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Intricacies of Development: The Impact of Maternal Experience and Isolation on the Social Development of Juvenile Male Rats

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ABSTRACT

Reproductive experience induces changes in females. Parity-related differences in maternal treatment of offspring can induce enduring changes in offspring. The relationships between maternal experience, early social isolation, and development were explored in rodents in this experiment. Male rats were weaned from multiparous (MP) and primiparous (PP) mothers and placed into isolation or social housing for four weeks. They were then observed in a social-interaction test. Social behavior and neural oxytocin and vasopressin were assessed post-testing, while corticosterone levels were measured across the four weeks. Weaning was extremely stressful for all offspring. PP- and MP-raised pups exhibited differences in social behavior, and trends towards different hormonal and neural profiles. The isolation paradigm was examined in a multidimensional fashion, revealing novel and provocative associations. This study further clarified some of the many ways that offspring development may be altered.

I certify that I have read this thesis and find that, in scope and quality, it satisfies the requirements for the degree of Master of Arts in Psychology.

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INTRICACIES OF DEVELOPMENT: THE IMPACT OF MATERNAL EXPERIENCE AND ISOLATION ON THE SOCIAL DEVELOPMENT OF JUVENILE MALE RATS

By

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One of the greatest challenges mammals face is arguably that of giving birth to and raising their offspring. When the female mammal reproduces, she encounters a great number of demands that must be fulfilled in order to reap the benefits of her genetic investment (Kinsley et al., 1999). Successful reproduction involves the mother's completion of her various maternal responsibilities, such as nesting, nursing, and foraging (Kinsley et al., 1999), and her ability to cope with the stress of mothering. A honing of her cognitive abilities during pregnancy and motherhood may promote successful reproduction.

Reproductive experience (RE) in the mother, or maternal experience, refers to the experience of pregnancy, parturition, and post-parturition interactions with offspring. In the female rat, RE facilitates many strong and long-lasting modifications, and also introduces a reciprocal relationship between the mother and infant (Fleming, O'Day, & Kraemer, 1999; Kinsley et al., 1999). Modifications as a result of RE will "enhance, refine, or tune maternal responsiveness towards subsequent offspring" (Fleming et al., 1999, p. 682). Each successive birthing experience is unique and may contribute to the variations in a female's treatment of her offspring.

Our study investigates the impact of the RE of mother rats on pup development. Of specific interest is the possibility of an effect of the mother's RE on the behavioral, brain, and endocrine measures of her pups that impacts their social development. In our study, social isolation was introduced as a well-established variable that has been shown to influence the social behavior of young rats (e.g., Panksepp, 1981). The interaction between the mother's RE and social isolation was also examined to ascertain whether maternal experience may attenuate any effects of isolation. Despite the evidence that the effects of multiple RE are additive, it is still unclear whether the effects of multiple RE in the female rat will produce long-term changes in her offspring. Our study seeks to further clarify the impact of the mother-infant relationship on social development.

Before our study is presented however, the following section (a comprehensive literature review) will examine the body of research pertaining to our study. The literature review first discusses a number of ways in which first-time mothers differ from more experienced mothers on ancillary measures. It then introduces research supporting the argument that there are differences between these classes of mothers in their exhibition of maternal behavior and their treatment of offspring. Third, it presents evidence pertaining to the various ways that an offspring's development can be impacted by these differences in maternal behavior and treatment. Next, it establishes social isolation as an interesting paradigm to have included in this developmental study. Finally, the literature review concludes with an overview of brain oxytocin (OT) and vasopressin (AVP). Our study is then presented and discussed. As our study was largely exploratory in nature, the literature review will hopefully assist to link the constructs of maternal experience, development, and isolation in the reader's mind.

Literature Review

Pregnancy and pup-exposure change the mother

Ancillary maternal modifications of the mother rat occur in many areas that contribute to the eventual survival of her offspring. Primiparous (PP; one pregnancy), inexperienced mothers and multiparous (MP; two or more pregnancies), experienced mothers differ on a number of ancillary measures, with the latter showing great improvements (e.g. spatial ability and foraging behavior) over the former. Gatewood et al. (2005) found that MP female rats had better spatial ability and memory-retention in a dry-land-maze foraging task than PP and nulliparous (NP; virgin) females. Similarly, in the dry-land version of the Morris water maze, maternal females took significantly less time than NP females to recall and obtain a food reward (Kinsley et al., 1999). Better foraging contributes to less time spent searching for food and more time spent with pups. Improvements in spatial ability were also reported in radial-arm maze tests, where MP females made significantly more correct choices than their age-matched NP counterparts (Kinsley, 1999). Love et al. (2005) found that maternal experience had an effect upon long-term memory performance but not on short-term memory in probe tests (testing memory for previously baited wells without sensory cues associated with a food reward; long-term memory) of previous experience in a dry-land maze. Maternal experience led to enhanced memory for food locations in probe tests one week following training, and this effect was present through testing up to 13 months of age. This long-term effect is corroborated by other recent findings that indicate that oxytocin has an influence on long-term memory and long-term potentiation but not on working memory in females, possibly acting as the mediator between improved reference memory in mothers (Tomizawa et al., 2003). However, Love et al. (2005) also found that at 17 months of age, NP rats outperformed PP rats during one dry-land maze test. This points towards an age-effect of parity on the duration long-term memory, and should be further explored.

Experienced and inexperienced mothers differ not only on behavioral measures, but on neuroendocrine measures as well. Rima et al. (in prep., 2006) found a significant difference in corticosterone (CORT) concentrations between NP (high levels CORT) and MP (low levels CORT) groups after exposure to a predatory threat, following a learned-fear paradigm. It appears that a female's physiological stress and fear in response to an acute stressor greatly decrease following RE. Further investigation is needed in this case to decipher if a female's hormonal stress response is continually modified with each new reproductive experience. Byrnes & Bridges (2006) found that younger PP females exhibited fewer anxiety-like behaviors when compared to NP controls, but that in older females, the PP females were more anxious than NP females. In this case, it was concluded that RE does significantly alter anxiety-like behaviors and that this may be a function of endocrine function or age. Their results differ slightly from other findings. Love et al. (2005) found that rats with maternal experience exhibited decreased emotionality (i.e., they spent a higher percentage of time in the open arms of an elevated plus maze) and a *lifelong* decrease in anxiety when compared to NP rats, as measured by amount of time spent contact with novel stimuli. As there are conflicting results on the effect of parity on long-term anxiety, further research is needed.

Wartella et al. (2003) also show that RE changes the female's response to her environment and found that pregnant or parous females are bolder and less anxious than virgins. Parous females had fewer episodes of freezing and more exploration in an open field test than did NP females. Decreased c-fos activation in the CA-3 region of the hippocampus and the basolateral amygdala (BLA) of parous females in comparison to virgins implies that the maternal brain is modified in order to make it less responsive to stressful events (Wartella et al., 2003). Decreased stress reactivity is adaptive for the mother in many ways. Elevated stress levels and sustained anxiety consume energy and waste critical time that could be devoted to preparing for or caring for young, foraging, or other important behaviors.

Gatewood et al. (2005) found that parous female rats had fewer hippocampal deposits of deleterious amyloid precursor proteins when compared to virgin females. This implies that motherhood may have a protective effect upon the brain especially in relation to certain neurodegenerative diseases such as Alzheimer's disease. These effects seemed to remain intact for 6, 12, 18, and 24 months of age, showing a long-term effect of RE on learning, memory and the brain.

There are other hormonal and physiological changes that occur during a first pregnancy in animals (Young & Insel, 2002). Many of these exist in perpetuity, making the mother biochemically different for each of her subsequent pregnancies. A number of studies show parity-related reductions in prolactin secretion. Byrnes and Bridges (2005) found that RE leads to a reduction in the prolactin surge on the afternoon of proestrus (the time immediately before estrus). Only PP females who had undergone both pregnancy and had lactational experience with her pups experienced this change. The length of the lactation experience contributed to a greater decrease in prolactin. This effect has also been shown in humans, and Musey et al. (1987) found that a first pregnancy will lead to a long-term (12-13 year) decrease in basal prolactin secretion when compared to nulliparous individuals. Other hormones are impacted by previous RE as well. While estrogen has not been found to differ between NP and PP pregnant rats, Bernstein et al. (1986) showed that the percentage and amount of free estradiol were significantly higher in the early part of a woman's first pregnancy than at a comparable point in her second pregnancy.

Additionally, neurotransmitters and neurohormones appear to differ between experienced and non-experienced mothers. Byrnes, Byrnes, & Bridges (2000) established that there is increased dopamine activity in the forebrain regions and hypothalamus as a result of parity, and that this effect lasts for at least several weeks post-partum. Pedersen, Caldwell, Walker, Ayers, & Mason (1994) found that there was a parturition-associated rise in oxytocin binding in the ventral tegmental area (VTA) and the medial preoptic area (MPOA), and that an oxytocin-antagonist in the VTA or MPOA and a vasopressin-antagonist in the MPOA blocked the mother's performance of pup retrieval and her assuming a nursing posture over her pups. Increased OT seems to be related to the activation and performance of maternal behaviors. More research is necessary before concluding that a first-time mother is both physiologically and behaviorally different from mothers with RE. However, it is possible that long-term changes made in the mother's physiology influence how she confronts each successive mothering experience.

In addition to pregnancy, mere exposure to offspring can change a mother as well. Lambert et al. (2005) found that pup exposure is equally as important to the development of maternal behavior as the pregnancy experience. In a spatial DLM task, PP females exposed to pups had faster latencies to find food in a DLM than PP females without pup exposure. In a probe trial to assess search strategy in the absence of food-related cues, PP females with pup exposure spent significantly more time in proximity to previously baited wells than did PP females without pup exposure. Rats with pup exposure found the food reward significantly faster than rats without pup exposure. Additionally, Lambert et al. (2005) found that pup exposure enhanced foraging in the DLM in NP females as well.

Inexperienced mothers, who are experiencing an influx of circulating hormones for the first time, will gather the pups, build a nest for them, crouch over them, and nurse them. These maternal behaviors persist for a number of weeks, even after hormonal regulation ceases. Initial hormones introduced to the mother's system stimulate the induction of maternal behavior, which is then sustained by other mechanisms. New mothers of many species come to recognize the odors, touch, and behaviors of their infants quickly after birth. These olfactory and somatosensory cues allow the pups to eventually acquire a reinforcing value for the mother. The pups will become increasingly reinforcing to the mother as she interacts more with them. This is evidenced by the fact that maternally experienced females show increased bar-pressing for pup-exposure in comparison to inexperienced females (Fleming et al., 1999). It seems that pup stimuli have intrinsic effects upon the mother's nervous system and her behavior (Fleming et al., 1999).

Experienced and inexperienced mothers treat their young differently

Not only are rodent PP and MP mothers different in the aforementioned ancillary areas, it is possible that these mothers differ in maternal behavior as well. Experienced mothers may have an advantage over first-time mothers in the practice of "mothering" itself: they already have RE and exposure to pups, and the previously mentioned modifications. "Neural activity brought about by pregnancy and the presence of pups may literally *reshape the brain*, fashioning a more complex organ that can accommodate an increasingly demanding environment" (Kinsley et al., 1999, p. 137). It is clear that many components of maternal experience (pregnancy, pup exposure, etc.) unite to produce modifications in the female that allow her to adaptively handle the demands placed on her as she cares for her offspring (Lambert et al., 2005; Wartella et al., 2003). Some of these modifications are apparent in the female's exhibition of maternal behavior.

Maternal behavior has been extensively studied, and rodents provide a good model to study the complex factors that govern these behaviors. In rodents, the interaction between mother and pups occurs within the context of a nest-bout, in which the mother will gather her litter under her and exhibit a number of maternal behaviors (Liu et al., 1997). Retrieving occurs after parturition, when the pups are scattered around the nest, and the more maternal the rat is, the faster she will gather her pups together (Rees, Lovic, & Fleming, 2004). Another important behavior is pup licking and grooming, as it provides stimulation for the young pups. Pup anogenital licking is important to ensure urination, defecation, and plays a role in sexual development of males. Crouching refers to a posture where the mother is positioned over most of the pups in some capacity (hovering, low crouch, high crouch, supine) and actively licks them, moves nest material, self-grooms, or moves pups within the litter. While crouching, pups have access to her teats and milk, and this close proximity provides temperature regulation and protection from the environment for the pups. Nest-building is another behavior that the mother performs in order to provide a place to conceal her pups (Rees et al., 2004). There is a body of research from a number of different species supporting the theory that experienced and inexperienced mothers perform their maternal duties differently and treat their young differently.

