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Effects of repeated pup exposure on behavioral, neural, and adrenocortical responses to pups in male California mice (*Peromyscus californicus*)

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ABSTRACT

In biparental mammals, the factors facilitating the onset of male parental behavior are not well understood. While hormonal changes in fathers may play a role, prior experience with pups has also been implicated. We evaluated effects of prior exposure to pups on paternal responsiveness in the biparental California mouse (*Peromyscus californicus*). We analyzed behavioral, neural, and corticosterone responses to pups in adult virgin males that were interacting with a pup for the first time, adult virgin males that had been exposed to pups 3 times for 20 min each in the previous week, and new fathers. Control groups of virgins were similarly tested with a novel object (marble). Previous exposure to pups decreased virgins' latency to approach pups and initiate paternal care, and increased time spent in paternal care. Responses to pups did not differ between virgins with repeated exposure to pups and new fathers. In contrast, repeated exposure to a marble had no effects. Neither basal corticosterone levels nor corticosterone levels following acute pup or marble exposure differed among groups. Finally, Fos expression in the medial preoptic area, ventral and dorsal bed nucleus of the stria terminalis was higher following exposure to a pup than to a marble. Fos expression was not, however, affected by previous exposure to these stimuli. These results suggest that previous experience with pups can facilitate the onset of parental behavior in male California mice, similar to findings in female rodents, and that this effect is not associated with a general reduction in neophobia.

1. Introduction

In both females and males of some rodent species, parental behavior (i.e., nurturant behavior toward immature individuals) can occur outside of typical reproductive conditions via continuous or repeated exposure to infants, a process called "concaveation" or, more commonly, "sensitization." Adult, sexually naïve (i.e., virgin) female rats (*Rattus norvegicus*), for example, typically avoid pups upon first exposure, but display maternal behaviors after repeated or continuous exposure (Bridges et al., 1972; Fleming and Rosenblatt, 1974; Jakubowski and Terkel, 1985a, 1985b; Lonstein et al., 1999; Quadagno et al., 1974; Reisbick et al., 1975; Rosenblatt, 1967; Stern and Mackinnon, 1976; Wiesner and Sheard, 1933). In contrast to rats, adult virgin female house mice (*Mus musculus*) frequently exhibit maternal behavior upon their first exposure to pups and are often described as "spontaneously maternal" (Gandelman, 1973; Leussis et al., 2008; Martín-Sánchez et al., 2015; Noirot, 1969; Stolzenberg and Rissman, 2011; Stolzenberg et al., 2012); however, repeated or continuous exposure to pups can increase measures of maternal behavior in virgin females even more (Alsina-Llanes et al., 2015; Brown et al., 1999; Ehret and Koch, 1989; Ehret et al., 1987; Pedersen et al., 2006). Virgin female prairie voles (*Microtus ochrogaster*) may attack, ignore, or care for foster pups at first exposure (Bales et al., 2007; Lonstein and De Vries, 2001), and exposure to pups in adolescence increases some aspects of maternal care in adulthood (Lonstein and De Vries, 2001), similar to rats (Stern and Rogers, 1988). In virgin female Syrian golden hamsters (*Mesocricetus auratus*), continuous exposure to pups often changes infant-directed behavior from infanticidal to maternal within a few days (Noirot and Richards, 1966; Swanson and Campbell, 1979).

Effects of repeated or continuous exposure to pups on paternal care by male rodents have received less attention than those on maternal care. Adult virgin male rats (Bridges et al., 1972; Jakubowski and Terkel, 1985b; Rosenblatt, 1967), mice (Ehret et al., 1987), and golden

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hamsters (Swanson and Campbell, 1979) can be sensitized to show parental care, with sensitization latencies longer than those of females. These species, however, may not be optimal models for understanding paternal behavior, as male rats, mice, and golden hamsters do not typically provide care for their offspring under naturalistic conditions. In the approximately 5–10% of rodents in which fathers provide care for their offspring in the wild (Dewsbury, 1985; Kleiman and Malcolm, 1981), almost nothing is known about the effects of prior exposure to pups.

The California mouse (*Peromyscus californicus*) is a monogamous, biparental rodent in which fathers spend as much time as mothers caring for offspring (e.g., huddling, grooming, and retrieving pups) and typically care for unrelated pups during experimental exposure, while adult virgin males may either attack, ignore, or care for experimentally presented pups (Chauke et al., 2012; de Jong et al., 2009, 2010; Gubernick and Addington, 1994; Gubernick and Alberts, 1987; Jasarevic et al., 2013; Rosenfeld et al., 2013). Cohabitation with a younger litter increases the likelihood of males behaving paternally toward an unrelated pup in young juveniles (35–45 days of age), but not in older juveniles (55–65 days) or adults (160 days) (Gubernick and Laskin, 1994). However, whether pup exposure during adulthood alters infant-directed behavior in adult male California mice is not known.

