Behavioural Brain Research 318 (2017) 18–23

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Maternal separation exacerbates Alzheimer's disease-like behavioral and pathological changes in adult APPswe/PS1dE9 mice

Jianjun Hui[a,c], Gaifeng Feng[a,b,*], Caifeng Zheng[a,c], Hui Jin[a,b], Ning Jia[a,b]

[a] Department of Human Anatomy, Histology and Embryology, School of Basic Medical Sciences, Xi'an Jiaotong University Health Science Center, 76# West Yanta Road, Xi'an, Shaanxi 710061, China
[b] Key Laboratory of Environment and Genes Related to Diseases, Xi'an Jiaotong University, Ministry of Education of China, 76# West Yanta Road, Xi'an, Shaanxi 710061, China
[c] Department of Emergency Surgery, Ankang City Central Hospital, 85# Jinzhou Street, Ankang, Shaanxi 725000, China

HIGHLIGHTS

- Maternal separation exacerbates adult APPswe/PS1dE9 mice learning and memory deficits.
- Maternal separation increases senile plaques deposition in cortex and hippocampus in APPswe/PS1dE9 mice.
- Maternal separation decreases cholinergic neurons in nucleus basalis of Meynert in APPswe/PS1dE9 mice.

ARTICLE INFO

Article history:
Received 29 May 2016
Received in revised form 17 October 2016
Accepted 19 October 2016
Available online 19 October 2016

Keywords:
Alzheimer's disease (AD)
Maternal separation(MS)
Senile plaque
Cholinergic neuron

ABSTRACT

Alzheimer's disease (AD), the most common neurodegenerative disorder that gradually destroys memory and cognitive abilities in the elderly, makes a huge emotional and economic burden on the patients and their families. The presence of senile plaques and the loss of cholinergic neurons in the brain are two neuropathological hallmarks of AD. Maternal separation (MS) is an animal paradigm designed to make early life stress. Studies on wild type rodents showed that MS could induce AD-like cognitive deficit and pathological changes. However, the effects of MS on AD susceptible population or AD animal models are still unclear. In the present study, male APPswe/PS1dE9 transgenic mice were separated from dam and pups 3 h per day from postnatal day 2 to day 21. After weaning, all animals were housed under normal conditions (4 mice per cage). At 9-month age, MWM tests were performed to evaluate the learning and memory abilities. Then the pathological changes in the brain were measured by histology staining. The results showed MS mice had more severe deficit of learning and memory. Compared to the control, there were more senile plaques in cortex and hippocampus, fewer cholinergic neurons in nucleus basalis of Meynert in MS mice. These results indicate that MS exacerbates Alzheimer's disease-like behavioral and pathological changes in APPswe/PS1dE9 mice.

1. Introduction

Alzheimer's disease (AD) is a progressive, irreversible brain disorder which is clinically characterized by an accelerated loss of memory and deterioration of other cognitive functions, including learning ability [1,2]. With the elderly population increasing steadily, AD is becoming a huge emotional and economic burden on the patients, their families and the society as a whole [3,4].

Senile plaques and loss of cholinergic neuron are two neuropathological hallmarks of AD [5,6]. The principal constituent of senile plaques is amyloid-β protein (Aβ), which is generated from amyloid precursor protein (APP) and plays a crucial role in AD development [7–10]. AD patients exhibit selective loss of cholinergic neurons in the brains, especially in nucleus basalis of Meynert [11]. Both the increased Aβ and loss of cholinergic neuron are believed to contribute to cognitive decline in AD [12,13].

Although the exact etiology of AD remains unknown, clinical and animal experimental researches suggested that stress was a risk factor for AD [14,15] and stress-related psychiatric disorders had been identified as a risk for developing AD [16–22].

* Corresponding author at: Department of Human Anatomy, Histology and Embryology, School of Basic Medical Sciences, Xi'an Jiaotong University Health Science Center, 76# West Yanta Road, Xi'an, Shaanxi 710061, China.
E-mail address: gaifeng.feng@mail.xjtu.edu.cn (G. Feng).

http://dx.doi.org/10.1016/j.bbr.2016.10.030
0166-4328/

Maternal separation (MS) is an animal paradigm designed to mimic repeated exposure to stress during early life [23,24]. Stress during early life could disrupt the maternal-infants relationship and lead to long-lasting deleterious effects on brain neurodevelopment and on the network of biological systems [25]. Studies on wild type mouse and rat showed that MS could induce AD-like cognitive deficit and pathological changes [26–29]. So we speculated that MS might exacerbate these abnormal changes in the AD susceptible population, especially those who had familial Alzheimer disease (FAD) inherent gene. To prove this, in the present study, APPswe/PS1dE9 double transgenic mice were used to explore the effects of MS on their behavioral and pathological changes.

2. Material and methods

2.1. Animals

In this study, the experimental procedures involving animals were performed in accordance with the guidelines for the Regulations of the Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China. All efforts were made to minimize animals' suffering.

APPswe/PS1dE9 mice were purchased from the Model Animal Research Center of Nanjing University (Nanjing, China). Mice were raised at 22–25 °C and 50–60% relative humidity under a 12-h light/dark cycle, with food and water available ad libitum. When they were 12 weeks of age, mice were mated (1 male mouse and 2 female mice in each cage). Males were removed from females 2 weeks after mating, and pregnant females were separated into individual cages. Nesting material was provided 1–2 days prior to the day of birth.

