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Effects of Environmental Enrichment and Physical Activity on Neurogenesis in Transgenic PS1/APP Mice

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Abstract

Rodents exposed to environmental enrichment show many differences, including improved cognitive performance, when compared to those living in standard (impoverished) housing. The purpose of the present study was to determine if a selective increase in neurogenesis occurred in cognitively-protected Tg mice raised in an enriched environment compared to those reared in physical activity housing. At weaning, double Tg APP+PS1 mice were placed into one of three environments: complete environmental enrichment (CE), enhanced physical activity (PA), or individual, impoverished housing (IMP). At 9–10 months of age, Tg mice were injected with BrdU (100 mg/kg BID) followed by euthanasia either 24 hrs or two weeks after the last injection. Unbiased estimates of BrdU positive cells in the hippocampal subgranular zone revealed a significant increase in cellular proliferation in Tg mice raised in CE or PA compared to Tg mice reared in IMP housing. However, counts of BrdU birth-dated cells two weeks after labeling showed no difference among the three groups, indicating decreased survival of cells in those groups (CE and PA) with higher cellular proliferation rates in the neurogenic niche. Counts of calretinin-expressing cells, a marker of immature neurons, also indicated no difference among the three groups of mice. In view of our prior study showing that enhanced cognitive activity (but not enhanced physical activity) protects Tg mice against cognitive impairment, the present results indicate that increased generation and survival of new neurons in the hippocampal dentate gyrus is not involved with the cognitively-protective effects of complete CE in Alzheimer's transgenic mice.

Keywords

Alzheimer's Disease; Proliferation; Hippocampus; Environmental Enrichment; Physical Activity; Neurogenesis

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1. Introduction

Neurogenesis (the generation of new neurons) is an intrinsic feature of the developing central nervous system. While the majority of neurogenesis occurs in early development, it continues throughout adulthood in two specific neurogenic niches, the subventricular zone (SVZ) and the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus (HPC). In normal mice, enhanced physical activity (PA) or complete environmental enrichment (CE= enhanced physical and cognitive activities) increases neurogenesis in the DG of the HPC and improves cognitive performance (Brown et al., 2003; Kempermann et al., 1997; Van et al., 1999b; Van et al., 1999a). Indeed, even aged mice exhibit increased hippocampal neurogenesis and cognitive benefits when provided CE housing (Kempermann et al., 1998; Kempermann et al., 2002), indicating considerable plasticity in the normal aging brain. The above studies involving “normal” rodents collectively suggest that neurogenesis may be involved in the cognitive protection provided by either PA or CE. It is important to underscore, however, that the normal aging brain does not model Alzheimer’s disease (AD). As such, any human relevance of these normal rodent studies is limited to the very mild loss of short-term memory (e.g., age-associated memory impairment; AAMI) that most humans can expect as a normal consequence of aging.

A number of epidemiologic and longitudinally-based studies have suggested that there is reduced risk of developing Alzheimer’s (AD) in individuals that partake in “active” lifestyles, including social, physical, and/or cognitive activities (Wilson et al., 2002; Colcombe et al., 2004b; Colcombe et al., 2004a). Although such human studies are informative, they cannot determine unequivocally which activities (social, physical, or cognitive) are most important for cognitive protection against AD. However, well-controlled studies can be done in APP transgenic mouse models of AD to address this issue. These transgenic (Tg) mouse models for AD develop β -amyloid-containing neuritic plaques and cognitive impairment as they age (Arendash et al., 2007; Ethell et al., 2006; Cracchiolo et al., 2007; Arendash et al., 2004b). Rearing such Alzheimer’s Tg mice in CE has universally been reported to protect against cognitive impairment (Jankowsky et al., 2005; Costa et al., 2007; Wolf et al., 2006; Cracchiolo et al., 2007). Moreover, our original report demonstrated that CE provides wide-spread cognitive benefits to “aged” Tg mice, suggesting a treatment potential for cognitive stimulation in human AD (Arendash et al., 2004a).

By contrast, cognitive benefits of enhanced physical activity in Alzheimer’s Tg mice have only been reported by one group (Adlard et al., 2005). Importantly, cognitive benefits of physical activity were non-existent when these investigators appropriately used “immobile” running wheels as their control (Nichol et al., 2007). As well, three other laboratories, including our own, have found no cognitive benefits of physical activity housing in Alzheimer’s Tg mice (Wolf et al., 2006; Cracchiolo et al., 2007; Richter et al., 2008). In this context, we have recently reported that only those AD Tg mice raised in complete environmental enrichment (e.g., enhanced cognitive activity) are protected from otherwise certain cognitive impairment during aging – Tg mice raised in enhanced social and/or physical activity housing were not cognitively protected (Cracchiolo et al., 2007). Moreover, only Tg mice raised in CE exhibited a reduction in brain A β deposition. We thus concluded that life-long cognitive activity is likely to be much more important than other activities for protection against AD in humans.