Research on primates has shown a number of differences between PP and MP mothers in terms of their treatment of offspring. In general, first-time mothers appear to be considerably less competent and motivated than second or third-time mothers and it seems that parity and the associated experiences (exposure to the young) are beneficial to the survival of the offspring and the performance of the female as a successful mother (Fairbanks, 1993; Fleming et al., 1999). Mitchell and Stevens (1968) investigated mother-infant interactions of rhesus monkeys in the context of a mildly stressful social situation (confrontation with another unknown adult-infant pair in an observation cage). No differences were found between the PP and MP mothers in terms of how often the subject mothers observed or interacted with the unknown adult-infant pair. However, while in this stressful situation, PP mothers looked more frequently at the human observers (who were located outside of the cage) than MP mothers. The inexperienced PP mothers seemed to perceive the observers as a threat, as they also exhibited significantly higher frequencies of lip-smacking, threatening, barking, and fear grimacing than did MP mothers when oriented towards the observers. Moreover, PP mothers stroked and petted their infants more frequently and for a longer duration of time than MP mothers. The MP mothers, on the other hand, spent significantly more time than the PP mothers exploring the non-social aspects of the environment. The authors concluded that PP monkey mothers are generally more anxious and are overly concerned for their infant's welfare. They exhibit this difference by paying their infants more attention than MP mothers do, and being more protective over any possible intruders in the environment than MP mothers are (Mitchell and Stevens, 1968).

Tanaka (1989) examined free-ranging Japanese macaques and found that the greatest influence upon the mother-infant relationship in these animals was parity, whose influence was greater than that of maternal rank and infant gender. The researcher concluded that primiparous mothers are "not entirely prepared to care adequately for their offspring" (Tanaka, 1989, p. 489). It was found that during the first month after birth, when an infant rooted in the MP mother's chest, she would accept it immediately, where as PP mothers often detached the searching infant from their chest (similar to the behavior of nulliparous females), as if they were unaware of the appropriate maternal response to the infant. After a few months, the difference between PP and MP mothers was no longer found. The PP mothers seemed to have learned the suckling behavior from interactions with their own infants, finding a suitable reaction to the infants' crying for milk. In the first and sixth months of observations, MP mother-infant dyads were more independent, with the MP mother prompting the infant's independence more frequently than PP dyads. MP and PP mothers also differed in their responses to their infants' attempts to stay near or cling to them. PP mothers (like nulliparous females) tended to sit on rocky surfaces, unaware of their infants' need for assistance when not clinging to them, and rarely embraced them, causing their infants to fall down from the rocks. MP mothers tended to sit on flat surfaces, seemingly knowledgeable that the rocky outcrops would not allow the mother to rest as she would have to hold onto the clinging infant. The MP mothers knew that they could rest on the flat ground without the infant needing to attach for support and without falling. After eight months, these differences were no also longer evident, again suggesting a period of adjustment for PP mothers.

Interest in the possible differences between first-born and later-born human children sparked researchers Mitchell, Ruppenthal, Raymond, and Harlow (1966) to explore the long-term effects of parity on infant rearing in rhesus monkeys. Monkeys raised by PP and MP mothers were behaviorally tested in pre-puberty in a social task with adults alone, with age-mates alone, and with infants alone. It was found that MP-raised monkeys had significantly more instances of hostility, but that monkeys raised by PPs directed hostility towards juveniles significantly more frequently. MP-raised monkeys had higher median frequencies for self-play, social play, and social exploration than the PP-raised group. Additionally, the PP-raised group exhibited significantly more stereotyped pacing and more frequent verbal "coos" than the MP-raised group. It is possible that the depressed play rates in PP-raised monkeys could be caused by differences in the mothering skills of the PP and MP mothers.

There is evidence for parity and experience-based differences in mothering skills in other species as well. Wang and Novak (1994) found that the experiential histories of monogamous prairie vole mothers impacted the development of their young. Multiparity significantly altered maternal behavior patterns, the development of the offspring, and overall reproductive success. MP mothers spent more time in the nest and exhibited more maternal care for offspring than did PP mothers, while PP mothers spent more time in locomotion and more time in passive-alone behavior outside the nest than did MP mothers. There were also observable differences between the litters of MP and PP mothers in terms of development, as litters of MP parents had visible fur and opened their eyes significantly earlier than the litters of PP parents. Survival rates for the litters were different as well, and despite no differences in litter size at birth between the two groups, MP-raised pups had higher survival rates than PP-raised pups. As the prairie vole is monogamous in nature (in contrast to the polygamous montane vole; Insel, 1997), it was not surprising that the fathers of these offspring had high levels of parental behavior as well. However, PP and MP fathers did not differ on any of the measured dimensions, implying that the improvement with parity occurs exclusively within the female.

The importance of early maternal experience with offspring appears to be crucial for establishing maternal responsiveness in mothers. In rabbits, it was found that PP females that were allowed full contact with their litter at parturition displayed nursing behavior 80 percent of the time in days 1-5 after birth, compared with only 33 percent if contact at parturition was prevented until 24 hours after birth (Gonza'lez-Mariscal et al., 1998). The discrepancy implies that if initial attachment does not take place for PP mothers, they may not initiate nursing behavior. This contrasted greatly with the MP mothers, who exhibited 100 percent nursing behavior regardless of whether they were given immediate access to their young (Gonza´lez-Mariscal et al., 1998). Experience raising a previous litter allows for a greater maternal responsiveness, even when deprived of immediate pup contact at parturition. A study comparing the maternal behaviors of experienced nulliparous and PP rats found that for most of the behavioral measures (including pup-retrieval, crouching, licking, genital-licking), rats who have had undergone both the experience of pregnancy *and* undergone at least one day of maternal experience after parturition performed higher than inexperienced animals without previous pregnancies, and higher than animals who had a previous pregnancy but no interactions with the young (Fleming & Sarker, 1990). This indicates that the maternal experience obtained postpartum is key in initiating maternal responsiveness. Similar results were found by Bridges (1975), who showed that in order for a mother to express certain components of maternal behavior (retrieving and grouping of pups), continuous pup-exposure is necessary and pregnancy itself is not enough to elicit full maternal behavior. MP animals have had both pregnancy and considerable pup-experience, whereas PP animals are novices. Maternal responsiveness appears to only increase with subsequent pregnancies. Moltz and Wiener (1966) investigated the impact of ovariectomy (OVX) on the maternal behavior of female rats of varied RE exposed to a litter of infant pups. Their research revealed that in MP-OVX rats, the maternal response remains after removal of the ovaries, and 19 out of the 20 MP-OVX mothers accepted and reared their adopted litters successfully, compared to only 10 out of the 20 PP-OVX mothers. The remainder of the PP mothers either cannibalized their adopted litters or allowed them to remain scattered around the cage. Finally, Mann and Bridges (1992) showed that MP rats were less sensitive to infusions of the endogenous opioid β-endorphin into their MPOAs than lactating PP rats. When MP mothers were exposed, they were more maternal and performed retrieving more frequently than did PP mothers exposed to the same β-endorphin. Also, MP but not PP mothers were insensitive to naloxone, an opiate antagonist, which suppresses prolactin release in response to suckling. This study shows that the sensitivity of the MPOA to opioid regulation of maternal care will change after repeated RE (multiparity). There seems to be a change in neural sensitivity to this peptide that may regulate the expression of maternal behavior.

> "Changes in the sensitivity of the opioid system within the MPOA may underlie some of the changes in behavior responsivity in multiparous animals. Since multiparous rats are generally thought to be 'better' mothers, it may be that a decrease in sensitivity to opioids which can interfere with active parenting may functionally buffer the female from external stressors and make her a more resilient mother" (Mann & Bridges, 1992, p. 246).

Despite the large body of research on the effects of parity on mothering ability, the information on parity and pup-outcome is sparse. There are no known studies to date that examine the difference in social behavior between MP and PP-raised rodent pups.

Maternal care influences infant development

Multiparous, experienced mothers of different species treat their offspring differently than do primiparous, inexperienced animals. There is reason to believe that these differences may have an impact on the offspring's post-weaning development. A number of studies have shown that the post-natal interactions between mother and infant have widespread effects on offspring development. Interaction with the mother and living in the nest context provide the infant with a rich learning environment (Fleming et al., 1999).

> "It may be surprising that rather subtle variations in maternal behavior have such a profound impact on development. However, for a rat pup… the social environment in the first days of life is defined by the mother and littermates. The mother serves as the primary link between the environment and the developing animal" (Francis & Meaney, 2002, p. 766).

An attentive mother will have a different impact on her offspring than a dismissive mother.

Research on attachment provides a good introduction to how a mother-infant bond can mediate an infant's later behaviors. The importance of the mother-infant bond to a child's development was first explored extensively by John Bowlby. He believed that in order for a person to be mentally healthy, "the infant and young child should experience a warm, intimate, and continuous relationship with his mother (or permanent mother substitute) in which both find satisfaction and enjoyment" (Bowlby, 1951, as cited in Bretherton, 1992, p. 761). Tracy & Ainsworth (1981) found that human infants placed into a strange or novel situation exhibited various individual differences in their reactions. This variation was dependent upon the style of the mother's bestowed affection. Mothers of infants who showed more aversion to bodily contact (hugging/cuddling) had infants who were anxiously attached and avoidant, whereas mothers of infants who showed more bodily contact had securely attached infants. Infant attachment style and adult personality are linked to the emotional quality of maternal responsiveness to the infant. Social learning during this period is critical in the formation of attachments and later social bonds.

In both humans and other species, development is directly affected by a number of early-occurring factors. A rat pup's environment is a powerful and influential force on its development. Brunson et al. (2005) found that a model of chronic psychological stress (poverty), depriving mothers and their infants of nesting/bedding material on days 2-9 after parturition, impacted the short-term maternal care of and the long-term development of the offspring. The limited nesting material condition resulted in abnormal nurturing behavior in the mothers, including reduced nursing and grooming of her pups. At the end of the stress period, the pups had enlarged adrenals, increased CORT, and modest weight loss compared to the normally-reared control group, an effect which dissipated by middle age (12 months).

Spatial memory was not different between the two groups at 4-5 months of age, but serious impairments in this area emerged for the early-life-stressed rats at 12 months of age (Brunson et al., 2005). Early-life-stressed animals did not reduce platform-finding time on successive trials on a Morris water maze task, and they did not seem to remember the location of the platform from day-to-day. The two groups were comparable on anxiety in an elevated plus maze test, but differed significantly in an object recognition procedure, with the early-life-stressed rats failing to distinguish a novel object from one they had seen the previous day, whereas controls spent twice as long examining the new object. These behavioral differences were echoed in examination of the subjects' brains. In the CA3 region of the hippocampus, the aging early-life-stressed rats had a modest decrease in axonal excitability, a massive depression in synaptic responses and ability to follow high-frequency stimulation, and an increase in the excitability of pyramidal neurons. In the CA1 region of the hippocampus of these rats, synaptic physiology was preserved, but long-term potentiation (LTP) was interrupted. In this study, post-parturition environment and variations in maternal care were both variables, and the changes observed in the infants may have been in response to either of these variables, or a combination of both (Brunson et al., 2005).

In rodents, the postnatal period is likened to "extra-uterine gestation", as a number of factors (including infant heart rate, respiratory rate, rate of protein synthesis, and endocrine state) are entirely under maternal control during this time (Insel, 1997, p. 732). For example, maternal tactile stimulation of her pups appears to regulate their growth parameters. Pups whose mothers fail to groom suffer from suppressed growth hormone release and decreased protein synthesis (Schanberg & Field, 1987). Other variations in the maternal care patterns of rodents have been found to influence offspring behavioral and neuroendocrine development. Evidence for the nongenomic transmission across generations of various behaviors has been found, especially for the maternal behaviors of licking, grooming, and arched-back nursing (LG-ABN). More specifically, female rats who receive higher levels of LG-ABN stimulation from their mothers as infants before weaning showed significantly more LG-ABN behavior than those of low LG-ABN mothers (Francis, Diorio, Liu, & Meaney, 2002). The interaction between the mother and infant during the weaning period is critical.

