Int. J. Devl Neuroscience 27 (2009) 747–755

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu

Environmental and tactile stimulation modulates the neonatal handling effect on adult rat spatial memory

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ARTICLE INFO

Article history:
Received 20 June 2009
Received in revised form 2 August 2009
Accepted 25 August 2009

Keywords:
Handling
Novelty
Tactile stimulation
Context
Spatial memory
Corticosterone

ABSTRACT

Handling of rat pups promotes their adult cognitive performance. However, new data suggest that individual components of the handling procedure, like exposure to novelty or tactile stimulation, have distinct lasting effects on behaviour. In this study we examined the interaction of early novelty exposure with a varying amount of tactile stimulation on spatial recognition memory and corticosterone secretion of adult male and female rats. A split litter design was used and the experimental animals were also compared to animal facility reared controls. The experiment was conducted in two phases. In the first phase, we examined the effect of novel or home environment during the 15-min of neonatal handling, following 10 back-strokes. Tactile stimulation of 10 back-strokes combined with novelty exposure, enhanced novel arm discrimination in a Y-maze task in adult rats of both sexes compared to their siblings that stayed at home, as well as to the animal facility reared controls. In the second phase, additional back-stroking (total of 20 back-strokes) reduced the Y-maze performance of males neonatally exposed to novelty, while the same treatment enhanced the performance of their siblings that stayed at home. Basal corticosterone levels, determined 1 week post-Y-maze, were significantly increased only in the novelty exposed/10 back-stroked females compared to same sex non-handled controls. In contrast, 10 back-strokes combined with the home cage environment increased corticosterone in males. Increase to 20 back-strokes reversed the impact of neonatal environment on corticosterone levels.

These data suggest that the nature and intensity of the individual components of a mild early life manipulation, like handling, are critical in modifying aspects of adult memory performance and basal adrenocortical function.

1. Introduction

There is accumulating evidence that adult rats exposed as pups to early life experience during the perinatal period show altered behaviour and neuroendocrine function (Champagne et al., 2009; de Kloet et al., 2005). For instance, an adverse experience, like prolonged maternal separation of 24 h, impairs later life cognitive performance and enhances adrenocortical and emotional reactivity. On the contrary, mild manipulations, like neonatal handling, can lead, via developmental programming, to animals with a better coping with stressful situations later in life (Champagne et al., 2009; DeNelsky and Denenberg, 1967; Lehmann et al., 2002; Levine, 1957; Meaney et al., 1985; Plotsky et al., 2005). Such handled animals, which are separated from their mother daily for 15 min over the first 3 weeks of life, exhibit as adults reduced anxiety. In response to a mild stressor they have a lower output of corticosterone, prolactin and adrenaline (Levine et al., 1967; Meaney et al., 1985; Meerlo et al., 1999; Nunez et al., 1996). On the other hand, handled animals have increased levels of glucocorticoid receptors in the hippocampus and frontal cortex (Meaney and Aitken, 1985; Meaney et al., 1994; Viau et al., 1993). Accordingly, their limbic–hypothalamic–pituitary–adrenal (LHPA) axis feedback sensitivity to corticosterone is enhanced, providing a more efficient termination of the stress response. The aforementioned features of handled animals have been linked to their generally improved performance in cognitive tasks (Kosten et al., 2007; Stamatakis et al., 2008).

Neonatal handling consists of at least three different manipulations: brief maternal separation (up to 15 min), touching (tactile stimulation) by the experimenter, and exposure to novelty. Firstly, the short term maternal separation eliminates olfactory cues as well as the tactile stimulation by the mother, for which the latter compensates at reunion (Pryce et al., 2001b; Villescas et al.,

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0736-5748/$36.00 © 2009 ISDN. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.ijdevneu.2009.08.013

1977). Secondly, touching by the experimenter during the separation can, to some extent, mimic the maternal tactile stimulation, but the various protocols are different in this respect. Finally, the additional exposure to novelty offers new olfactory and visual stimulation (from 2nd week on). Although the effect of neonatal handling on the adult animal's physiology and behaviour has been extensively studied since the mid-1950s (Levine, 1957), only few studies have examined the impact of its separate components.

The established handling paradigm (Meaney et al., 1985) was modified by introducing a split litter design, for studying the impact of exposure to novelty separately (Tang, 2001). In this model, all pups are experiencing daily a short term separation from their mother as well as the experimenter's touching. However, only half of the pups in each litter are exposed individually to novelty, while the rest are left in their home cage. The adult male rats, exposed to novelty exposure as part of this handling protocol, exhibited as adults enhanced spatial and non-spatial memory (Tang, 2001; Tang et al., 2006) and showed increased hippocampal LTP (Tang and Zou, 2002). More recently, by using the same paradigm (Benetti et al., 2007), it was shown that brief exposure of pups to an unfamiliar environment produced in adult males an attenuated fear and stress response, as well as diminished sexual behaviour. Females have been scarcely studied in this paradigm. Juvenile and adult female rats, that had experienced as pups prolonged absence of the mother while being placed in a novel environment, were less reactive to stressors as compared to female pups that stayed in their nest during maternal absence (Rees et al., 2006). However, others did not observe this difference using the same protocol (Pryce et al., 2001a).

