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Short communication

Acute restraint stress produces behavioral despair in weanling rats in the forced swim test

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ABSTRACT

Stressful experiences in the rat during early life increase the vulnerability to later signs of behavioral despair in adulthood, reflected in increased immobility in the forced swim test (FST). However, the possible immediate effects of stress in weanling rats have only been partially described. The present study tested whether a single session of mild restraint stress modifies immobility in the FST in 21-day-old Wistar rats. After evaluating any possible changes in locomotion using the open field test (OFT), the latency and total duration of immobility were assessed in a single FST session. Regardless of gender, mild restraint stress significantly reduced crossings in the OFT, shortened the latency to the first period of immobility, and increased immobility in the FST compared with a control group devoid of stress. We conclude that a single mild physical stress session, as early as postnatal day 21, produces signs of behavioral despair.

1. Introduction

Early exposure to stress in rodents produces behavioral changes in adulthood. For example, the interruption of dam–pup interactions from postnatal day (PND) 1 through PND14 reduces locomotion, increases immobility in the forced swim test (FST), reduces the time spent in the open arms of the elevated plus maze, reduces serotonin levels in the hippocampus, and reduces serotonin transporter levels in the dorsal raphe nucleus in adulthood (Lee et al., 2007). Similarly, prolonged social isolation (PND30-70) also increases immobility in the FST, reduces grooming, and increases exploration behavior (Brenes-Sáenz et al., 2006). Additionally, early exposure (PND22-24) to predator odors later produces low emotional reactivity (Tsoory et al., 2007). Adult rats display behavioral despair and anxiety-like behaviors when they are previously exposed to stress during the juvenile period (Avital and Richter-Levin, 2005). By contrast, scarce studies have dealt with the immediate impact of stress in very young animals (Maslova et al., 2002), which may lead to the supposition that very young animals are not able to display any immediate behavioral repercussions of stress. Therefore, we subjected PND21 rats to a single mild restraint stress session, with the hypothesis that such stress exposure is sufficient to produce measurable signs of behavioral despair in a single FST session in weanling rats.

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2. Materials and methods

2.1. Animals

Seven sexually naive female adult Wistar rats were individually placed in acrylic boxes for 1 week with an experienced adult Wistar male for mating. Males were removed from the cages once the presence of a seminal plug was confirmed. We obtained litters of 8–11 rats that were gender-classified on PND21. During gestation and after delivery, the dam and pups remained undisturbed for 21 days and were handled as little as possible when changing the bedding material. On PND21, pups were weaned and maintained for the next 2 days in groups of 4–5 siblings per cage in the housing facilities under a 12 h/12 h light/dark cycle (lights on at 7:00 AM) with free access to water and food. We strictly followed the Guide for the Care and Use of Laboratory Animals (1996) promulgated by the National Institutes of Health and Mexican law requirements (Norma Oficial Mexicana NOM-062-ZOO-1999). This protocol was also approved by the Ethical Committee of the Biomedical Research Institute (UNAM).

2.2. Groups

Upon weaning, all rats were visually confirmed to be able to consume a rodent diet (Harlan 2018S Teklad Global Rodent Diet). Four groups were formed: two control groups (Ctrl) that remained undisturbed (♂ Ctrl, $n = 13$; ♀ Ctrl, $n = 13$) and two groups of PND21 rats that were subjected to restraint stress (♂ RS, $n = 17$; ♀ RS, $n = 18$). A similar number of animals was assigned to each experimental group that were carefully selected from different litters, with only 2–3 rats from the same litter assigned to the same group.

2.3. Restraint stress

On PND21, rats from the RS group were individually placed for 6 min into a rectangular transparent acrylic box (8 cm × 7 cm base, 7 cm height). Tests were performed between 9:00 AM and 11:00 AM. In this small cage, the rats were able to see outside, but their movements were restricted by the walls. No electric foot-shocks or any other stressful procedures were applied. The duration of restraint stress (6 min) was determined according to previous reports in which rats of a similar age were subjected to physical stress (Overmier and Murison, 1991; Kim et al., 1999; Matsumoto et al., 2005). The testing room was illuminated with white light (40 lux) (Sharp and La Regina, 1998). The rats from the control group were taken directly from the housing facilities and subjected to the open field test (OFT). The OFT was performed immediately after the stress session in the other groups.

2.4. Open field test

The OFT was used to determine the effect of RS on exploration. Animals were placed in a corner of an opaque Plexiglas box (20 cm height, 44 cm× 33 cm base divided into 12 squares 11 cm × 11 cm each) where they freely explored the cage during a 5 min videotaped session. Locomotor activity was assessed by counting the number of times the animal crossed any square. A rat stepping from one square to another with its rear legs was considered a square crossing. Immediately after the OFT, the rats were subjected to the FST.