In cross-fostering studies, when the biological female and male offspring of low LG-ABN mothers were paired after birth with high LG-ABN mothers, the offspring were significantly less fearful under conditions of novelty than any offspring raised by low LG-ABN mothers. This effect held true in the reverse as well, and when pups born of a high LG-ABN mother were paired with low LG-ABN mothers, the offspring paired with the low LG-ABN mothers during weaning were more fearful during conditions of novelty (Francis et al., 2002). This suggests that a mother's behavior and not her genetic contribution are impacting the pup's development.

The frequency of performance and duration of LG-ABN stimulation may also be influenced by environmental factors. In particular, the impact of post-natal handling of pups increases the frequency of LG-ABN behavior in the mother and decreases the offspring's response to stress (Francis et al., 2002). Post-natal handling of low LG-ABN offspring significantly increased the frequency of maternal LG-ABN behavior to the same level exhibited by mothers that were originally high on LG-ABN behavior. Even more telling was the fact that these offspring then became high LG-ABN mothers towards their own offspring even though their biological mothers were low LG-ABN mothers. This supports a nongenomic transmission of behavior between mother and infant.

These and other naturally occurring variations in maternal care in the earliest stages of development also impact responses to stress in the infant rat. Adrenal glucocorticoids and catecholamines act as a hormonal frontline of defense against stressors and are released as a product of the hypothalamic-pituitary-adrenal axis (HPA) (Meaney et al., 1996). Briefly, the HPA axis consists of the hypothalamus, pituitary gland and adrenal gland. These organs produce the various peptides involved in the stress response. Corticotropin-releasing hormone (CRH) and arginine vasopressin-1a (AVP-1a) are released from parvocellular neurons from the paraventricular nucleus of the hypothalamus (PVNh) to start the chain of events. These peptides elicit the secretion of adrenocorticotropin hormone (ACTH) from the pituitary gland, which provokes the release of adrenal glucocorticoids. Levels of glucocorticoids are an indicator of the level of stress that the organism is undergoing. Glucocorticoids stimulate increased blood glucose, increasing the availability of energy substrates, and decrease immunological responses, making the body ready for "fight or flight." However, prolonged exposure to glucocorticoids is very damaging to the system and is also highly associated with psychopathology (Meaney et al., 1996), and it is thus important for the stress response to be able to be "turned off" as soon as the stressor is removed. Glucocorticoids must be able to inhibit subsequent ACTH from the pituitary (negative-feedback), and the focus for this glucocorticoid negative-feedback inhibition is to decrease the release of CRF and AVP from the neurons in the parvocellular region of the PVN. The negative-feedback system is regulated by glucocorticoid receptors (GR) in the hippocampus. The neurons of the PVNh act as a target center for stress mechanisms (Insel, 1997; Meaney et al., 1996).

HPA response development is shaped partially by events occurring early in an organism's life, and these early environmental factors influence activity at the level of the PVNh CRF and AVP neurons (Meaney et al., 1996). Naturally occurring diversity in maternal behavior will affect the development of individual differences in behavior and neuroendocrine responses to stress (Liu et al., 1997). "If the behavioral and endocrine responses to stress are indeed variable among individuals, it is likely that the organism's development, and in particular, the quality and quantity of mother-infant interactions are influential" (Bardi, Bode, Ramirez, & Brent, 2005, p. 264). The development of the infant nervous system (Fleming et al., 1999) and stress response (Liu et al., 1997) depend on interactions with the mother both before and after birth, as well as the post-natal rearing environment (Brunson et al., 2005).

In primates, it has been found that differences in maternal behavior influences the behavioral and endocrine responses of juveniles. In baboons, a study of juvenile individual differences during a stress test (15 minute isolation) found that these infants had a multidimensional behavioral response (Bardi et al., 2005). The dimensions discriminated between infants that exhibited externally and internally directed behaviors, and also discriminated between infants that exhibited active and passive behaviors. Here it was found that cortisol levels were associated with active behaviors (running away or "flight" behavior). Although maternal baseline cortisol levels during the perinatal period were not significantly related to juvenile post-stress cortisol levels, there were variations in the juveniles that corresponded to differences in their mothers' behavior. Mothers who displayed high levels of maintaining contact and directing affiliation towards the infant had juveniles who exhibited less active responses to the stress test: they had higher levels of vigilance (rapid glancing at the surroundings), and resting (motionless with eyes shut) or displacement (scratching, muzzle wiping, brow wiping, startling) activities. Mothers who displayed high levels of stress behaviors had juveniles that were more active during the stress test, exhibiting more locomotion (running, jumping, climbing, walking), and higher cortisol levels. Here the behavioral responses of the juveniles in the stress-isolation test were associated with interactions that they had with their mother (Bardi et al., 2005).

In rodents, a mother's behavior and her interactions with her pups have been found to essentially program her offspring's HPA responses to stress. Mothers who exhibited high LG-ABN behavior had offspring who showed significantly reduced plasma ACTH and CORT responses to restraint stress tests in comparison to offspring from low LG-ABN mothers (Liu et al., 1997). This effect held into adulthood, as the greater the frequency of maternal licking and grooming, the lower the HPA response to stress in adulthood. It appears that the more nurturing a mother is during the wean-period, the better equipped her offspring are to deal with stress across the lifespan.

Offspring of high LG-ABN mothers also have an increased sensitivity to the inhibitory effects of glucocorticoids on stress-induced HPA activity. Offspring from low and high LG-ABN mothers were given bolus injections of CORT hours before acute restraint stress. The treatment of CORT suppressed the plasma ACTH response to the stress more so in offspring of high LG-ABN than offspring of low LG-ABN (Liu et al., 1997). The inhibition of CRH by the glucocorticoid negative-feedback system is essential in order to halt the HPA circuit. Liu et al. (1997) also found that CRH mRNA expression in parvocellular neurons of the PVNh was significantly decreased in offspring of high LG-ABN mothers in comparison with offspring of low LG-ABN mothers, and was also significantly correlated with the frequency of maternal licking and grooming during the first 10 days of life. The more licking/grooming the animal received, the less reactive the infant was to stress. Differences in CRH seem to be mediated by an increased hippocampal GR expression. Offspring of high LG-ABN mothers had higher GR mRNA expression in their hippocampus than did low LG-ABN mothers, which would result in an increased feedback inhibition of CRF and AVP synthesis and reduced ACTH released from the pituitary during stress (Liu et al., 1997). Based on the current data, it appears that those animals that have experienced high levels of stimulation early in development have an attenuated stress response.

Natural variations in a mother's behavior before and after birth cause long-term changes in the behavioral, neural, and hormonal mechanisms of offspring (Fleming et al., 1999). Mothers without previous RE are different than mothers with RE. These mothers, through their differential treatment of their offspring, will help to program their infants' HPA functioning (Meaney et al., 1996).

Social isolation

Social interactions are essential for both physical and psychological health across development. Loneliness and social isolation have an impact on a wide range of developmental factors, including the ability to deal with stress (Cacioppo et al., 2002). Cacioppo et al. (2002) studied the effects of isolation and loneliness in male and female undergraduate college students. They found that individuals who were lonely and had fewer social interactions actually had lower basal heart rates and lower heart rate reactivity, and reported greater anxiety, anger, and shyness, less sociability, less optimism, and poorer social skills. Lonely individuals had poor sleep quality and greater daytime dysfunction due to sleepiness. Additionally, the endocrinal profiles of lonely individuals were found to be different than those of social individuals. While salivary cortisol levels across the day were not different across the groups, correlations revealed that the individual's daily cortisol level was significantly correlated with loneliness when the individual had been chronically isolated or lonely (Cacioppo et al., 2002). This finding in humans is very interesting, as research in rodents also has revealed that there is a difference between isolated and social animals both in terms of their endocrine profile and their behavior, although some of the findings are contradictory in nature.

Viveros, Hernandez, Martinez, and Gonzalez (1988) examined rats that had been isolated from 22-150 days post-weaning, and found that housing condition did not modify basal CORT levels, but there was a trend for isolated offspring to have a slightly higher CORT level in comparison to socially housed offspring. After a stress test (foot shocks), only socially housed offspring had a significant increase from basal CORT levels. The authors speculate that this represents an improved adaptive mechanism in these socially housed rats to respond in a suitable fashion to intense stress (Viveros et al., 1988). Mar Sanchez, Aguado, Sanchez-Toscano, and Saphier (1998) also investigated the effects of hormone levels on isolated/early weaning and control (socially housed/normal weaning) rats. They found that at 75 days of age, the isolated offspring had lower basal plasma CORT and a reduced HPA response to restraint stress. A possible confounding factor of this study, however, is the fact that the weaning times were different for the isolated and control groups. Replication is necessary before concluding that isolation causes lower basal plasma CORT. Finally, Weiss, Pryce, Jongen-Relo, Nanz-Bahr, and Feldon (2003) found that isolation effects only occurred in male rats, and that there was no difference in basal CORT among isolated and social groups. There was, however, an increase of ACTH in isolated rats both basally and following stress. This indicates an increase HPA response to restraint stress, contradicting the previous study (Mar Sanchez et al., 1998). There is conflicting evidence on whether isolated or socially housed offspring have higher concentrations of CORT. Additionally, patterns of CORT release across development, without the addition of a stressor, have not been studied previously.

Isolation rearing of rats is commonly used in investigations of social behavior. Rats, in particular, are extremely sensitive to isolation during their juvenile stages (starting immediately post-weaning), as these animals are generally very gregarious. However, no studies to this date have examined the interaction between social isolation and maternal condition. Rats who are isolated during this sensitive period exhibit a decrease in social activity for the rest of their lives, even if they are returned to group-rearing after puberty (Arakawa, 2003; Arakawa, 2005). Arakawa (2005) asserts that play behavior during the juvenile stage is "important for the regulation of social stress responses to changing social circumstances in adulthood" (p. 231).

Play behavior in young rats increases from 18-28 days of age, peaking between 32-40 days of age, and decreases gradually after this point to no play at 58 days of age (Panksepp, 1981). Gaining experience during development through play behavior may be a "source process for adult social competence" (Panksepp, 1981, p. 327). A lack of social play and social-deprivation in juvenile rats can lead to persistent behavioral deficits in the adult rat, especially as full synaptic density is not completed until after weaning (Lapiz, et al., 2003). Interestingly, isolated rats do exhibit higher levels of play behaviors in social interaction tests than do socially housed rats (Panksepp, 1981). Socially housed rats have already had the ability to determine their place within in a social structure (dominant or subordinate), whereas during a social interaction test, isolated offspring have their first experience with such a structure (Panksepp, 1981). Ikemoto & Panksepp (1992) found that isolated offspring have an increased appetite for social play over socially housed offspring. After food-deprivation, juveniles were placed in a T-maze and were able to make a choice between a food reward (Froot Loops) or a social reward (playing with another rat). Isolated offspring made more choices for social interaction than did socially raised offspring.

Varlinskay, Spear, and Spear (1999) found that in rats, social behavior was impacted by previous social experiences and housing conditions. They found that adolescent rats isolated after weaning until 38-42 days of age showed increased levels of social investigation of a novel animal in a social interaction test, played more with the other animal, and spent more time in contact with the other animal. The greatest frequency of these behaviors was found when the novel animal that they were exposed to was a socially housed animal previously, in comparison to when the isolated animal was placed with another isolated animal. Not only did the subjects' own experience impact the social interaction, but the experience of the novel animal also played a role. Somewhat contrary to Varlinskay et al.'s (1999) findings is that of Van den Berg, Van Ree, and Spruijt (1999), showing that isolation from days 22-25 post-weaning caused changes in behavior in adulthood (at 12 weeks of age). Specifically, they found that in adulthood, isolated rats exhibited decreased social behaviors (decreased anogenital sniffing, following, and social exploration) when compared to socially housed offspring. More work must be done in this area before drawing conclusions about the long-term effects of isolation in rats.

