



## Running exercise delays neurodegeneration in amygdala and hippocampus of Alzheimer's disease (APP/PS1) transgenic mice



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### ABSTRACT

Alzheimer's disease (AD) is an age-related neurodegenerative disease. Post-mortem examination and brain imaging studies indicate that neurodegeneration is evident in the hippocampus and amygdala of very early stage AD patients. Exercise training is known to enhance hippocampus- and amygdala-associated neuronal function. Here, we investigated the effects of exercise (running) on the neuronal structure and function of the hippocampus and amygdala in APP/PS1 transgenic (Tg) mice. At 4-months-old, an age before amyloid deposition, the amygdala-associated, but not the hippocampus-associated, long-term memory was impaired in the Tg mice. The dendritic complexities of the amygdalar basolateral neurons, but not those in the hippocampal CA1 and CA3 neurons, were reduced. Furthermore, the levels of BDNF/TrkB signaling molecules (i.e. p-TrkB, p-Akt and p-PKC) were reduced in the amygdala, but not in the hippocampus of the 4-month-old Tg mice. The concentrations of A $\beta$ <sub>40</sub> and A $\beta$ <sub>42</sub> in the amygdala were higher than those in the hippocampus. Ten weeks of treadmill training (from 1.5- to 4-month-old) increased the hippocampus-associated memory and dendritic arbor of the CA1 and CA3 neurons, and also restored the amygdala-associated memory and the dendritic arbor of amygdalar basolateral neurons in the Tg mice. Similarly, exercise training also increased the levels of p-TrkB, p-AKT and p-PKC in the hippocampus and amygdala. Furthermore, exercise training reduced the levels of soluble A $\beta$  in the amygdala and hippocampus. Exercise training did not change the levels of APP or RAGE, but significantly increased the levels of LRP-1 in both brain regions of the Tg mice. In conclusion, our results suggest that tests of amygdala function should be incorporated into subject selection for early prevention trials. Long-term exercise protects neurons in the amygdala and hippocampus against AD-related degeneration, probably via enhancements of BDNF signaling pathways and A $\beta$  clearance. Physical exercise may serve as a means to delay the onset of AD.

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### 1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. Pathologically, AD is characterized by extracellular deposition of A $\beta$  peptides in the amyloid plaques and intracellular accumulation of hyperphosphorylated tau in the

neurofibrillary tangles. Biochemical and morphological studies suggest that neuronal synaptic connections are greatly reduced in AD brains (Larson, Lynch, Games, & Seubert, 1999; Small, Mok, & Bornstein, 2001). The most frequent and early clinical symptom of AD is memory impairment, in particular short-term memory (Albert et al., 2011; Thies & Bleiler, 2013).

Atrophy of the medial temporal lobe, a critical region involved in memory formation, is a recognized marker for AD (Duara et al., 2008; Jobst et al., 1994). Volumetric measurements of the hippocampus and amygdala, both residing in the medial temporal lobe, have been used to predict the cognitive status of the elderly

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(den Heijer et al., 2006) and to assist the clinical diagnosis of AD (Callen, Black, Gao, Caldwell, & Szalai, 2001; Heun et al., 1997; Tang, Holland, Dale, Younes, & Miller, 2014). Hippocampal and amygdalar memory impairments are already evident in the prodromal stage of AD (Palmer et al., 2007). In patients with MCI due to AD, atrophy (Fjell et al., 2010; Mizuno, Wakai, Takeda, & Sobue, 2000; Poulin, Dautoff, Morris, Barrett, & Dickerson, 2011; Roh et al., 2011) and deposition of amyloid plaques (Haroutunian et al., 1998; Markesbery et al., 2006) in the hippocampus and amygdala are more pronounced than in normal individuals of similar ages. Furthermore, the levels of A $\beta$ 42, total tau and p-tau in the cerebrospinal fluid (CSF) in patients with MCI are known to correlate with volumes of the hippocampus and amygdala (Fjell et al., 2010). These findings suggest that degeneration in the hippocampus and amygdala are crucial in clinical AD symptom manifestation. However, most scientific efforts focus only on hippocampal degeneration and leave amygdala less explored.

Increased physical activity has been shown to improve cognitive performance in humans at various ages (Hillman, Erickson, & Kramer, 2008). Exercise is also known to improve the ability of AD patients to perform daily activities (Rolland et al., 2007). In animal studies, running improves the performance of hippocampus- and amygdala-associated learning and memory (Lin et al., 2012; Liu et al., 2009; van Praag, 2009). The beneficial effect of running exercise is, at least partially, contributed by the enhanced BDNF–TrkB signaling pathway (Lin et al., 2012; Liu et al., 2009). Therefore, we hypothesize that exercise training could delay or prevent AD progression by protecting neurons in the hippocampus and amygdala.

