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Experiencing early life maternal separation increases pain sensitivity in adult offspring

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ABSTRACT

Maternal separation is a widely accepted model for studying long-term behavioral changes produced by events during early life and its association with changes in pain sensitivity. Thus, our objective was to evaluate sensitivity to pain, under different stimuli in adult male and female rats that had undergone early life maternal separation. Animals were subjected to maternal separation from postnatal day (PND) 2–15. Maternal behavior and litter weight were evaluated during this period. Sensitivity to pain was assessed in offsprings during adulthood by exposing them to stimuli, including formalin (5%; 20 µl), a hot plate, and the electronic von Frey test, 4, 7, 10, and 24 h after the administration of saline or Freund's complete adjuvant (CFA) injection. Maternal separation did not affect maternal behavior or litter weight during PND 2–15. However, experiencing maternal separation increased pain sensitivity in the rats subjected to formalin by increasing number of flinches and licking time Further, females appeared more sensitive than males to thermal stimuli, as they showed a decrease in latency in the hot plate test. In this test, male and female offsprings that were exposed to early life maternal separation and received the CFA injection also showed a reduction in latency to react to the painful stimuli. In the von Frey test, there was a reduction in withdrawal threshold in maternal separation animals injected with CFA, thus demonstrating a greater sensitivity to the mechanical stimuli. In conclusion, experiencing early life maternal separation increased pain sensitivity in adult offsprings. Thus, our data are important to understand the impact of environmental influences, such as stressful life events during critical developmental periods, on pain vulnerability.

1. Introduction

In mammals, including rodents, the mother is the provider of food for the offsprings. In addition, a complex mother-infant interaction emerges post-birth, which extends beyond nutritional needs. The mother provides the essential temperature for the pups in the nest, and is responsible for visual, olfactory and auditory development in the offspring. This occurs for an extended period during postnatal development, when maternal care is essential for proper development in the offsprings (Pryce et al., 2001; Costa et al., 2013).

Normal offspring development is dependent on mother-infant interactions, and a reduction in these interactions through maternal separation can lead to impairments in cognition, emotion, and even neurogenesis in adulthood (Kuhn et al., 1990; Raceková et al., 2009). Maternal separation is a widely accepted model of maternal deprivation to study long-term behavioral changes produced by events during early life, and is considered an early life stress model (Wu et al., 2014). It has been shown to induce significant changes in animal behaviors such as abnormal response to stress as well as painful stimuli (Bernardi et al., 1986; Kwok et al., 2014).

The social environment plays a critical role in shaping the brain and programming behavior throughout life, and experiencing stressful events during early life can negatively affect the development of neural transmission systems, such as circuits, which are involved in developing a response to painful stimuli (Lupien et al., 2009; Uhelski and Fuchs, 2010; Jennings et al., 2014; Amini-Khoei et al., 2015).

Children who experience early life stress have an increased risk of chronic pain in adulthood (Jones et al., 2009; Von Korff et al., 2009). In addition, maternal separation during the first 2 weeks postpartum for 3 h daily showed reduced sensitivity to pain in female rats when assessed using a hot plate (Weaver et al., 2007). On the other hand, the combination of two early life stress events (neonatal isolation and social

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deprivation) does not induce thermal or mechanical hypersensitivity to pain. Furthermore, maternal separation in female, but not male rats, showed increased sensitivity to mechanical stimuli in the von Frey test (Burke et al., 2013). In contrast, other studies demonstrated that maternal separation did not alter thermal or mechanical thresholds in rats placed on a hot plate, by tail-flick and on the von Frey tests (Kalinichev et al., 2001; Uhelski and Fuchs, 2010).

Although research shows that maternal separation can impair pain sensitivity, there are also controversial studies on pain sensitivity in adult animals exposed to early life stress. In addition, there are no studies demonstrating the influence of maternal separation on different noxious stimuli in male and female offsprings. Thus, our objective was to evaluate sensitivity to pain, under different stimuli (mechanical, thermal and chemical), in adult male and female rats that had undergone maternal separation during the early periods of development. For this, complete Freund's adjuvant (CFA) inflammatory pain model was used and pain sensitivity was assessed using a hot plate and the von Frey test. In addition, the sensitivity to pain under a chemical stimulus was evaluated in the formalin test. Maternal behavior and litter weight were monitored during the period of maternal separation to exclude possible confounding factors such as changes in maternal care and malnutrition due to maternal separation.

2. Materials and methods

2.1. Animals

Adult Wistar nulliparous female and male rats at approximately 8 weeks of age were obtained from the Central Animal Facility of the Federal University of Alfenas and were housed in a temperature-controlled room (22 °C) on a 12:12 h light-dark cycle (lights on at 7:00 h) with ad libitum access to the water and standard laboratory rat chow. All experimental procedures followed the Ethical Principles in Animal Research adopted by the Ethics Committee on the Use of Animals at the Federal University of Alfenas (protocol 32/2016). The experiments were performed in accordance with good laboratory practice protocols and quality assurance methods.