Social play is not the only aspect of behavior that is impacted by isolation-rearing. Wongwitdecha & Marsden (1996) reported that rats reared in isolation were more aggressive (biting, boxing) more frequently in a social interaction test. Additionally, these researchers reported that isolated rats were less sensitive to anxiolytic effects of diazepam than were rats reared socially; while diazepam decreased aggression in both social and isolated groups, it only decreased passive interactions (sitting near/lying in contact with novel rat) in the social group, with no effect in the isolated group. Isolated rats continued to seek social contact even after being exposed to diazepam. Additionally, isolated juvenile rats were found to have increased initial startle reactivity and a reduced prepulse inhibition to stimuli in comparison to socially housed controls (Varty, Paulus, Braff, & Geyer, 2000; Weiss et al., 2003). Isolated offspring are more startled by stimuli in their environment and are less able to cope with repeated startling stimuli. Weiss et al. (2003) also found that males who had been socially isolated had an anxiogenic profile in tests on an elevated-plus maze.

There are also conflicting reports on the effect of isolation on exploration. Arakawa (2005) found that isolation during the juvenile stage of development in rats will inhibit open-field exploration, an effect that is maintained into adulthood. Specifically, isolated rats in an open-field test have an increased tendency to avoid the center of the field (Arakawa, 2003). This contradicts the findings of Varty et al. (2000) who reported that isolated rats exhibited increased exploratory activity over socially housed animals when placed in a chamber designed to measure behaviors through metal touch-plate sensors. Similarly conflicting evidence has been found for the effects of isolation on self-grooming (Niesink & Van Ree, 1982; Van den Berg, Van Ree, Spruijt, & Kitchen, 1999). More research is necessary on the effect of isolation on exploratory behavior and self-grooming.

Oxytocin and Vasopressin

As previously discussed, AVP-1a released by parvocellular neurons in the PVNh is critically important to arousal and the stress response via the HPA axis. When an individual is exposed to stress, the HPA axis is not the only site of activation; stress also activates the hypothalamo-neurohypophyseal system (HNS). There are four classes of neurohypophyseal hormone receptors: oxytocin (made in the PVN and supraoptic (SON) nucleui), AVP-1a (found in the brain), AVP-1b (found in the pituitary), and AVP-2 (found in the kidney) (Insel, 1997). Evidence suggests that both OT and AVP (type 1b) are manufactured by HNS magnocellular secretory neurons in the PVN and then, after exposure to a stressor, are released from the posterior pituitary gland into the blood (Engelmann et al., 2006; Insel, 1997; Neumann et al., 2000).

OT has been shown to attenuate both stress-induced neuroendocrine and molecular responses of the HPA and HNS axes in both male and female rats (Neumann et al., 2000; Windle, Shanks, Lightman, & Ingram, 1997) and humans (Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003). OT released in response to stress can act as a buffer for the system, contributing to decreased glucocorticoid levels. Paredes et al. (2006) found that rabbits that had been isolated and exposed to repeated stressors had a significant increase in PVNh OT when exposed to a social experience test. Neumann et al. (2000) found that in both male and female rats, endogenous OT in the PVNh appears to regulate the neuroendocrine stress response. Administration of an OT-antagonist into the PVNh increased the activity of the HPA axis, as shown by higher basal and stress-induced levels of ACTH and CORT. It is thought that endogenous release of OT acts as an inhibitor of the HPA axis, and that increased levels of OT may actually decrease ACTH and CORT levels (Neumann et al., 2000), providing for an inverse relationship between these variables. This effect has proved true in humans as well, and Heinrichs et al. (2003) found that social support and the central OT system interact to lower cortisol concentrations in humans during psychological stress tests.

Other research has shown that OT and AVP are implicated in pro-social behavior, pair-bonding, social development, and social recognition (Insel, 1997; Young & Wang, 2004). OT and AVP have emerged as critical mediators of partner preference and monogamous behavior in monogamous prairie voles and it seems that these systems may be linked closely to the dopamine reward system in the nucleus accumbens as well (Insel, 1997; Young & Wang, 2004). It appears that in female prairie voles, OT release is essential for the formation of pair bonds, while AVP is essential for the formation of pair bonds in males (Insel, 1997; Young & Wang, 2004).

Most pertinent to our study is research showing that infant attachment also seems to be somewhat dependent upon the regulation of OT and AVP. Central exogenous administration of OT to rat pups at 6-8 days of age decreased their number of ultrasonic vocalizations (USV) in reaction to social isolation, with no effect on locomotor activity or coordination (Insel & Winslow, 1991). These USVs signal distress and are associated with vulnerability and are usually followed by maternal retrieval. Animals with higher levels of endogenous OT may have decreased numbers of USVs in comparison to animals with lower levels. Similarly, administration of central AVP to infant rats also decreased the number of pup USVs, reduced locomotor activity, and decreased the latency to express a response to negative geotaxis (the amount of time it takes for a pup to turn 90° on a 30° inclined plane). The changes in vocal behavior seemed to be mediated by the AVP-1a receptor type (Winslow & Insel, 1993). "Exogenous administration of OT or AVP reduces separation response of rat pup, consistent with the possibility that these binding sites are responsive to their respective peptides, and that these peptides have a role in either attachment or the separation response" (Insel, 1997, p. 732).

The Current Study

The goal of the current study is to further elucidate the relationship between a mother's experience (whether it is her first litter or if she has had many litters) and the hormonal, neural, and social behavioral responses in her pup. This study is exploratory in nature and uses an isolation paradigm to experimentally manipulate test subject pups from PP and MP mothers. Males were used in this study, as the effects of isolation have been primarily shown in male rats (Arakawa, 2005).

Male pups from PP and MP mothers (the maternal group) were either isolated during their peak play-ages (from 21-49 days of age) or housed socially during this time. Fecal samples were collected at three points (0 weeks, 2 weeks, and 4 weeks post-weaning), and were processed for CORT levels in order to assess the pup stress response. Subjects were behaviorally tested in a social interaction task and scored on affiliation, following, social exploration, leaving, self-grooming, and exploration, and then sacrificed. Brain tissue was processed for AVP and OT. Mar Sanchez et al. (1998) failed to find a difference in the expression of OT and AVP cells in isolated and socially housed rats after exposure to a severe restraint-stress test; this study seeks to extend this previous research. We examined OT and AVP cells in rats that were isolated or socially housed and undergone a social interaction test, with the idea that the social interaction test would be more pertinent to these neurohormones than a severe restraint test.

It was hypothesized that the previous experience of a mother (PP or MP) would impact her pup's performance in a social interaction test, her pup's CORT levels, and neural expression of OT and AVP in the PVNh of her pup. It was also hypothesized that pups from MP mothers may have an attenuated response to the isolation period, reflecting an interaction between maternal experience and isolation. Establishment of differences between the pups of PP and MP mothers would allow for further research into specific mechanisms and the various ways that maternal treatment might differ.

In replication of previous studies (e.g. Panksepp, 1981), it was hypothesized that isolated offspring would show longer durations of affiliative behavior and following and higher frequencies of social investigation behavior than socially housed offspring. Self-grooming and exploration were expected to differ between the offspring in the isolated and social groups, but due to the conflicting evidence in the research (e.g. Arakawa 2005; Niesink & Van Ree, 1982; Van den Berg, Van Ree, Spruijt, & Kitchen, 1999; Varty et al., 2000), no specific directional hypotheses were made. If isolated juveniles were to express more social behavior, then it was expected that socially housed juveniles would spend more time remaining motionless in the absence of social behaviors. Leaving behavior (reflecting a desire to be distant from the other animal) may be different between isolated/social and PP/MP groups, but no directional hypotheses were made.

It was also hypothesized that pups from PP and MP mothers would differ in their behavior, specifically on affiliation, following, social investigation, and leaving. As this study was exploratory in nature, no specific directional hypotheses were made, although a number of possibilities were considered. For example, pups from PP mothers may spend more time affiliating, following, and may have more social investigation than pups from MP mothers, reflecting a potential lack of early maternal stimulation due to the mother's inexperience and their own desire for more contact experience, in a fashion similar to isolated animals. It is also feasible that MP-raised pups would engage in more of these behaviors if their mothers were somewhat less attentive overall. Perhaps the MP mothers had already habituated to the mothering experience and did not act as nervous or overly attentive, as has been shown in primates (e.g. Mitchell & Stevens, 1968), thus potentially stimulating their offspring less than the PP mothers. It was not expected that PP- and MP-raised pups would differ on self-grooming and exploration.

An investigation of the OT and AVP expression in the PVNh after the social interaction test was expected to show that there were differences between expression in isolated and social offspring. There were a few possibilities considered to account for a possible difference. It was considered plausible that there would be more OT and AVP expressed in isolated offspring than in those who were housed socially, in reflection of the hypothesized longer durations/higher frequencies of affiliative behaviors. As OT/AVP expression may also reflect an indication of neurohormone secretion, it was also considered that isolated offspring may express less OT/AVP, reflecting a recent release from the receptors and a depletion of these peptides. PP- and MP-raised pups were expected to differ on brain OT and AVP expression although the direction was unknown, so no directional hypotheses were made.

Similarly, although there were expected to be differences between MP and PP pups in terms of CORT levels across time, no specific hypotheses were made in this area and analyses performed were exploratory in nature. It was also expected that isolated offspring would differ from socially housed offspring on CORT levels, although conflicting evidence in the research (e.g. Viveros, Hernandez, Martinez, & Gonzalez, 1987; Weiss, Pryce, Jongen-Relo, Nanz-Bahr, & Feldon, 2003) made a non-directional hypothesis preferable in this case as well. Additionally, it was expected that there would be an effect of time on the CORT levels across the three periods when samples were gathered.

We expected that there would be interactions between maternal condition and experimental condition on behavioral, brain, and hormone data. It was hypothesized that having a MP mother may help an isolated offspring to elicit an attenuated response to the isolation paradigm. It was also hypothesized that subjects would exhibit a multidimensional response that would not be immediately visible in parametric analyses. It was hoped that analysis of this multidimensional response would help to uncover other similarities and differences between offspring from different maternal conditions (PP or MP mother) placed into different experimental conditions (isolation or social housing). No known study to date has explored infant's social development in this way, using both an isolation paradigm and maternal experience. This exploratory experiment was important in uncovering relationships between maternal experience and offspring hormones, neurohormones, and behavior as they relate to social development.

Method

Animals

Fourteen female Sprague-Dawley rats originally purchased from Zivic-Miller Laboratories, Inc. (Zelionople, PA) and maintained as breeding stock were used in the present study (N=14) to generate male test subjects (N=28). All animals were housed in 20 x 45 x 25 cm polypropylene cages, the floors of which were covered with corncob bedding. The tops of the cages were wire lids with sections for food and water. Animals were housed at the University of Richmond's Animal Facility vivarium. They had access to water *ad libitum*, were fed a daily commercially available diet (Purina Rat Chow), and were kept under a reverse light/dark cycle (lights off at 6:30 am, lights on at 4:30 pm). Human contact with these rats was limited to delivery of the food and water and cleaning of the cages. All animal maintenance and the procedures used in this study were strictly conducted according to the standards and guidelines set forth by the University of Richmond Institutional Animal Care and Use Committee (IACUC) and those prepared by the National Institutes of Health (NIH).

Procedure for mating and the generation of reproductive groups and test subjects

The females were randomly assigned to one of two groups: primiparous (PP; one mating/one pregnancy and lactation) and multiparous (3-MP; three matings/three pregnancies and lactations). Fourteen males were used for breeding. Mating commenced immediately at sexual maturity for the 3-MP females. These females underwent three rounds each of mating (~1-1.5 weeks), gestation (~21 days), and post-parturition (~21 days). The first and second litters of the 3-MP females were kept with the mother until weaning on postnatal day 21±1. Following each weaning, the 3-MPs were given between 10 days and two weeks recovery before re-mating and were housed individually until their next round of mating.

