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Enrichment improves cognition in AD mice by amyloid-related and unrelated mechanisms

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Abstract

Lifelong cognitive stimulation is associated with a lower risk of Alzheimer's disease (AD), but causality is difficult to prove. We therefore sought to investigate the preventative potential of environmental enrichment (EE) using mice expressing both human mutant presenilin-1 and the amyloid precursor protein (PS1/PDAPP). At weaning, mice were placed into either an enriched or standard housing environment. Behavioral testing at 4.5–6 months showed that environmentally enriched PS1/PDAPP mice outperformed mice in standard housing, and were behaviorally indistinguishable from non-transgenic mice across multiple cognitive domains. PS1/PDAPP mice exposed to both environmental enrichment and behavioral testing, but not to EE alone, showed 50% less brain β-amyloid without improved dendritic morphology. Microarray analysis revealed large enrichment-induced changes in hippocampal expression of genes/proteins related to Aβ sequestration and synaptic plasticity. These results indicate that EE protects against cognitive impairment in AD transgenic mice through a dual mechanism, including both amyloid dependent and independent mechanisms.

Keywords: Alzheimer's disease; Environmental enrichment; Amyloid; Microarray

1. Introduction

Alzheimer's disease (AD) is a common neurodegenerative disorder characterized by parenchymal β-amyloid deposition, neurofibrillary tangle formation, neuronal loss, and cognitive decline. A lifelong pattern of high mental activity [68] and educational attainment [56] correlates with lower risk of AD and may be protective. Furthermore, high levels of linguistic ability early in life are associated with a reduced risk of the disease [47,53]. These studies suggest that extra "cognitive reserve" developed throughout life may help buffer against the development of later dementia. However, the extent to which cognitive stimulation (i.e. environmental enrichment, EE) protects against AD remains difficult to assess in humans because: (1) retrospective studies cannot unequivocally isolate environmental enrichment from other factors affecting cognition over a lifetime, and (2) although short-term cognitive stimulation of AD patients has been shown to be beneficial [36], long-term intervention in humans is impractical. Furthermore, epidemiological human studies give no insight about the potential mechanisms by which EE may protect against AD.

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It is known from previous studies that non-transgenic rodents subjected to cognitive stimulation (i.e. EE) perform better in water mazes, exhibit increased dendritic branching and dendritic spine formation [11,22,61], increased synaptogenesis [45], and increased neuronal plasticity-related gene expression [43], while exhibiting decreased levels of apoptotic cell death [70]. The key question about whether EE intervention can protect against AD pathology, or its associated mental decline, was addressed in the present study using a transgenic mouse model of the disease. The mice chosen express both the human mutant amyloid precursor protein ($hAPP^{V717F}$) and mutant presenilin 1 ($hPS1^{M146L}$) genes, which result in moderate brain β-amyloid plaque deposition and significant behavioral impairment by 5–6 months of age. At weaning, mice were placed into either standard housing or a continuous enriched environment. Half of the animals in each housing condition were behaviorally tested between 4.5 and 6 months of age, while the other half remained in their respective housing to control for effects of behavioral testing on neuropathology and gene expression.

Our previous experiments determined that an EE paradigm used therapeutically in aged APPsw transgenic mice with moderate Aβ plaque deposition provided cognitive benefits without affecting the amyloid plaque burden [2]. The results in that study suggest that consistent, intensive cognitive stimulation could provide considerable benefit to mild-moderate stage AD patients. Here we sought to determine the extent to which pre-emptive EE protects memory, impacts Aβ deposition, and affects dendritic morphology. To characterize these changes at the molecular level, hippocampal gene expression was also analyzed using a whole mouse genome microarray. We found that PS1/PDAPP mice raised in EE outperformed those raised in standard housing (SH) across a variety of cognitive tests, in which they were statistically indistinguishable from non-transgenic (NT) mice. Interestingly, brain Aβ deposition in Tg mice was not affected by EE alone. However, EE in combination with an extensive behavioral testing paradigm (an additional enriching experience), resulted in large reductions in brain Aβ deposition. Although the extent of dendritic branching in cortex/hippocampus was unchanged by enrichment, Tg mice given EE did exhibit increased expression of synaptic plasticity-related genes as well as increased expression of transthyretin (a known Aβ sequestering molecule).

In addition to our current and previous environmental enrichment experiments, two other groups have reported on the neuropathological and/or behavioral effects of EE in Alzheimer's transgenic mice. Jankowsky et al. (2003) reported that raising female APP + PS1 mice in a potentially stress provoking EE (i.e., involving frequent addition/removal of different-aged animals) actually increased brain Aβ deposition [30]. In 2005, the same group confirmed their initial findings of EE-induced increases in brain Aβ deposition for female APP + PS1 mice, while surprisingly also reporting associated protection against cognitive impairment [29]. Recently, Lazarov et al. (2005) reported that discontinuous enrichment sessions in male APP + PS1 mice induced a decrease in brain Aβ deposition and favorable changes in gene expression associated with learning, memory, and neurotrophic actions. These opposite effects of enrichment/novelty on brain Aβ deposition have been attributed to the fact that one study involved males while the other utilized female mice. The different results could also be explained by Jankowsky et al. employing continuous EE while Lazarov et al. utilized discontinuous EE sessions. Furthermore, different APP + PS1 transgenic lines were utilized in the two studies. The present study circumvents these confounding issues by placing equal numbers of male and female mice in each housing condition, and by utilizing continuous housing conditions which more appropriately mimic humans living a lifestyle of consistent cognitive enrichment. Moreover, the gene microarray analysis of the present study was done in animals with already moderate amyloid burdens. This would appear to be more relevant to aging humans who have lived a cognitively-stimulating lifestyle than the microarray analysis done by Lazarov et al., which was conducted in young APP transgenic mice after a considerably shorter period of novelty sessions and well before overt Aβ deposition. Our results show a profound protective effect of EE in both male and female mice, and favorable changes in gene expression related to cognitive function. Reductions in Aβ burden also occurred, but only after an intensive 6-week period of behavioral testing was added to 4.5 months of EE.

2. Materials and methods

2.1. Construction of transgenic mice

All procedures involving experimentation on animal subjects were done in accord with the guidelines set forth by the University of South Florida's Institutional Animal Care and Use Committee. Heterozygous PDGF-hAPP(V717F) mice [Swiss-Webster × C57BL/6] were crossed with PDGF-hPS1(M146L) heterozygotes [Swiss-Webster × C57BL/6] to generate mice with an $APP^{+/-}$, $PS1^{+/-}$ genotype. All offspring were screened by PCR to identity the PDGF-hAPP [19] and the PDGF-hPS1 gene [16]. Mice were also screened for the retinal degeneration (rd) mutation (which causes blindness) and found to be negative for this mutation.

2.2. Environmental enrichment

At weaning, mice were place into two groups that were exposed to standard single housing (PS1/PDAPP $n = 32$; NT = 23) or environmentally enriched housing (PS1/PDAPP $n = 27$; NT $n = 19$) for 4.5–5.5 months. All standard housed (SH) animals were housed in shoe box cages (6.5″ side, 10.5″ long, and 5.5″ high) with static microisolator tops under climate-controlled conditions on a 12 h light/12 h dark cycle, fed Harlan Teklad Global Diet #2018 and provided with tap water ad libitum. Although some enrichment studies in