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Birth spacing in the mouse communal nest shapes adult emotional and social behavior

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ABSTRACT

The interactions with the mother and with peers are among the most relevant early environmental factors shaping adult brain function and behavior. In order to investigate the role of these factors, we exploited a novel early manipulation, the Communal Nest (CN), consisting in a single nest where three mothers give birth, keep their pups and share care-giving behavior from birth to weaning. In particular, we reared CD-1 swiss mice in three different CN conditions, each one characterized by a different interval between the three deliveries (Birth Spacing) of 3, 5 or 7 days (respectively, CN±3, CN±5, CN±7). Length of birth spacing affected maternal behavior, CN±7 pups receiving the highest levels. At adulthood, mice reared in the different conditions showed differences in emotional response and social skills. In the plus maze test, short birth spacing was found to be associated with enhanced emotionality, CN±3 mice showing highest levels of anxiety-like responses in the plus maze compared to the other two CN groups. In the social interaction test, the strategies to achieve dominance differed among the three groups. While CN±3 mice appeared to have a more aggressive strategy, displaying high levels of attack behavior in the first encounter, CN±5 and CN±7 mice displayed a more affiliative strategy based on social investigation. Overall, these findings show that birth spacing shapes the early mouse social environment and, in turn, affects the development of social skills and emotional responses.

1. Introduction

The early environment has been shown to exert a major impact in determining the developmental trajectories of the organism. A key role has been attributed to the mother, representing for the mammalian infant the most important source of early experiences. Indeed, altricial mammals depend on her for essentially all of their needs, especially feeding [1]. Changes in maternal behavior have been related to modifications in the physiological and behavioral response of the offspring later in life [2–5]. Studies carried out in different species, ranging from non-human primates to laboratory rodents, have shown that high levels of maternal behavior are associated with reduced emotionality and improved social skills in the adult offspring, in addition to lower hypothalamic-pituitary-adrenal (HPA) axis activation following stress [2]. These results have been interpreted according to the maternal mediation hypothesis [6], stating that the quantity and quality of maternal behavior is a key factor in shaping the adult individual. More recently, an increasing amount of data suggests that factors other than the mother mediate the long-term consequences of the early environment [7–10]. For instance, interaction with peers appears extremely relevant. Indeed, according to their number, gender and age, they determine the quality and quantity not only of peer interactions but also of interaction with the mother [10–12]. In non-human primates, peer interactions during plastic post-natal phases are of fundamental importance for the development of sophisticated social behavior and emotional responses [13–15]. In rodents, though it has been reported that high social complexity is a feature of the ecological niche of the developing pup [16,17], peer interaction and its consequences on the adult individual have been rarely investigated [12,18–20]. For instance, it has been shown that litter gender composition affects behavior at long-term, mice reared in a balanced sex ratio condition showing higher levels of exploration compared to mice reared in isosexual litters [12].

In the mouse communal nest (CN), mothers combine their litters in a single nest and nurse them. In this condition, mothers take care of the pups in an apparent indiscriminate fashion, though this is still a debated issue [21–23]. Furthermore, more than one mother can be simultaneously on the nest (in a CN procedure involving three mouse mothers, when the nest is attended, an average of 1.5 mothers has been observed [24]). The CN occurs when deliveries are either synchronous or not, since it has also been observed when the discrepancy in age of pups was of 12 days [25]. When deliveries are asynchronous, birth spacing—i.e., the interval between deliveries—has an impact in characterizing the early social environment of the pup.

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Birth spacing affects pup development mainly by modulating two different features of its early environment: maternal behavior and peer competition. Indeed, the number and age of pups present in the nest may determine the amount of care-giving behavior provided by dams [12,26,27] as well as the competition among pups to access limited resources [28–31].

The aim of the present study is to investigate the effects of variations of the social complexity of the mouse nest environment on pup development. In particular, we evaluated the effect of birth spacing, analyzing the long-term consequences on emotional and social behavior of being reared in a CN in which three CD-1 mouse mothers give birth 3, 5 or 7 days apart (respectively, CN±3, CN±5, CN±7). The birth spacing determines the age difference of the three litters belonging to the same CN. In the CN±3 condition, on the day the last litter was born, the other two litters are respectively, 3- and 6-days old. For the CN±5 and CN±7 conditions, the other two litters are, respectively 5- and 10-days old and 7- and 14-days old (Fig. 1). Previous studies exploiting the synchronous CN manipulation have shown that, when tested in the social interaction test, in which the experimental mouse faces a conspecific in a neutral cage, adult mice reared in CN have more elaborate social competences, promptly playing a given social role. Furthermore, CN mice display an increased anxiety-like response in the elevated plus maze [24,32,33]. Our hypothesis was that, depending upon birth spacing, these behaviors would be differently affected. In particular, we expected that the CN±3 group that, among the CN conditions here investigated, is the closest to synchronous CN, would show more elaborate social competences and higher levels of anxiety-like behavior compared to the CN±5 and CN±7 groups. The results are discussed in terms of the role of the many features of the early environment, including maternal behavior, in shaping the developing organism [7,10].