2.2. Experimental design

Female rats were time-mated by placing them with sexually experienced male rats (2 females to 1 male). The day on which sperm was observed during vaginal lavage was designated as gestation day zero, and the parturition day was considered as postnatal day (PND) 0. On PND 1, all litters were culled to 8 pups (4 males and 4 females). For all procedures, we used 20 pregnant dams that were divided into 2 experimental groups (n = 10 per group): maternal separation at PND 2–15 (MS; group in which the pups were isolated from the mother) and control (non-MS; group in which the pups were not isolated from the mother). During this period, maternal behavior of the dams and weight of the litters were monitored. After weaning in PND 22, the offsprings were separated based on sex into cages with up to 4 animals per cage until they reached adulthood at approximately 8 weeks. Offsprings (40 males and 40 females of each group) were used to assess sensitivity to painful stimuli on formalin test and CFA-induced inflammatory pain through hot plate and von Frey electronic tests. To avoid a litter effect, one male and one female rat from each mother were used in each experimental group for behavioral testing during adulthood and all animals only participated in a single experiment. To avoid hormonal interference in nociceptive responses in female offsprings, the tests were only carried out on females during the diestrus period of their estrous cycle.

2.3. Maternal separation

Maternal separation was performed daily from PND 2–15 in the morning (9:30 a.m. to 12:30 p.m.) for 3 h, for up to 2 weeks. During the separation procedure, each pup was placed in a single compartment without shavings in an incubator at 30 °C. After separation, the pups were returned to their original cage. For animals that did not undergo maternal separation, litters were left undisturbed (adapted from Lai et al., 2008; Wu et al., 2014; Nishinaka et al., 2015).

2.4. Evaluation of maternal behavior

Observations of maternal behavior were made from PND 2–15 in dams from both experimental groups (n = 10 per group) during two periods: in the morning before maternal separation (between 08:00 to 09:30) and in the afternoon after maternal separation (between 15:00 to 16:30). In each session, the behavior of each mother was scored every 3 min (30 observations per period per day, for a total of 60 observations per mother per day) in the following parameters: maternal behavior parameters − licking pups (either its body surface or its anogenital region), nursing pups in an arched-back posture, “blanket” posture in which the mother lays over the pups, passive posture in which the mother is lying either on her back or side while the pups are nursing, nest building, maternal self-grooming (breast stimulation through self-cleaning); and non-maternal behavior parameters − feeding, exploring the cage housing, not exploring and no maternal self-grooming. Data are reported as the total percentage of the maternal behavior and non-maternal behavior (number of observations in which the target behavior was recorded divided by the total number of observations × 100) (Champagne et al., 2003; Costa et al., 2013; Vilela et al., 2013).

2.5. Litter weight assessment

The weight of litters was monitored during the period of maternal separation (PND 2–15) in the litters that were isolated and in the control litters (n = 10 per group). After analyzing maternal behavior and before maternal separation, the weight of each litter was recorded for later analysis.

2.6. Formalin test

Rats from both groups (MS and non-MS) were injected with formalin for this experiment. A subplantar injection of formalin (diluted to 5% in sterile 0.9% saline; 20 µl/paw) was administered to the right hind paw of the animals. Immediately after the formalin injection, animals were placed in an open plexiglass box (10 × 20 × 24 cm), which permitted observation. Pain response was characterized by rapid jerks of the injected paw and by slight biting or licking. After the formalin injection, the rats displayed the behavior typical to this model, i.e., it held the injected paw just off the floor. During this period, spontaneous flinching of the injected paw was also observed. Flinching is readily discriminant behavior that can be characterized as a rapid and brief withdrawal or flexion of the injected paw. This pain related behaviors was quantified by counting the number of finches and licking time behavior directed towards the right hind paw every 5 min for up to 60 min (Tjølsen et al., 1992; Yamamoto et al., 2002).

2.7. Hot plate test

Rats from the MS and non-MS groups were injected with saline or CFA (100 µl) for this experiment. The hot plate test was carried out to assess the effects of early life stress on the thermal nociceptive threshold. Rats were placed on a 50 ± 0.5 °C hot plate inside a contained with polyacrylic walls and before saline or CFA injection, to avoid tissue damage, a cut-off time of 20 s was employed. The latency of a discomfort reaction, such as licking of the paws or jumping, was recorded for each animal before or 4, 7, 10 and 24 h after administration of saline or CFA (Yamamoto et al., 2002).