz Biotechnology, Santa Cruz, CA, USA), monoclonal mouse anti-NeuN (1:400; Chemicon, Billerica, MA, USA), monoclonal mouse anti-nestin (1:400; Millipore, Billerica, MA, USA) and polyclonal goat anti-tubulin (TUB; 1:250; Santa Cruz Biotechnology) for 72 h at 4°C. Immunofluorescence secondary antibodies used in this study were Cy3-TM3 donkey anti-rat (1:500; Jackson ImmunoResearch, West Grove, PA, USA), Cy5 donkey anti-goat (1:250; Jackson ImmunoResearch), Cy5 donkey anti-mouse (1:250; Jackson ImmunoResearch), and Cy2-conjugated streptavidin (1:250; Jackson ImmunoResearch).

Stereological analysis

Quantification of positive cell markers within the DG of the hippocampus was performed by design-based stereology (StereoInvestigator 8; MBF Bioscience, Williston, VT, USA) using an optical fractionator applying the N x V ref method (for a review, see refs. 53, 54), as used previously (55), with some modifications. For the analysis of total BrdU, DCX, and GFAP cells, every 6th section was taken for immunohistochemical and stereological analysis. The DG was traced at low magnification (×10), and all cell counts were performed at high magnification (×63; Zeiss AX10 microscope; Carl Zeiss Ltd., Hertfordshire, England). The sampling parameters were as follows: counting frame width W = 100 μm, counting frame height Y = 100 μm, sampling grid size X = 148 μm, sampling grid size Y = 210 μm, disector height Z = 20 μm, and section periodicity = 6. For the analysis of NeuN+ and S100B+ cells, every 3rd section was taken for immunohistochemical and stereological analysis. The section periodicity was changed to 3, with sampling grid sizes X and Y = 100 μm, and counting frame sizes X and Y = 100 μm.

Two-step protein extraction for dot-blot analysis and Western blot analysis

Two-step protein extraction was performed to obtain water-soluble fractions for oligomeric Aβ detection and detergent-soluble fractions for Western blot analysis. First, tissues from cortex and hippocampus were thoroughly homogenized in 1X PBS containing protease-inhibitor cocktail (Sigma-Aldrich) and ultracentrifuged at 100,000 g for 1 h at 4°C, as described previously (56). The supernatant was collected, and the protein concentration was calculated by the bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA). The pellet immediately underwent second-step extraction in ROLB buffer for phosphorylation-sensitive protein extraction (10 mM HEPES (pH 7.4), 0.5% Triton X-100, 80 mM β-glycerophosphate, 50 mM sodium fluoride, 2 mM sodium orthovanadate, 100 nM staurosorpine, 100 nM K252a, 50 nM okadaic acid, and 50 nM microcin (Calbiochem, Gibbstown, NJ, USA); mammalian protease inhibitor cocktail (Sigma-Aldrich); and phosphatase inhibitor cocktail II (Calbiochem)]. Protein quantification was performed using the BCA method (Pierce), and an equal concentration of proteins was used for immunoblotting. Membranes were blocked overnight at 4°C with polyclonal rabbit anti-APP 369 antibodies (1:2000; a generous gift from Dr. Sangram S. Sisodia, University of Chicago, Chicago, IL, USA), monoclonal mouse anti-phosphorylated Tau (PHF-1; 1:2500; ref. 57), monoclonal mouse anti-Tau5 (1:10,000; Chemicon), monoclonal mouse anti-kine- nin heavy chain (KHC; H2; 1:2000; ref. 11), monoclonal mouse anti-kinesin light chain (KLC; 63-90; 1:2000; ref. 58), monoclonal mouse anti-Tau5 (1:10,000; Chemicon), and monoclonal mouse anti-actin (1:5000; Chemicon). Secondary antibodies used for Western blots were rabbit anti-mouse horseradish peroxidase (1:5000) and protein A-peroxidase (1:1000; Pierce). For dot-blot analysis, 50 μg of water-soluble protein fraction was directly blotted onto the prewetted nitrocellulose membrane assembled within the dot-blot apparatus (Bio-Rad, Hercules, CA, USA). The membrane was later blocked with 5% nonfat milk/ TBST solution for 2 h at room temperature and incubated in primary antibody, polyclonal rabbit anti-amyloid oligomer (A11; 1:5000; Millipore) overnight at 4°C. Protein expression levels were quantified by densitometric analysis using ImageJ 1.41o software (National Institutes of Health, Bethesda, MD, USA).