The current study addressed the important issue of whether increased neurogenesis in the brain is “selectively” associated with (and perhaps involved with) the cognitive protection provided by complete environmental enrichment. We hypothesized that, if neurogenesis is required for the cognitive protection exclusively provided by complete CE, only Alzheimer’s Tg mice raised in that environment should show increased hippocampal neurogenesis – enhanced physical activity should be ineffective at increasing neurogenesis. However, we report a non-specific increase in hippocampal cell proliferation was evident for both housing environments.

Furthermore, the number of surviving BrdU-labeled cells and new neurons expressing calretinin was not different between the three groups of mice, suggesting neurogenesis does not contribute to the cognitive protection provided by CE to Alzheimer's Tg mice.

2. Results

In order to investigate the effects of environment on cell proliferation in the SGZ, BrdU was administered and mice were euthanized 24 hours later. A one-way ANOVA revealed a significant effect of environment [$F(2,6)=6.23$, $p=0.03$] suggesting that the number of BrdU-labeled cells varied depending on the environment in which the mice were housed. As can be seen in Figure 1A, CE and PA resulted in significantly more BrdU-labeled cells in the SGZ than an IMP environment ($p<.05$). These data suggest that both CE and PA significantly increase progenitor proliferation in the SGZ of the DG in APP+PS1 Tg mice. Without co-staining for specific phenotypes, the increase in BrdU incorporation may reflect proliferation of both neural stem/progenitor cells and other cells in the neurogenic niche, such as microglia and endothelial cells.

In order to investigate the effects of environment on survival of newly labeled progenitor cells of the SGZ, mice were euthanized 2 weeks after cellular birth-dating with BrdU injections. A one-way ANOVA failed to detect any effect of environment on numbers of BrdU-labeled cells in the granular cell layer of the DG [$F(2,6)=0.26$, $p=.78$]. As can be seen in Figure 1B, numbers of BrdU-labeled cells did not differ between CE, PA or IMP environment suggesting that regardless of manipulation, similar numbers of BrdU-labeled cells survived two weeks after birth-dating. However, it is interesting to point out the percentage of surviving BrdU-labeled cells differed dramatically as a result of environment. Less than 5% of BrdU positive cells survived in the CE and PA conditions compared to 32% in the IMP environment, reflecting higher cell turnover rates in the enriched environments.

To determine if CE and PA influenced development of new neurons in hippocampus, estimates of the number of calretinin-expressing cells in DG of hippocampus was performed (Figure 3). Calretinin is commonly used to label immature neurons as an index of neurogenesis in the DG (Brandt et al., 2003; Wolf et al., 2006). A one-way ANOVA failed to detect differences in the number of calretinin-expressing cells in the DG [$F(2,8)=0.155$, $p=0.86$], suggesting that housing in an CE, enhanced PA or an IMP environment does result in significant effects on neurogenesis in APP/PS1 mice (Figure 3A). In summary, APP/PS1 mice housed in a CE or with access to PA exhibit increased cellular proliferation in the neurogenic niche of the hippocampus, but these newly born cells do not persist for long, resulting in no difference between groups in the net total number of immature neurons residing in hippocampus.

3. Discussion

The present study focused on the effect of environmental enrichment- and physical activity-induced adult neurogenesis in the dentate gyrus of APP+PS1 transgenic mice. Using BrdU-labeling techniques, an enhancement in cellular proliferation in the DG was observed with both CE and PA as compared to an IMP environment. Next, the effects of CE and PA on cell survival (two weeks after BrdU administration) in the DG were investigated. No differences in numbers of surviving BrdU-labeled cells or in numbers of new neurons, indicated by calretinin-expressing cells, was found in the DG of APP/PS1 mice. Regardless of housing manipulation, the net total of newly-born hippocampal neurons remained constant. However, it is of interest to point out that the proportion of surviving BrdU-labeled cells differed between CE, PA, and IMP environments. The percentage of cells that survived was dramatically less in mice housed in either CE or PA compared to the IMP environment, suggesting that both CE and PA environments increase cell turnover in the dentate gyrus.

