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Research report

Early deprivation reduced anxiety and enhanced memory in adult male rats

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ABSTRACT

The effects of early deprivation (ED, which involves both dam and littermate deprivation) on anxiety and memory are less investigated in comparison with maternal separation (MS), and it is not yet clear how ED affects long-term potentiation (LTP) in the hippocampal Schaffer collateral pathway. By using a series of behavioral tests, enzyme-linked immunosorbent assay and field potential recording, we explored the effect of pre-weaning daily 3-h ED on anxiety, memory and potential mechanisms in adult male rats. Compared with control, ED rats spent longer time in open arms of elevated plus maze and in light compartment of light-dark transition box. Consistently, stress-induced blood plasma corticosterone level was also lower in ED rats. Moreover, ED rats showed better performance in social recognition and Morris water maze test. In accordance with results in memory tests, the threshold of LTP induction in hippocampal CA3–CA1 pathway of ED rats was also reduced. Our results indicate ED reduced anxiety, but enhanced social recognition and spatial reference memory. We suggest the diminished hypothalamic–pituitary–adrenal axis response and facilitated hippocampal LTP may contribute to the anxiety-reducing and memory-enhancing effects of ED, respectively.

1. Introduction

Infant–mother relationship consists a major part of environmental stimuli in early life, which can affect development of individual emotion, cognition and social behaviors. Clinical studies suggest adults with experience of childhood parental loss have sensitized response to stress, and are at higher risk of suffering psychiatric disorders such as depression and anxiety (Agid et al., 1999; Harris et al., 1986; Longstaffe, 1979; Tyrka et al., 2008).

Two paradigms of postnatal manipulation of infant–mother relationship in rats were mostly used in previous studies, which are early handling (EH, 10–15 min per day) and maternal separation (MS, 1–6 h per day). In these manipulations, pre-weaning rat pups were separated from dams as an intact litter for a certain time period per day for several days. EH rats have relatively lower anxiety level (Cannizzaro et al., 2006; McIntosh et al., 1999; Skripuletz et al., 2010), and display better performance in several memory tests (Kosten et al., 2007; Núñez et al., 1995; Peters et al., 1991; Pham et al., 1997; Pryce et al., 2003; Weiss et al., 2001). In contrast, anxiety-like behavior (Huot et al., 2001; Pascual and Zamora-León, 2007; Skripuletz et al., 2010; Wigger and Neumann, 1999) and memory deficits (Frankola et al., 2010; Huang et al., 2002; Hulshof et al., 2011; Huot et al., 2002; Oitzl et al., 2000) were reported in MS rats. Various neuroendocrinological and neuromorphological alternations were proposed to explain these effects (Huot et al., 2002; Kalinichev et al., 2002; Pascual and Zamora-León, 2007; Wigger and Neumann, 1999). However, early deprivation (ED), another paradigm which involves deprivation of not only the dam but also the littermates, was less investigated. In addition, to date, there is no report about how ED affects long-term potentiation (LTP) in the hippocampal CA3–CA1 pathway, which is a cellular mechanism that may underlie hippocampus-dependent learning and memory (Moser et al., 1998; Tsien et al., 1996; Villarreal et al., 2002), although a few studies focused on the impact of ED on entorhino-hippocampal and amygdalo-hippocampal LTP (Blaise et al., 2008; Kehoe and Bronzino, 1999).

In the present study, we examined the behavioral responses of ED by measuring anxiety and memory, and explored the underlying biochemical and cellular mechanisms in rats.

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2. Material and methods

2.1. Animals

Sprague Dawley rats aged 3–4 months were purchased from Shanghai Slac Laboratory Animal Co., Ltd. In China, and were maintained under a 12/12 h light/dark cycle with lights on at 7:00 AM. Temperature and humidity were kept at 22 ± 1 °C, 55 ± 5%, with *ad libitum* access to food and water. Rats were allowed to breed one week after arriving to the animal facilities. For breeding, one male and two female rats were co-housed per cage for one week, then female rats were separated as one per cage. All procedures were conducted according to Animals Act, 2006 (China).

2.2. Experimental groups and ED treatment

To determine which protocol for ED to be used in the present study, a preliminary study on time length of ED was first performed. Rats were treated with 1, 3 or 6-h of ED per day on postnatal day 1–21. Our results showed that 1 or 3-h ED did not affect the body weight compared with control rats, while 6-h ED significantly reduced the body weight (Jin et al., 2011). We therefore chose the 3-h ED protocol for the present study.