Electrophysiology

Mice (APPswe/PS1ΔE9 and NonTg) were sacrificed within 5 d after the termination of 1 mo differential experience (as above), and hippocampal slices were prepared as described (59). Slices were maintained at 35 ± 1°C in an interface chamber constantly perfused (1.0 ml/min) with medium containing 124 mM NaCl, 3 mM KCl, 1.2 mM KH2PO4, 26 mMNaHCO3, 10 mM Na-glucose, 2.5 mM CaCl2, 2.5 mM MgSO4, and 2 mM Na-ascorbate, gassed with 95% O2/5% CO2. Schaffer/commissural synapses were stimulated with twisted, bipolar electrodes placed in stratum radiatum (SR) of field CA1a or CA1c; field excitatory postsynaptic potentials (EPSPs) were recorded with a glass micropipette placed in SR of CA1b. Basal synaptic transmission was assessed by constructing input-output curves using stimulation currents of 2.5–160 μA. Paired-pulse facilitation was assessed with
interpulse intervals from 50 to 800 ms. LTP was induced by θ-burst stimulation (TBS) consisting of 2, 4, or 8 high-frequency bursts (100 Hz, 4 pulses) repeated at 200-ms intervals (5 Hz). The fEPSP slope was monitored at 20-s intervals for ≥10 min before and 60 min after TBS. LTP was assessed as the potentiation present 30 and 60 min after TBS.

Statistical analysis

Data are presented as means ± se. Histological and biochemical data were analyzed statistically using Student’s t-test or 2-way ANOVA analysis using Prism version 5.00 for Windows (Graph-Pad Software, San Diego, CA, USA). Electrophysiological data were analyzed by repeated measures ANOVA, with genotype and environmental condition as between-subjects factors and stimulus intensity (input-output curves) or inter pulse interval (paired-pulse facilitation) as within-subjects factors, or by factorial ANOVA (LTP) with genotype, environmental condition, and TBS burst number as between-subjects factors. Results were considered statistically significant when \( P < 0.05 \).

RESULTS

Reduced numbers of proliferating cells in the DG of FAD-linked APPswe/PS1ΔE9 can be rescued by experience in an enriched environment

To evaluate whether the extent of increase in the number of proliferating cells in the hippocampus after experience in an enriched environment is consistent across genotypes, we applied 2-way ANOVA analysis. Two-way ANOVA analysis showed a significant effect of housing conditions \((F_{1,16}=54.88, P<0.0001)\) and genotype \((F_{1,16}=10.84, P<0.01)\), with no significant interaction between the two parameters \((F_{1,16}=0.08642, P=0.7726)\), suggesting that the extent of increase in proliferating cells after enrichment is comparable in mutant and NonTg mice. This result suggests that the significant increase in cell proliferation after experience in an enriched environment is consistent across genotypes, such that APPswe/PS1ΔE9-expressing hippocampal cells have the capacity to proliferate to the same extent as wild-type cells when given the appropriate stimuli.

