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Maternal exposure to environmental enrichment before and during gestation influences behaviour of rat offspring in a sex-specific manner

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HIGHLIGHTS

- Female rats experienced EE during the pre-reproductive and gestational period.
- EE improves spatial learning in females before mating.
- Maternal exposure to EE affects offspring behaviour in a sex-specific manner.
- Maternal EE increases play behaviour and anxiety in males and learning in females.
- Maternal EE increases motility in male and female offspring.

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ABSTRACT

The beneficial effects of Environmental Enrichment (EE) applied immediately after weaning or even in adulthood have been widely demonstrated. Less is known about the possible changes in behaviour and brain development of the progeny following the exposure of dams to EE. In order to further investigate this matter, female rats were reared in EE for 12 weeks, from weaning until delivery. After having confirmed the presence of relevant behavioural effects of EE, both control and EE females underwent mating. Maternal behaviour was observed and male and female offspring were then administered a battery of behavioural test at different ages. EE mothers showed a decreased frequency of total nursing and, during the first 2 days of lactation, an increase in licking/grooming behaviour. Maternal exposure to EE affected offspring behaviour in a sex-specific manner: social play behaviour and anxiety-like behaviour were increased in males but not in females and learning ability was improved only in females. As a general trend, maternal EE had a marked influence on motility in male and female offspring in both locomotor activity and swimming speed. Overall, this study highlights the importance of environmental stimulation, not only in the animals directly experiencing EE, but for their progeny too, opening the way to new hypothesis on the heritability mechanisms of behavioural traits.

1. Introduction

The development of behaviour during ontogenesis is linked to the development of the structures of the nervous system and is affected by environmental conditions. It has been clearly demonstrated in rodents that exposure to environmental enrichment (EE), applied immediately after weaning or even in adulthood, has profound neurological and behavioural effects: EE stimulates neurogenesis and neuronal plasticity [1–4] by the production of brain neurotrophic factors including NGF and BDNF [5–7] and promotes neuronal branching, synaptogenesis and dendritic arborisation [8,9]. EE exerts positive effects on learning and memory, especially in tasks requiring spatial memory processes [7,10,11]. The effects of EE on emotional activity are more questionable [11–17] but the inconsistencies might be due to a number of factors including the length of exposure time to EE and the number of conspecifics in the cage (social enrichment).

Studies on the effects of EE have also been performed exposing mothers to EE and observing the outcomes in the progeny. Some of them focus on maternal exposure *during pregnancy* and the effects on male progeny, demonstrating better cognitive performance [18,19] and an increase of neurogenesis, synaptic markers and neurotrophic

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factors [19]. Other studies with the same time window of EE exposure, show an increased locomotor activity in the Open Field (OF) and an enhanced anxiety-like behaviour in the Elevated Plus Maze (EPM) both in male and female offspring [20,21]. Only two groups of research have studied the effects of EE exposure in the *pre-reproductive phase* in the rat. The first group reported an early development of complex motor skills in the pups associated with an increase of brain BDNF [22] and higher spatial performance in the MWM in the male offspring [23]. The other group found a decrease of anxiety behaviour in the EPM in the offspring of both sexes [24]. We previously had studied the effects of maternal exposure to EE *during lactation* and no modification of play behaviour, spatial memory and anxiety was observed in the adult offspring (see Supplementary Fig. 1).

The purpose of this study was to investigate the effects of a long period of EE maternal exposure on maternal care and on different behavioural traits in mothers and offspring of both sexes in the rat. We used a time window of EE including the pre-reproductive and gestation phases. Both of them are very vulnerable periods of life, due to the fact that two important processes occur during these periods: gametogenesis in the first and embryogenesis in the second one. Besides, there aren't studies that consider such a long period of exposure. Thus, studying both mothers and male and female offspring behaviours we evaluated whether a gender-specific behavioural profile transmission to the progeny exists and whether maternal behaviour was affected as well. To this aim, female rats were reared in an EE from weaning until delivery and their behaviour in the MWM and EPM assessed prior to mating. Maternal care was studied and female and male offspring of different ages were subjected to a battery of tests investigating social play behaviour, spatial learning and anxiety. Further, a hierarchical position test was performed to evaluate if the dominant or subordinate character is related either to maternal enrichment or to emotional and cognitive traits [25,26].

2. Materials and methods

Thirty-one female and fourteen male Wistar rats at post-natal day (PND) 21 were purchased from Charles River (Italy). All rats were housed in a controlled-temperature room (21–23 °C, humidity 40–50%) and maintained on a 12-h light/dark cycle (light on 07:00 am); food (Standard Diet Charles River 4RF21, Italy) and water were available *ad libitum*. This study was carried out in accordance with the Italian Animal Welfare legislation (art. 4 and 5 of D.L. 116/92) that implemented the European Committee Council 106 Directive (86/609/EEC).

