

Research report

# Effects of brief and long maternal separations on the HPA axis activity and the performance of rats on context and tone fear conditioning

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## Abstract

Previous studies show that early life events result in neurobehavioural alterations that may be either beneficial or detrimental to the stress response. Given the close relationship between corticosterone secretion and mnemonic processes, the purpose of the present study was to investigate the effects of brief (BMS, 15 min) and long maternal separations (LMS, 180 min) on memory tasks in adult rats, assessed by context and tone fear conditioning. At adulthood, males were evaluated for behavioural and hormonal reaction to the training environment, being tested for context fear conditioning; tone fear conditioning; and learning curve in the context fear conditioning, in which rats were daily re-exposed to the context, followed by a brief footshock and in the last day of the experiment (day 5) animals were exposed to the context. Corticosterone and ACTH plasma levels were determined in naïve rats (basal) or 5, 25 or 45 min after each test. Peak ACTH and corticosterone levels were similar among the groups after context fear conditioning; however, levels of CTL rats remained elevated for a longer time. In the learning curve of context fear conditioning, both BMS and LMS rats exhibited less freezing behaviour than CTL rats, without differences in hormone secretion. There was neither an association between activity of the HPA axis and performance on memory tasks nor different motivational properties of the tasks on the HPA axis between BMS and LMS rats, i.e., both manipulations lead to similar performance in hippocampus-dependent and independent memory tasks.

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## 1. Introduction

Throughout the life of mammals, the continuous interaction between genetic and environmental factors seems to determine the development of neurobiological aspects of the stress response and coping behaviour. Among the environmental factors, disruptions of the mother–infant relationship are one of the most powerful, in as much as the stress response may become adaptive or mal-adaptive depending on the length of the disruption. Therefore, maternal presence not only serves as a primary source of warmth, nutrition, and licking, which are necessary for the pups survival, but it also regulates numerous physiological, behavioural and psychological processes [11,14,21,37,38,42].

Among the many paradigms of disruption of mother–infant relationship and their long-term consequences, possibly the best characterized ones are the 15 and 180 min maternal separations, hereby referred to as brief and long maternal separation (BMS and LMS, respectively). On one hand, brief maternal separation usually results in hyporesponsiveness to stress and less fear and anxiety-like behaviours, and a more efficient negative feedback system [1,6,15,30]. On the other hand, pups submitted to long maternal separation (LMS) generally become hyperresponsive to stress, exhibiting elevated levels of hypothalamic corticotrophin-releasing factor (CRF) mRNA in the hypothalamic paraventricular nucleus (PVN), adrenocorticotropin hormone (ACTH) and corticosterone (CORT) responses to stressors, and enhanced anxiety-like behaviour [18,19,21].

The permissive role of glucocorticoids on learning and memory processes depends on optimal plasma levels of the hormone and on the balanced occupation of MR and GR [16,29], in as much as removal of corticosterone by adrenalectomy

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tomy, blockade of GR, or high levels of glucocorticoids have been associated with impairment of learning and memory tasks [7,24,28,33,34,36]. Therefore, the above-mentioned consequences of early life events, varying from resilience to vulnerability to stressor exposure, could be the consequence of increased hippocampal MR/GR mRNA ratio in LMS versus BMS rats, and therefore, influence memory formation.

Though not extensive, the literature on early life events and consequences on memory and learning has yielded to inconclusive results. Much of the inconsistency of findings lies in the use of different lengths of maternal separation, different rat strains, different memory tasks and different testing ages [20]. In general, 15 min of maternal separation in Long–Evans or Sprague–Dawley rats produce an increase in context learning conditioning in pre-pubertal and adolescent rats either tested 24 h or 15 days after the training, indicating a persistent effect on recall capacity [4]. When tested in a much advanced age (15-months-old), BMS rats perform better in the water Morris maze, a hippocampal-dependent task, and show less hippocampal neuronal loss [25], indicating that BMS prevents the decay in cognitive performance commonly observed with aging. Recently, the comparison between BMS and LMS males and females on both contextual and tone fear conditioning failed to show any remarkable effect of these two manipulations on freezing behaviour, although BMS males exhibited less ultrasonic vocalizations [17]. In Wistar rats, nonetheless, LMS (either 3 or 4 h) either improves the performance of male rats in the Morris water maze [32] or both males and females in avoidance learning [32,39] or leads to no change in behaviour [23].