More new neurons survive and incorporate in the DG of APPswe/PS1ΔE9 mice after enrichment

To determine whether the enrichment-induced increase in the number of proliferating NPCs is manifested by an

Figure 1. Environmental enrichment. A) Enrichment cage, composed of running wheels, colorful tunnels, and toys. Components in the cage were reconfigured every day to ensure novelty. Control mice were singly housed in standard laboratory cages. B) Experimental design. Mice were weaned at P21 and housed either 3 or 4 animals/cage (experimental group). Mice in the experimental group experienced enriched environmental conditions for 3 h every day. To examine cell proliferation and early differentiation in the SGL, mice were injected with BrdU on the last 3 d of enrichment (P58–P60) and sacrificed 3 h after the last injection (experimental group 1). To examine newly differentiated neurons in the SGL, animals were allowed to experience an enriched environment for another month and sacrificed at P90 (experimental group 2). Mice in the control groups were singly housed in standard laboratory cages and were subjected to same BrdU regimen.
increase in the number of new neurons, brain sections were immunostained with antibodies raised against neuronal differentiation markers (Fig. 3). The number of new neurons, as detected by the number of newly formed cells expressing doublecortin (BrdU+/DCX−) was significantly decreased in the SGL of APPswe/PS1ΔE9 mice maintained in standard housing, compared with wild-type mice housed in the same conditions (Fig. 2A), suggesting that APPswe/PS1ΔE9 mice exhibit impaired neuronal differentiation in the hippocampus. However, after enrichment, the number of new neurons increased dramatically in both APPswe/PS1ΔE9 and their NonTg wild-type littermates (Fig. 3A, C). The extent of enrichment-induced increase in the number of new neurons relative to the number of new neurons in the corresponding standard-housing groups was comparable in both groups, as determined by 2-way ANOVA, demonstrating that the enriched environment affects both genotypes to the same extent (F₁,₁₆=0.6603, P=0.4284) and that experience in an enriched environment can rescue impaired formation of new neurons in the hippocampus of APPswe/PS1ΔE9 mice.

To examine whether the increase in the number of newly formed neurons is manifested by a greater number of mature neurons in the DG of APPswe/PS1ΔE9 mice after enrichment, mice were injected with BrdU from P58 to P60 and subjected to enriched environmental conditions for 1 mo. Control groups were subjected to the same BrdU regimen and maintained in standard housing conditions for 1 mo. All animals were sacrificed at P90 (Fig. 1B, group 2). The number of newly formed mature neurons, as detected by the number of cells expressing NeuN (BrdU+/NeuN−; Fig. 3B, D), was significantly increased in the granular layer of the DG of both NonTg and APPswe/PS1ΔE9 mice after experience in an enriched environment (Fig. 3B), and the extent of increase was comparable in both genotypes (F₁,₁₂=1.353, P=0.2673). In agreement with a previous report (25), the number of BrdU+/NeuN+ cells observed was not significantly different between NonTg and APPswe/PS1ΔE9 mice maintained in standard housing conditions, suggesting that the reduced number of BrdU+ cells in APPswe/PS1ΔE9 mice is due to impaired proliferation and/or differentiation rather than a decrease in NPC survival.

Number of new astrocytes in the brains of APPswe/PS1ΔE9 mice increases after enrichment

Examination of the number of newly formed astrocytes using antibodies raised against GFAP (Fig. 4A, D) revealed that APPswe/PS1ΔE9 mice maintained in standard housing did not exhibit a significant decrease in the number of newly
formed astrocytes (BrdU\(^+\)/GFAP\(^+\); Fig. 4A). A significant increase in the number of newly formed astrocytes (BrdU\(^+\)/GFAP\(^+\)) in both APP\(\text{swe}/\text{PS1}\Delta\text{E9}\) and their NonTg wild-type littermates was observed after experience in an enriched environment (Fig. 4A). In contrast, examination of the number of newly formed S100\(^+\) cells, a marker for a subset of astrocytes and microglia (62; Fig. 4D), revealed no significant increase in the number of these cells in APP\(\text{swe}/\text{PS1}\Delta\text{E9}\) mice after experience in an enriched environment (Fig. 4B), suggesting that stimuli of the complex environment up-regulate a selective target population of NPCs in the DG. To further characterize these BrdU\(^+\)/GFAP\(^+\) cells, brain sections were coimmunolabeled with the neural stem cell markers nestin and Sox2. We show that BrdU\(^+\)/GFAP\(^+\) newly formed astrocytes colocalize with nestin and Sox2, suggesting that these BrdU\(^+\)/GFAP\(^+\) cells represent NPCs (Fig. 4F, G).

