Companion retards the development of memory deficit in APP/PS1 mice

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Abstract

Alzheimer’s disease (AD), a progressive neurodegenerative disease, is the most common cause of dementia in aged person. Studies have demonstrated beneficial effects of social support on aging. This study is to examine whether companion retards AD development in APP/PS1 mice. APP/PS1 mice weaning at 6 months old were randomly assigned to group that cohouse with 1, 3, or 6 month-old WT mice for 3 months. Until at the age of 9 months, APP/PS1 mice were tested for fear-conditioning paradigms to test contextual and cue memory. In addition, APP/PS1 mice of group housing (no new companion treatment) were used as a control. We found that improvement of memory was higher in companion with 1-month WT mice than in other groups. Therefore, we choose 1-month WT mice as our companion model. We divided bad or good memory of aged APP/PS1 mice as unsusceptible or susceptible to companion group. The results show that $\frac{A\beta_{42}}{A\beta_{40}}$ ratio and calpain activity were significantly lower in the susceptible APP/PS1 mice than in the unsusceptible groups. These results suggest companion attenuates the increased $A\beta_{42}/A\beta_{40}$ ratio, calpain activity and rescues the memory deficit in APP/PS1 mice.

Keywords: Alzheimer’s disease; APP/PS1 mice; companion
Introduction

Alzheimer’s disease (AD) is a progressive neurodegenerative disease. It is the most common cause of dementia in aged person (Snyder et al., 2005). AD patients have declarative memory impairments and even the capacities for reasoning and language slip away (Selkoe, 2002). The pathological hallmarks of AD are amyloid plaques, comprising the Aβ, and neurofibrillary tangles. According to the amyloid hypothesis, Aβ is the key mediator of neuronal degeneration of AD (Citron, 2004; Hardy and Selkoe, 2002). In addition, Alzheimer dementia is characterized by emotional disturbances, notably apathy and social withdrawal.

Studies on human and other species have demonstrated beneficial effects of social interaction on aging (Carey, 2001; Omholt and Amdam, 2004). Short-lived Drosophila mutants of the antioxidant enzyme Cu/Zn superoxide dismutase displayed a robust lifespan extension upon cohousing with active flies or younger age (Ruan and Wu, 2008). These evidences suggest that social support or housing conditions can affect one’s behavior. Therefore, the purpose of this study is to detect whether companion inhibits AD development in aged APP/PS1 mice.

To date, at least 14 mammalian calpains, cytosolic calcium-activated cysteine proteases, have been identified (Huang and Wang, 2001). Two major forms, calpain 1 and calpain 2, also known as μ-calpain and m-calpain, have been linked to disease AD
(Nixon, 2003). Recently, calpain actions on the GluR1 subunit of AMPA receptors (Yuen et al., 2007), amphiphysin I (Wu et al., 2007) and suprachiasmatic nucleus circadian oscillatory protein (Shimizu et al., 2007), have been shown to modulate synaptic activity and memory formation. Therefore, we also investigated whether calpain is involved in the companion effect.
Materials and methods

Animals

APP/PS1 mice were obtained from the Jackson Laboratory (West Grove, PA, USA). APP/PS1 mice expressing a chimeric mouse/human amyloid precursor protein (Mo/HuAPP695swe) and a mutant human presenilin 1 (PS1dE9) were backcrossed to C57BL/6J strain for six generations to create APP/PS1 transgenic mice. All studies on animals were approved of the Institutional Animal Care and Use Committee of the College of Medicine, National Cheng Kung University.

Fear conditioning

Fear conditioning occurred in 30×24×21 cm specially designed chamber (Med Associates, St. Albons, VT) that was equipped with a shock floor, house light, and speaker mounted on the wall through which tone presentations were delivered. The shock floor consisting of stainless-steel rods was connected to a shock generator for foot shock delivery. The house light provided illumination during all sessions. All switches were controlled by FreezeScan software (Clever Systems, Reston, VA).

