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MODELING PATERNAL ATTENTIVENESS: DISTRESSED PUPS EVOKE DIFFERENTIAL NEUROBIOLOGICAL AND BEHAVIORAL RESPONSES IN PATERNAL AND NONPATERNAL MICE

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Abstract—With the exception of parturition and lactation, male California deer mice (*Peromyscus californicus*) exhibit the same parental responses toward offspring as conspecific females. A closely related species, *Peromyscus maniculatus*, however, rarely exhibits paternal responses. In the current study, a comparative species approach was used to assess paternal responses in both *Peromyscus* species with varying levels of paternal experience (biological fathers, pup-exposed virgins, and pup-naïve virgins). Of special interest was the persistence of the males to direct their attention toward a distressed pup housed in a small enclosure (i.e., a barrier existed between males and pups). In addition to pup-directed responses, non-pup-directed responses such as grooming, resting and jumping were recorded. Subsequently, all animals' brains were assessed for fos-immunoreactivity (ir) in several areas previously associated with the paternal brain circuit. Overall, *P. californicus* exhibited more pup-directed responses as well as less fos-ir in brain areas involved in emotional integration and processing such as the insula and anterior cingulate. In addition to increased activation of emotional regulatory areas, *P. maniculatus* males, observed to direct their behavior away from the pup, exhibited higher fos-ir in the nucleus accumbens (involved in goal acquisition), perhaps due to a heightened motivation to avoid the pups. Interestingly, experience with pups altered the lateral septum and amygdala activation of *P. maniculatus* to levels similar to *P. californicus* biological fathers. Finally, fos-ir was increased in the medial preoptic area, involved in the maintenance of maternal behavior, in the biological fathers of both species. Thus, although biological predispositions toward pup-directed behaviors were observed in *P. californicus* males, evidence of a few shifts toward the paternal neural activation profile was apparent in *P. maniculatus* males. Specifically, modifications in fear responses and social processing may represent the cornerstones of the gradual shift from social tentativeness to social attentiveness in the presence of pups.

Key words: paternal attentiveness, *Peromyscus* model, vasopressin, fos-immunoreactivity, social affiliation, paternal neural circuit.

INTRODUCTION

Compared to the minimal parental attention young reptiles receive, mammalian offspring require extensive parental care for survival. The reallocation of resources away from *self* toward *other* in parenting responses represents a significant step toward the emergence of social and cooperative behavior (Lambert, 2012). In maternal mammals, for example, offspring are viewed as an extension of the mother's *internal milieu* with resources generously dispensed to energy-demanding offspring (Lambert and Kinsley, 2008; Schulkin, 2011).

Whereas mammalian mothers are most often characterized as effective caregivers, paternal responses are more variable. Although maternal mammals often benefit from assistance from various forms of alloparents such as affiliative adult females or older offspring, only about 5% rely on paternal assistance (Kleiman and Malcolm, 1981). For the few mammalian species known to exhibit paternal responses, behaviors range from infrequent babysitting observed in baboons to constant nurturing observed in owl monkeys (Wright, 1984; Dixson, 1994; Fernandez-Duque et al., 2009; Hrdy, 2009; Wolovich et al., 2010). Interestingly, a wide range of responses are sometimes observed within a single species, as evidenced in humans. Generally, paternal mammals are divided into two categories: *facultative* fathers provide minimal care, only contributing to the mother's workload in demanding contexts, and *obligate* fathers provide essential care for offspring (Rosenblatt and Snowdon, 1996; Hrdy, 2009).

The extreme variability observed in paternal responsiveness across mammalian species provides an opportunity to evaluate critical variables involved in parenting responses in order to identify key neurobiological variables associated with the emergence and maintenance of social attentiveness and caregiving responses. To address this question, several biparental species such as prairie voles (*Microtus orchagoster*) and California deer mice (*Peromyscus californicus*) have

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Abbreviations: AVP, arginine vasopressin; BNST, bed nucleus of the stria terminalis; ir, immunoreactivity; MDS, multidimensional scaling; MPA, medial preoptic area; PBS, phosphate-buffered saline; PEVs, pup-exposed virgins; RE, reproductive experience.

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been investigated. In paternal *P. californicus* mice, increased levels of prolactin and oxytocin have been observed (Gubernick and Nelson, 1989; Gubernick et al., 1995); further, castration reduced paternal responsiveness but had no impact on aggression (Trainor and Marler, 2001). On the other hand, experimental manipulations of *Phodopus campbelli* suggested that the co-variation of hormones and paternal behavior might not be necessary to express paternal behavior (Wynne-Edwards and Timonin, 2007). Nevertheless, in their natural habitats, removal of males from the family context decreases offspring survival; thus, in *P. californicus*, paternal care of the young has clear adaptive significance for this as well as other monogamous species (Gubernick and Teferi, 2000; Jašarević et al., 2012).

