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Research report

Repeated cognitive stimulation alleviates memory impairments in an Alzheimer's disease mouse model

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ABSTRACT

Alzheimer's disease is a neurodegenerative disease associated with progressive memory and cognitive decline. Previous studies have identified the benefits of cognitive enrichment on reducing disease pathology. Additionally, epidemiological and clinical data suggest that repeated exercise, and cognitive and social enrichment, can improve and/or delay the cognitive deficiencies associated with aging and neurodegenerative diseases. In the present study, 3xTg-AD mice were exposed to a rigorous training routine beginning at 3 months of age, which consisted of repeated training in the Morris water maze spatial recognition task every 3 months, ending at 18 months of age. At the conclusion of the final Morris water maze training session, animals subsequently underwent testing in another hippocampus-dependent spatial task, the Barnes maze task, and on the more cortical-dependent novel object recognition memory task. Our data show that periodic cognitive enrichment throughout aging, via multiple learning episodes in the Morris water maze task, can improve the memory performance of aged 3xTg-AD mice in a separate spatial recognition task, and in a preference memory task, when compared to naïve aged matched 3xTg-AD mice. Furthermore, we observed that the cognitive enrichment properties of Morris water maze exposer, was detectable in repeatedly trained animals as early as 6 months of age. These findings suggest early repeated cognitive enrichment can mitigate the diverse cognitive deficits observed in Alzheimer's disease.

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1. Introduction

Alzheimer's disease (AD), a neurodegenerative disease that primarily afflicts the elderly population, is associated with progressive memory and cognitive decline. The pathological features of the postmortem AD brain; include extra- and intra-cellular depositions of amyloid-β (Aβ) species, intracellular accumulations of hyperphosphorylated tau protein, neuroinflammation, and irreversible neuronal and synaptic degeneration. These pathological hallmarks are prominently found in brain regions heavily involved in the learning and memory process, including the neocortex, hippocampus and amygdala (Chabrier et al., 2014; DeKosky and Scheff, 1990; Hardy and Revesz, 2012; Querfurth and LaFerla, 2010; Scheff et al., 1990).

Epidemiological and clinical evidence suggests that education, occupation, and an active lifestyle, involving enhanced social, physical, and mental components, can improve cognitive function in healthy older people, and are protective against a general reduction rate of memory decline and the development of AD, in part by an attenuation of disease symptoms, and a slowing of disease progression in patients (Baker et al., 2010; Fratiglioni et al., 2004; Friedland et al., 2001; Mayeux and Stern, 2012; Svensson et al., 2014; Yaffe et al., 2014). Until recently, the most common explanation for the observed beneficial effect of a cognitively and physically active lifestyle, were that such activities enhance cognitive reserve, and enable patients to compensate for cognitive decline without affecting AD-related neuropathology (Le Carret et al., 2005). In this regard, several studies using AD animal models have reported that cognitive and physical stimulation, in the form of repeated learning or environmental enrichment, enhances performance of cognitive

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tasks, prevents AD disease progression, and results in a significant reduction of cerebral Aβ plaques and amyloid angiopathy (Ambree et al., 2006; Billings et al., 2007; Greenough et al., 1972; Herring et al., 2008; Lazarov et al., 2005; Mirochnic et al., 2009). Specifically, our group previously showed that repeated training in the Morris water-maze (MWM) spatial recognition task, produces learning improvements for newly acquired platform locations, and reduces tau and Aβ pathology in 3xTg-AD animals (Billings et al., 2007).

In the present study, we sought to determine whether the beneficial effects observed after multiple MWM training episodes, were specific to that task, or if they could alleviate memory deficits found in other hippocampal-dependent spatial memory task. In addition, we also sought to determine if the beneficial effects of repeated MWM training episodes could extend beyond hippocampal-dependent memory to benefit memory processes that require more involvement of the cortex. Thus, 3xTg-AD mice were trained every three months in the MWM until 18 months of age, and subsequently tested in the Barnes maze spatial memory task, followed by the more cortical-dependent novel object recognition memory task. Our results indicate that our MWM training paradigm not only improved cognitive performance in the Barnes maze spatial task, but importantly, it also ameliorates more cortically-dependent memory deficits found in the novel object recognition task. These findings suggest that the cognitive benefits found after periodic cognitive enrichment throughout aging in one spatial memory task, can also alleviate the deficits found in other spatial memory dependent task, and non-spatial memory task. Thus, our findings support the idea that constant cognitive stimulation could be a part of an integrative treatment to delay memory decline in AD patients (Chapman et al., 2015; Fratiglioni and Wang, 2007; Kelly et al., 2014).

