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Effects of neonatal paternal deprivation or early deprivation on anxiety and social behaviors of the adults in mandarin voles

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ABSTRACT

This study examined whether neonatal paternal deprivation (PD: father was removed and pups were raised just by mother) or early deprivation (ED: pups were raised by both parents except separated from not only the dam but also the peers for three hours a day from PND 0 to 13) has long-term effects on anxiety and social behaviors of adult mandarin voles. Newborn mandarin voles of F2 generation were randomly assigned to one of three groups: bi-parental care (PC: pups were raised by both parents), PD and ED. The parental care behaviors of F1 generation were observed at the age of 0, 13 and 21 days (PND 0, 13, 21) of F2 generation of PC and PD groups. Moreover, each mandarin vole of F2 generation received an open field test and a social interaction test on PND 70 and PND 75, respectively. No significant differences of parental behavior were observed between mothers and fathers from PC families, showing typical parental behavior of socially monogamous rodents. In addition, no significant differences of maternal behaviors were found between mothers from PC and PD families, indicating no maternal compensation towards pups for the absence of the paternal care. In the open field test, mandarin voles from both PD and ED families displayed higher levels of anxiety and lower locomotor activity, relative to offspring of PC family. In the social interaction test, both PD and ED mandarin voles also showed lower levels of social behavior and higher levels of anxiety. Thus, both PD and ED significantly increase anxiety and reduce social behavior of adult mandarin voles, suggesting that variation in parental investment may lead to variation in anxiety and social behaviors in rodents with different mating systems.

1. Introduction

Parental care is an important early environmental factor associated with behavioral and neuroendocrinal development of offspring. It consists of various complex behaviors that provide early social environment as well as nourishment, warmth, tactile stimulation and protection. Denenberg et al. (1973, 1977) found that early handling can increase exploratory activity and influence the hypothalamo-pituitary-adrenal (HPA) axis in the rabbit (Denenberg et al., 1973, 1977). The study on puppy found that gentling during early puppyhood was advantageous to the emotional development and welfare (Gazzano et al., 2008). In mammals, mothers are the primary source of nutritional resources and behavioral stimulation for the offspring and these mother–infant interactions are the primary source of social stimulation and then induce long-lasting changes in offspring phenotype (Champagne and Curley, 2005; Branchi, 2009). The HPA axis of the developing rat is also, in part, regulated by maternal factors. Active licking and grooming suppress neural activation and alter the pattern of corticotrophin-releasing factor (Levine, 2001). Previous research suggests that, for rodents, the increase in intensity of maternal care that follows handing of newborns is responsible for the physiological responses (Levine, 1975). Therefore, manipulation of the mother–infant relationship has been used extensively to investigate the developmental impact on offspring.

Furthermore, previous studies suggest that early in development, experiences such as maternal separation (MS: pups were raised by both parents except separated from the dam but not the peers) can induce neuroendocrine changes and produce short and long-term effects on the offspring (Cirulli et al., 2003; Pryce and Feldon, 2003; Weaver et al., 2004). Such changes include depression- and/or anxiety-like behaviors in rats later life (Lee et al., 2007) and long-term gender-dependent effects on behavior and HPA axis status (Slotten et al., 2006). Neonatal MS also led to neurochemical and behavioral changes linked to stress-sensitive brain regions such as the paraventricular region of the hypothalamus, the hippocampus and the lateral amygdala (Giachino et al., 2007). However, in contrast to most of the studies, Millstein and Holmes

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(2007) suggest that MS does not provide a model of early life stress effects on the anxiety- and depression-related behaviors in mice. Early deprivation (ED), which is different from MS, is the separation of pups from not only the dam but also the peers. Recent study has reported that similarly to MS, ED can increase anxiety (Rees et al., 2006). Deprivation of mother–pup contact or decreased frequency of contact directs development along a path involving heightened stress response and decreased social behavior (Champagne and Curley, 2005). Artificial licking-like tactile stimulation to rat pups reduces behavioral indices of anxiety and improves social learning (Gonzalez et al., 2001). These studies suggest that early parental investment plays important roles in the development of offspring behaviors.

In some social rodent species, fathers are also a rich source of sensory and emotional stimulation that is important for offspring development (Ovtscharoff et al., 2006). Such paternal behavior includes huddling, licking and grooming, body nosing, nose–nose contacts, nosing-the-nape of-the-neck and riding on the fathers back and so on (Reynolds and Wright, 1979). In California mice, paternal grooming promotes the development of novel object recognition (Bredy et al., 2004) and paternal behavior in general can influence the development of aggression and the associated neural substrates (Frazier et al., 2006). Further, a lack of paternal care can affect the synaptic development in the anterior cingulate cortex, which may underlie the mechanism of emotional behavior (Ovtscharoff et al., 2006). Paternal behavior also can increase alloparental behavior and decrease passive-alone behavior of juvenile prairie voles (Wang and Novak, 1994). Although the effects of paternal behavior on recognition, aggression, alloparental behavior have been studied in the past decade, the developmental effects of paternal behavior on anxiety and social behavior of adult animals are unclear.