Taken together, these results suggest that experience of APP\(\text{swe}/\text{PS1}\Delta\text{E9}\) mice in a complex environment rescues neurogenic impairments exhibited by the transgenic mice maintained in standard housing. Environmental enrichment induces an increase in neurogenesis in the transgenic mice that is comparable to the response observed in wild-type NonTg littermates.

**Environmental enrichment attenuates amyloid pathology by reducing oligomeric A\(\beta\) levels in the cortex and hippocampus of FAD-linked APP\(\text{swe}/\text{PS1}\Delta\text{E9}\) transgenic mouse**

The hippocampus is one of the earliest brain areas exhibiting amyloid deposits and neurofibrillary tangles and is most heavily affected in AD (14). Thus, it would be reasonable to assume that impaired neurogenesis in the SGL of the DG may result, in part, from altered amyloid and tau metabolism. Notably, in addition to impairing cognitive function and neurotoxicity (3, 63), oligomeric A\(\beta\) was shown recently to impair the proliferation of human embryonic stem cells (64). Because
impaired neurogenesis and rescued neurogenic response were observed in APPswe/PS1E9 mice maintained in standard housing and an enriched environment, respectively, at 2 mo of age (Figs. 2–4), 2–3 mo before onset of deposition, we examined levels of soluble oligomeric Aβ in the brains of these mice. For this purpose, we prepared brain protein extract from the cortex and the hippocampus of FAD-linked APPswe/PS1E9 mice that were maintained in standard housing conditions or exposed to an enriched environment, then performed a dot-blot analysis using the conformation-specific oligomeric Aβ antibody, A11 (56).

As predicted, we observed a significant reduction in the levels of soluble oligomeric Aβ in both the cortex and hippocampus of APPswe/PS1E9 mice that experienced an enriched environment (Fig. 5). This result is in agreement with our previous studies suggesting that the experience of APPswe/PS1E9 mice in an enriched environment reduces the extent of amyloid deposition and steady-state levels of Aβ (40) and suggests that experience in an enriched environment up-regulates mechanisms that either inhibit formation of oligomeric Aβ, or enhance their degradation or clearance through up-regulation of neprilysin (NEP) enzymatic activity (40). These experiments further suggest that after experience in enriched environmental conditions, NPCs in APPswe/PS1E9 mice are exposed to lower levels of neurotoxic oligomeric Aβ species.

**Enrichment enhances hippocampal LTP**

To determine whether enrichment stimulates synaptic plasticity in APPswe/PS1E9 transgenic mice, electrophysiological recordings were made in the CA1 field of hippocampal slices prepared from APPswe/PS1E9 mice after 1 mo in the enriched environment or maintenance under standard housing conditions. Recordings were also