Importantly, the present study found that CE, PA and IMP environments resulted in equal numbers of calretinin-expressing cells in the granular cell layer, indicating that the maturation of neurons in the granular cell layer of the dentate is tightly regulated. Even as CE and PA may increase the ability of the brain to increase proliferation of new cells in the DG, homeostatic mechanisms keep the final number of new neurons in hippocampus relatively stable. In any event, we conclude from these data that, in AD transgenic mice, an increase in cell turnover in the neurogenic niche occurs as a result of exposure to either physical activity or enhanced cognitive activity. Since we had previously shown that only enhanced cognitive activity (but not physical activity) protects against cognitive impairment (Cracchiolo et al., 2007), our results suggest that neither cellular proliferation, nor increased survival of new neurons is required for the cognitively-protective effects of complete environmental enrichment. This conclusion is consistent with a recent study showing that blockade of neurogenesis in normal mice does not prevent the cognitive-enhancing effects of complete EE (Meshi et al., 2006).

There are no other reported studies using double tg APP/PS1 mice to investigate the effects of housing on neurogenesis. However, one study in a tg strain of mouse with a single mutation (APP23) found no difference between CE, PA, and standard-housed tg APP23 mice in the number of surviving BrdU-labeled cells within DG 4 weeks after BrdU injection, similar to the present findings (Wolf et al., 2006). However, in comparison to APP23 Tg mice housed in PA or standard housing, CE increased numbers of calretinin-expressing cells in the SGZ of the DG. This result contrasts with the present study which shows that numbers of calretinin-expressing cells in Tg APP/PS1 mice was not increased in CE and PA relative to IMP environments. An important reason for the difference in results may be attributed to the use of different strains of Tg mice; double Tg APP/PS1 mice were used in the present study whereas the Tg APP23 strain was used in the prior study. In addition, mice in that study underwent behavioral testing prior to euthanasia. A battery of behavioral testing introduces a confounding variable with potential to impact neurogenesis. A less likely explanation is that the length of time between birth-dating of cells and counting of immature neurons was shorter (2 weeks) in the present study.

In “normal” mice and rats, CE and PA have both been demonstrated to have a positive effect on proliferation of neural progenitors in the dentate gyrus of the hippocampus and both housing environments result in cognitive benefits (Kempermann et al., 1997; Van et al., 1999b; Van et al., 1999a). Thus, an increase in hippocampal cellular proliferation induced by CE and PA would appear to occur in both normal and Alzheimer’s Tg mice. However, normal mice show cognitive benefit through *both* housing environments, while Alzheimer’s Tg mice exhibit cognitive protection *only* through CE housing – PA housing is insufficient. Although epidemiologic/longitudinal human studies have reported protective effects of cognitive and/or physical activities against AD (Friedland et al., 2001; Larson et al., 2006) the relative importance of these activities to protect against AD cannot be unequivocally determined in humans for several reasons: (1) retrospective human studies cannot be controlled and rely on recall, (2) well-controlled longitudinal studies over decades are impractical and usually do not establish that baseline performance levels are the same for each group, and (3) the impact of cognitive versus physical activities cannot be evaluated independently from each another, nor can these activities be unequivocally isolated from other life style factors (i.e., diet) that can impact AD risk.

Although current Alzheimer’s Tg models have limitations in interpreting their findings relative to humans, results from the present study and our prior work (Costa et al., 2007; Cracchiolo et al., 2007; Arendash et al., 2004a) suggest that both cognitive and physical activities induce proliferation of cells in DG of hippocampus without a net increase in new neurons, but only enhanced cognitive activities provide cognitive benefits against AD.

4. Materials and Methods

Subjects and general protocol

All mice contained a mixed background of C57B6, B6D2F1, SJL, and SW. Mice were generated from a cross between male mice, heterozygous for the mutant APPK670N, M671L gene (i.e., the APPsw, Swedish mutation derived from Tg2576 mice), and mutant PS1 (6.2 line) females bearing the M146V mutation. Mice were initially genotyped at the time of weaning and then had a confirmatory genotyping at 4 months of age. Double transgenic (APPsw+PS1) mice were randomly divided into the various housing groups where they lived through testing (see below). Mice were housed and maintained in a specific pathogen-free facility under a 14/10 h light–dark cycle, with *ad libitum* access to rodent chow and water.