The mandarin vole (*Microtus mandarinus*) is a socially monogamous rodent that is widely distributed in China (Tai et al., 2001; Tai and Wang, 2001). This species offers another interesting model for the study of developmental influences on social behaviors. Monogamy in mammals is defined by a suite of complex social interactions (Cushing et al., 2003; Kleiman, 1977) including adult male and female pair sharing a nest and home range and preferentially copulating with the mate, male participating in parental care and vigorous defending of the nest against intruders (Young et al., 1998). In contrast, polygamous rodents are relatively asocial, nest typically in isolated burrows and breeds promiscuously; breeding partners do not form a pair-bond after mating; males are not parental; and females abandon the offspring in the second or third postnatal week (Young et al., 1998). Variation in anxiety and related behaviors were also found in rodents with different mating systems. For example, employing the elevated plus maze test, Stowe et al. (2005) found that socially monogamous male prairie voles entered the open arms more frequently, remained there longer, and showed a higher level of overall locomotor activity than did polygamous male meadow voles (Stowe et al., 2005). Parental care varies widely between monogamous and polygamous rodents (Hayes and De Vries, 2007). From these reports, it is hypothesized that different parental investments in closely related vole species with different mating systems cause different emotional and social behaviors.

The goal of this research was to examine whether variation in parental care plays an important, but different, role in emotional and social development of offspring associated with different mating system. Bi-parental care (PC) occurs just in monogamy, while PD is similar to parental care in polygamy (Young et al., 1998). In summary, the present research aimed to answer the following questions: (1) Whether father mandarin voles display paternal cares in the PC family; (2) whether mothers in the PD family compensate for the absence of paternal care from their mate by increasing maternal care towards pups; and (3) whether different parental investment (PC, PD and ED) induces different long-lasting effects on emotional and social behaviors in offspring.

2. Materials and methods

2.1. Subjects

Animals used in this study were laboratory-reared F1 or F2 generation that originated from a wild population in Henan province in China. Every three months, the mandarin voles were supplied from wild to keep the genetic pool big in the outbred wild mandarin vole colony. Every individual was numbered to avoid mating of brother and sister. Animals were maintained on a 12-h light:12-h dark cycle and allowed free access to food (carrot and rabbit chow), water and cotton nesting material in polycarbonate cages (44 cm × 22 cm × 16 cm). All the procedures were approved by the Animal Care and Use committee of Shaanxi Normal University and were in accordance with the Guide for the Care and Use of Laboratory Animals of China.

2.2. Treatments

F1 generation: 20 female and 20 male mandarin voles, which were the first generation of the wild population, have been bred and raised in the home cage and left undisturbed. At 21 days of age, they were weaned and housed in same-sex sibling pairs. At 70 days of age, female and male mandarin voles were paired for 20 non-sib couples. The male mandarin voles were marked by cutting a bundle of hair on their backs. Mandarin voles of F1 generation were randomly assigned to two groups. For bi-parental care families, all family members were housed in their home cage and left undisturbed until pups were weaned. For neonatal paternal deprivation families (PD), the father was removed immediately after the pups were born and the mother weaned the pups on her own.

F2 generation: These animals were bred by the F1 generation. At PND 0, whole litters were randomly assigned to one of the three groups (PC, PD or ED) according to their family. In PC and PD groups, pups grew up in the PC or PD families that were treated as above. In the ED group, pups were removed from their natal cages for 3 h (09:30–12:30 h) on each of PND 0–13 and placed inside an incubator at 32 °C. Pups were physically separated from one another during ED by placing them in individual holding compartments of the humid incubator with their own natal nesting material which they were familiar with. At the end of the daily separation periods, pups were returned to the home cage nest and stayed with their parents. At PND 21, pups were weaned and housed in same-sex sibling pairs.

2.3. Behavioral test

Parental Behavior Observation: At PND 0, 13 and 21 of offspring, parental behavior of mother (PC = 10 or PD = 10) and father (PC = 10) mandarin voles of F1 generation was observed in their natal cages. Two pups of one litter were remained as stimulus while other pups were placed inside an incubator at 32 °C. Before the formal test, one hour observation was carried out on some litters to make sure 15 min was adequate to reflect the longer time periods. At the beginning of the test, the tested animal was placed on the opposite side of the nest separated by clapboard in the middle of the cage. After 1 min separation, the clapboard was removed and the total durations of following behaviors were recorded for 15 min (McGuire and Novak, 1986; Yu and Fang, 2003): Huddling (sitting over the pups with an arched-back posture) (Stern and Johnson, 1990), licking the pups, retrieving the pups, lactating, sniffing the pups, nesting (carrying cotton or tidying the nest), nonkyphotic (sitting flat on a pup or next to it, while touching, but without an arched back) (Bales et al., 2006) and nonsocial behaviors.