The PP group was mated similarly to the 3-MP group, but their first and only mating experience occurred at the same time as the 3-MP's third round of mating. The resulting litters (PP's first and 3-MP's third) were weaned on postnatal day 21±1. At the time that they gave birth to the subjects in this study, all females were aged between 195 and 205 days of age.

Immediately following weaning at 21±1 days of age, two male pups each from PP (N=14) and 3-MP (N=14) mothers were selected randomly to become test subjects. The subject pups (N=28) were randomly assigned to two groups. One male from each mother was assigned to an isolation (I) condition, and the other male from the same mother was assigned to a social housing (S) condition. Those rats assigned to the I-condition were housed singly for four weeks (27-28 days). Those rats assigned to the S-condition were housed with another one of their wean-mates (also male) for four weeks (27-28 days). Arakawa (2003) showed that for rats in the juvenile stage, a long (14 day or more) isolation was more effective than a short (1 day) isolation, and that any isolation longer than 39 days showed no more of a significant effect upon open-field behavior than isolation for a lesser amount of time. The four-week (28 day) period was chosen as a feasible middle-ground length of time between 14 and 39 days. The isolated animals were placed in a room separate from the rest of the laboratory animals in order to reduce the effects of scent or audition. Isolated and socially housed rats were housed in identical cages. They received the exact same treatment, feeding schedule, and light schedule throughout the experiment. To distinguish the rats from one another when socially housed, the use of permanent marker on the confederate rats' tail allowed for him to be differentiated from the subject animal.

Some of the remaining male PP pups in the litters were used across litters as "novel pups". All novel pups in this study were from PP mothers and were socially housed throughout their lives. These pups, paired with subject rats in behavioral testing, were unfamiliar to the subjects (i.e. from different litters).

All animals were subject to standardized handling during delivery of food and water and cleaning of the cages, as well as collection of fecal samples at specific times. Behavioral testing commenced after four weeks in their respective conditions.

Behavior

Apparatus. Behavioral testing required the use of an aquarium (12 x 24 x 18-inch glass structure with a top made out of wire mesh netting) to serve as the novel environment. It provided enough space for the animals to interact without interruption from the environment, and yet allowed for a limited enough space for the observer to capture all the nuances of the social interaction. The aquarium's floor was covered in the same corncob bedding as is used in the cages in an effort to keep the animal's environment consistent with what is experienced on a day-to-day basis. The aquarium was cleaned with alcohol between tests to control for odors arising from previous tests.

Testing required a laptop computer with the Observer data collection system (Noldus Information Technology, Sterling, Virginia). Additionally, a video camera was used to record the behavioral sessions. Red-light lamps, undetectable to the rats, were used to illuminate the room for the researcher when starting the video camera. The behavioral testing also required the use of "novel pups" that were unfamiliar to the subjects (age-matched, but from a different litter).

Procedure. To observe behaviors after the four-week period (at 49±1 days of age), subjects were placed in an aquarium (a novel environment) with a novel age-matched male animal for a total of 18 minutes of behavioral testing. Subjects were tested individually and between the hours of 8 a.m. -10 a.m. (procedure similar to Niesink & Van Ree, 1982).

Testing occurred in the Gottwald Animal Facility's behavioral testing rooms (roughly 8 x 8 feet each). To distinguish the rats from one another during testing, the novel rat's tail was colored with permanent marker to allow them to be differentiated. The sessions were 18 minutes long and were videotaped via a non-obtrusive camera. The camera recorded one testing session at a time, and was placed directly in front of the aquarium, roughly 3 feet away. The researcher entered the room to place the animals in the cage and to turn on the camera and subsequently exited to allow the animals to behave naturally.

The resulting behavioral videotapes were transferred onto DVD and scored using the Observer data collection system by an observer blind to the subjects' experimental and maternal conditions.

The following behaviors were scored: affiliation, self-grooming, exploration, following, motionlessness, social investigation, and leaving. A further description of the behavioral ethogram can be found in Table 1. Within two hours post testing, the animals were sacrificed and brain samples were obtained.

Tissue Preparation

Apparatus. Neural processing of brain tissue for OT and AVP assessment required the use of the following substances: sodium pentobarbital, phosphate buffered saline (PBS), 4% paraformaldehyde (PF), and clearing solutions (alcohols and xylenes). Primary and secondary antibodies for OT and AVP were purchased. The animals were sacrificed via transcardial perfusion, so the appropriate materials were necessary (surgical tools, needles, etc.). A cryostat was used to cut the brain sections. Microplate wells, slides, and cover-slipping materials were also necessary. A microscope with the Bioquant Image Analysis System (R&M Biometrics, Nashville, TN) was used.

Procedure. Standard immunohistochemical (IHC) techniques were used to process the brains for OT and AVP immunoreactivity (IR), using tagged antibodies to locate these receptors within the PVN of the hypothalamus following perfusion and fixation. The animals were first killed by an overdose of sodium pentobarbital soon after behavioral testing. They were then transcardially perfused, pumping the vascular system first with PBS for 2-3 minutes, followed by cold 4% PF for about 20 minutes.

The brain was post-fixed in PF for two to four hours, followed by an overnight immersion in 20% PBS-sucrose solution. By using the Paxinos and Watson (1986) atlas of the rat brain as a reference, the PVN, the area of interest, was identified. Brains were then blocked for the area of interest (the PVN of the hypothalamus) and 40µ sections were cut in the cryostat (Zeiss Microm) at -16°C. Nine sections per brain per stain were put into micro-plate wells, alternating between wells destined for OT and AVP, for a total of 18 sections taken for staining per brain. Remaining sections were used for positive and negative controls, and placed in separate micro-plate wells.

Following cryo-sectioning, the tissues were washed for 10 minutes in a 5% DMSO/PBS solution, for 20 minutes in a 3% H_2O_2, 1% normal goat solution, a series of five five-minute PBS washes, and finally in a blocking solution for two hours. The sections were exposed to primary antisera (for OT, a concentration of 1:6,000 was used, and for AVP a concentration of 1: 2,000 was used; Chemicon/Millipore, Temecula, California) and incubated for 24 hours at 37°C. Next, after six PBS washes, the tissues were incubated for two hours in a solution containing biotinylated secondary antibody (Chemicon/Millipore; 1:600 for OT and AVP). After additional washes, sections were exposed to the Avidin-Biotin Complex (Vector Laboratories, Burlingame, California) for one hour and then DAB-nickel sulfate for five minutes. After washing, sections were mounted on subbed (gelatinized) slides and left to dry. After they were dry, they were exposed to clearing solutions (alcohols and xylenes) and cover-slipped with CitraMount mounting medium (Polysciences, Inc., Warrington, Pennsylvania).

Image Analysis and Quantification. Although nine sections were taken from each animal, only the brain tissue that was adequately stained (as compared to negative controls) was quantified in this study. All stained tissue was analyzed using the Bioquant Image Analysis System (R&M Biometrics, Nashville, TN) and a Zeiss-Axioplan microscope fillet with color video camera and computer interface. These sections were quantified using the Field Area Array of Bioquant (Nashville, TN) which uses light thresholding to identify stained cells. Darker areas contrast with the light background and indicate stained tissue. All counts were performed by observers blind to the experimental conditions, ensuring that their counts were not biased.

The area of interest, the PVN, was identified by using obvious landmarks (i.e. the third ventricle) at low (40X) magnification. The PVN was identified as being to the immediate right and left of the dorsal tip of the third ventricle. Tissue sections were analyzed for the total area stained at 100X magnification. As the area of the PVN was greater than one screen of vision (which covered approximately 450 x 600 μm), each hemisphere was divided into three separate sections. To quantify the one hemisphere of the tissue, the corner of the field of vision was placed at the most dorsal part of the third ventricle. This was considered to be the starting point, with coordinates: 0 μm, 0 μm. To obtain the first screen of measurement, the screen was moved ventrally 400 μm (downwards from the starting point) with coordinates: 0 μm, 400 μm (labeled "A" in Figure 1). Stained cells were measured in this first screen. The second screen was found by moving laterally (away from the midline) 600 μm to the coordinates: 600 μm, 400 μm. After measuring the stained tissue in this area, the screen was moved back to the first screen (0 μm, 400 μm) and then ventrally 450 μm (one screen), to the coordinates: 0 μm, 850 μm. This procedure was repeated for the other hemisphere until a total (sum) value of stained area within the PVN was obtained. The total stained area (in square microns) within the PVN was measured and then averaged out across the nine sections (or the number of appropriately stained sections) of OT staining and nine sections of AVP staining. The result was one value of average stained area per brain for both OT- and AVP-stained tissue.

Hormone Assay

Apparatus. Processing of the fecal samples for corticosterone levels required the use of a Corticosterone-Correlate Enzyme-Immuno-Assay (EIA) Kit – 96 wells (Assay Designs, Ann Arbor, Michigan), glass test tubes, a centrifuge, a microplate reader, and a microplate shaker.

Procedure. In order to measure hormone levels, 0.1g of fresh fecal samples were collected in duplicate from the mothers on the day of weaning (Day 21±1 postpartum) and in duplicate from the subjects at three points throughout the experiment: immediately upon placement into their experimental condition I or S condition (21 days of age), after two weeks (35 days of age), and immediately before behavioral testing at the four-week mark (49 days of age). Corticosterone was then measured using a CORT enzyme immunoassay kit (Assay Designs, Ann Arbor, MI). One sample per subject was dissolved in 1 mL of 100% methanol and was then homogenized and vortexed for CORT extraction. The extracted CORT was centrifuged for 10 minutes and dissolved in an assay buffer (tris buffered saline) before it was incubated in donkey anti-sheep IgG antibody. The assay procedure followed the Assay Designs protocol (Cat. # 900-097). The sensitivity of the assay was determined to be 26.99 pg/mL. A linearity measure of a measured CORT concentration versus a known CORT concentration in a sample containing 4,111 pg/mL CORT resulted in a slope of 0.931 with a correlation coefficient of 0.999. Inter- and intra-assay variations were 8.4 % and 8.2% respectively. The cross reactivity on the assay was 100% for corticosterone, 21.3% for deoxycorticosterone and 21% for desoxycorticosterone. Each sample was analyzed in duplicate for each of the three time periods examined, and a single mean value in pg/mL was used for statistical analysis.

Statistical Analysis

Three types of data analysis were performed. First, behavioral data were compared between groups using factorial ANOVAs in which fixed factors were experimental group and maternal group, and the dependent variable was the behavior of interest. Corticosterone values were analyzed using both factorial and repeated measures ANOVAs. Two outliers found to be outside of two standard deviations of the mean were removed for parametric analyses (time 3 value was removed from subject 19 and time 2 value was removed from subject 25; these two subjects had average corticosterone levels composed of two rather than three numbers). After removal of the outliers, these data were normally distributed and the assumption of sphericity for ANOVAs was not violated, so the Sphericity Assumed statistic was used. Second, in addition to comparisons between groups using parametric statistics, correlational analyses were used to examine the relationship between the latency and frequency of engaging in specific behaviors, neural OT and AVP, and average CORT measures.

Finally, behavioral data, brain data, and CORT data were subjected to multi-dimensional scaling (MDS) analysis techniques. A description of the MDS technique is included for readers unfamiliar to the procedure. MDS is a set of mathematical techniques that allow researchers to reduce data to a certain number of dimensions that represent the "hidden structure" of databases (Kruskal & Wish, 1978). MDS refers to a class of techniques that use proximities (similarities or dissimilarities) as input, and, using correlations, will subsequently output a spatial representation of the data points on a map in two or higher dimensional space (a geographic configuration of points; a map). Each point on the map corresponds to one of the objects (either subjects or variables), and this configuration reflects the "hidden structure" of the database and generally makes the data much easier to understand and interpret (Kruskal & Wish, 1978).

MDS can be used as either an exploratory technique to identify unrecognized dimensions affecting behavior, or as a way to get comparative evaluations of objects when the specific bases of comparison are unclear or unknown (Hair, Anderson, Tatham, & Black, 1998). The larger the dissimilarity or the smaller the similarity between two objects (their proximity value; how close they are to each other in space), the further they should be in the spatial map. When plugging different variables in space (two-dimensions or more), there is not generally a perfect fit, and a statistic called s-stress measures this lack of fit for the configuration. Stress values can range from 0 (perfect fit, except when assumptions are violated) to 1 (very poor fit), so smaller stress values (ranging from 0 to .15) point towards a better fit of the data to the configuration at hand. S-stress is often called a "badness of-fit-measure", as larger values point towards a worse fit for the data (Stalans, 1995).