2. Materials and methods

2.1. Animals and breeding procedures

Thirty male and 60 female mice of an outbred CD-1 Swiss-derived strain (ICR) weighing 25–27 g were purchased from a commercial breeder (Harlan, 20050 Correzzana, MI, Italy). Upon arrival at the laboratory, the animals were housed in an air-conditioned room (temperature 21±1 °C, relative humidity 60±10%) with lights off from 08.00 to 20.00 h. Males and females were housed in same-sex groups of 6 individuals in 42×17×14×cm Plexiglas boxes with a metal top and sawdust as bedding, and with pellet food (Enriched standard diet, Rieper, Vandoies, BZ, Italy) and tap water *ad libitum*.

All animal handling and experimental procedures were performed according to European Community guidelines (EC Council Directive 86/609) and the Italian legislation on animal experimentation (Decreto L.vo 116/92).

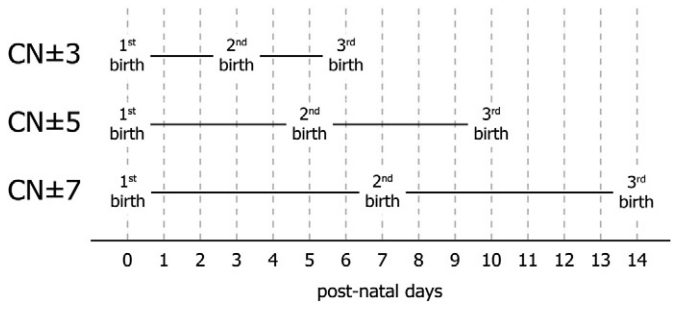

Fig. 1. Schematic of birth-spacing. In all CN conditions, each one of three mothers gives birth to a litter, all three in a single cage. The three mothers keep their litters together in a single nest and share care-giving behavior from birth to weaning. The three CN conditions, CN±3, CN±5 and CN±7, differ for the time interval between the three deliveries (i.e., birth spacing) that was 3, 5 or 7 days, respectively. In other words, in the CN±3 conditions, on the day the last litter was born, the other two litters were respectively, 3- and 6-days old. For the CN±5 and CN±7 conditions, the other two litters were, respectively 5- and 10-days old and 7- and 14-days old.

2.2. Rearing conditions

After one week of acclimatization to the animal room, the animals were used to form breeding groups, made up by 1 male and 2 females, housed in 33×13×14×cm Plexiglas boxes. Vaginal plugs were checked twice a day (at 09.00 h and 19.00 h) from the first day after breeding group formation. The male was removed from the breeding cage around pregnancy day 16. According to the expected day of delivery (calculated on the basis of the day of vaginal plug detection), the females were combined in trios, five days before the first delivery and assigned to one of three experimental groups: CN±3, CN±5, CN±7. The number of trios was 5 per group, for a total of 15 mothers per group. In the three experimental groups, the deliveries of the females of each trio were, respectively, 3, 5 or 7 days apart (Fig. 1). Vaginal plug observation allowed to have actual deliveries consistent with expected deliveries. The female trios were kept in 42×17×14×cm Plexiglas boxes. On the first day after birth, each litter was culled to four males and four females. Pups were weaned on PND 25 and males of each litter were housed in groups of 4 animals in 42×17×14×cm Plexiglas boxes. Females were not used in this study. For each test, from each CN cage, a total of 6 subjects were selected (2 mice for each age).

At adulthood (5 months of age) mice were tested. All tests were carried out between 0930 and 1530 h, i.e., during the dark period. Animals were transferred to the experimental room at least 40 min before the test in order to let them acclimatize to it. Animals were weighed at culling (PND 1), on PND 43 and at 5 months of age.

2.3. Maternal behavior

For each CN group, maternal behavior has been scored daily, over four sessions, for 27 days starting on the day after the first delivery occurred. This period was chosen so that at least the time from PND 1 to PND 13 for all litters in all CN conditions was covered. In each session, for each cage (n=5 in each group), data were collected using one-zero sampling, over 8 observations. For each cage, each observation was 5 min after the previous one. Each session lasted around 40 min. The experimenter recorded whether the behavior was present or not during the observation; different behavioral items could occur during the same observation. The sessions started at, 9.00, 12.00, 17.00, 21.00. Only the last session was during the dark phase of the 12:12 cycle, and was performed under dim red light illumination. For each cage, maternal behavior was scored as the sum of the maternal behavior of the three mothers.

Maternal behavior has been analyzed according to previous works [34–36]. In particular, the following behaviors were scored: *Arched-back nursing*: the dam is immobile and in a high upright dorsal arch posture supported by rigid fore- and hind limbs, the head is depressed, the trunk and limbs are bilaterally symmetrical, and pups are attached to the nipples; *Blanket nursing*: the dam is over the pups, relatively immobile, bilaterally symmetrical, with the head not depressed, and in a low dorsal arch posture supported by rigid fore- and hind limbs or in a low dorsal arch posture supported by rigid fore limbs or rigid hind limbs or lies flat on top of the pups with little or no limb support; *Passive nursing*: the dam body is lying down on her side with more than one pup usually attached to the nipples; *Licking*: licking of the pup body; *Ano-genital licking*: licking concentrated on the ano-genital region of the pup; *Retrieval*: the dam picks up the pup gently with the incisors by its dorsal skin and carries it to the nest; *Digging*: digging in the sawdust, moving it around using the snout and/or both the forepaws and hindpaws, mostly moving around the cage and sometimes changing the arrangement of the substrate material; *Rearing*: the animal stands on its hind limbs, often sniffing; *Moving*: the animal moves around the cage, actively exploring; *Eating*: