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Research report

The different effects of maternal separation on spatial learning and reversal learning in rats

Qiong Wang[a], Man Li[a], Wei Du[a], Feng Shao[a,*], Weiwen Wang[b,**]

[a] Department of Psychology, Peking University, Beijing 100871, China
[b] Key Laboratory of Mental Health, Institute of Psychology, Chinese Academy of Sciences, Beijing 100101, China

HIGHLIGHTS

- MS increased locomotor activity in the open field of all three ages of rats.
- MS induced less anxiety-behaviors in the open field of adolescent rats.
- MS slightly disrupted spatial learning of MWM in adolescent and young adult rats.
- MS improved reversal learning of MWM in adolescent and young adult rats.

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ABSTRACT

Early postnatal maternal separation (MS) can play an important role in the development of psychopathologies during ontogeny. In the present study, we investigated the effects of repeated MS (4 h per day from postnatal day (PND) 1 to 21) on locomotor activity and anxiety behavior in open field, spatial learning and reversal learning in Morris water maze of male and female juvenile (PND 21), adolescent (PND 35) and early adult (PND 56) Wistar rats. The results indicated that MS increased locomotor activity of rats across all ages and reduced anxiety behavior of adolescent rats in open field test. MS also increased swim distance in spatial learning and decreased escape latency in reversal learning in adolescent and early adult rats. Additionally, for socially reared rats, there was increased spontaneous locomotion with age, decreased reversal learning ability with age. The present study provides novel insights into the consequences of MS and demonstrates unique age-dependent changes at the behavioral levels.

1. Introduction

Adverse early life events are considered risk factors for the development of psychiatric diseases [1,2]. In rats, maternal separation (MS), which deprives pups of their mothers, has often been used as a model for adverse early life experiences [3,4]. MS has been demonstrated to induce behavioral and cognitive abnormalities, such as increased locomotor activities [5,6], anxiety-related behaviors [7,8], prepulse inhibition (PPI) deficits [9,10], and impaired spatial learning in the Morris water maze test [11,12].

Reversal learning measures cognitive flexibility, which is defined as the ability to rapidly adjust established patterns of behavior according to changing circumstances [13]. Reversal learning has been considered a suitable model for measuring the cognitive rigidity in schizophrenia and other neuropsychiatric disorders. It has been demonstrated that adverse life experiences, such as social isolation, can disrupt the reversal learning performance of rats [14–18]. However, it is unclear whether MS can affect reversal learning in animals. To date, only one study has reported that a single 24 h MS at postnatal day 8 reduced reversal learning in the Morris water maze (MWM) of adult CD1 mice [19]. Successful reversal learning performance depends on normal prefrontal cortico-striatal functioning, which includes the prefrontal cortex (PFC) [20,21] and nucleus accumbens (NAc) [22,23]. It is well known that these brain regions are related to the neural substrates of neuropsychiatric diseases.

It has been demonstrated that there are age-related differences among several behavioral and physiological repertoire. For

* Corresponding author at: Department of Psychology, Peking University, 5 Yiheyuan Road, Beijing 100871, China. Tel.: +86 10 62755262; fax: +86 10 62761081.
** Corresponding author at: Key Laboratory of Mental Health, Institute of Psychology, Chinese Academy of Sciences, 4A Datun Road, Beijing 100101, China. Tel.: +86 10 64864516; fax: +86 10 64872070.
E-mail addresses: shaof@pku.edu.cn (F. Shao), wangww@psych.ac.cn (W. Wang).

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example, elevations in emotional reactivity, reward processing, locomotor and explorative activity follow an inverted U shape, with the peak occurring during adolescence [24,25]. While the ontogeny of rat social play is characterized by an inverted-U-shaped function, with a peak existing between 32 and 40 days of age, especially in 35-day rats [26,27]. Furthermore, the dopaminergic activity in the prefrontal cortex has been reached a peak during adolescence [28].

Furthermore, other studies have indicated that the developmental period of animals may be another important factor for the MS effects. For example, Roceri et al. reported that MS produced a short-term-up-regulation of Brain-derived neurotrophic factor (BDNF) expression in the hippocampus and PFC when measured on postnatal day (PND) 17 and a reduction of BDNF expression in the PFC at adulthood [29]. Our lab identified that long term MS (one daily period of 4 h from PND 1 to 21) had different effects on the serotonergic activity in juvenile, adolescent and young adult rats [30]. However, to date, there is no study that has investigated the effects of MS on spatial learning and reversal learning in juvenile, adolescent and young adult rats.

Thus, a MS procedure was utilized for this research in a dynamic and developmental view. In the present study, we aimed to investigate the effects of repeated MS (4 h/day from PND 1 to 21) on the open-field activity, spatial learning and reversal learning of the MWM in juvenile (PND 21), adolescent (PND 35) and early adult (PND 56) rats [31].