Our goal in using MDS was to produce a configuration of the variables that represented the data, was interpretable, and had a low s-stress level for two dimensions. A dimension represents both the number of coordinate axes (X, Y, Z, etc.) and the number of underlying factors or interpretable directions in the database (Kruskal & Wish, 1978). In choosing the number of dimensions for analysis, there are a few general rules that should be followed in MDS. First, the number of variables in the model should correspond to the number of dimensions in that, for each dimension of the data, there should be at least four variables entered into the model (Kruskal & Wish, 1978). In a two-dimensional (2-D) analysis, there should thus be eight or more variables entered into the MDS. Second, the s-stress value should be taken into consideration; the lower the stress-value, the better the fit is. However, the interpretations of solutions found in more than three dimensions is difficult and may not be worth any improvement in fit (Hair et al., 1998), so the ability to interpret the data is the third factor that one should take into consideration when deciding on dimensionality. Finally, an index of fit of the model (*r* squared value or RSQ) can be taken into account as the amount of the variance that is explained within the model. The greater the variance that is explained (levels .80 to 1), the better the model is. All of these factors were taken into account in our MDS analysis, and two dimensions were chosen.

Nonmetric unfolding MDS was used to produce a map of stimulus variables (behaviors, brain data, and hormone data, in this case), created from the averaged inter-variable proximities gathered from the subjects, and a map of the subjects themselves. Superimposing these maps together gives a complete picture and allows for individual proximities to be mapped onto particular variables or groups of variables. In this case, there were four groups (isolated-primiparous, social-primiparous, isolated-multiparous, and social-multiparous), and the use of nonmetric MDS is valuable as it allows for the association of subject groups to be mapped onto particular variables of interest.

MDS techniques have been very useful in the past for the analysis of social relationships in non-human primates (Bardi et al., 2005; McCormack, Sanchez, Bardi, & Maestripieri, 2006), and were used in a similar capacity in our study. MDS techniques were found to be appropriate for our dataset. An important point to reiterate is that the supplementary information that is provided by the MDS analysis includes information about the *latent* configuration of the dataset. The complete picture provided by MDS is not possible to see with the use of only the standard parametric statistical techniques, as MDS takes into account the inter-relationships that occur between variables. Our study used non-metric unfolding MDS to examine patterns of association between subjects' groups and the incorporated variables (durations of affiliative behavior, following, self-grooming, exploration, and the motionless state, average corticosterone levels, average oxytocin levels, and average vasopressin levels). Individual scores across each variable were transformed into z-scores before we commenced MDS analysis.

All data were analyzed using SPSS version 13.0 and 15.0.

Results

Parametric Analyses

Behavior. There were no significant main effects or interactions found between groups for the following behaviors: self-grooming, following, and exploration. However, there was a significant difference in the means of total duration (in seconds) of affiliation between offspring in the isolated ($M = 262.74$, *Standard Deviation,* $SD = 94.16$) and social ($M = 115.78$, $SD = 76.43$) conditions ($F(1, 24) = 19.19$, N = 28, $p < .001$; see Figure 1). Additionally, there was a significant difference between offspring in the isolated ($M = 282.44$, $SD = 97.28$) and social ($M = 420.62$, $SD = 101.98$) conditions in the total duration (in seconds) they remained motionless ($F(1, 24) = 12.9$, N = 28, $p = .001$; see Figure 2). There were no significant main effects for maternal group on affiliation and motionlessness, and no interactions.

The frequency of social investigations was significantly different between both the experimental groups ($F(1, 24) = 17.65$, N = 28, $p < .001$; see Figure 3) and the maternal groups ($F(1, 24) = 6.43$, N = 28, $p = .02$; see Figure 4), although there were no significant interactions ($F(1, 24) = .64$, N = 28, $p = .43$). Isolated offspring ($M = 49.86$, $SD = 11.98$) had more social investigations than social offspring ($M = 33.29$, $SD = 10.802$). Also, pups from MP mothers ($M = 46.57$, $SD = 13.21$) had significantly more social investigations than did pups from PP mothers ($M = 36.57$, $SD = 13.41$). Finally, pups from MP mothers ($M = 38.43$, $SD = 12.64$) exhibited more instances of leaving behavior (Figure 5) than did pups from primiparous mothers ($M = 29.36$, $SD = 11.06$; $F(1, 24) = 4.12$, N = 28, $p = .05$).

Brain quantification. An analysis of variance revealed that there were no significant main effects of experimental condition or maternal condition and there were no significant interactions in stained brain oxytocin (square microns) across the groups.

In an analysis of variance of square microns of brain vasopressin levels, it was found that there was no significant main effects of experimental condition ($F(1, 24) = .264$, N = 28, $p = .162$) and no significant interactions ($F(1, 24) = .490$, N= 28, $p = .49$). There was, however, a trend for MP-mothered offspring ($M = 4643.11$, $SD = 2376.17$) to have more square microns of vasopressin than PP-mothered offspring ($M = 3193.28$, $SD = 1912.13$; $F(1, 24) = 3.012$, N = 28, $p = .095$), although it was not significant at the 0.05 alpha level.

Hormone Assay. In an examination of the hormones of the subjects' mothers, collected on the day of weaning, a one-way analysis of variance found that there was no significant difference between the corticosterone levels of the MP and PP mothers on the day of weaning ($F(1, 12) = .208$, N = 14, $p = .66$).

The three samples of corticosterone that were gathered for each subject were averaged. A factorial analysis of variance revealed that there were no significant differences in average corticosterone levels between animals across maternal condition ($F(1, 24) = .06$, N = 28, $p = .80$) or experimental condition ($F(1, 24) = 1.852$, N = 28, $p = .19$).

To compare the three samples across time, a repeated-measures ANOVA was conducted and revealed that there was a significant effect of time on average corticosterone levels ($F(2, 48) = 4.12$, N = 28, $p = .02$). Corticosterone concentration levels (Figure 6) in fecal samples on the day of weaning were 544.48 (*SD* = 398.97), while two weeks later, the concentration levels were 1059.54 (*SD* = 780.46), and four weeks later were 980.15 (*SD* = 864.49). There was no significant effect of experimental condition on corticosterone levels (*F*(2, 48) = .80, N = 28, *p* = .46), and no significant interaction between maternal condition and experimental condition on corticosterone levels (*F*(2, 48) = .05, N = 28, *p* = .95). There was, however, a trend (see Figure 7) pointing towards a main effect of the pups' maternal condition on corticosterone levels over time (*F*(2, 48) = 2.82, N = 28, *p* = .07), although it was not significant at the .05 alpha level.

Correlational Analyses

Correlations were run between behavioral, brain, and hormone data. The results of these correlations are reported in Table 2. Affiliative behavior was positively correlated with following (*r*(26)= .47, *p* = .01) and social investigation (*r*(26) = .67, *p* < .001), while it was negatively correlated with being motionless (*r*(26) = -.72, *p* < .001).

Following behavior was negatively correlated with being motionless (*r*(26) = -.47, *p* = .01). Following and social investigation were positively correlated (*r*(26) = .50, *p* = .007), and social investigation was negatively correlated with being motionless (*r*(26) = -.43, *p* = .02).

Leaving was positively correlated with social investigation (*r*(26) = .52, *p* = .005). Leaving was also negatively correlated with average corticosterone levels (*r*(26) = -.39, *p* = .04). Finally, explorative behavior was positively correlated with average oxytocin levels (*r*(26) = .44, *p* = .02).

Multidimensional Scaling (MDS) Analyses

The following measures were considered for the MDS analysis initially: durations of affiliative behavior, following, self-grooming, exploration, and the motionless state, frequency data for social investigation and leaving, as well as average corticosterone levels, average oxytocin levels, and average vasopressin levels. In one model attempted, all 10 variables were entered (see Figure 8). The 2-dimensional (2-D) model yielded a stress score of S-stress = 0.110 and an RSQ value of 0.95. These statistics indicate a good fit of the data and a high amount of variance explained. This model was, however, extremely difficult to interpret. There was not a clear-cut division between the subjects on parity or housing differences. Additionally, the correlations contradicted the parametric data. While isolated animals showed significantly higher levels of affiliation in parametric analyses, this 2-D solution shows affiliation, social investigation, leaving, and following to be highly correlated with *socially* housed animals, not isolated animals.

In order to correct for this difficulty in interpretation, social investigation and leaving were not included in the final analysis. Inclusion of eight variables is adequate for a 2-D solution, as there should be at least four variables included for each dimension. Social investigation and leaving were correlated with each other, as well as affiliation and following. For this reason, we decided to exclude them from the final MDS analysis in order to preserve the number of variables at eight, and to obtain a model that was easier to interpret given the parametric analyses.

The final 2-D solution (see Figure 9) yielded a stress score of S-Stress = 0.111 and an RSQ value of 0.95. The combination of stress score and RSQ values shows that the data are not being distorted to fit the model and that the model explains a large percentage of the variance between the cases.

Dimension 1 is positively correlated with self-grooming, following, affiliation, and vasopressin, and represents the dichotomy "internally focused" (seeking-social or self stimulation) / "externally focused" (not seeking-social/self stimulation) of the animals. Dimension 2 is positively correlated with motionlessness, self-grooming, and corticosterone, and represents the relative activity and interactivity of the animals. This continuum ranges from non-interactive/non-active to interactive/active behavior.

A positive, linear combination of the two dimensions (shown by the diagonal line drawn to separate the graph into two sectors) provided two sectors (A and B). Three variable clusters also emerged. The closer the variables were on the map, the higher the association between the variables. A cluster located in sector "A" included motionlessness, corticosterone, and self-grooming. A second cluster emerged in sector "B", which was composed of affiliation, following, and vasopressin. Divided across the two sectors was the final cluster which was composed of oxytocin and exploration.

The linear combination of the two dimensions differentiated between isolated animals and socially housed animals. Although there were a number of differences between offspring on maternal condition based on parametric analyses, these relationships were not reflected in the MDS map. Socially housed animals were located in sector A significantly more often than isolated animals (79% were located in sector A; $\chi^2(1, N = 28) = 9.14, p < .01$). These animals were more likely to be motionlessness, have higher basal corticosterone levels, and engage in more self-grooming. Isolated animals were significantly more often located in sector B (79% were located in sector B; $\chi^2(1, N = 28) = 9.14, p < .01$). This implies that isolation lends itself to higher levels of affiliative behavior, following, and vasopressin. The third variable cluster of oxytocin and exploration was found to fall in between the two sectors, along the linear regression line, and included members of both isolated and social groups.

Discussion

The results of this study confirm previous findings concerning the effect of isolation on the behavior young juvenile rats (e.g. Panksepp, 1981). The isolated rats in our study exhibited significantly longer durations of affiliation and engaged in significantly more social investigations than socially-housed rats, confirming hypotheses. Also, the hypothesis that socially housed rats would remain motionless for a longer total duration during the testing period was confirmed. Isolated and socially-housed animals did not differ on instances of leaving behavior. Contrary to contradictory previous research showing differences between animals on self-grooming and exploration measures in different directions (Arakawa 2005; Niesink & Van Ree, 1982; Van den Berg, Van Ree, Spruijt, & Kitchen, 1999; Varty et al., 2000), no differences were found between isolated and social offspring on self-grooming or exploration in this experiment. The discrepancy between previous studies, as well as this study, may be due to differences in methodology. These behaviors should be re-examined in subsequent studies before any firm conclusions are drawn.

The hypothesis that pups from PP and MP mothers would differ on duration of affiliation and duration of following was not found to be true in our study and there were no significant differences in behavior on these measures. As hypothesized, there was no difference between the two groups on self-grooming and exploration. However, MP-pups had significantly more social investigations and more instances of leaving than did PP-pups, supporting hypotheses that the offspring would differ on these measures. MP-raised pups entered the vicinity of the other animal to investigate them more frequently and also left the vicinity of the other animal more frequently as well. Although levels of affiliation did not differ between the maternal groups, pups from experienced mothers were more inclined to investigate the other animal before engaging in affiliative behavior. They were also more likely to remove themselves from affiliation more frequently. This may indicate more cautiousness and independence.