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**Figure 4.** A selective increase in the number of glia in the brains of APPswe/PS1E9 mice after experience in an enriched environment. A) Number of BrdU+/GFAP+ newly formed astrocytes in the DG is greatly increased in both NonTg and APPswe/PS1E9 enriched groups compared with standard-housing controls. *P < 0.0001, **P = 0.0058; Student’s t test. B) No significant increase in the number of BrdU+/S100β+ cells was observed after experience in an enriched environment in the NonTg or the APPswe/PS1E9 group (P=0.5945 and P=0.2575, respectively), suggesting that enrichment induces signaling that up-regulate specific subtypes of the glial population. Data are means ± se. C) Comparison of the extent of increase in the number of lineage-specific BrdU+ cells examined in the DG of mice that experienced enriched environmental conditions or standard housing. D, E) Representative confocal image of cells coexpressing BrdU+ (red) and GFAP+ (green) (D) and BrdU+ (red) and S100β+ (green) (E) in the DG of APPswe/PS1E9 mice. F, G) Proliferating astrocytes in the SGL of APPswe/PS1E9 mice colocalize with neural stem cell markers. BrdU+/GFAP+ cells coimmunostain with sox2 (F) and nestin (G). Scale bars = 20 μm (D, E); 10 μm (F, G).
made from NonTg littermate mice treated similarly. Enrichment experience had no significant effect on input-output curves (Fig. 6A) or paired-pulse facilitation (Fig. 6B, C) in APPsw/PS1ΔE9 or NonTg mice. LTP was induced by TBS consisting of 2, 4, or 8 bursts. LTP after 2 bursts was small and unreliable and was not analyzed further. The results from 4- and 8-burst TBS are shown in Fig. 7. A 3-way analysis of variance was conducted to determine the contributions of number of bursts, genotype, and housing conditions on the degree of LTP measured 60 min post-TBS. No main effects of genotype ($F_{1,64}=0.12$) or burst number ($F_{1,64}=0.08$) were observed; however, there was a significant main effect of housing condition ($F_{1,64}=8.03$, $P<0.01$). No significant interactions were observed between any of the independent variables. These results indicate that experience of mice in an enriched environment enhanced LTP in both NonTg and APPsw/PS1ΔE9 transgenic mice (Fig. 7).

Environmental enrichment attenuates Tau pathology by reducing hyperphosphorylated tau levels

We reported previously that neurogenic areas in the brains of transgenic APPsw/PS1ΔE9 mice exhibit...
upregulated levels of phosphorylated tau protein, as detected by PHF-1 immunoreactivity (19). Hyperphosphorylation of tau reflects elevated neuronal kinase activities in AD brains (8–11), and these misregulated kinases may be detrimental when it comes to NPC proliferation, neuronal development, and maturation. We therefore examined whether the experience of APPswe/PS1/H9004E9 mice in an enriched environment modulates phosphotransferase activities by analyzing tau phosphorylation. For this purpose, we prepared protein extracts from the cortex and the hippocampus of enriched-environment and standard-housing APPswe/PS1/H9004E9 mice using ROLB buffer, then examined protein expression by Western blot analysis.

Using anti-phosphorylated tau PHF-1 (Ser-396 and Ser-404; ref. 57) antibody, we observed a significant reduction in levels of tau phosphorylation (Figs. 8 and 9). To examine whether enrichment-induced reduced tau phosphorylation results from a decrease in steady-state levels of tau isoforms or in phosphorylation, we reprobed the membrane with the phosphorylation-independent Tau 5 antibody. In both hippocampus and cortex, no significant change in expression level of tau isoforms was observed between brains of standard-housing and enriched-housing APPswe/PS1/H9004E9 mice using ROLB buffer, then examined protein expression by Western blot analysis.

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DISCUSSION

This study examined the hypothesis that brain plasticity can be induced in FAD-linked APPswe/PS1/H9004E9 transgenic mice, reducing neuropathology and rescuing brain structure and function. Although several studies have examined the effect of enriched environmental conditions on AD neuropathology (40–47), the controversial observations of these studies called for further examination of the effect of an experience in a...
complex environment on amyloid metabolism. Investigations have been focusing on the effect of enrichment on the extent of amyloid deposition, the “end product” of amyloid pathology, whereas the effect of enrichment on oligomeric Aβ or on tau pathology is still unclear. Billings and colleagues have shown that involuntary repeated spatial water-maze training decreases amyloid pathology and tau pathology in 3xTg-AD mice (67). Whether voluntary experience in enriched environmental conditions exerts similar effects is not known. Likewise, the effect of enrichment on other critical aspects of hippocampal plasticity in FAD mice is poorly understood. Our study was aimed at addressing these issues, adding novel insight into the effect of experience in a complex environment on hippocampal plasticity in FAD mice.

