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Behavioral effects in adolescence and early adulthood in two length models of maternal separation in male rats

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HIGHLIGHTS

- MS21 produces anxious behavior in adolescence and adulthood.
- MS21 impairs recognition in adolescence and adulthood.
- MS10 deteriorates associative/emotional learning only in adolescence.
- MS10 protects against anhedonic-like behaviors.

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ABSTRACT

Maternal separation (MS) is an extensively used early life stress model. There is some variability in the MS lengths used. Maternal separation leads to emotional and behavioral alterations such as anxiety, despair, or memory problems. We performed MS in Wistar rats with two length models from postnatal day 1 until day 10 and from postnatal day 1 until day 21 during 4 h per day in both groups. We performed a test battery of a wide range of behaviors to measure anxiety, despair, prepulse inhibition, recognition memory, and associative memory both in adolescent and adult subjects. We found that the longer model leads to anxious behavior and impairs recognition in adolescence and adulthood whereas the shorter one deteriorates associative/emotional learning only in adolescence and protects against anhedonic-like behaviors. In our opinion, these results can be explained by the fact that different lengths lead to different profiles: the longer one is an anxious profile, whereas the shorter one is more impulsive.

1. Introduction

More than 50% of children worldwide are exposed to early stress [1]. Early psychosocial stressors could result in neuropsychiatric disorders such anxiety, depression, and post-traumatic stress disorder (PTSD) [2–4]. Subjects who suffered early stress could also show memory and attention problems [5–7]. Due to the high prevalence and the severe consequences of early stress in human beings, animal models of early post-natal stress have been developed. One of the most used consists in repeated separations of the pups from the mother: maternal separation (MS) [8]. Nonetheless, there are some variations in the way that different researchers perform MS. Different authors use different separation lengths, for example, some of them finish on postnatal day (PND) 10 [9] whereas others finish on PND 14 [10–13] or on PND 21 [14–16].

Due to the variability in MS models, only sometimes it can be found the same alterations that in early stressed humans. MS leads to anxious behaviors [15,17,18] but not all the MS models reach this effect, especially the shorter ones [19,20]. MS produces depressive-like behaviors [18,21] but not all the studies found differences in immobility (a measure of depression) [22]. Another impairment found in MS models is a reduction of prepulse inhibition (PPI) [23,24].

MS could also lead to memory impairments such as associative memory deficits in fear conditioning [13] but, with shorter MS lengths, some authors found more freezing (better associative memory related to fear conditioning) [25]. Some researchers also found object recognition problems in adulthood [19,26]. This recognition impairment lasts until old age [27]. However, using a longer MS protocol, recognition alterations have not been found [16].

The length of the MS protocols may be one of the keys to disentangle these controversial results. In the first two weeks of rodent

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life, neurodevelopment is still in progress. Proliferation, migration, and differentiation processes are still occurring within the encephalon, in which different areas have different sensitive periods of development [1]. Exploring the effects of two lengths of MS could be useful because it allows us to compare how the moment in which the stress is suffered affects different systems. The 10 first days could be particularly interesting because they include almost all the hyporesponsive period to stress. This period, in which the subject is less responsive to stress, takes place between PND3 and PND14 in rats [28]. It seems that early stress interrupts this period, and leads to long-term increases in the stress response [29]. We chose 10 first days because some interesting developmental processes start just after this period: the granular hippocampal cells migration occurs between postnatal days 10–25 in rodents [30] and allopregnelonone levels reach its peak in PND10. Neonatal allopregnelonone levels in the second week of life seem to be important for the maturation of dopaminergic systems and GABAergic thalamocortical connections [30]. Another interesting period is the first 21 days, because this length includes the entire time in which the rats need maternal care (from birth to weaning).

The aim of this study was to compare in adolescence and adulthood the effects of two lengths of early MS over a wide range of behaviors. These behaviors were emotion-related, such as anxiety and depression, or learning-related, like alertness (the simplest kind of attention) and memory (recognition and associative types).

Only a few works compare two lengths of MS [31,32] none of them explore the MS effects on two developmental periods. Also, we explore a wide range of behaviors, using an extended battery of test not employed in prior studies.

2. Materials and methods

2.1. Animals

A total of 60 Wistar male rats (220–350 g at the end of the experiment) were taken from the animalarium at Oviedo University. All the animals had ad libitum food and tap water and were maintained at a constant room temperature (22 +/−2 °C), with a relative humidity of 65–75% and an artificial light-dark cycle of 12 h (08:00-20:00/20:00-08:00). The procedures and manipulation of the animals used in this study were carried out according to the Directive (2010/63/EU), Royal Decree 53/2013 of the Ministry of the Presidency related to the protection of animals used for experimentation and other scientific purposes.