Beginning at 6 weeks of age, transgenic mice of the same gender were moved from standard social cages to one of the following housing environments: impoverished environment (IMP; n=7), enhanced physical activity (PA; n=5), or into complete enrichment (CE; n=7). Each housing group was gender-balanced at the beginning of the study. Animals living in an IMP environment were housed individually and had access to food and water within their standard Plexiglas mouse cage (6.5" wide, 10.5" long, 5.5" high). Mice in the PA group were social housed by gender in cages that were 7" wide, 11" long, and 5" high with each cage equipped with two running wheels. CE mice also were socially housed by gender and provided running wheels for enhanced physical activity. However, they also had enhanced cognitive activity compared to IMP and PA housing. A 110 L Sterilite container (19" wide, 32" long, 13.5" high), with an inner "CrittterTrailTWO" rodent house, was used as housing for the CE group. Housing for this group not only involved various crawl-tubes and platforms within the rodent house, but also involved running wheels and toys in the courtyard surrounding the rodent house; these items were changed weekly for novelty. To provide additional cognitive stimulation, CE mice were also placed in novel, complex environments for at least 1 h three times a week over the course of the study. At 8.5 to 9.5 months of age, mice were injected with BrdU (100 mg/kg BID) followed by anesthesia either 24 hours or two weeks after the last injection. Mice were anesthetized with Nembutal, then transcardially perfused with 0.9% saline and brains of the PS1/APP mice were removed for histology. All procedures used were reviewed and approved by the USF Institutional Animal Care and Use Committee (IACUC).

Immunohistochemistry and cell counts

Mice were euthanized with Nembutal, perfused transcardially with 0.9% saline followed by 4% phosphate buffered paraformaldehyde. The brain was then removed and placed in 4% paraformaldehyde in 0.1M phosphate buffered solution (PBS, pH 7.4) over night, then embedded in paraffin with 24 h processing. At the level of the hippocampus (bregma –2.92 mm to –3.64 mm), serial 5- μ m sections (150 μ m apart) were made from each mouse brain using a sliding microtome. Sections were deparaffinized using xylene and ethanol washes followed by antigen retrieval with citrate buffer. For BrdU immunohistochemistry, sections were denatured using 2N HCl and neutralized in 0.15M borate buffer then washed in PBS. Sections were then treated in 3% H₂O₂ solution to block endogenous peroxidase, washed in PBS then blocked in PBS+ (PBS, 10% normal goat serum, 1% 100x Triton X, 10% BSA) for 1 hour at 4°C. Sections were incubated overnight at 4°C in rat monoclonal anti-BrdU (AbD Serotec, Raleigh NC, #OBT0030G, 1:100) diluted in PBS. Sections were washed in PBS, incubated in biotinylated anti-rat IgG (H+L), made in goat (Vector Laboratories, Burlingame CA, #BA-9400, 1:200) followed by ABC reaction (Vector Laboratories, Burlingame CA) for 45 minutes, then rinsed and treated with Pierce DAB Enhancement Solution (Pierce Biotechnology, Rockford, IL). For calretinin immunofluorescence, sections were blocked in PBS+ (PBS, 10% normal goat serum, 1% TX-100, 1M Lysine) for 1 hour at 4°C and incubated overnight at 4°C in rabbit anti-calretinin (Swant International, 1:2500) in PBS. Sections were

washed in PBS and incubated in goat anti-rabbit IgG Alexa Fluor 488 (Invitrogen, #A11008) and coated with vectorshield mounting medium (Invitrogen).

Unbiased estimates of cell proliferation and neurogenesis were made by counting immunoreactive cells (BrdU and calretinin) in serially sectioned hippocampus according to the method previously described (Shors et al., 2001; Shors et al., 2002). Total number of BrdU and calretinin cells was estimated by counting positively labeled cells in every 12th section of hippocampal tissue under a 40X objective. The number of positively labeled cells counted in every 12th section was then multiplied by the number of intervening sections to obtain the total number of BrdU and calretinin positive cells in the DG. The number of positively labeled cells counted in every 12th section was then multiplied by the number of intervening sections to obtain the total number of BrdU and calretinin positive cells in the DG. Since the entire region of interest analyzed is limited by the anatomical boundary of the dentate gyrus, and the number of “positive” cells is small, there was no need to randomly select areas for the “counting box” as is required in stereological estimates of cell number in structures with very large numbers of “positive” cells distributed across large expanses of tissue such as cerebral cortex (Kempermann, 2006).