A total of 23 litters were used in the present study, with 7–12 rat pups per litter. Neonatal male rat pups were selected, and were assigned to different experimental groups with a within litter design, *i.e.* in each experiment, half of pups in each litter were selected as control group, and the rest half served as ED group. The control rats were not disturbed except for weekly cage cleaning. The ED treatment started from postnatal day 1 (ED rats), each of them was taken out from the home cage, weighed, and then put in a smaller cage with heating pads (26 cm × 20 cm × 14 cm maintained at 30 °C, with clean bedding material) to separate from dam and littermates for 3 h before put back to the home cage. ED pups were kept in standard IVC (individually ventilated cage) system in a different room to prevent ultrasound communication between individual pups, or between pups and their dam. The remaining pups (control group) stayed with the dam during the separation period. These procedures were repeated daily until postnatal day 21. All rats were weaned on postnatal day 21 and randomly housed with 4 per cage. All procedures were performed between 11:00 AM and 2:00 PM.

To avoid the carry over effect between tests, different batches of rats were used in each of all the following experiments except that one batch of rats were used for light–dark transition test and then for Morris water maze after one week of interval.

2.3. Behavioral tests

Behavioral tests were conducted when rats were 3 months old. All behavioral tests were performed in separate rooms between 9:00 AM and 5:00 PM, with room temperature at 22 ± 2 °C. Rats were brought into the room 1 h before the experiment began.

2.3.1. Open field

The open field tests were performed in a coverless box (50 cm × 50 cm × 45 cm, Coulbourn Instruments, Lehigh Valley, PA, USA) and the luminosity was 80 lux. Rats were placed individually into the center of the box and were allowed to explore in the box for 15 min. Their activity were monitored and the number of moves, total distance traveled and time spent in the center region were automatically calculated (Tru Scan Activity System, Coulbourn Instruments, Lehigh Valley, PA, USA).

2.3.2. Elevated plus maze

Elevated plus maze apparatus, made from opaque plastic, consisted of two open arms (50 cm × 9 cm), two enclosed arms (50 cm × 9 cm × 39 cm) and a central area (9 cm × 9 cm), elevated 70 cm above the ground (Jiliang Co., Ltd., Shanghai, China). The luminosity level was 70 lux in the central area and the open arms, and 55 lux in the enclosed arms. The activity of the rats were monitored for 5 min, and time spent in the open arms as well as entries into different arms was automatically calculated by video tracking system (ANY-maze, Stoelting Co., Wood Dale, IL, USA).

2.3.3. Light–dark transition

The light–dark box (50 cm × 50 cm × 45 cm, Coulbourn Instruments, Lehigh Valley, PA, USA) was divided by an opaque plexiglass with an aperture between two compartments. The light compartment was illuminated by strong light (400 lux). Rats were individually placed at the center of the light compartment facing away from the aperture, and were allowed to explore freely in the box for 30 min. The activity of the rat were monitored and the time spent in the light compartments as well as number of transitions was automatically calculated by Tru Scan Activity System (Coulbourn Instruments, Lehigh Valley, PA, USA).

2.3.4. Social discrimination

The protocol is as described previously (Dong et al., 2012). The experiment was carried out in uncovered rat cages (50 cm × 36 cm × 28 cm). In the training session, rats were habituated to a clean rat cage (with clean bedding material but without food and water) and the experiment room for 30 min, and then a naïve juvenile male rat (1 month old) was put into the cage. The activity of the rat was tracked by a video camera, and the time spent on sniffing, grooming, climbing or closely following the juvenile rat in 3 min was recorded. The test session began 30 or 60 min after the training session, in which the same juvenile male rat and a novel juvenile male rat were put into the same cage. The rat was again allowed to explore freely for 3 min and the time spent on exploring either of the two juvenile rats were recorded. The ratio of the amount of time spent exploring the novel juvenile rat over the total time spent exploring the two juvenile rats was calculated as the novel investigation index.

2.3.5. Morris water maze

The protocol is similar to that described previously (Wang et al., 2009). The water maze apparatus is a circular pool (1.5 m in diameter, 54 cm in height) filled with black-dyed water at 22 ± 1 °C. The pool was surrounded by a black curtain attached with several different extra-maze cues. The experimental procedure includes pre-training, training and probe test. During pre-training, a visible platform was located at the center of the pool throughout 4 trials. Rats were trained to swim and get on the visible platform (15 cm in diameter). The escape latency to the visible platform was recorded and swim speed was calculated. During training, rats were trained to find the hidden platform which is submerged 1 cm below the water level and placed at the center of one of the quadrant. Training consisted of 4 sessions (4 trials/session/day). Escape latency to the hidden platform of each rat were recorded. If rats did not find the platform in 90 s, they were guided and placed on the platform, and escape latency were recorded as 90 s. Rats were allowed to stay on the platform for 15 s before placed back to the home cage. A probe test was carried out after the training sessions were completed, in which rats were allowed to swim for 90 s in the absence of the platform. The time spent in each quadrant was recorded. The navigation routes of the rats were tracked and further analyzed by WaterMaze Software (Coulbourn Instruments, Lehigh Valley, PA, USA).