On the training day, mice were transported to a behavioral room. After a 1h habituation period in the room, mice were placed in the training chamber for 120 s. After the acclimation period, mice were presented with a 20 s pure tone (3 kHz) that
coterminated with 3 s foot shock (0.75 mA). This tone-foot shock pairing procedure was repeated 4 times with 40 s inter-trial interval (ITI). After the last tone-shock pairing, mice were given to explore the context for 2 min before removal from the training chamber. The second day, 24h after training, mice were returned to the training chamber 3 min without exposure the tone or foot shock to measure context fear test. At the end of the contextual test, mice were returned to their home cage.

During training and contextual fear test, the chamber was cleaned with 75 % ethanol before each mouse. The behavior procedure of mice was recorded by video camera and freezing was measured automatically using FreezeScan software. Freezing was defined as the absence of any movement except for respiration. Freezing levels are presented as percent time spent freezing.

Aβ ELISA assay

The concentration Aβ of hippocampal and amygdala tissues was detected by Aβ₄₀ or Aβ₄₂ Colorimetric ELISA kits (Invitrogen, Carlsbad, CA). The extraction buffers and experimental protocol were processed according to the manufacturer’s instructions (Johnson-Wood, et al 1997). AEBSF was added to prevent degradation of Aβ peptides in the extraction buffers. The absorbance was read with Microplate Reader. All were run in triplicates.
**Calpain activity**

Calpain activity was measured using Ac-LLY-AFC as the substrate provided by the calpain activity assay kit (Calbiochem, San Diego, CA) according to the manufacturer’s instructions. Hippocampi or amygdala were homogenized in supplied extraction buffer and centrifuged at 10,000×g for 1 minute. Equal amount of supernatants and the fluorogenic calpain substrate Ac-LLY-AFC were transferred to a 96-well microplate and then incubated for 1 h at 37°C in the dark. Upon cleavage of calpain substrate Ac-LLY-AFC, the fluorogenic protein (AFC) releases yellow-green fluorescence at an excitation/emission wavelength of 400/505 nm. Calpain activity was measured as relative fluorescence intensity and all samples were run in triplicates.

**Statistical Analysis**

All values were given as mean ± SEM. One way ANOVA and Newman–Keuls *post hoc* comparisons were used to analyze the differences in freezing responses among companion treatment in unsusceptible or susceptible group. The level of significance was *p* < 0.05.
Results

Companion affects fear memory

APP/PS1 mice weaning at 6 months old were randomly assigned to cohouse with 1, 3, or 6 month WT mice for 3 months. Until APP/PS1 mice at the age of 9 months, we use fear-conditioning paradigms to test contextual and cue memory. In addition, APP/PS1 mice of group housing (no new companion treatment) were used as a control (Figure 1). As shown in figure 2a, contextual freezing response of companion treatment may help improve contextual fear memory. We next assessed memory for cue. We also found that companion treatment may affect cue-induced freezing response (Figure 2b).

To divide bad or good memory as unsusceptible or susceptible to companion

In figure 2, we found that some APP/PS1 mice with companion treatment can improve fear memory but others can not. Therefore, we determined to separate bad or good memory as unsusceptible or susceptible group. We set upper limiting value of control group’s freezing response as dividing line (Figure 3). After using dividing line to separate bad or good memory, we found that improved ratio with 1-month WT mice was higher than other companion groups (Figure 4). Therefore, we used 1-month WT mice as companion model.
Companion decreases $A\beta_{42}/A\beta_{40}$ ratio in susceptible group

APP/PS1 mice were killed at the age of 9 months, hippocampi (Figure 5a) and amygdala (Figure 5b) were dissected and the ratios of $A\beta_{42}/A\beta_{40}$ were determined by sandwich ELISA. We found that there was no significant difference in $A\beta_{42}/A\beta_{40}$ ratios between control and unsusceptible groups ($p>0.5$). However, the ratios of $A\beta_{42}/A\beta_{40}$ were significantly lower in susceptible group than other groups both in hippocampi and amygdala.