2.1. Experimental design

On arrival in our facility, 15 female rats were randomly assigned to Environmental Enrichment (EE) and 16 females to Standard rearing Conditions (SC). The enrichment began on the day of arrival and continued for 12 weeks. Females reared in EE were housed in a group of five in a large cage (65 × 62 × 41 cm, L × W × H) equipped with two floors connected by a ladder (PRS, Italy). The cage contained wood shavings, a 25 cm running wheel and a shelter (a tunnel in which the rat can enter) that were kept stable in the cage throughout the enrichment period. Moreover, the cage was supplied with other three objects including coloured plastic toys (different kind of balls, nylon bones, kongs, tunnels), different wood toys (bricks, ladders, cubes), and nesting materials (paper, wood wool, Nestlets®) that were rotated every day and completely substituted twice a week. All cages were provided with a video camera monitoring system (24/24 h), counter system for wheel, food and water (PRS, Italy) (for an image of the cage see Supplementary Fig. 2). Females reared in SC were paired-housed in standard cages (43 × 23 × 15 cm, L × W × H).

From PND 60, six weeks after the beginning of enrichment, the Morris Water Maze (MWM) test and Elevated Plus Maze (EPM) were performed both in EE and SC female rats.

At PND 80, females were mated for 1 week with standard-reared male rats: two females and one male per standard cage, five females and two males per enriched cage. Afterwards, males were removed and females were maintained in their home cages (standard and enriched) throughout pregnancy. Four days before parturition, SC and EE females were individually housed in standard and enriched cages, until offspring were weaned.

Body weight, food and water intake were recorded weekly until delivery.

A schematic overview of the experimental design is reported in Fig. 1.

2.2. Experimental groups of pups

The day of birth was counted as day 0 (PND 0); on PND 1, the number of offspring per dam was counted, sexed, weighed and culled to eight pups, four males and four females. For each kind of maternal rearing conditions (SC and EE), two groups of male and female pups were obtained. In order to avoid offspring exposure to EE, at PND 1, the enriched cage was modified: the rotating wheel was blocked and all the toys were removed from the cage. Weaning took place on PND 21: dam was removed and the offspring were placed in a clean cage, maintaining their respective housing condition (they remain with their littermate) until PND 33. At this age, in order to allow the establishment of hierarchical relationships, two or three offspring per litter were randomly selected, for each sex, and housed in Colony cages (larger standard cages: 80 × 50 × 38 cm, L × W × H) combining experimental groups as follows: 10 rats per cage, 5 male offspring of EE mothers and 5 male offspring of SC mothers or 5 female offspring of EE mothers and 5 female offspring of SC mothers. They were maintained under these housing conditions until the end of the study. As illustrated in Fig. 1, offspring were sequentially tested in several behavioural paradigms at different ages. A total of 120 male and female offspring (30 animals per group) were tested.

2.3. Assessment of maternal behaviour

The behaviour of each dam was observed five times per day (60 min per session) from the 2nd to the 11th day after delivery. The observation sessions occurred at 6 a.m., 9 a.m., 12 a.m., 4 p.m., and 8 p.m.; the first and last sessions were undertaken during the dark phase of the daily cycle, and were performed under dim red light illumination. For each session, the behaviour of each female was scored every 4 min (75 observations per day). The frequency of the following behaviours was scored: (*i*) mother licking and grooming any pup (L/G), (*ii*) mother nursing pups in an arched-back posture (ABN), (*iii*) passive nursing (the mother lays over the pups or the mother is lying either on her back or side while the pups nurse), (*iv*) total nursing that included ABN and passive nursing, and (*v*) nest building (mothers moved bedding in the nest and elsewhere).

2.4. Social play behaviour

The behavioural assessments were performed in a sound-attenuated, lighting- and temperature-controlled room, as previously reported [27,28]. Briefly, the testing arena consisted of a Plexiglas cage measuring 40 × 40 × 40 cm with approximately 2 cm of wood shavings covering the floor. Animals were habituated and tested during their light cycle, but under red light conditions. At PND 30-32, animals were individually habituated to the test cage for 10 min on each of the two days prior to testing. On the test day, the animals were socially isolated for 3.5 h before testing, to enhance their social motivation and thus facilitate the expression of social play behaviour. This isolation period has been shown to induce a half-maximal increase in the amount of social play behaviour [29]. At the time of testing, each pair (two similarly treated animals) was placed in the test cage and allowed to interact for 15 min.