In this study, we aimed to investigate the effect of exercise on neurodegeneration in the hippocampus and amygdala of young APP/PS1 double transgenic (Tg) mice, to mimic the prodromal stage of AD. We chose mandatory treadmill running as the exercise paradigm in order to control the precise intensity and duration of the exercise. The Tg mice were investigated at four months of age, before the typical onset time of amyloid deposition which occurs at around 6-months-old. The wild-type (Wt) littermates served as controls. After 10 weeks of exercise training, the learning and memory performance, dendritic morphology and synaptic protein expressions were examined. We chose the Pavlovian fear conditioning task to evaluate learning and memory performance because this task allowed us to assess both hippocampus- and amygdala-associated learning and memory functions (Phillips & LeDoux, 1992). Single neurons in the hippocampal CA1 and CA3 regions and the basolateral amygdala (BLA) were labeled by fluorescent dye injection to determine the changes in dendritic arborization. As BDNF/TrkB signaling pathways are known to regulate neuroplasticity, the levels of BDNF/TrkB and their downstream signaling proteins (i.e. PKC, AKT and ERK) in the hippocampus and amygdala were measured. Finally, the concentrations of A $\beta$  (40 and 42), APP, RAGE, and LRP1 were also determined.

## 2. Materials and methods

### 2.1. Animals

All experimental protocols were carried out in accordance with the National Institutes of Health Guidelines for animal research (Guide for the Care and Use of Laboratory Animal) and approved by the National Cheng Kung University Institutional Animal Care and Use Committee. All efforts were made to minimize the number of animals used and their suffering. Congenic male and female APP/PS1 double transgenic [B6.Cg-Tg (APP<sup>swe</sup>, PSEN1<sup>dE9</sup>) 85Dbo/Mmjax] mice were purchased from the Jackson Laboratory (Bar Harbor, ME). The Tg mice express a chimeric mouse/human amy-

loid precursor protein (APP695<sup>swe</sup>) and a mutant human presenilin 1 (PSEN1<sup>dE9</sup>) with the exon 9 deletion under the control of mouse prion promoter elements (Jankowsky et al., 2004). The mice were bred and maintained on a B6C3 background. All mice were housed in a controlled environment (temperature 23  $\pm$  1  $^{\circ}$ C; 12 h light/12 h dark cycle, light cycle begins at 06:00) in the Laboratory Animal Center of National Cheng Kung University with unrestricted access to food and water. Shortly after weaning, these mice were genotyped using a protocol provided by the Jackson Laboratory. Body weights were measured every three days.

Four sets of the mice were used in this study. The first set ( $n = 6$ ) was subjected to behavior test (see below Pavlovian fear conditioning tasks). The second set ( $n = 6$ ) was used to record the body weights and to collect the brains and soleus muscles for protein level determinations and citrate synthase activity assays, respectively. The third set ( $n = 6$ ) was isolated for A $\beta$ <sub>40</sub> and A $\beta$ <sub>42</sub> quantifications. The fourth set ( $n = 5$ ) was used to examine the neuronal morphology. Mice of the first set were killed by CO<sub>2</sub> euthanasia after the behavior test, while the other three sets were anesthetized with ethyl carbamate (Urethane, Sigma, St. Louis, MO, 1.2 g/kg, i.p.) and perfused with 50 ml of cold normal artificial CSF (aCSF) for subsequent analyses. There were few occasions mice were excluded from the study because they were not compliant with the training or injured during the training.

### 2.2. Exercise protocols

The detailed protocol of treadmill exercise (Ex) training has been described elsewhere (Lin et al., 2012). At the age of six weeks, mice were first subjected to a one-week familiarization course to reduce handling- and environment-related stimuli, followed by a nine-week Ex training course. Starting from the second week, mice of the Ex group ran on a leveled motor-driven treadmill (Model T510E, Diagnostic and Research Instruments Co., Taoyuan, Taiwan) at the speed of 10 m/min for 20–60 min/day (with an increment of 10 min/day), five days per week for the first week. During the second to fourth weeks mice ran on the treadmill for 60 min per day at the same speed (10 m/min), five days per week. The treadmill speed increased 1 m/min per month reaching 12 m/min at the end of the training period. The front end of the treadmill was covered with a dark cloth to attract the mice to run forward. A gentle tail touch was sufficient to keep the mice running and the entire training process was carried out without tail shock. The sedentary (Sed) groups were placed on the treadmill for ten minutes each day without receiving any exercise training.

### 2.3. Measurement of citrate synthase activity

The training effect of Ex was determined by measuring citrate synthase activity in the soleus muscle as previously described (Wu et al., 2007). Briefly, the soleus muscle samples were homogenized in ice-cold 0.1 M of Tris–HCl containing 0.1% Triton X-100 buffer, pH 8.3, centrifuged at 14,000g for 15 min at 4  $^{\circ}$ C, and the protein concentration of the supernatant was adjusted to 1.5  $\mu$ g/ $\mu$ l. After adding dithionitrobenzoate, acetyl CoA and oxaloacetate to the supernatant, citrate synthase activity was determined at 412 nm and expressed as  $\mu$ mole of substrate utilized per minute per mg of protein.

### 2.4. Pavlovian fear conditioning tasks

Pavlovian fear conditioning and subsequent testing were performed to evaluate memories associated with hippocampus and/or amygdala. The responses to contextual conditioning reflect the functions of hippocampus and amygdala, while the responses to cued conditioning mainly depend on the function of amygdala.

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