2.5. Forced swim test

Rats were individually placed into a rectangular pool (50 cm × 44 cm base, 60 cm height) filled with clean water (25 ± 1 °C). The water was 14 cm deep, which was sufficient for observing PND21 rats floating while immobile at an inclination of 0–30° from the surface of the water. As soon as the rats were placed in the pool, they swam vigorously, with clear dexterity, and constantly moved through the pool. The variables evaluated during the 5 min session were the latency to first immobility, defined as the time elapsed between the introduction of the rat to the pool and the first period of immobility, and the total immobility time, defined as the sum of all periods of immobility. Immobility was determined when the rat floated for more than 1 s, making minimal movements with no displacements and keeping its head above the water surface (Porsolt et al., 1977). At the end of the test, rats were placed in a dry and clean cage and were subsequently returned to their housing facilities. Two independent observers measured the latency to the first period of immobility and total immobility time in videotaped sessions consistent with previous reports (Contreras et al., 2001, 2008; Gutiérrez-García et al., 2007; Rodríguez-Landa et al., 2007).

2.6. Statistical analysis

Videotapes from the OFT and FST sessions were reviewed until reaching 100% agreement by trained observers who were blind to the experimental treatments. Results were analyzed by specially designed software that allowed semiautomated analysis. Data were analyzed by two-way analysis of variance (ANOVA) to determine differences between gender (males and females) and groups (Ctrl and RS) and interactions between factors when at least one factor reached significance. Variables evaluated were crossings in the OFT and immobility latency and total immobility time in the FST. Results are expressed as mean ± standard error of the mean. Only differences with $p \leq 0.05$ were considered statistically significant, in which case Student–Newman–Keuls *post hoc* test was used.

3. Results and discussion

3.1. Open field test

Gender differences in locomotor activity occur after puberty when gonadal hormone receptor activity is stimulated by hormonal priming (Blizard et al., 1975). Thereafter, female adult rats display greater locomotor activity than males (Alonso et al., 1991; Frye and Wolf, 2002). Comparisons of the gender factor ($F_{1,57} = 2.461$, $p = 0.122$) indicated that total crossings were similar between males (42.9 ± 4.36) and females (52.5 ± 4.30). As expected, we did not observe any differences between males and females in locomotor activity on PND21. In contrast, we observed changes depending on whether rats received stress. ANOVA revealed significant differences in the OFT between groups ($F_{1,57} = 6.222$, $p = 0.016$), in which crossings in RS rats were lower (40.1 ± 4.00) than in Ctrl rats (55.4 ± 4.64). The interaction between factors was not significant ($F_{1,57} = 0.040$, $p = 0.841$). RS groups displayed fewer crossings on PND21, similar to adult rats that exhibit reduced locomotor activity immediately after unavoidable electric footshock stress (Gutiérrez-García et al., 2007). Thus, locomotor activity is a sensitive measure of mild stress on PND21, regardless of gender.

3.2. Forced swim test

3.2.1. Latency to first period of immobility

We used the FST to test behavioral despair, an adult-based model. In this model, the latency to the first period of immobility is considered an indicator of the magnitude of the first effort of rats to solve the demanding situation represented by the FST (Contreras et al., 1998; Espejo and Miñano, 1999). Two-way ANOVA did not reveal gender differences (male, 182.8 ± 19.00 s; female, 236.5 ± 36.29 s; $F_{1,57} = 2.850$, $p = 0.097$), but the latency to the first period of immobility was shorter in the RS groups than in the Ctrl groups ($F_{1,57} = 7.620$, $p < 0.008$; Fig. 1). The interaction between factors did not reach statistical significance ($F_{1,57} = 3.376$, $p = 0.071$). The latency to the first period of immobility was shorter in the RS groups than in controls, indicating that a single RS session is sufficient for generating an immediate effect in the FST on PND21,

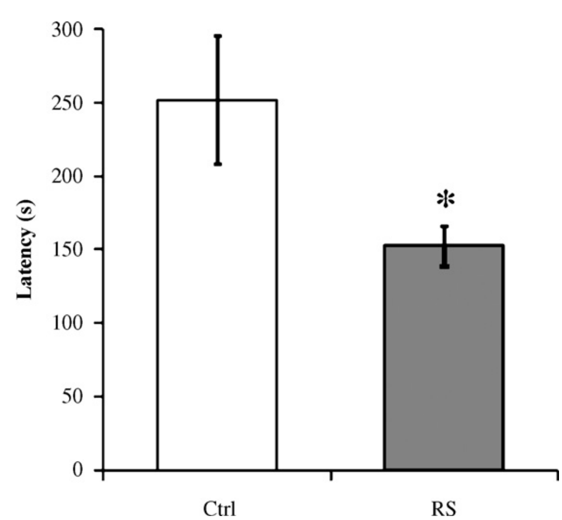

Fig. 1. The effects of stress on the latency to the first period of immobility in the forced swim test. Latencies were shorter in restraint stress (RS) animals than in control rats (Ctrl). $^{*}p < 0.008$, Student–Newman–Keuls *post hoc* test.