2.2. Maternal separation

On postnatal day 1(PND1), all pups were numbered by cutting toe (s) and their genomic DNAs were collected from the tail and toe (s) biopsies. Then APP/PS1dE9 double transgenic mice were genotyped by PCR analysis of genomic DNAs. On postnatal day 2 (PND2), we selected 10 litters which had paired male APP/PS1 mice, and only one pair of mice in each litter was used in the experiment. For each pair of the selected pups, one is randomly assigned to MS mouse, and the other is the control. MS was performed as previously described with little modification [30,31]. In brief, MS pups were separated from their dams for 3 h (09:00 a.m.–12:00 p.m.) from PND 2 to 21. During separation, pups were transferred to an adjacent room. Each pup was placed individually in a plastic beaker with breathable lids. The plastic beakers were warmed with a heated blanket to maintain the temperature at 33–35 °C. After 3 h of separation, the pups were returned to their home cage. In the control group, pups were left undisturbed except changing the bedding in their cages weekly. After weaning on PND22, all animals were housed under normal conditions (4 mice per cage, 22–25 °C and 50–60% relative humidity, a 12-h light/dark cycle, with food and water available ad libitum).

2.3. Behavioral tests

When mice were 9-month old, Morris Water Maze (MWM) tests were performed to measure their learning and memory as we described before [32]. In brief, the test was conducted in a pool (120 cm diameter) which was filled with water (23 ± 1 °C). The pool was imaginarily divided into four quadrants. The test was divided into 2 trials: 5-day spatial navigation trial (assessing learning) and one day probe trial (assessing memory). In the spatial navigation trial, an escape platform, located constantly at 4th quadrant, was submerged 1 cm beneath the water surface. In each spatial navigation trial, the time to reach platform from the start location was recorded as latency. Each trial lasted a maximum of 60 s. If the mouse failed to find the platform within 60 s, it was manually guided to the platform and allowed to stay on the plate for 10 s, and the latency was recorded 60 s.

24 h after the last spatial navigation trial all mice were given the probe trial, in which the platform was withdrawn, in order to assess the final strength of memory traces. In probe trials, time spent in target quadrant and platform region, platform crossings (the number of times that the mice crossed the location of the platform), the swimming distance and speed were measured.

Performance in all tasks was recorded and analyzed by a computer-based video tracking system and image analyzing software (Chengdu TME Technology company, Chengdu, China).

2.4. Detection of senile plaques in cortex and hippocampus

After the water maze test, mice were anesthetized with 10% chloral hydrate (300 mg/kg) intraperitoneal injection, and perfused transcardially with 0.9% NaCl followed by 4% paraformaldehyde (pH 7.4). Brains were removed and postfixed in 4% paraformaldehyde for 24 h and subsequently incubated in 30% sucrose till sunk for cryoprotection. Coronal sections of 30 um thickness were collected and stored at 4 °C in PBS until use.

For senile plaques staining, two series sections (containing the dorsal hippocampus) with 150 μm distance between the sections (one of every 5 series sections) were used. One set of sections were performed using standard free-floating labeling procedures for immunohistochemistry. In brief, endogenous peroxidase in tissue was blocked by treating with 3% H_2O_2 in PBS for 20 min. Nonspecific background staining was blocked by incubation in 5% normal goat serum or normal rabbit serum, 0.3% Triton X-100 for 30 min. Brain sections were incubated with primary antibodies ($A\beta_{1\text{-}42}$, 1:1000, Cell Signaling Technology, USA) overnight at 4 °C, rinsed 3 times with PBS, followed by biotinylated anti-rabbit secondary antibody for 1 h. After PBS rinsing, sections were incubated in avidin-biotin complex (Boster biotechnology company, China) for 1 h at room temperature. The reaction was developed using 3,3′-diaminobenzidine (DAB) substrate kit (Boster biotechnology company, China). The control experiments with primary antibody omitted resulted in negative staining.

Fibrillar Aβ deposits were visualized using Thioflavin S (Th-S) staining in another set of mouse brain sections [32]. Brain sections were washed with Tris buffer 3 times and stained for 5 min with a solution of 0.05% Th-S in 50% ethanol. After that, sections were washed in 50% ethanol and Tris buffer, then covered using Vectashield (Vector Laboratories).

The staining was observed under microscope. Percent of immunopositive area or Th-S staining area (% Field Area) (immunopositive area (or Th-S positive area)/total image area) was determined for amyloid plaques of the cortex, hippocampus from per animal by averaging images. The percentage of occupied area was calculated using the image analysis software Image-Pro Plus [33].

2.5. Detection of cholinergic neurons in the nucleus basalis of Meynert

Choline acetyltransferase (ChAT), the enzyme responsible for the biosynthesis of acetylcholine, is the most specific indicator for monitoring the functional state of cholinergic neurons in the nervous systems. So ChAT immunohistochemistry staining were used to detect the cholinergic neurons in the brain. One series of sections (containing the nucleus basalis of Meynert) with 150 μm distance between the sections (one of every 5 series sections) were