During prolonged (24 h) maternal separation, artificial tactile stimulation of male pups, especially in the anogenital region, has been shown to reverse the central effects of separation in rats, while additional feeding restored corticosterone secretion (Pauk et al., 1986; Suchecki et al., 1993; van Oers et al., 1998). Tactile stimulation can also enhance the maturation of cortical neurons (Schapiro and Vukovich, 1970), improve passive avoidance response (Zhang and Cai, 2006) and spatial working memory (Zhang and Cai, 2008). In paradigms of 1 h maternal separation, tactile stimulation of the dorsal area has been shown to reverse the effect of separation on anxiety-like behaviour and pain sensitivity in adult rats of both sexes (Imanaka et al., 2008). The lasting behavioural effects of tactile stimulation during the handling paradigm have not been studied.

The aim of this study was to examine the impact and the interaction of early novelty exposure with a varying amount of tactile stimulation on spatial recognition memory and basal corticosterone secretion of adult male and female animals (Fig. 1A for an overview). Therefore the experiment was conducted in two phases. We first examined the effect of novel or home environment, following 10 back-strokes, on the above parameters. The outcome was further investigated with an additional amount of tactile stimulation (total of 20 back-strokes) preceding the daily novelty or home exposure. In both phases, a split litter design was used. The experimental animals were also compared to non-handled, animal facility reared (ARF) controls.

2. Experimental procedures

2.1. Subjects

18 female and 6 male Wistar rats (obtained at 6 weeks of age from Hellenic Pasteur Institute, Athens, Greece) were used for breeding. After a habituation period of 2 weeks, 3 females were put in a cage with one male rat (macrolon-polycarbonate type IV cages with wire lid; 60 cm × 38 cm × 20 cm; containing sawdust bedding). Pregnancy was determined by vaginal smears obtained every morning and pregnant females were transferred in individual cages (home cage: macrolon-polycarbonate type III cages with wire lid; 42.5 cm × 26.6 cm × 18.5 cm) containing sawdust. The day of birth was defined as postnatal day 0 (=PND 0). We accepted litter with a maximal excess of one male or female and our litter size was 7–9 pups. The pups of experimental groups were exposed to neonatal manipulations from PND1 to PND21. In the control litters, the pups remained undisturbed with their dams in the housing room until the time of behavioural testing, receiving only the animal facility rearing (change of cage once per week; starting at PND10). In total, offspring of 13 litters was used in this study.

All animals were weaned at PND22, group housed with the same sex littermates and received the normal animal facility rearing afterwards. At PND90 they were

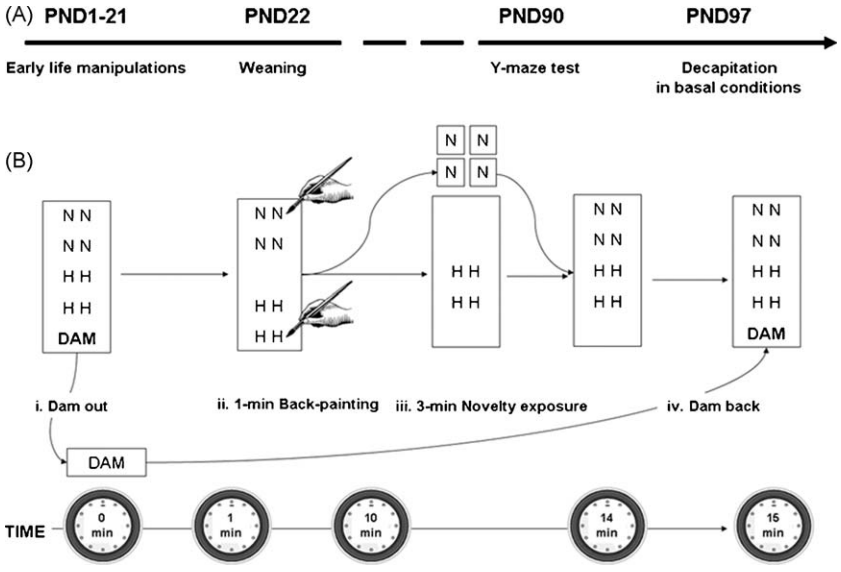

Fig. 1. Experimental methods. (A) Time line of longitudinal study. (B) Sequential steps of early life manipulations. (i) "Dam out". The dams were transferred out of the litter. This step took approximately 1 min (as indicated in the clock). (ii) Back-painting (tactile stimulation): back-stroking, consisting of passages of a marker tip over the pups' back, was used as daily tactile stimulation; started 1 min after the dam was removed and lasted 1 min per animal. For all the pups of a litter, this step took approximately 9 min (as indicated in the clock). (iii) Novelty exposure (environmental stimulation): exposure to novelty was performed according to Tang (2001) and started 10 min after the dam was removed. A split litter design was used so that half of the animals in each litter (painted with a certain color during stroking) were put individually in "novel" cages (macrolon-polycarbonate 18 cm × 20 cm × 14 cm; containing fresh sawdust bedding), under heating lamps for 3 min (Novel animals). The other half of the litter (painted with another color during stroking) remained for 3 min in the home cage under heating lamps (Home animals). After 3 min the Novel pups were transferred back to their home cage. This step took approximately 4 min (as indicated in the clock). During the transfer of Novel (back and forth), the home pups were also equally touched. (iv) "Dams' back" (dams' reunion): Finally, the dams were returned in the home cage. This step took approximately 1 min (as indicated in the clock).