To the best of our knowledge, no comparison between hippocampus-dependent and independent tasks and the profile of hormonal secretion has been examined in adult rats submitted to early life manipulations. Thus, in view of the effects of early life events on glucocorticoid receptors (specially in the hippocampus) and its role in memory formation, we investigated the activational properties of context (a hippocampus-dependent task) and tone fear conditioning (a hippocampus-independent task) on the HPA axis, in order to establish a possible relationship between the animals' performance in memory tasks and their hormone response to reminders of aversive situations.

## 2. Material and methods

All experimental procedures described were submitted to and approved by the Ethics Committee in Research of Universidade Federal de São Paulo (CEP 1194/04), being in accordance with the NIH guidelines for use and care of animals.

### 2.1. Subjects

Wistar rats, from the animal colony of the Department of Psychobiology were used for breeding, which consisted of placing two nulliparous females and one experienced male for 10 days, males being rotated twice within this period. Females remained two/cage for another week, and they were placed individually in home-cages filled with sawdust bedding. The animal facility is maintained under controlled light/dark (lights on between 7:00 and 19:00) and temperature conditions ( $23 \pm 2^\circ\text{C}$ ). Cages were inspected twice/day for the presence of pups. The day of birth was designated post-natal day (pnd) 0.

### 2.2. Separation procedures

On pnd 1, litters were culled to five males and three females and distributed into one of the three following groups: (1) brief maternal separation: pups were separated from the mother for 15 min, from pnd 2 to 14; (2) long maternal separation: pups were separated from the mothers for 180 min, from pnd 2 to 14; (3) non-handled control (CTL): pups remained with their mothers for the entire weaning period, which lasted until pnd 22.

Each litter was removed from the home-cage and placed in a different cage, filled with sawdust. The cage was placed on top of a heating pad set at  $30\text{--}33^\circ\text{C}$ . Once a week, during the manipulation period, half of the sawdust in the home-cage was replaced by clean bedding; this procedure was also adopted for CTL litters. At the end of the manipulation period (from pnd 15 on), mother and litter remained in the home-cage without any perturbation, except for the animal facility routine cage cleaning, until weaning day, when pups were separated by gender, remaining with their siblings. Only males were used in this study ( $N = 5/\text{cage}$ ).

### 2.3. Apparatus

The training apparatus for both tasks consisted of a black acrylic box (22 cm long  $\times$  21 cm wide  $\times$  22 cm high). The walls were black with some visual white patterns. The floor was made of iron rods through which the shocks used as unconditioned stimulus (US) could be delivered. The lid was made of transparent acrylic, permitting the behavioural observation. The box was connected to an automatic Ugo Basile 7551 shock delivery source. The tone fear conditioning test was carried out using a transparent cylindrical test chamber, 35 cm in diameter  $\times$  60 cm high, with a transparent lid, with small wholes. The apparatuses were placed in different rooms, being connected to a buzzer located outside the conditioning apparatus and outside the cylindrical chamber (80 dB) activated by a manual switch and used as the conditioned stimulus (CS).

### 2.4. Experimental procedures

At 3 months of age, different sets of CTL, BMS and LMS rats were submitted to the following experiments:

#### 2.4.1. Experiment 1: ACTH and corticosterone responses to training

Twenty-four animals ( $N = 8/\text{group}$ ) were randomly selected and trained in the conditioning apparatus, by placing each rat individually in the black box of the apparatus where they remained for 2 min. After this period, a series of five footshocks (0.6 mA, 1 s long and 30 s apart from each other) was initiated. The time that each rat remained in freezing, defined as complete immobility and absence of whisker movements and sniffing [5], was continuously recorded, minute by minute for 5 min, by an observer, blind to the group condition. Immediately after the end of the training session, rats were decapitated and trunk blood was collected in pre-cooled tubes containing EDTA.

#### 2.4.2. Experiment 2: contextual fear conditioning

The task was carried out on 2 consecutive days. On the first day (training), the animals were submitted to the same protocol as in Experiment 1. At the end of the training session, the animals were removed from the apparatus and placed back in their original home-cages. On the testing day (24 h later), contextual conditioning was assessed by placing each animal in the same training context, i.e. the black acrylic box without presentation of US. Freezing/min was taken as a measure of contextual conditioning [41].

#### 2.4.3. Experiment 3: tone fear conditioning

The task was carried out on 2 consecutive days. On the first day (training), the animals were individually placed in the black box of the apparatus and 2 min later, a series of five tone-shock pairings was presented. The tone (conditioned stimulus, CS) lasted 5 s and during the last second a 0.6 mA footshock (unconditioned stimulus, US) was delivered. The 30 s interval between pairings was maintained. Thirty seconds after the last footshock, the animal was removed from the apparatus and placed back in its original home-cages. Twenty-four hours later (test day), each animal was placed inside a cylindrical test chamber

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