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Early handling effect on female rat spatial and non-spatial learning and memory☆

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ABSTRACT

This study aims at providing an insight into early handling procedures on learning and memory performance in adult female rats. Early handling procedures were started on post-natal day 2 until 21, and consisted in 15 min, daily separations of the dams from their litters. Assessment of declarative memory was carried out in the novel-object recognition task; spatial learning, reference- and working memory were evaluated in the Morris water maze (MWM). Our results indicate that early handling induced an enhancement in: (1) declarative memory, in the object recognition task, both at 1 h and 24 h intervals; (2) reference memory in the probe test and working memory and behavioral flexibility in the "single-trial and four-trial place learning paradigm" of the MWM. Short-term separation by increasing maternal care causes a dampening in HPA axis response in the pups. A modulated activation of the stress response may help to protect brain structures, involved in cognitive function. In conclusion, this study shows the long-term effects of a brief maternal separation in enhancing object recognition-, spatial reference- and working memory in female rats, remarking the impact of early environmental experiences and the consequent maternal care on the behavioral adaptive mechanisms in adulthood.

1. Introduction

Mother–infant interactions during the first weeks after birth influence the development of physiology and behavior (Levine, 1957). Exposure to "moderate stress" in early life appears to be advantageous for the infant by honing greater skills to adapt to psychological and physiological stressors in adulthood. According to the theory firstly proposed by Smothermann and Bell (1980), the outcome of postnatal manipulations is affected by changes in maternal behavior directed toward the offspring during reunion after a given period of mother–infant separation (Denenberg, 1964, 1999). Brief daily separation of the pups from the mother, a form of early handling (EH), enhances the intensity of maternal grooming, licking and arch-back nursing toward male pups (Moore and Morelli, 1979; Liu et al., 1997), thus fostering their behavioral and endocrine responses to environmental challenges in the adult offspring (Francis and Meaney, 1999; Liu et al., 1997; Meerlo et al., 1999; Cannizzaro et al., 2007). In particular, long-lasting influence of early-life experience on neuroendocrine and behavioral consequences to threatening situations has been considered to involve changes in the hypothalamic-pituitary-adrenal (HPA) axis (Heim et al., 1997). The impact of EH procedures on adult behavior appears to be more profound if the manipulation occurs during the early postnatal period (Meaney et al., 1988), when the HPA axis response to stress is dampened. Indeed, from the first postnatal day (PND) corticosterone concentrations decrease dramatically and remain at low levels until approximately day 14. In addition, it has also been reported that concentration of pituitary adrenocorticotropin (ACTH) and hypothalamic corticotrophin-releasing hormone (CRH) are also low during the first two postnatal weeks (Walker et al., 1986; Levine, 2001), hence scaling down HPA axis reactivity to stress (Korosi and Baram, 2010; Sánchez et al., 2001; Francis and Meaney, 1999; Plotsky and Meaney, 1993). Interestingly, dams show a predominant inclination in directing more attention to male offspring in the form of anogenital licking, primarily because of

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early androgen production, which appears to play a key role in the growth and development of sensory, motor, autonomic, endocrine, and nervous systems (Moore, 1992; Moore and Morelli, 1979; Richmond and Sachs, 1984). Consistently several studies report that early rearing conditions during brain development affect cognitive functions in adult male offspring, since rats that received early handling manipulation typically display better performance on various maze tests, object recognition tasks (Bilbo et al., 2006; Bredy et al., 2003a; Kosten et al., 2007; Lehmann et al., 2002; Liu et al., 2000; Tang, 2001) and in the "Can Test" (Cannizzaro et al., 2005, 2006). The polymorphism of neuroendocrine processes and behavioral responses following EH procedure include gender-related differences; however very few studies (Oomen et al., 2009, 2011; Pryce and Feldon, 2003; Schenk and Morris, 1985) exist on the complex interactions among early handling exposure to brief maternal separation, mother–infant interaction, sex-specific behaviors, and environmental challenges in female adult rats. Thus, the present study was aimed at investigating the long-term consequences of brief maternal separation on discrete cognitive functions such as object recognition-, spatial reference- and working memory in order to better evaluate the impact of early environmental experiences and the consequent maternal care on the behavioral adaptive mechanisms in female rats.