2. Materials and methods

2.1. Experimental subjects and maternal separation

Male and female adult Wistar rats were obtained from the Academy of Chinese Military Medical Science and were housed under controlled environmental conditions (ambient temperature 22 °C, 12 h light/12 h dark cycle, lights on at 7:00 a.m.) with free access to food and water. The experiments were performed in accordance with the guidelines of the Beijing Laboratory Animal Center and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23).

The rats were mated to produce litters that consisted of 8–12 pups. This MS procedure has been previously described [32]. Briefly, the pups were randomly separated into two experimental groups. The MS procedure was performed on one group (maternal separation, MS group, 48 pups), and the second group was left undisturbed from PND 1 to 21 (non-maternal separation, NMS group, 48 pups); each group had 24 male and 24 female pups. All experimental subjects were derived from different mothers. The MS procedure consisted of separating the rat pups from their mothers for 4 h per day from PND 1 to 21. This separation was regularly performed each day between 10:00 and 14:00. During these 4 h of separation, each pup was maintained separately from its littermates on heated sawdust at 28–30 °C. The dams of the separated litters remained in the home cage during the 4 h pup isolation period. The pups from the control litters remained in the cage with the dam and litter during this period. After weaning at PND 21, 16 MS (8 males and 8 females) and 16 NMS (8 males and 8 females) animals were examined in the behavioral tests. The other siblings were allocated to different cages (4 animals per cage). Then, at PND 35, another 16 MS (8 males and 8 females) and 16 NMS (8 males and 8 females) animals were examined in the behavioral tests. At PND 56, the last 16 MS (8 males and 8 females) and 16 NMS (8 males and 8 females) animals were examined in the behavioral tests.

2.2. Open field test

The testing apparatus included a circular arena of 180 cm in diameter with a 50 cm high wall. The central area of the open field was defined as a circular arena of 60 cm in diameter in the middle zone of the apparatus. The test room had a dim illumination (40 W) to decrease the anxiety of the rats. An animal was placed in the center of the field, and the horizontal activity (distance traveled) and time spent in the central arena were recorded for 5 min and analyzed by a computer-based system (Etho Vision; Noldus Information Technology, Wageningen, Netherlands). The open field was cleaned after each test.

2.3. Morris water maze test

2.3.1. Apparatus

Testing was conducted in a circular pool with a 150 cm diameter and filled to a depth of 22 cm with water (23 ± 2 °C). A circular Plexiglas platform (8 cm diameter) was placed 2 cm beneath the water level at different locations depending on which test was currently employed. The water was made cloudy by adding milk. Distinctive visual cues were set up on the wall surrounding the pool. A video camera was positioned above the water maze. The swim paths of the rats were tracked, digitized, and stored for later behavioral analysis using Etho Vision 3.1 (Noldus). The water maze was divided into four logical quadrants—north, south, east, and west—that served as starting positions for the rats. All animals were tested in the spatial learning and reversal learning.

2.3.2. Spatial learning

The spatial learning task consisted of 4 days of acquisition using the hidden platform. This was followed by a probe test on the fifth day without the platform. The platform was fixed in the middle of the west quadrant, 45 cm from the maze wall. During the first 4 days, four swim trials were given per day, in which each animal was released from a different quadrant in each trial. This was done in a pseudo-random manner and the start quadrant used was varied across the sessions. A maximum of 60 s was allowed for each trial. If the rat did not find the platform within 60 s, it was guided to the platform and allowed to remain there for 10 s. After each training trial, the rats were dried with paper towels and returned to their home cages for 50 s before the next trial, so the intertrial interval was approximately 60 s. Both the latency to escape onto the platform and distance traveled were recorded.

2.3.3. Reversal learning

After the spatial learning task was completed in each subject, they were examined using the reversal learning test. For this test, the platform was located in a novel position in the middle of the east quadrant, 45 cm from the wall opposite to the location used for the spatial memory task. The rats were also tested for four trials separated by 60-s inter-trial interval for 4 days. Otherwise, this test was similar to the spatial learning test.

2.4. Statistical analyses

All data are shown as the mean ± standard error of the mean (SEM). The analyses were performed using SPSS 16 software. The Open field test results were analyzed using multivariate analysis of variance (MANOVA). Repeated measures MANOVA was used for the analysis of MWM escape latencies with rearing condition, sex and age as the independent factors and test day as the within-subject factor. Comparisons with two and three groups were analyzed using Student's *t*-test and one-way analysis of variance (ANOVA) followed by LSD post hoc tests, respectively. The significance level was defined as $p < 0.05$.