Susceptible to company group ameliorates memory impairment by regulation of calpain activity

Calpains are calcium-dependent enzymes that determine the fate of proteins through regulated proteolytic activity. Calpains have been linked to the modulation of memory and are key to the pathogenesis of AD (Trinchese et al., 2008). We dissected hippocampi (Figure 6a) and amygdala (Figure 6b) to detect calpain activity. The data show that calpain activity was no difference between control and unsusceptible group. However, calpain activity was significantly lower in susceptible group than in other groups ($p<0.001$).
Discussion

The study using cohousing paradigm reveals that the relationship between companion and the improvement of AD. Our data demonstrate that when cohousing with 1-month WT mice, aged APP/PS1 mice significantly retard the impairment of memory, providing an evidence for the beneficial effects of companion on AD development.

An immediate question of the study would be whether this improvement effect is mediated by social interaction. Social activities are important for Alzheimer's patients. We have observed that aged APP/PS1 mice cohoused with 1-month WT mice gain significantly higher improved ratio of freezing responses than other groups. Therefore, we cannot exclude the possibility of this result might high social interaction between aged APP/PS1 mice and young mice. We also found that cue-dependent memory can be improved after companion treatment. The results suggest that companion might affect physical condition such as emotion. Our results indicate that cohousing with 1-month WT mice helps inhibition of AD development, corroborating the enduring notion that AD may be benefited by an appropriate social environment.

In summary, we divided bad or good memory of aged APP/PS1 mice as unsusceptible or susceptible to companion group. The results show that Aβ42/Aβ40 ratio and calpain activity were significantly lower in the susceptible APP/PS1 mice
than in the 9-month (control) and unsusceptible groups.
References


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Figure 1. To design experimental procedure. When weaning of 6 months, APP/PS1 mice were randomly assigned to company with 1, 3 or 6-month WT for 3 months until APP/PS1 mice at the age of 9 months when behavioral test were performed. As a control, APP/PS1 mice of group housing (no new companion) were used.
Figure 2. Freezing responses of cohousing with different age of WT mice. (a) 6-month old APP/PS1 mice were randomly assigned to cohous with WT mice for 3 months. The mice received 4 tone-shock pairings and 24 h later freezing responses was measured as an index for memory retention. For contextual fear memory, freezing response was significantly lower in the 9-month APP/PS1 mice (n=30). (b) One hour after contextual fear test, mice were placed back into the novel context with altered visual, tactile and odor cues and tested for amygdala-dependent fear learning (n=15 in each companion group).
Figure 3. To divide bad or good memory as unsusceptible or susceptible to companion. We set upper limiting value as dividing line to divide bad (unsusceptible to companion) or good memory (susceptible to companion).
Figure 4. Improved ratio was higher by companion with 1-month WT mice
We set upper limiting value of contextual (a) or cue fear memory (b) to divide unsusceptible or susceptible group.(c) For contextual test, amelioration ratio of memory impairment in APP/PS1 mice were significantly enhanced by companion with 1-month WT mice.(d) APP/PS1 mice retard impairment of amygdala-dependent fear learning in companion with 1-month WT mice.
Figure 5. Aβ 42/40 ratio was deceased in susceptible group. Mice were sacrificed at the age of 9 months, hippocampi (a) and amygdala (b) were dissected, and ratio of $Aβ_{42}/Aβ_{40}$ was determined by sandwich ELISA (n=4 in each group). Susceptible group exhibited significantly lower ratio of $Aβ_{42}/Aβ_{40}$ compared with other groups. *** $p<0.001$ vs. 9MTg mice. ###$p<0.001$, ##$p<0.01$ vs. unsusceptible group Tg mice.
Figure 6. Susceptible to company group ameliorates memory impairment by regulation of calpain activity. Whole cell lysates of hippocampi (a) or amygdala (b) from control, unsusceptible and susceptible APP/PS1 mice were prepared and assayed for calpain activity. Calpain activity was significantly lower in the susceptible APP/PS1 mice than in the 9-month and unsusceptible APP/PS1 mice (n=5~6). **p<0.01, ***p<0.001 vs. 9MTg, ###p<0.001 vs. unsusceptible group APP/PS1 mice.