This experiment extended previous research conducted on the impact of isolation by comparing the OT and AVP levels of isolated and social animals after a social-isolation task. Previously, OT and AVP levels were examined in isolated and socially-housed rats after being exposed to a highly-stressful restraint test (Mar Sanchez et al., 1998). Our study found that there was a lack of differentiation between isolated and social subjects in OT/AVP expression in parametric analyses, which did not support hypotheses. This implies that although isolation does have a relatively important impact on social behavior (i.e. affiliation), this difference does not appear to be directly reflected in the expression of OT and AVP. OT and AVP have been shown to be linked to pair bonding, social recognition, and affiliation (Insel, 1997; Young & Wang, 2004). It is likely that although isolated animals have altered social behavior, there is another mechanism moderating the differences in affiliative behavior. Uncovering the neural mechanisms governing this difference in behavior would be interesting to investigate in future work.

Although there were no differences between isolated animals and social animals on parametric analyses of brain data, a link between AVP and social behaviors was supported by the MDS analysis, placing it in close proximity to both affiliation and following. It is likely that AVP, and not OT, was more highly associated to the affiliative behaviors in this experiment, as AVP has been shown to be more important to males in terms of social behavior, while OT has been shown to be more important to females (Insel, 1997). Additionally, data from brain quantification suggests that MP-pups might have higher levels of AVP in their PVNh, supporting the hypothesis that PP- and MP-raised pups would differ on neural measures, although this finding is only at the trend level (p = .10) and must not be over-interpreted.

Contrary to previous research showing that isolated and social animals have different basal corticosterone (Mar Sanchez et al., 1998), parametric tests did not differentiate between isolated and social animals on basal corticosterone levels. There was also no difference in CORT levels for isolated and social animals over time. The MDS map does, however, point towards basal CORT as being more associated with animals that are socially housed. Previous studies have shown that after stress tests, socially housed rats and not isolated rats have significant increases in CORT from basal levels (Viveros et al., 1988), although this does not directly explain the association of CORT to the socially housed offspring on the MDS map. Other studies have shown that after isolation, rats have lower basal plasma CORT and a reduced HPA response to restraint stress (Mar Sanchez et al., 1998), contradicting the previously cited study (Viveros et al., 1988). It would have been interesting to obtain blood plasma CORT after the social interaction test to further explore the possibility of a differential reaction to stressors. The inconsistent effects of isolation rearing on glucocorticoid activity may be due to the different ages at the onset of isolation, the duration of isolation, and the differing types of isolation that may occur. An experiment removing all sensory and social stimuli from an animal (i.e. total sensory deprivation) may have different outcomes than an experiment that only removes the social stimuli (i.e. just placing the animal in a cage alone), allowing the animal to still hear and smell other animals in the environment.

The hormone data in this study also show that the weaning experience is extremely stressful and that this effect persists for at least four weeks post-weaning, regardless of housing condition or maternal condition (see Figure 6). Basal corticosterone levels were significantly different over time; they doubled in the second week post-weaning over the initial reading on the day of weaning and these elevated levels remained in the fourth week post weaning. Cook (1999) found that different patterns of weaning in the rat had a significant impact on the adult rat's responsiveness to stress. Specifically, pups that experienced a gradual lengthening of time of separation from the mother from days 21-30 of postnatal care had significantly lower levels of corticosterone in response to a restraint stress in adulthood than animals abruptly removed from their mothers at 21 days of age. However, animals left in the cage with the mother until 30 or 40 days of age had the highest levels of corticosterone (Cook, 1999), suggesting that the more attached the animals become to the mother, the more stressful it is to be removed from them later. Removal from the mother at 21 days of age is a typical laboratory procedure and it appears that this procedure provides an important overt early life effect involving mother-pup interactions (Cook, 1999).

Our data also point towards an interesting interaction between corticosterone and the maternal condition of pups over time. It appears that while pups from PP and MP mothers had similar levels of corticosterone on the day of weaning, the patterns in subsequent weeks are starkly different. PP-pups had a spike of corticosterone levels in the second week post-weaning, almost doubling in number from the levels at weaning. In the fourth week post-weaning, these pups had evidence of a sharp decrease in levels, and the fourth week levels are comparable to the first week levels. This implies that PP-pups initially (week 2) had higher stress after being placed into their experimental condition, but they habituated to their condition (either isolation or social) after two weeks and their corticosterone levels returned to lower levels in the fourth week post-weaning.

This contrasted the CORT pattern for the MP-pups. The MP-pups exhibited a steady increase in corticosterone levels during the four weeks in their experimental conditions. Although at week 2, PP-pups are higher on CORT, at week 4 PP-pups were actually lower on CORT than MP-pups. This is contradictory to initial hypotheses, which posited that MP-pups would actually have lower CORT levels than PP-pups. No differences were found in terms of whether the offspring were housed socially or isolated, showing that the observed differences between PP- and MP-pups were solely in response to the weaning experience.

A number of possible explanations exist. The elevated levels in MP-raised pups may reflect the fact that these animals had more contact in the nest and after removal from the nest at weaning, are having a harder time dealing with this. Moore & Morelli (1979) found that mother rats spend more time licking the anogenital regions of male than female pups. It is possible that the elevated levels of licking in males are further differentiated by maternal experience. Perhaps these MP-offspring were extremely attached to their mother and are having great difficulty dealing with the separation. On the other hand, these elevated levels may not necessarily reflect a debilitating effect on the MP-offspring's stress-response. Perhaps the elevated levels are due to an improved adaptive mechanism, allowing the MP-pups to respond appropriately to the stress of weaning, but manifesting in a steady increase in CORT levels.

The aforementioned CORT differences between PP- and MP-pups are interesting, but caution must be made in interpretation, as these are only trend data approaching significance ($p = .07$). Before any conclusions are made, corticosterone levels between PP- and MP-pups should be investigated further to determine if there is, indeed, an effect in this direction. The current study was limited in its sample size of 28 subjects. A larger study focusing on the pattern of pups' hormone differences post-weaning would be useful in this case. Additionally, it is clear that the current data represent a small segment of the overall picture of hormone functioning in juveniles. A long-term study examining these hormone effects over time and different age groups would be ideal.

Contrary to hypotheses, there were no significant interactions between maternal condition and experimental condition on any analyses run. It was expected that isolation would be robust-enough of an effect to differentiate between PP-pups and MP-pups as well on this level. The fact that isolation did not seem to interact at all with parity implies that the effects of parity on later social interactions and development may be more complex and manifest in unexpected ways. According to the current experiment, it seems that having an experienced mother does not help to alleviate the impact of isolation on social behavior.

Taken together, the parametric analyses of maternal experience demonstrate that there may be a difference between pups raised by experienced versus inexperienced mothers, and that this effect is visible up to four weeks post-weaning. MP-raised pups have more social investigations, more leaving behavior, a trend towards more AVP expression, and a trend towards higher levels of CORT in the fourth week post-weaning. These results are very promising and should be further investigated in the future.

An examination of multiple variables concurrently with the MDS technique provided a more complete characterization of the behavioral and physiological indicators of the development of juvenile rats exposed to isolation than previously available. No known study has examined an isolation paradigm's effect on development in this manner, including brain, hormone, and behavioral data. The first MDS map (see Figure 8.) explored included ten variables (affiliation, following, social investigation, leaving, motionlessness, exploration, self-grooming, AVP, OT, and CORT). It was hoped that by including variables that were found to be different between pups of PP and MP mothers (social investigation and leaving), a division between these two groups would be clear on the MDS map. This was not supported by the data, and in this initial MDS map, there was no clear-cut distinction between the pups of PP and MP mothers. Also, the MDS solution pictured in Figure 8. is problematic as it does not allow for easy interpretation of the data. This map is contradictory to both the parametric analyses run in this experiment, and previous research on the subject. Isolated animals have been consistently found to have higher levels of affiliative behavior (e.g. Panksepp, 1981), yet the MDS solution pictured in Figure 8. places affiliative behavior, social investigation, and following in close proximity with primarily *socially* housed animals.

For these reasons, and after attempting a number of other possible models, the variables social investigation and leaving were eliminated from the final MDS map. These two variables were chosen for elimination because correlations showed them to be highly correlated with one another as well as with affiliation. The final MDS (see Figure 9) included eight variables and was much easier to interpret. This map was the best model to explain the overall variance in the dataset. The effect of isolation seemed to be more powerful than parity, as both the initial MDS (10 variables) and the final MDS (eight variables) failed to find a clear distinction between the subjects in terms of parity. Although there were interesting differences between the pups of PP/MP moms on parametric analyses, more work is necessary to replicate and extend the current results.

The final MDS map shows isolated offspring to be associated with higher levels of affiliation, following, and AVP. In terms of the dimensions, isolated animals are more internally focused and are more active as they seek out social stimulation. Socially housed offspring are associated with motionlessness, self-grooming, and higher CORT. The dimensions show that social animals are more externally focused and are not necessarily seeking out social or self-stimulation (non-interactive). They may, however seek out stimulation through exploration. The social animals are also relatively non-active and spend more time motionless.

Isolated offspring may be trying to make up for their lack of social experience by elevating their affiliation levels. The fact that following is also included on the isolated side could imply that these offspring are persistently dependent on the novel animal and desperate for contact; even when the novel animal moves away from the isolated offspring, they will follow them until they achieve affiliation. The social offspring's correlation to motionlessness could imply independence; these animals do not have the driving need to affiliate with the novel rat, and are content to amuse themselves in non-social ways.

The placement of the variables on the MDS map merits discussion. The MDS map shows basal corticosterone to be in close proximity (highly associated with) self-grooming. In rodents, self-grooming may be an indication of, or response to, stress. Self-grooming in rats has been linked to number of activities, including social contact, sexual behavior, exploratory behavior, and sleeping (Spruijt, Van Hooff, & Gispen, 1992). Novelty and stress may also increase self-grooming behavior in rats. However, it has been found that the performance of self-grooming may be more a reflection of habituation to a stressful situation and the process of de-arousal, than enhanced fear itself (Spruijt, Van Hooff, & Gispen, 1992). In this case, self-grooming in the behavioral task was related to basal corticosterone levels taken before the behavioral task (the fecal samples were all gathered prior to the social interaction test). Perhaps social animals had a greater stress response to the novelty of the social task itself, and exhibited more self-grooming as a result during habituation. This is merely speculation and would require plasma corticosterone readings during the test itself for corroboration.

The correlation and close proximity of OT and exploration on the MDS map was also interesting. In addition to being highly related to one another, OT and exploration were also negatively correlated to corticosterone levels. OT has been found to have anxiolitic-like effects on neuroendocrine and behavioral systems. Windle, Shanks, Lightman, and Ingram (1997) found that when rats were given central infusions of OT, they had significantly more entries into the open arms of an elevated plus maze, and spent significantly more time in the open arms of the maze. Here, a greater amount of time spent in the open arms of the maze reflects a lower level of anxiety. Additionally, central administration of OT in rat pups was found to decrease the number of rat ultrasonic vocalizations (Insel & Winslow, 1991). These vocalizations signify distress and elicit maternal retrieval; a decrease in vocalizations represents a decrease in distress. Although these experiments involved the infusion of OT centrally, it is possible that higher levels of endogenous OT may also be linked to lower levels of anxiety. Neumann et al. (2000) infused an OT antagonist into the PVNh of both male and female rats and found that this significantly increased the activity of the HPA axis, as indicated by enhanced secretion of ACTH and corticosterone into the blood. They concluded that endogenous OT has a gender-independent inhibitory effect on basal and stress-induced ACTH release.