2. Materials and methods

2.1. Transgenic mice

All animal experimental procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California, Irvine. All mice were housed with food and water ad libitum under a 12 h dark/light cycle. In this study, we utilized 3, 6, 9, 12, 15, and 18-month old 3xTg-AD mice. The characterization of 3xTg-AD mice has been described previously (Oddo et al., 2003). Briefly, two independent transgenes encoding human APP_{Swe} and human tau_{P301L} (both under the control of the mouse Thy1.2 regulatory element) were co-microinjected into single-cell embryos harvested from homozygous mutant $PS1_{M146V}$ knockin (PS1-KI) mice, all on a mixed 129SvJ/C57BL/6 background, were used for all experiments (Oddo et al., 2003).

2.2. Morris water maze

3xTg-AD mice ($n = 15$ per group) were trained in the Morris water maze (MWM) every three months (3, 6, 9, 12, 15 and 18 months of age) with 4 training trials per day for as many days as needed to reach the training goal of swimming to the submerged platform (escape latency) within 15 s as previously described (Martinez-Coria et al., 2010). The apparatus used for all water maze tasks was a circular aluminum tank (1.5 m diameter) painted white and filled with water maintained at 26–29 °C. Position of the hidden platform MWM was changed for every training episode to avoid bias for the platform location (i.e training 3 months (quadrant 1 Northeast) 6 months (quadrant 2, Northwest), 9 months (quadrant 3 Southeast), 12 months (quadrant 4, Southwest)). Following training at each episode, mice underwent a 24 h probe trial, in which the hidden platform was removed and the latency to cross the location of the hidden platform was measured. We compared the latency to reach the platform between repeatedly trained 3xTg-AD with 10 aged-matched naïve mice (Fig. 1A).

2.3. Barnes maze

At the conclusion of the final MWM probe at 18 months, repeatedly MWM trained 3xTg-AD mice were subsequently trained on the Barnes Maze (BM) along with behaviorally naïve age and sex matched 3xTg-AD mice. The BM was conducted as previously described (Clinton et al., 2007): 4 training trials per day for 5 days and test was performed 24 h after the last training trial.

2.4. Novel object

Following BM training and probe testing, 18 month old repeatedly MWM trained 3xTg-AD mice was trained in a novel object recognition task, as previously described (Martinez-Coria et al., 2010). Briefly, animals were trained by letting them explore two identical objects placed at opposite ends of the arena for 10 min. 24 h later, mice were tested for 3 min with one copy of the familiar object and one novel object of similar dimensions. Recognition index (RI) represents the percentage of time mice spent exploring the novel object. Together with the repeatedly MWM trained group, another two sets of animals were trained on the recognition task: naïve mice, animals trained only on Barnes maze (BM trained).

2.5. Statistical analysis

All data are expressed as mean ± SEM. All the quantitative data were analyzed by Student's *t*-test (two-groups) or repeated-measures ANOVA (multiple groups/multiple training days) with a Bonferroni's post hoc test. Briefly, Morris water maze and barnes maze trainings were analyzed by repeated measure ANOVA followed by pairwise comparisons. Barnes maze test was analyzed by t-test and object recognition RI was analyzed by one-way factorial ANOVA. All significant values were set to $p < 0.05$. All statistical analysis was performed using Prism (GraphPad, La Jolla, CA).

3. Results

3.1. Repeated Morris water-maze training alleviates subsequential spatial learning and memory deficiencies in 3xTg-AD mice

We sought to examine the effects of repeated MWM training on succeeding spatial learning. A group of 3xTg-AD mice was trained in the MWM task every 3 months from age 3 months to 18 months. At each time point, our repeat group of mice was compared with an independent aged and sex-matched naïve 3xTg-AD group (Fig. 1A). Our findings revealed that 3xTg-AD mice subjected to multiple training episodes showed significant learning improvements at 6, 9, 12, 15, and 18 months of age compared to naïve animals. As such, repeat trained mice, from ages 6 to 18, required fewer days to reach performance criterion (<15 s to find the platform) (Fig. 1B). These effects were especially pronounced at 15 and 18 months of age, where repeatedly trained 3xTg-AD showed the greatest learning differences when compared to naïve 3xTg-AD mice (Fig. 1B)

To investigate for potential changes in long-term memory in repeatedly MWM trained 3xTg-AD mice, a probe trial was conducted 24 h following the last training trial at each 3 month episode. During the probe trial, the submerged platform was removed from its training location, and the latency for the animal to cross the former location of the platform was measured. Repeatedly trained 3xTg-AD mice exhibited a significant improvement in long-term