To incorporate environmental enrichment as a therapeutic intervention or a preventive measure for AD, it is inevitably important to examine the effect of environmental enrichment on aged animals. Several studies have examined the effects of physical activity and environmental enrichment on neurogenesis in 5-mo-old and older mice after amyloid deposition, using different AD transgenic mouse models (24, 45, 68). Results of these studies demonstrate that environmental enrichment up-regulates hippocampal neurogenesis (24, 68, 69).

Equally important is the examination of the effect of environmental enrichment in young FAD mice with neurogenic impairments, when the brain lacks amyloid deposition, inflammation, neuronal degeneration, and other major pathologies that occur later in life and cause secondary alterations in neurogenesis. Impairments in neurogenesis at this stage are the direct result of the expression of mutant proteins that cause the disease. Unfortunately, very little information is available on the status of neurogenesis preonset of amyloid deposition and whether environmental enrichment can rescue neurogenic impairments at this stage. Our results demonstrate that the experience of these FAD-linked APPswe/PS1ΔE9 mice in enriched environmental conditions significantly reduces oligomeric Aβ accumulation and abnormal tau hyperphosphorylation (the neurotoxic precursors that constitute AD hallmarks), overcomes compromised neurogenesis, and potentially enhances neuronal function, as manifested by increased expression of critical axonal transport players and enhanced LTP.

First, we show that experience in enriched environmental conditions rescues impaired neurogenesis in the DG of transgenic mice harboring FAD-linked APPswe/PS1ΔE9 by promoting NPC proliferation, neuronal differentiation, selective astrocyte differentiation, and neuronal maturation. It is now well established that in adult rodents, exposure to environmental enrichment induces neurogenesis in the SGL of the DG (48, 70). We show that impaired neurogenesis in hippocampus of APPswe/PS1ΔE9 mice occurs long before amyloid deposition, implying that amyloid deposition is not a primary cause for impaired neurogenesis in these animals.

In addition, we show that the stimuli provided by enriched environmental conditions can successfully reverse impaired neurogenesis in these mice early in the pathogenesis of the disease. Previously, Choi et al. (49) showed that experience of FAD-linked mice harboring PS1 variants in an enriched environment enhanced neu-

Figure 8. Environmental enrichment attenuates tau pathology by reducing hyperphosphorylated tau levels in the cortex. A) Representative Western blot analysis of protein extracts from the cortex of standard-housing (SH) and enriched (EE) APPswe/PS1ΔE9 mice. FL-APP, FL-APP using anti-APP-C-terminal 369 antibody; Tau5, total tau protein using Tau5 antibody; PHF-1, phosphorylated-tau protein using PHF-1 monoclonal antibody; KHC, kinesin-1 heavy chain using KHC (H2) antibody; KLC, kinesin-1 light chain using KLC (63–90) antibody. B) Densitometric quantification of protein expression levels in the cortex of standard-housing and enriched mice, as detected by Western blot analysis. Data are means ± se (arbitrary units).
rogenesis in mice expressing PS1 human wild type but not in mice expressing mutant PS1/H9004E9 or PS1M146L. Unlike in APPswe/PS1/H9004E9 mice, hippocampal neurogenesis is not impaired in the PS1/H9004E9 or PS1M146L mice to begin with. Therefore, the neurogenic mechanism activated in the two studies may be significantly different. In addition, PS1/H9004E9 and PS1M146L transgenic mice exhibit significantly lower levels of Aβ than APPswe/PS1/H9004E9 mice and no amyloid deposition later in life (40).