Statistical Analysis

Separate one-way analyses of variance (ANOVA) were used to determine the effects of environment (IMP, CE, PA) on each dependent measure (BrdU proliferation, BrdU survival, calretinin). Bonferroni post hoc analyses were used to isolate effects. All statistical analyses were determined significant at the 0.05 alpha level.

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Reference List

- Adlard PA, Perreau VM, Pop V, Cotman CW. Voluntary exercise decreases amyloid load in a transgenic model of Alzheimer's disease. *J Neurosci* 2005;25:4217–4221. [PubMed: 15858047]
- Arendash GW, Garcia MF, Costa DA, Cracchiolo JR, Wefes IM, Potter H. Environmental enrichment improves cognition in aged Alzheimer's transgenic mice despite stable beta-amyloid deposition. *Neuroreport* 2004a;15:1751–1754. [PubMed: 15257141]
- Arendash GW, Jensen MT, Salem N Jr, Hussein N, Cracchiolo J, Dickson A, Leighty R, Potter H. A diet high in omega-3 fatty acids does not improve or protect cognitive performance in Alzheimer's transgenic mice. *Neuroscience* 2007;149:286–302. [PubMed: 17904756]
- Arendash GW, Lewis J, Leighty RE, McGowan E, Cracchiolo JR, Hutton M, Garcia MF. Multi-metric behavioral comparison of APPsw and P301L models for Alzheimer's disease: linkage of poorer cognitive performance to tau pathology in forebrain. *Brain Res* 2004b;1012:29–41. [PubMed: 15158158]
- Brandt MD, Jessberger S, Steiner B, Kronenberg G, Reuter K, Bick-Sander A, von der BW, Kempermann G. Transient calretinin expression defines early postmitotic step of neuronal differentiation in adult hippocampal neurogenesis of mice. *Mol Cell Neurosci* 2003;24:603–613. [PubMed: 14664811]
- Brown J, Cooper-Kuhn CM, Kempermann G, Van PH, Winkler J, Gage FH, Kuhn HG. Enriched environment and physical activity stimulate hippocampal but not olfactory bulb neurogenesis. *Eur J Neurosci* 2003;17:2042–2046. [PubMed: 12786970]
- Colcombe SJ, Kramer AF, Erickson KI, Scalf P, McAuley E, Cohen NJ, Webb A, Jerome GJ, Marquez DX, Elavsky S. Cardiovascular fitness, cortical plasticity, and aging. *Proc Natl Acad Sci U S A* 2004a;101:3316–3321. [PubMed: 14978288]
- Colcombe SJ, Kramer AF, McAuley E, Erickson KI, Scalf P. Neurocognitive aging and cardiovascular fitness: recent findings and future directions. *J Mol Neurosci* 2004b;24:9–14. [PubMed: 15314244]