2. Methods

2.1. Animals

Wistar rats were housed with free access to food and water and maintained on a 12 h on/off cycle (8:00–20:00 h) at a constant temperature (22–28 °C) and humidity (55 ± 10%). Pairs of primiparous females of 120 days of age were mated with one male of 150 days of age. The day on which sperm was detected in the vaginal smear was defined as gestational day (GD) 1. Pregnancy was determined by weighing and palpation. Seven days before delivery, 8 pregnant dams were individually housed in sawdust-containing plastic cages (40 cm × 60 cm × 20 cm). All litters born within a 2-day period were randomly divided into 2 experimental groups: early handling (EH) and Control (CTR) rats. The suitable control group for the handling procedure was the one left completely undisturbed. In this group – in contrast to the EH group – dams were never stimulated to provide bursts of sensory stimulation to the pups by brief experimentally induced separations that take place during routine animal facility care (Caldji et al., 2000; Korosi and Baram, 2010). Immediately after birth and on weaning time, no significant differences in number, morbidity, mortality or weight gain were observed among the different groups. On weaning time, at the postnatal day (PND) 21, 16 female pups (8 belonging to EH group and 8 to control group) were separated from males and randomly housed in groups of 2 per cage and maintained under standard laboratory conditions until they reached adulthood.

2.2. Experimental procedure

On test days, the animals were brought into the laboratory at least 60 min before the experimental session to allow them acclimatize. The experiments were carried out in a sound-isolated room between 9:00 and 14:00 and conducted in accordance with the regulations of the Committee for the Protection and Use of Animals of the University of Palermo, following current Italian rules on animal experimentation (D.L. 116/92) and European directives (2010/63/EU).

2.3. Handling procedures

From PND 2 until PND 21, litters were daily separated from the mothers. The separation procedure consisted in removing the mother from the nest and temporarily placing her in a separate cage. At the same time, in order to disallow ultrasound vocalizations, pups were moved into a different chamber at 30–32 °C and individually placed into sawdust-containing small plastic cups for 15 min. At the end, mothers and pups were reunited in their home cages. The separation procedure was always performed in the same room, at the same time (10:00 h) and by the same experimenter (Cannizzaro et al., 2005, 2006, 2007).

2.4. Object recognition test

At PND 55, EH and CTR rats were tested for four consecutive days in an open field arena, according to the object recognition (OR) task protocol (Bevins and Besheer, 2006; Taglialatela and Hogan, 2009). At day 1 and 2, 5 min habituation sessions were performed at 10.00 a.m., in order to let the animals freely explore the arena. Open field apparatus uses a square box, 44 cm wide, 44 cm long and 20 cm high. Twenty-four hours after the last habituation session, at day 3, rats were subjected to a 5-min training session when they were presented two identical, non-toxic objects (i.e. two metal cans) which were placed against a wall in the open field arena. To prevent coercion to explore the objects, rats were released against the center of the opposite wall with its back to the objects. The time spent on exploring each object was recorded using ANY MAZE Video Tracking System, (Ugo Basile, Italy); a 2 cm^2 area surrounding the object was defined such that nose entries were recorded as time exploring the object. After the training session, animals were placed in their home cage for 1 h retention interval. Then, animals were returned to the arena containing two objects: one was identical to the familiar one but previously unused (to prevent olfactory cues and the necessity to wash objects during experimentation), the other was a novel object (metal, glass or hard plastic items). Time spent on exploring each object was recorded along 5-min session. At Day 4, after 24 h retention, rats were tested again with two objects: the familiar one and an other novel object. Objects were randomized and counterbalanced across animals. Again, time spent on exploring each object was recorded along 5-min session. The objects and arena were thoroughly cleaned with 70% isopropanol at the end of each experimental session.

The recognition index (RI), which is the time spent on investigating the novel object, divided by the total amount of exploration time of the novel and familiar objects, relative to the total object investigation [RI = TN/(TN + TF)], is a measure of novel object recognition (Benice et al., 2006; Broadbent et al., 2010; Oliveira et al., 2010; Sarkisyan and Hedlund, 2009) and the main index of retention (Botton et al., 2010; Gaskin et al., 2010; Mumby et al., 2002; Piterkin et al., 2008; Schindler et al., 2010). If RI percentage is higher than 50%, it indicates more time spent on enquiring into the novel object, whereas less than 50% indicates that time was prevailingly spent on exploring the familiar object, and 50% indicates a null preference.

2.5. Morris water maze

The Morris water maze (MWM) apparatus used in these experiments is a circular, light-blue swimming pool with the following dimensions: diameter 160 cm; walls 70 cm high. It was filled with tap water to a depth of 50 cm. The water temperature was carefully maintained at 23 ± 2 °C. The pool was divided into four quadrants (NW, NE, SW and SE) of equal size by using the ANY MAZE system to quadrisect the pool. A removable squared escape platform (10 cm × 10 cm) could be positioned in the quadrants,