It becomes apparent that both APP and PS1 play major roles in neurogenesis in the adult brain, regulating proliferation, survival, and differentiation of neural stem and progenitor cells. In addition, increasing evidence suggests that misregulation or dysfunction of these molecules compromises these processes or alters neurogenesis (for review, see ref. 23). Given our observations showing the rescue of impaired hippocampal neurogenesis after experience in an enriched environment, it would be reasonable to assume that signals provided by the enriched environment down-regulate the pathological effects that mutant APP and PS1 exert on neurogenesis. Indeed, concomitant to enhanced neurogenesis, the experience of APPswe/PS1ΔE9 mice in enriched environmental conditions significantly reduces FAD-linked neuropathology, that is, levels of oligomeric Aβ and hyperphosphorylated tau.

Using conformation-specific antibodies, our study is the first one to demonstrate that an enriched environment significantly reduces the neurotoxic oligomers of Aβ that primarily accumulate in the hippocampus and cortex. A few reports have examined the impact of environmental enrichment on Aβ levels and amyloid deposition in transgenic mice, revealing variable effects of enrichment on these processes (41–46). Detailed examination of these studies reveals critical differences in experimental design, such as composition of the enriched environment, access to running wheels, duration of differential experience, treatment relative to onset of deposition, age, gender, type of transgenic mice, and more (for detailed analysis of these studies and a comparative description, see ref. 71).

It should be noted that in most FAD transgenic mice used in these studies, there is an age-dependent increase in amyloid deposits. The extent of amyloidosis, its quantification, and the cause-and-effect pathological interpretation might be challenging as animals get older and secondary effects (e.g., inflammation) become significant. By using young animals, well before onset of deposition, the current study was designed to avoid any complications that might result from these mechanisms. Our results strongly support the conclusion that the experience of APPswe/PS1ΔE9 mice in enriched environmental conditions reduces levels of Aβ. They also suggest that one of the mechanisms by which experience in an enriched environment down-regulates levels of amyloid deposition is by reducing levels of oligomeric Aβ.

In addition, we show, for the first time, that an enriched environment attenuates the other neuropathological hallmark of AD, namely, hyperphosphorylation of tau. Demars et al. (19) showed previously that tau is hyperphosphorylated in both neurogenic areas of
the brain in APPswe/PS1ΔE9 mice. Here we show that PHF-1 immunoreactivity, indicative of tau phosphorylation, is dramatically reduced in the brains of APPswe/PS1ΔE9 mice after experience in an enriched environment. The manifestation of attenuated tau phosphorylation is po-tentially 2-fold, supporting the function and integrity of mature neurons and promoting neurogenesis. The fact that kinases known to produce hyperphosphorylation of tau, like GSK3 and CK2, will also inhibit fast axonal transport led us to examine whether markers of axonal transport were also enhanced. Our results show that indeed, expression of kinesin-1—the major motor of anterograde axonal transport in neurons—is increased in the brains of these mice, supporting our hypothesis. Furthermore, reduction of oligomeric Aβ can also have a positive impact in axonal function and transport because the perfusion of oligomeric Aβ (but not fibrillar or unaggregated Aβ) dramatically inhibits both directions of axonal transport (11). Increases in neuronal impulse activity have long been known to affect fast axonal transport (72, 73) as well as the synthesis of neuronal proteins, raising the possibility that the enhanced delivery of synaptic components by fast anterograde axonal transport may contribute to the attenuation of pathological aspects of AD with exposure to an enriched environment.

Previous efforts suggested that an increase in the Aβ-degrading enzyme NEP may underlie enrichment-induced reduction in steady-state levels of soluble and insoluble Aβ. In addition, a battery of genes were identified that are modulated in the hippocampus and cortex of transgenic mice after experience in an enriched environment. Intriguingly, these genes play a role in neuronal survival, neurogenesis, and amyloid metabolism (40). Our results are in agreement with these observations. We show that while steady-state levels of FL-APP do not change after enrichment, levels of oligomeric Aβ are down-regulated, suggesting that experience in an enriched environment may modulate Aβ clearance and degradation rather than APP production.

