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Research report

Maternal separation exaggerates spontaneous recovery of extinguished contextual fear in adult female rats

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HIGHLIGHTS

- MS exaggerated spontaneous recovery of extinguished contextual fear in female rats.
- MS had no effect on contextual fear learning and extinction as well as innate fear.
- MS impaired LTP in IL mPFC layer2/3-layer5 and hippocampal SC-CA1 pathways.

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ABSTRACT

Early life stress increases the risk of posttraumatic stress disorders (PTSD). Patients with PTSD show impaired extinction of traumatic memory, and in women, this occurs more often when PTSD is preceded by child trauma. However, it is still unclear how early life stress accounts for extinction impairment. Here, we studied the effects of maternal separation (MS, postnatal day 2 to 14) on contextual fear extinction in adult female rats. Additionally, to examine changes in synaptic function affected by MS, we measured long-term potentiation (LTP) in prefrontal cortex and hippocampus in vitro, both of which have been implicated in fear extinction. We found that adult female rats had been subjected to MS exhibited significant spontaneous recovery of fear to the extinguished context. Furthermore, MS exposure resulted in LTP impairment in both infralimbic prefrontal cortex layer 2/3-layer 5 and hippocampal SC-CA1 pathways. Interestingly, no obvious effects of MS on contextual fear conditioning, fear recall as well as extinction training and recall were observed. Innate fear in the elevated plus maze or open field test remained nearly unaffected. These findings provided the first evidence that MS may exaggerate spontaneous recovery after contextual fear extinction, for which LTP impairment in the medial prefrontal cortex and hippocampus may be responsible, thereby possibly leading to impaired extinction associated with PTSD.

1. Introduction

Clinical studies indicate that early life stress has been consistently associated with increased risk of stress-related neuropsychiatric disorders such as PTSD in adult [1–3]. Patients with PTSD typically exhibit exaggerated and persistent fear responses to the reminders of the traumatic events, a symptom which is widely studied via fear extinction in animal models. Fear extinction is the reduction in conditioned responses that occurs when the conditioned stimulus no longer predicts the unconditioned stimulus [4–6]. Our understanding of the mechanisms underlying fear extinction impairment may be valuable to elucidate the pathophysiology of PTSD.

Maternal separation (MS), a well-established animal model of early life stress, has been reported to have enhanced [7–9], decreased [10,11] or no effects [12,13] on innate fear. In contrast, the effects of MS on conditioned fear have been less well characterized. Previous studies conducted to assess conditioned fear also have yielded conflicting findings. Fear conditioning has been shown to be impaired [14,15] or enhanced [16] or unaffected [17,18] by MS. In these studies the effects of MS on extinction of fear memory have been poorly assessed. Moreover, reports indicate that the effects of MS on innate fear and fear conditioning are gender-dependent [11,14]. Females respond strongly to early life stress

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and suffer from memory impairment in adulthood [16,19], indicating that females are affected functionally by early life stressors. However, few studies have clearly addressed the effects of MS on extinction of fear memory in females.

Studies have revealed that brain regions including the prefrontal cortex and hippocampus appear vulnerable to stress [20,21], and both the brain regions are involved in fear extinction [22,23]. The medial prefrontal cortex (mPFC) is critically responsible for extinction memory consolidation and recall [24–28]. The hippocampus (HPC) plays a vital role in contextual modulation of fear extinction [29–31]. MS has been reported to reduce the dendritic spine density and disorganize the synaptic composition in mPFC [32–34], and alter dendritic arborization and spinogenesis in HPC [35–37]. These changes in synaptic structure maybe impact on long-term potentiation (LTP), a cellular mechanism well-known to underlie learning and memory. However, there are still few evidences related to how the MS modifies LTP in mPFC and HPC in females.

Therefore, the present study was conducted to examine the long lasting effects of MS on fear extinction in adult female rats. The animals that had been subjected to MS were tested in a 16-day paradigm of contextual fear, which assessed the acquisition, recall, extinction and extinction recall as well as the subsequent spontaneous recovery of contextual fear. Furthermore, LTP changes in the local circuit of mPFC or HPC were examined attempting to elucidate the possible underlying cellular mechanisms relate to the effects of MS.

