Physiology & Behavior xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Physiology & Behavior

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

Postpartum repeated separation from pups affects the behavior and neuroendocrine parameters of mandarin vole fathers

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HIGHLIGHTS

- Brief pups separations increase anxiety-like behaviors of fathers.
- Long time pups separations increased depression-like behaviors of fathers.
- BPS increased sniffing and self-grooming, but reduced attacking and climbing.
- LPS increased quiescent, but suppressed social interaction in fathers.
- LPS and BPS altered levels of central OT and AVP, and serum corticosterone.

ARTICLE INFO

Article history:
Received 14 April 2014
Received in revised form 4 November 2014
Accepted 5 November 2014
Available online xxxx

Keywords:
Mandarin vole
Pups separation
Anxiety
Depression
Social behaviors
OT
AVP
CORT

ABSTRACT

Repeated separation from pups results in anxiety and depression-like behaviors in mothers. This level of attachment has also been established between fathers and pups in monogamous rodents. We hypothesized that brief and lengthy separation from their pups would affect emotion, social behavior and neuroendocrine parameters in socially monogamous male mandarin voles (*Microtus mandarinus*). The results indicate that brief pup separation (BPS) of 15 min/day significantly reduced the percentage of time spent in the central area, total distance and total transition in open field tests. BPS resulted in increased sniffing and self-grooming in fathers, but reduced attacking and climbing. Long pup separation (LPS) of 3 h/day suppressed attacking, sniffing, no-social investigating and digging in fathers, but increased time in immobile in social interaction and forced swimming tests. LPS upregulated levels of central oxytocin (OT) and vasopressin (AVP), serum corticosterone (CORT); BPS increased central OT and serum corticosterone only. These findings show that BPS and LPS are critical stressors for fathers and alter anxiety and depression-like and social behaviors in monogamous mandarin voles. These changes in behaviors may be associated with alteration in OT, AVP and CORT.

1. Introduction

Social stress influences behavioral and neuroendocrine functions associated with mood disorders [1–3]. Depression is an important mood disorder and can be caused by various conditions, such as endocrine abnormalities, cancer, drug abuse and chronic stress [4]. Stressful social experiences can also induce depressive disorders [5]. For example, prolonged separation from a partner or loss of a partner increases the probability of depression [6,7]. Daily 'handling' or brief separation (15 min) of rat pups from their mothers (BMS) during postpartum weeks 2–3 decreases acute stress-induced anxious behavior and hypothalamic–pituitary–adrenal (HPA) axis activation of offspring during adulthood [8,9]. In contrast, long daily periods (3–5 h) of maternal separation (LMS) during the postnatal period in rats increases acute stress-induced anxious behavior in adult offspring [10] and anxiety and depression-like behaviors in mothers [5,11,12]. Interestingly, some studies suggest that repeated brief maternal separation from pups, but not prolonged separation, may also be stressful for Wistar rat mothers [13].

Fathers provide high levels of paternal care and heavily interact with pups in socially monogamous rodents [17,18]. Like maternal deprivation, paternal deprivation also leads to increased anxiety, decreased parental care and sociability [18,19] and affects recognition and pair bonding in this kind of rodents with close attachment [20,21]. Although there is strong evidence that the presence of fathers has a significant effect on offspring, no study has addressed whether separation from pups has any effect on fathers. One recent study also demonstrated that mandarin vole pups can elicit significant reward value to their fathers similar to cocaine [22]. Given the different effects that different length of separations from pups have on mothers, we hypothesized that the separation of pups from fathers for different duration daily

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http://dx.doi.org/10.1016/j.physbeh.2014.11.017
0031-9384/

may also produce different effects on levels of anxiety, depression and social behavior in fathers.

The HPA axis participates in the progress of anxiety and depressive symptoms [23–25], and antidepressants focus on the HPA axis [26]. If pup separation can affect depression and anxiety-like behaviors, we predicted that these effects would be associated with changes in HPA activity. Both oxytocin (OT) and vasopressin (AVP) are mainly synthesized in the supraoptic nucleus (SON) and paraventricular nucleus (PVN). OT and AVP are involved in emotion and social behavior via interaction with stress hormones in the HPA axis [27–32]. For example, AVP can promote the excretion of adrenocorticotropic hormone (ACTH) and the activation of AVP neurons may be associated with HPA axis functional excitement [31]. These two peptides can also modulate emotional and social behavior via binding with their receptors in other brain regions [30]. Corticosterone (CORT) is yet another component of the stress response and the presence or absence of CORT to repeated stress may be associated with different responses across several markers [35]. In one study, 4 weeks of long maternal separation (3 h/day) from their litters increased plasma CORT concentrations in rat dams compared with brief separation (15 min/day) [12], however, in another study, eight random days of brief maternal separation (15 min/day) induced higher plasma CORT levels than controls and long maternal separation from their litters (4 h/day) [13]. Here, we predict that repeated separation from pups for different lengths of time alters levels of anxiety and depression, and neuroendocrine parameters differently in fathers.