- Costa DA, Cracchiolo JR, Bachstetter AD, Hughes TF, Bales KR, Paul SM, Mervis RF, Arendash GW, Potter H. Enrichment improves cognition in AD mice by amyloid-related and unrelated mechanisms. *Neurobiol Aging* 2007;28:831–844. [PubMed: 16730391]
- Cracchiolo JR, Mori T, Nazian SJ, Tan J, Potter H, Arendash GW. Enhanced cognitive activity--over and above social or physical activity--is required to protect Alzheimer's mice against cognitive impairment, reduce A β deposition, and increase synaptic immunoreactivity. *Neurobiol Learn Mem* 2007;88:277–294. [PubMed: 17714960]
- Ethell DW, Shippy D, Cao C, Cracchiolo JR, Runfeldt M, Blake B, Arendash GW. A β -specific T-cells reverse cognitive decline and synaptic loss in Alzheimer's mice. *Neurobiol Dis* 2006;23:351–361. [PubMed: 16733088]
- Friedland RP, Fritsch T, Smyth KA, Koss E, Lerner AJ, Chen CH, Petot GJ, Debanne SM. Patients with Alzheimer's disease have reduced activities in midlife compared with healthy control-group members. *Proc Natl Acad Sci U S A* 2001;98:3440–3445. [PubMed: 11248097]
- Jankowsky JL, Melnikova T, Fadale DJ, Xu GM, Slunt HH, Gonzales V, Younkin LH, Younkin SG, Borchelt DR, Savonenko AV. Environmental enrichment mitigates cognitive deficits in a mouse model of Alzheimer's disease. *J Neurosci* 2005;25:5217–5224. [PubMed: 15917461]
- Kempermann G. *Adult Neurogenesis*. Oxford University Press; New York, NY: 2006.
- Kempermann G, Gast D, Gage FH. Neuroplasticity in old age: sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. *Ann Neurol* 2002;52:135–143. [PubMed: 12210782]
- Kempermann G, Kuhn HG, Gage FH. More hippocampal neurons in adult mice living in an enriched environment. *Nature* 1997;386:493–495. [PubMed: 9087407]
- Kempermann G, Kuhn HG, Gage FH. Experience-induced neurogenesis in the senescent dentate gyrus. *J Neurosci* 1998;18:3206–3212. [PubMed: 9547229]
- Larson EB, Wang L, Bowen JD, McCormick WC, Teri L, Crane P, Kukull W. Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older. *Ann Intern Med* 2006;144:73–81. [PubMed: 16418406]
- Meshi D, Drew MR, Saxe M, Ansorge MS, David D, Santarelli L, Malapani C, Moore H, Hen R. Hippocampal neurogenesis is not required for behavioral effects of environmental enrichment. *Nat Neurosci* 2006;9:729–731. [PubMed: 16648847]
- Nichol KE, Parachikova AI, Cotman CW. Three weeks of running wheel exposure improves cognitive performance in the aged Tg2576 mouse. *Behav Brain Res* 2007;184:124–132. [PubMed: 17698211]
- Richter H, Ambree O, Lewejohann L, Herring A, Keyvani K, Paulus W, Palme R, Touma C, Schabitz WR, Sachser N. Wheel-running in a transgenic mouse model of Alzheimer's disease: protection or symptom? *Behav Brain Res* 2008;190:74–84. [PubMed: 18342378]
- Shors TJ, Miesegaes G, Beylin A, Zhao M, Rydel T, Gould E. Neurogenesis in the adult is involved in the formation of trace memories. *Nature* 2001;410:372–376. [PubMed: 11268214]
- Shors TJ, Townsend DA, Zhao M, Kozorovitskiy Y, Gould E. Neurogenesis may relate to some but not all types of hippocampal-dependent learning. *Hippocampus* 2002;12:578–584. [PubMed: 12440573]
- Van PH, Christie BR, Sejnowski TJ, Gage FH. Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proc Natl Acad Sci U S A* 1999a;96:13427–13431.
- Van PH, Kempermann G, Gage FH. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat Neurosci* 1999b;2:266–270.
- Wilson RS, Mendes de Leon CF, Barnes LL, Schneider JA, Bienias JL, Evans DA, Bennett DA. Participation in cognitively stimulating activities and risk of incident Alzheimer disease. *JAMA* 2002;287:742–748. [PubMed: 11851541]
- Wolf SA, Kronenberg G, Lehmann K, Blankenship A, Overall R, Staufenbiel M, Kempermann G. Cognitive and physical activity differently modulate disease progression in the amyloid precursor protein (APP)-23 model of Alzheimer's disease. *Biol Psychiatry* 2006;60:1314–1323. [PubMed: 16806094]

Figure 1. Measures of cell proliferation and survival in the dentate gyrus

APP+PS1 transgenic mice were housed in either an impoverished environment (IMP), enhanced physical activity environment (PA) or complete environmental enrichment (CE) for 7–8 months. **A)** Proliferation was assessed by quantifying the total number of BrdU-labeled cells (y-axis) in the SGZ 24 hr after BrdU administration. The mice raised in CE and PA exhibited significant increases in number of BrdU-labeled cells compared to IMP. *Indicates significant difference from IMP ($p < 0.03$). **B)** Cell survival was evaluated by estimating the total number of BrdU-labeled cells in the DG two weeks after BrdU administration. No differences in the number of surviving BrdU-labeled cells were observed as a result of environmental manipulation.

Figure 2. Photomicrographs of representative sections of BrdU immunoreactive cells
BrdU+ staining in dentate gyrus of APP/PS1 Tg mice housed in an IMP environment (A low magnification; B magnification), PA (C low magnification; D magnification) or CE (E low magnification; F magnification).

Figure 3. Adult hippocampal neurogenesis

A) Neurogenesis was assessed by counting the total number of calretinin-immunoreactive neurons throughout the entire dentate gyrus of the hippocampus. There was no significant difference in calretinin-expressing neurons in APP/PS1 Tg mice housed in an enriched environment (CE), with enhanced physical activity (PA) or in an impoverished (IMP) environment. Representative photomicrograph of calretinin-immunoreactive cells (green) in the subgranular zone of the dentate gyrus low magnification (**B**; 50 μ m) and high magnification (**C**; 20 μ m).