2.2. Maternal separation

Litters were randomly assigned to undergo maternal separation or to be reared under animal facility rearing (AFR) conditions. Litters with more than 10 animals were culled to 10. For MS, litters were separated from dams for 4 h per day, as other authors [14,23], starting at 10:00 h and ending at 14:00 h. The MS group was separated from PND 1 to PND 21 whereas the MS10 group was separated from PND 1 to PND 10. The reason for using 4 h of MS per day was to avoid short-term MS. If the MS is too short (15 min per day), it is not enough to produce deleterious effects. This brief MS could even lead to positive effects, which is why some authors call this very brief MS "Handling" [33,34]. This positive effects make us chose AFR as reference group over handling.

Each separation consisted of removing the dams from the home cage and placing them in an adjacent cage while the pups were kept together in a new cage. The litters remained together during the separation time in an incubator (30 °C, 55–65% relative humidity). After the separation period, the dam and the litter were returned to the home cage (first, the litter). Control litters were reared under

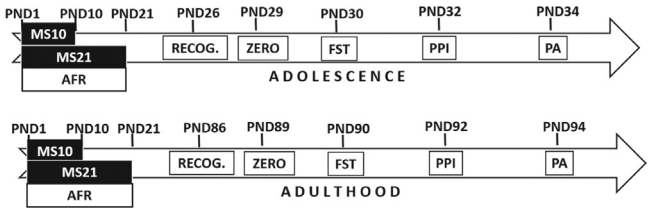

Fig. 1. (A) Timeline of the behavioral test in adolescence. (B) Timeline of the behavioral test in adulthood. From PND 1 to PND 10, the MS10 animals were separated from their dams (10 males for the adolescent group and 10 for the adult group) From PND 1 to PND 21, the MS21 animals were separated from their dams (10 males for the adolescent group and 10 for the adult group) or only disturbed for the weekly cleaning (10 AFR males for the adolescent group and 10 AFR males for the adult group). PND = Post-natal day, MS = Maternal separation, RECOG. = Recognition memory test, ZERO = Zero maze test, FST = Forced swim test, PPI = Prepulse inhibition test, PA = Passive avoidance test.

standard animal facility rearing (AFR) conditions, disturbed only by animal facility practices, once a week, until weaning. On PND 21, the animals were weaned and segregated by sex and only males were selected for the study. Therefore, 6 groups of male animals were included in the experiment, two control groups or AFR (n = 20) and two experimental groups: MS10 (n = 20) and MS21 (n = 20). Ten animals per group were tested at each age: 30 in adolescence (AFR n = 10, MS10 n = 10, MS21 n = 10) and 30 in adulthood (AFR n = 10, MS10 n = 10, MS21 n = 10).

2.3. Experimental procedures

The behavior of the animals was tested between PND 26 and PND 34 (adolescent results) and between PND 86 and 94 (adult results). All behavioral tests were conducted sequentially in the same order in adolescence and adulthood (see Fig. 1). The rationale to use the above order was to put the less stressful test first. Other researchers in the field use similar sequences to test the animals [16,35].

2.3.1. Object recognition

On PND 26–28/86-88, object exploration was assessed in a square open field (100 × 100 × 40 cm) with an open roof, placed in a rectangular room with several distal cues and one proximal cue, which consisted of a small circular white sticker located on the east wall of the open field. The open field was made of grey fiber glass and contained two diffuse white lights placed at the sides of the room, providing an illumination density of approximately 10 lx at the center of the open field. A video camera connected to a video recorder was mounted above the field to store the sample and test trials on video files for off-line analysis. After each trial, the apparatus was thoroughly cleaned with a 75% ethanol solution.

Four different objects made of combinations of plastic pieces of four different colors (green, yellow, orange, and blue) were used. For habituation, two different objects (13 × 13 × 16 cm) were made with the four available colors, whereas for recognition testing, two different objects (in triplicate) were made. All objects had sufficient weight to ensure that the rats could not displace them and they were counterbalanced within each group to avoid possible preference effects. Thus, the order of the particular objects used in the tests was reversed for half of the rats in all the experiments. This counterbalancing ensured that the target object in any given pair was reversed. After each trial, objects were thoroughly cleaned with a 75% ethanol solution in order to remove odor cues.

All animals were habituated to the open field on two consecutive days before the test day. On the first day of habituation, groups of 5–6 rats were placed together in the open field and allowed to explore it for 6 min without objects. Afterwards, rats were given another 6 min exploration session, but this time, only one rat at a