Mandarin voles (*Microtus mandarinus*) are socially monogamous rodents widely distributed in China. They form enduring pair attachments and display high levels of bi-parental care [17,18,36,37]. Here, we investigated the impacts of short-term separation and long-term separation from pups on behavioral and neuroendocrine parameters in fathers. We measured the effect of separation on fathers using an open field test, same-sex social interaction test and forced swimming test. Levels of serum OT and CORT, and OT and AVP expression in the PVN were also examined after forced swimming test.

2. Materials and methods

2.1. Subjects

The mandarin vole is a socially monogamous rodent [36] widely distributed in China [37,38]. The animals used in this study were bred and born in a colony reared at the College of Life Sciences, Shaanxi Normal University, Xi'an, China. The colony was established with wild animals from Lingbao City, Henan and is replenished each year. Breeding pairs and litters were housed in polycarbonate cages (44 cm × 26 cm × 16 cm) and maintained on a reversed light:dark 14:10 cycles (lights on at 20:00 h) and at 25–28 °C. Hardwood shavings and cotton were provided as substrate and bedding. Rabbit chow (Laboratory Animal Center, Xi'an Medical University), carrot and malt were provided ad libitum. Mandarin voles used in our experiments were laboratory-reared F2 generation animals. All procedures were in accordance with the Guide for the Care and Use of Laboratory Animals and were reviewed by the Animal Care and Use Committee at Shaanxi Normal University.

2.2. Procedures

Male ($n = 30$) and female ($n = 30$) voles were randomly assigned into one of the three groups (10 male–female pairs in each group): no pup separation (NPS); brief pup separations (BPS) for 15 min/day; or long pup separations (LPS) for 3 h/day. Males and females were paired randomly and primiparous. The day of delivery was designated as postpartum day 0. Litters were culled to 3 to avoid the litter size effect. Pup separation commenced on postpartum day 2. In the LPS group, pups were separated from their parents for 3 h once a day between 9:00 h to 12:00 h for 14 consecutive days. In the BPS group, pups were separated from their parents for 15 min once a day between 9:00 h to 9:15 h for 14 consecutive days. Pups removed from their cages were placed together with litter mates in their own small box in a thermostat water bath at 32 °C during the separation period. In the NPS group, pups were not separated from their parents but handled as in the other two groups. On postpartum day 21, all pups were permanently removed from the parental cages. Cages and bedding were cleaned every three days. All procedures were conducted by the same person during the experimental period. Behavioral tests were performed between 14:00 h and 18:00 h and following a sequence we designed so that the test of least stress was first. The fathers with second litter was given birth were excluded from the data analysis.

2.3. Behavioral tests

2.3.1. Open-field test

Open-field tests were conducted the day after weaning. Spontaneous motor activity and the anxiety-like behavior of fathers were assessed in an open-field chamber consisting of a square arena (50 cm × 50 cm × 25 cm) made of gray glacial polyvinyl chloride, brightly and evenly illuminated by six 60 W lamps mounted 2 m above the arena. The area was divided into 25 quadrants (nine central and 16 peripheral). Subject voles were individually placed into the center of the open-field and allowed 5 min to explore. During this period, time spent in the central and peripheral zones, total distance traveled during the experiment and the number of crossings between quadrants were recorded using a digital video camera and Videomot2 (TSE Systems, Bad Homburg, Germany). After each test, the father was returned to his home cage and the apparatus was cleaned using 70% ethanol.

2.3.2. Male–male social interaction

Male–male social interaction tests were performed in polycarbonate cages (44 cm × 26 cm × 16 cm) 3 days after the open field test. At the beginning of the test, the focal animal was placed in the center of the cage to habituate for 10 min. Then, a strange male stimulus animal of the same age and size was put in the cage and was fixed to the chamber lid with a tether and permitted to move throughout half the chamber. Each stimulus animal was used for only one subject. Light intensity was approximately 200 lx during the experiment. At the end of the test, the focal animal was returned to its home cage and the substrate in the apparatus was replaced by clean sawdust. The total duration and frequency of the following behaviors for the focal animal were recorded for 15 min: sniffing the stimulus vole (include head, body or anogenital region); self-grooming (cleaning the fur or scratching); digging substrate; non-social investigation (sniffing substrate); climbing the wall; attacking (active fighting between two voles such as wrestling, biting or chasing) and being immobile. The social interaction test was recorded using a digital video camera. The duration and frequency of behaviors were scored from the video footage by an observer blind to experimental treatment using Observe 5.0 (Noldus, Wageningen, Netherlands).

2.3.3. Forced swimming test

To evaluate the degree of a depressed-like state in mandarin vole fathers, we performed a modified forced swimming test (FST) [39]. On postpartum day 25, paternal voles were put into a vertical plexiglass cylinder (height 30 cm, diameter 18 cm) containing approximately 25 cm of water maintained at 25 °C. The cylinder was rinsed thoroughly and filled with clean water prior to testing each animal. Once the test began, the duration of the following behaviors was recorded during the last 5 min of the 6-min testing period: swimming, struggling, climbing and immobility (the vole floated in an upright position and made only small movements to keep its head above water). Each animal was removed after 6 min, dried with a towel and placed in a heated

Please cite this article as: Kong L, et al, Postpartum repeated separation from pups affects the behavior and neuroendocrine parameters of mandarin vole fathers, Physiol Behav (2014), http://dx.doi.org/10.1016/j.physbeh.2014.11.017