The current study adds another novel aspect to the mechanism underlying enrichment-induced reduction in neuropathology by showing that the experience of APPswe/PS1ΔE9 mice in an enriched environment modulates phosphotransferase activities. Hyperphosphorylation of tau reflects elevated neuronal kinase activities in AD brains (8–11). Down-regulation of these processes suggests that kinase and phosphatase signaling are modulated after experience in an enriched environment.

Increasing evidence suggests that oligomers of Aβ peptide, but not the monomeric forms, can potentially disrupt LTP and cognitive functions (3, 74, 75). Our electrophysiological studies of NonTg mice and APPswe/PS1ΔE9 mice treated similarly yielded three interesting results. First, no differences in input–output curves or paired-pulse facilitation were attributable to genotype or enrichment. Second, LTP was not significantly reduced in the FAD-linked transgenic mice housed under standard conditions at these early times. As noted above, these mice were tested at relatively young ages (i.e., before the development of amyloid plaques). Nevertheless, the transgenic mice did have measurable levels of oligomeric Aβ and reduced neurogenesis (in the DG). These effects are apparently insufficient to interfere with synaptic transmission and LTP in the CA1 field. Third, enrichment enhanced LTP in both APPswe/PS1ΔE9 and control mice. This is the first evidence that enrichment experience enhances LTP in FAD-linked transgenic mice, although a similar effect was noted previously in wild-type mice (76). The mechanisms for this enhancement are not known but could involve enrichment-stimulated production of brain-derived neurotrophic factor (77), a growth factor that facilitates LTP induction (78, 79) and was previously shown to be upregulated in APPswe/PS1ΔE9 mice after enrichment (40) by regulating the endocytosis of NMDA receptors from the plasma membrane (80, 81), by enhancing neurogenesis (70, 82), or perhaps by enhancing axonal transport.

Numerous studies have shown that FAD-linked transgenic mice with amyloid pathology have impairments in learning and memory paradigms, including acquisition of long-term spatial memory (40, 47, 83–91), spatial reversal learning (92), use of spatial working memory (47, 83, 85, 86, 88), acquisition of social recognition memory (89), object recognition memory (90), and contextual fear conditioning (91). In some cases, the severity of the impairment has been correlated to Aβ levels in the brains of individual mice (86, 93, 94) and treatments aimed to reduce Aβ levels (e.g., immunization with Aβ or Aβ antibodies) can reverse learning deficits (84, 85, 95, 96). Most of these tasks depend on the hippocampus, and the deficits in neurogenesis or hippocampal synaptic plasticity (i.e., LTP) are possible causes for the learning deficits in these transgenic mice.

Although studies have suggested that the experience of FAD mice in an enriched environment attenuates cognitive deficits, the mechanism underlying this improvement remains controversial and largely unknown (43, 44, 47). Our study suggests a few possible mechanisms. One possibility that derives from our work is that enhanced cognition after enrichment may result in improved LTP, rescued neurogenesis, or both. This would be consistent with the recent data showing that intracellular oligomeric Aβ blocks synaptic transmission when directly injected in the presynaptic terminals (97). In addition, increasing evidence suggests that newly formed neurons integrating in the granular layer of the DG play a role in hippocampus-dependent function (for reviews, see refs. 98, 99). Rescue of the impaired neurogenic phenotype in APPswe/PS1ΔE9 mice by environmental enrichment may underlie, at least in part, its effect on LTP and cognitive function of these mice. Finally, enhanced axonal transport concomitantly with reduced levels of both oligomeric Aβ and hyperphosphorylated tau may contribute to improved neuronal function and enhanced cognition.

In summary, this study provides strong evidence that
the experience of FAD transgenic mice in enriched environmental conditions enhances brain plasticity and attenuates FAD neuropathology. The results add empirical support to the assumption that stimulation of cognitive, physical, and social activity is an effective prophylaxis against AD.

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REFERENCES


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