The observation of distinct differences among closely related species provides an opportunity to utilize the comparative species approach to identify neurobiological mechanisms associated with paternal responses (Lambert et al., 2011; Franssen et al., 2011a). Compared to *Peromyscus maniculatus*, for example, *P. californicus* paternal mice exhibit decreased activation of brain areas associated with fear and anxiety upon reuniting with their pups following a 24 h separation. Further, enhanced arginine vasopressin (AVP)- and oxytocin-immunoreactivity (ir) in the paraventricular nucleus of the hypothalamus, as well as restructuring of mature neurons in the hippocampus, were observed in *P. californicus* paternal mice (Lambert et al., 2011). Focusing on just *P. californicus* males, past research has indicated increased activation of the medial preoptic area (MPA), a structure associated with the expression of maternal behavior, in the presence of pups (Lee and Brown, 2002; de Jong et al., 2009; Pereira and Morrell, 2011).

Given the noted differences in paternal responsiveness displayed by the two closely related *Peromyscus* species, the purpose of the current study was to build on past research in our lab investigating paternal responses in the presence of pups with additional research investigating males' motivation to approach and contact a distressed (isolated) conspecific pup restrained in a small enclosure. Thus, with no female present, would males direct attention toward the restrained pup or toward themselves or other aspects of the environment? Accordingly, biological fathers, pup-exposed virgin (PEV) males (foster males) and pup-naïve virgin males of both species were evaluated in the presence of distressed pups. Subsequently, an analysis of the activation of various brain areas (via c-fos-ir) was conducted to determine characteristic neural response activation patterns throughout the males' brain regions associated with pup-, self-, or escape-directed responses. In order to streamline neural investigations in the current study, the brain areas of interest were grouped in the following response categories: Interoception and Integrated Emotional Processing (cingulate and insula); Fear, Social Defense and Anxiety [amygdala, lateral septum, and bed nucleus of the stria terminalis (BNST)] and Motivation [nucleus accumbens and MPA]. Although each of these brain areas is involved in multiple responses, this categorization enabled us to focus on the general areas of emotional regulation, fear/anxiety, and motivation in the current investigation. More specific information about these areas and relevant functions is included in the ''Discussion'' section.

To further understand the relationships among the variables associated with social attentiveness and social tentativeness in the distressed pup context, multidimensional scaling (MDS) analysis was conducted to complement more traditional statistical analyses. On the basis of previous studies (de Jong et al., 2010; Lambert et al., 2011), it was hypothesized that *P. californicus* males would demonstrate a significantly higher rate of pup-directed behaviors than *P. maniculatus*. Additionally, these behavioral changes were expected to be differentially associated with activation patterns in specific brain areas involved in fear responses, emotional regulation, and motivation.

EXPERIMENTAL PROCEDURES

Animals

Forty-four adult male mice (22 *P. californicus* and 22 *P. maniculatus*), ranging from 6 to 12 months of age, were obtained from the *Peromyscus* Genetic Stock Center at The University of South Carolina (Columbia, SC, USA). First-time fathers (six males per species) were housed together with their families in 29 cm × 18 cm × 12.5 cm wire-lidded cages with filter top lids, lined with a mixture of (1/4) in. corncob and aspen bedding (Harlan Teklad, Madison, WI, USA). Control virgins (eight males per species) and PEVs (eight males per species) were housed in pairs in similar cages. Each cage contained one nestlet for enrichment purposes. Animals were provided food (#2018 Harlan Teklad, Madison, WI, USA) and water *ad libitum*. The animals were maintained on a 16:8 light–dark schedule with lights on at 0500 h. Animals were maintained in accordance with the Randolph-Macon College (RMC) Institutional Animal Care and Use Committee.

All animals were given at least 7 days to habituate to the colony room at RMC prior to the onset of testing, described below. At this time, animals were exposed to their respective ''paternal'' condition for 5 days; that is, fathers remained with their families and the virgin males were housed together two per cage to control for social contact. PEV males were exposed to unfamiliar pups daily, as described in ''Pup-exposure protocol'' section. Because the PEVs were placed in a novel cage for pup exposure sessions, fathers and pup-naïve virgins were also placed in a similar cage for the same amount of time to control for novelty.

Two days prior to testing, all animals were exposed to the wire mesh enclosure (subsequently used to enclose pups) in their home cages for 30 min. These enclosures (see Fig. 1) were 3.8 cm in height and 11.1 cm in circumference; when used in testing, this apparatus separated the pup from the male but provided plenty of room for the pup to move around during the brief testing session.

Pup-exposure protocol. Following a 10-min habituation period to the parental exposure cage, the males were exposed to one unrelated alien conspecific pup for 10 min, once a day for 5 consecutive days. Exposure sessions commenced at 0900 h. The pups used in the pup-exposure protocol were housed with both parents and these ''donor families'' were not involved in the experiment in any other way. Pups were approximately 6 days of age. Donor families remained consistent throughout the exposure period. If the same pup was used for multiple tests, the pup was returned to its home