2. Materials and methods

2.1. Animals

Pregnant Sprague-Dawley (SD) female rats (Animal House Center, Kunming Medical University, Kunming, Yunnan, China), were carefully fed to generate the female litters for the present study. Pregnant females were housed individually in a temperature and humidity-controlled environment with a 12:12-h light/dark cycle (light on at 07:00 a.m.) and given ad libitum access to standard rats chow and water. Each cage of pregnant females was checked for delivery twice a day (10:00 a.m. and 07:00 p.m.) and the day of birth was designated postnatal day 0 (PND 0). Dams with litters were left undisturbed until PND 2 and then randomly assigned to MS or the control (CTR). A total of 42 female pups were used in this study (22 MS females, and 20 CTR females). Animal care and experimental protocols were approved by the Committee for Animal Care at Kunming Institute of Zoology, Chinese Academy of Sciences, China.

2.2. Maternal separation

Maternal separation (MS) procedure was performed as described previously [9]. On the morning of PND 2 (9:00 a.m.), the litters were sexed and culled randomly to 6–10 pups per dam with a male: female ratio of approximately 1:1. Then, all the pups of each dam were randomly assigned to the CTR group, pups with their dam were left undisturbed except cage cleaning twice a week until weaning; or the MS group, pups were separated from their dam for 3 h daily (9:00 a.m.–12:00 a.m.) from PND 2 to 14. For the MS procedure, the dam was firstly removed into a new cage, and then all the pups of the dam were removed into another new cage, in the same rearing room. The dam was quickly returned into the home cage, but the pups were returned to the home cage at the end of the MS. At the weaning day on PND 22, five same-sex animals per cage of MS or CTR respectively were taken from different dams, and were only female pups used in the present study. For each study (behavioral test and LTP), no more than 2 animals were from the same litter. Experimental studies were conducted when the animals were at the age of 8–10 weeks.

2.3. Fear conditioning and extinction

2.3.1. Apparatus

Rodent fear conditioning chamber (30.5 cm × 24 cm × 24 cm; MED Associates) was enclosed in a ventilated and sound-attenuated box (63 cm × 43 cm × 63 cm; MED Associates). The chamber was constructed of aluminum (two side walls) and Plexiglas (rear wall, ceiling, and hinged front door). The grid floor of the chamber was composed of 19 stainless steel bars (5 mm in diameter). A programmed MED PC system controlled the ventilation fan and the number, duration and intervals of electric shocks. The behavior of the rats was recorded using a digital video camera on the ceiling of the sound-attenuated box.

2.3.2. Procedure

The entire procedure was conducted over 16 days with four experimental phases including contextual fear conditioning, extinction training, extinction recall and spontaneous recovery test. All the animals were allowed to habituate for at least one hour to the behavioral room from the rearing room before experiment on each day. Each group contains 8 rats taken from 4 to 5 dams.

Contextual fear conditioning (*day* 1): All animals were placed in the conditioning chamber and received five footshock (1.0 mA, 2 s) at 2 min intervals after a 2 min acclimation in the conditioning chamber. Rats were retained for another 2 min in the chamber after the last footshock. After conditioning, animals were immediately placed into their home cages and then returned to the rearing room.

Contextual fear recall and fear extinction training (*day* 2 *to day* 5): Approximately 24 h after fear conditioning, all conditioned animals went through extinction training session over four consecutive days from day 2 to day 5. During each extinction day, animals were exposed to the conditioned chamber for 10 min without footshock. The percentage of freezing during the first extinction training day on day 2 was considered as the recall of contextual fear.

Extinction recall (*day* 6): Approximately 24 h after the last extinction training day, all animals were returned to the extinction chamber for 4 min to assess fear extinction recall.

Spontaneous recovery (*day* 16): 10 days after extinction recall, all animals were re-exposed to the chamber for 4 min to examine the recovery of contextual fear.

Freezing behavior was video-recorded and quantified by an experienced observer blind to the experimental groups. The immobilization of rats except for respiration was regarded as freezing. The total time spent in freezing was recorded with a digital stopwatch. Fear response was scored as percentage of the time spent in freezing.

2.4. Elevated plus maze test

The elevated plus maze is 50 cm above the floor and consisted of two open arms (50 cm × 10 cm) and two enclosed arms (50 cm × 10 cm × 40 cm) with an open roof, arranged such that the two open arms were opposite to each other. A video camera was positioned above the apparatus to record the behavior of the animals. Each animal was placed in the central platform, facing to a closed arm. Each rat was tested for 5 min. An entry was counted when all four paws of the rat entered an open or closed arm. The maze was wiped with ethanol after each test. We measured the following parameters: the percentage of the time spent in the open arms, the percentage of open arms entries and numbers of open