In female rodents, one mechanism underlying the onset of maternal behavior in parturient mothers is suppression of fear-, anxiety-, and stress-related responses to infants. Inhibition of hypothalamic-pituitary-adrenal axis and neuronal responses to aversive stimuli occurs during late pregnancy and lactation, and facilitates expression of maternal care (Brunton et al., 2008; Lightman et al., 2001; Slattery and Neumann, 2008). In virgin males of some biparental rodent species, exposure to pups may dampen some stress-related responses: pup exposure decreases plasma corticosterone levels in response to a handling stressor in virgin male prairie voles (Kenkel et al., 2012). Similar stress response-dampening effects of pup exposure might occur in male California mice. In one study, repeated exposure to pups decreased males' behavioral responses to a novel-object open-field test (Bardi et al., 2011); however, other research has found few or no differences in stress response between fathers and virgin male California mice (Chauke et al., 2011, 2012; de Jong et al., 2013; Harris and Saltzman, 2013). The effects of repeated pup exposure on the acute neural and corticosterone responses to pups are unknown.

The aim of this experiment was to determine the effects of repeated pup exposure on behavioral, neural and corticosterone responses to pups in adult, virgin male California mice. To do so, we examined responses to an unfamiliar pup in virgin males that had or had not been exposed to a pup during the preceding week. To control for novelty, we also examined effects of repeated exposure to a novel object on virgin males' subsequent responses to the same object. Finally, we characterized behavioral, neural, and corticosterone responses to pups in new fathers as a positive control. We predicted that repeated pup exposure would increase paternal behavior in virgin males; alter neural responses to pups, as indicated by Fos expression, in brain regions associated with paternal care, stress and/or anxiety; decrease acute corticosterone responses to pups; and possibly decrease basal plasma corticosterone levels.

2. Methods

2.1. Animals

Fifty-three male California mice, descendants of mice purchased as adults from the Peromyscus Genetic Stock Center (University of South Carolina, Columbia, SC, USA), were used. Animals were housed in 44 × 24 × 20 cm polycarbonate cages containing aspen shavings and cotton wool for nesting material, with food (Purina Rodent Chow 5001) and water available ad libitum. Colony rooms were kept on a 14:10 light:dark cycle (lights on from 0500 h to 1900 h). At 27–33 days of age, prior to the birth of the next litter of siblings, animals were removed from their parents' cage and housed in groups of four same-sex, age-matched littermates and/or unrelated juveniles.

All procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* and were approved by the University of California, Riverside (UCR) Institutional Animal Care and Use Committee. UCR is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

2.2. Experimental design

In adulthood (161–231 days of age), each mouse either remained housed with one of the males from its original group of four (virgin males) or was paired with an unrelated female (new fathers). Thereafter, subjects were weighed twice per week to monitor health and to habituate animals to handling.

Beginning at least 14 days (26.59 ± 1.97 days, mean ± SE) after pair formation, virgin males underwent data collection over a 10-day period (days 1–10; Table 1). On day 1, we collected a basal blood sample (see below) from each animal at 1200–1500 h. On day 3, each mouse was exposed to either an unrelated pup or a control object (pup-sized glass marble), or underwent control handling procedures without being exposed to either stimulus. Each animal subsequently underwent the same pup-exposure, marble-exposure, or handling procedures on days 5 and 7. On day 8, a second basal blood sample was collected from all animals. Finally, on day 10, 21 virgin males underwent a 60-minute exposure to an unfamiliar pup; this included the 11 males that had previously been exposed to a pup on days 3, 5, and 7 (repeated-pup condition) and 10 males that had undergone control handling procedures on the same days (single-pup condition). Similarly, 22 males underwent a 60-minute exposure to a marble on day 10, including the 12 animals that had been exposed to a marble on days 3, 5 and 7 (repeated-object condition) and the 10 remaining mice that had undergone control handling procedures on those days (single-object condition). Finally, 10 breeding males (new fathers) were tested with an unrelated pup 5–7 days after the birth of their first litter, as a positive control. Immediately after the 60-minute pup exposure on day 10, all males were decapitated, and blood and brains were collected. Brains were subsequently analyzed for Fos using immunohistochemistry, and blood was assayed for corticosterone (see below).

Males in the 5 experimental conditions did not differ in age (183.24 ± 2.37 days, mean ± SE; $F[1,47] = 2.12$, $p = 0.940$, $\eta^2 = 0.15$; one-way ANOVA) or body mass (46.49 ± 1.0 g, mean ± SE; $F[1,47] = 0.82$, $p = 0.520$, $\eta^2 = 0.06$; one-way ANOVA) at the beginning of data collection on day 1.

2.3. Pup and marble exposure

On days 3, 5, and 7, virgin males in the repeated-pup and repeated-object conditions were removed from their home cage between 1200 h and 1500 h and isolated in a clean cage containing bedding, food and water. Animals were tested in new cages to allow testing of both cage mates around the same time under identical conditions. After a 10-minute habituation period, an unfamiliar, unrelated, 1- to 4-day-old pup or a marble was introduced into the corner of the cage farthest from the subject for 20 min. If a subject attacked a pup, the exposure was immediately concluded and the pup was euthanized with pentobarbital (ca. 200–300 mg/kg i.p.; Fatal-Plus, Vortech Pharmaceuticals, Dearborn, MI, USA). To control for effects of handling, subjects in the single-pup and single-object conditions were placed in a clean cage on days 3, 5, and 7 and allowed to habituate for 10 min, after which time a gloved hand touched the bedding in the corner farthest from the subject to mimic placement of a pup or marble. Subjects then remained in the cage for an additional 20 min before being transferred back to their home cage. All exposures were videotaped.

Pup and marble exposures on day 10 were conducted identically to the earlier exposures, except that animals in all 5 conditions were