The MDS results show the OT and exploration cluster to be located between the sector containing isolated offspring and sector containing socially housed animals, with animals from both groups clustering around them. It is possible that in our experiment some animals had higher endogenous OT in their system, and thus lower basal corticosterone levels. During the social interaction test, this could have caused them to not fall in a close proximity the other two clusters (motionlessness/self-grooming/CORT and affiliation/following/AVP), but closer to the exploration/OT cluster. They were not motionless and scared (negative correlation to CORT on dimension 2), they did not affiliate (negative correlation to AVP on dimension 1), but acted independently to explore their environment. This is all speculation however, and more research is needed on the impact of OT on social-interactions.

There were a number of limitations in the current study and a number of changes could make this study more comprehensive. First of all, this study lacked observation of mother-infant interactions within the nest, and relied instead on support in the literature to corroborate the hypothesis that behaviors during this period were different in PP and MP mothers. As this experiment was exploratory in nature, our wish was to establish that there was a possible effect on the infants before initiating in-depth work. This experiment does point towards interesting differences in the pups of PP and MP mothers, and nest observations of maternal behavior will be imperative in future studies.

Maternal stress hormones should be assessed during the weaning period to present a more complete picture of the mother's reaction to her litter. The current experiment only examined CORT on the last day of weaning, and found no differences. It is likely that by this time the PP mothers had habituated to their pups. Assessing maternal stress throughout the weaning period and relating this to pup CORT levels would be intriguing. Relating the OT expression in the PP and MP mothers to their maternal behavior and later pup behavior would also be a possible direction of future studies, as OT has been linked to maternal behavior in past research (e.g. Pedersen et al., 1994).

The developmental scope of this study could have been improved by focusing more intently on the period that the offspring were in their experimental condition (isolation or social housing). In this experiment, the only developmental variable included across the four weeks post-weaning were the three CORT measures. Obtaining more frequent CORT measurements throughout the experimental period may help to elucidate and clearly define the differences between PP- and MP-pups hinted at in this experiment. Observing behavior while the offspring are in their experimental condition would also help to provide a more comprehensive developmental picture of how isolation and maternal condition impact the juvenile rat's behavior.

Our study did not take into account the possible impact of the novel rats used in the social interaction test. All rats used as novel pups were socially housed PP-pups, and it is possible that this had an effect on the behavior of the subjects. Varlinskay et al. (1999) found that social behaviors are impacted both by intrinsic factors (previous social experience and housing conditions) and extrinsic factors (the social activity of the partner). Using different combinations of isolated and socially-housed rats may prove to alter the subjects' behavior, as in Varlinskay et al. (1999). It would also be interesting to look at female offspring in this paradigm, as only males were examined in this study, and it appears that maternal treatment of males and females differs in the first 18 days post-parturition (Moore & Morelli, 1979).

Our research has many implications. Although there were no distinctions in the overall MDS map between pups of PP and MP mothers, these animals did differ on behavioral measures. If subsequent studies confirm that pups of PP and MP rodent mothers have differing behavior (social investigation and leaving), it may be important to take the experience of the mother into consideration when obtaining laboratory subjects.

The experience of the mother may be yet another potential source of variability in the lab setting. Additionally, the effects of weaning should also be taken into account in the laboratory setting. As Cook (1999) suggests, different types of weaning can incur long-lasting differences in the rat's ability to cope with stress. Our study shows that the typical laboratory weaning is very stressful for juvenile rats, an effect which persists for at least four weeks post-weaning. Every effort should be made to ensure that weaning protocols across labs are uniform, as slight differences may impact offspring development.

Current research has examined the attenuating effects of OT on the stress response and both endogenous OT and administered OT have been shown to attenuate the stress response (Heinrichs et al., 2003; Neumann et al., 2000). Our research shows that a male rat's average stress response may be inversely related to both neural OT after a social interaction test and the amount of exploration the animal performs. Perhaps social anxiety and other disorders that involve difficulties with appropriate social behavior (for example, autism and Asperger's Syndrome) may be treated with OT administration in the future. More research should be done on the relationship between OT and CORT, both in animals and especially in humans, as our findings are correlational and do not necessarily imply causation.

Research in this area also has implications for studies in humans on birth order effects. Differences in first-borns and later-born children have been investigated in a number of areas including openness to new ideas and experiences, conscientiousness, agreeableness, neuroticism, extraversion, social attitudes, interpersonal relationships, et cetera (Sulloway, 1996). The mechanisms within the parent-child relationship that cause differences in offspring outcomes, beliefs, and attitudes are still largely unknown. The current research on maternal behavior and parity is frequently done in rodents. Rodents differ from non-human primates and humans, as they have multiple pups per litter each time they give birth, as compared with a typical birth of one offspring for primates and humans. It would be fascinating to extend previous primate studies showing differences between PP and MP mothers (e.g. Tanaka, 1989) to include brain and hormone data. The number of studies in the literature on primates that examine mechanisms to explain individual differences in offspring social development (e.g. Bardi et al., 2005) are still few and far-between. Although our study shows few differences between pups of PP- and MP-raised pups, differences between primates raised by mothers of differing amounts of RE seem to be more robust. Investigations into how having an experienced versus a non-experienced mother may impact primate infant development are very important to human research. Research on primates in this capacity would enhance what is known about the causes of and mechanisms behind order effects that occur in humans.

Learning more about how early social support impacts development, behavior, and hormones is an important direction in current research. Investigation further into the role that parents play in their offspring's early development should be a goal in future research. If even some differences in offspring can be traced back to maternal experience, actions should be taken to ensure that mothers can give their offspring the best possible early developmental experience. Additionally, this study has reemphasized the fact that isolation can have an astounding impact on offspring development. Simple alterations in the early environment, especially in relation to the availability of early social interactions, can change an organism's behavioral profile. Attention to the early developmental experience is crucial and efforts to improve this period may help to improve offspring social outcomes and reactions to stress.

In conclusion, this experiment has illustrated that there are some differences between PP- and MP-raised rat pups. The importance of these differences and reliability of our results must be established in future studies. Weaning was found to be a highly stressful experience for young rats, an effect which persisted for at least four weeks after normal weaning time. This study provided a broader clarification of the reciprocal relationship between a mother rat and her male offspring's hormonal response to weaning and later social behavior, opening the door to possible future studies in this area. Most importantly, the impact of isolation on behavioral, neural, and endocrine measures was further clarified in this study in a multidimensional analysis. This study extended the current literature on isolation's effect on social development. It is hoped that this research and promising future extensions will contribute to growing body of knowledge on offspring development.

References

Arakawa, H. (2005). Interaction between isolation rearing and social development on exploratory behavior in male rats. *Behavioural Processes, 70*, 223-234.

Arakawa, H. (2003). The effect of isolation rearing on open-field behavior in male rats depends on developmental stages. *Developmental Psychobiology, 43*, 11-19.

Bardi, M., Bode, A.E., Ramirez, S.M., & Brent, L.Y. (2005). Maternal care and development of stress response in baboons. *American Journal of Primatology, 66,* 263-278.

Bernstein, L., Depue, R.H., Ross, R.K., Judd, H.L., Pike, M.C., & Henderson, B.E. (1986). Higher maternal levels of free estradiol in first compared to second pregnancy: Early gestational differences. *Journal of the National Cancer Institute, 76*, 1035-1039.

Bretherton, I. (1992). The origins of attachment theory: John Bowlby and Mary Ainsworth. *Developmental Psychology, 28*(5), 759-775.

Bridges, R.S. (1975). Long-term effects of pregnancy and parturition upon maternal responsiveness in the rat. *Physiology and Behavior, 14*, 245-249.

Brunson, K.L., Kramar, E., Lin, B., Chen, Y., Colgin, L.L., Yanagihara, T.K., Lynch, G., & Baram, T.Z. (2005). Mechanisms of late-onset cognitive decline after early-life stress. *The Journal of Neuroscience, 25*(41), 9328-9333.

Byrnes, E.M., Byrnes, J.J., & Bridges, R.S. (2001). Increased sensitivity of dopamine systems following reproductive experience in rats. *Pharmacology, Biochemistry and Behavior, 68*, 481-489.

Byrnes, E.M., & Bridges, R.S. (2006). Reproductive experience alters anxiety-like behavior in the female rat. *Hormones and Behavior, 50*, 70-76.

Byrnes, E.M., & Bridges, R.S. (2005). Lactation reduces prolactin levels in reproductively experienced female rats. *Hormones and Behavior, 48*, 278-282.

Cacioppo, J.T., Ernst, J.M., Burleson, M.H., McClintock, M.K., Malarkey, W.B., Hawkley, L.C., et al. (2002). Lonely traits and concomitant physiological processes: the MacArthur social neuroscience studies. In Cacioppo, J.T., Berntson, G.G., Adolphs, R., Carter, C.S., Davidson, R.J., McClintock, M.K., et al. (Eds.) *Foundations in Social Neuroscience* (pp. 763-771). Cambridge, MA: MIT Press (839-852).

Cook, C. (1999). Patterns of weaning and adult response to stress. *Physiology & Behavior, 67*(5), 803–808.

Engelmann, M., Ebner, K., Landgraf, R., & Wotjak, C.T. (2006). Effects of Morris water maze testing on the neuroendocrine stress response and intrahypothalamic release of vasopressin and oxytocin in the rat. *Hormones and Behavior, 50*, 496-501.

Fairbanks, L.A. (1993). What is a good mother? Adaptive variation in maternal behavior of primates. In Kenrick, D.T., & Luce, C.L. Eds. (2004). The functional mind: Readings in evolutionary psychology. Boston: Pearson Education, Inc.

Fleming, A.S., & Sarker, J. (1990). Experience-hormone interactions and maternal behavior in rats. *Physiology and Behavior, 47*, 1165-1173.

Fleming, A.S., O'Day, D.H., Kraemer, G.W. (1999). Neurobiology of mother-infant interactions: Experience and central nervous system plasticity across development and generations. *Neuroscience and Biobehavioral Reviews, 23*, 673-685.

Francis, D.D., & Meaney, M.J. (2002). Maternal care and the development of stress response. In Cacioppo, J.T., Berntson, G.G., Adolphs, R., Carter, C.S., Davidson, R.J., McClintock, M.K., et al. (Eds.) *Foundations in Social Neuroscience* (pp. 763-771). Cambridge, MA: MIT Press.

Francis, D., Diorio, J., Liu, D., & Meaney, M. (2002). Nongenomic transmission across generations of maternal behavior and stress responses in the rat. In Cacioppo, J.T., Berntson, G.G., Adolphs, R., Carter, C.S., Davidson, R.J., McClintock, M.K., et al. (Eds.) *Foundations in Social Neuroscience* (pp. 797-803). Cambridge, MA: MIT Press.

Gatewood, J.D., Morgan, M.D., Eaton, M., McNamara, I.M., Stevens, L.F., Macbeth, A.H., Meyer, E.A.A., Lomas, L.M., Kozub, F.J., Lambert, K.G., & Kinsley, C.H. (2005). Motherhood mitigates aging-related decrements in learning and memory and positively affects brain aging in the rat. *Brain Research Bulletin, 66*, 91-98.

Gonza´lez-Mariscal, G., Melo, A.I., Chirino, R., Jime´nez, P., Beyer, C., & Rosenblatt, J.S. (1998). Importance of mother/young contact at parturition and across lactation for the expression of maternal behavior in rabbits. *Developmental Psychobiology, 32*(2), 101 – 111.

Hair, J.F., Anderson, R.E., Tatham, R.L., & Black, W.C. (1998). *Multivariate Data Analysis*. India: Pearson Education.

Heinrichs, M., Baumgartner, T., Kirschbaum, C., & Ehlert, U. (2003). Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. *Biological Psychiatry, 54*, 1389-1398.

Ikemoto, S., & Panksepp, J. (1992). The effects of early social isolation on the motivation for social play in juvenile rats. *Developmental Psychobiology, 25*(4), 261 – 274.

Insel, T.R., & Winslow, J.T. (1991). Central administration of oxytocin modulates the infant rat's response to social isolation. *European Journal of Pharmacology, 203*, 149-152.

Insel, T.R. (1997). A neurobiological basis of social attachment. *American Journal of Psychiatry, 154*, 726-735.

Kinsley, C.H., Madonia, L., Gifford, G.W., Tureski, K., Griffin, G.R., Lowry, C., Williams, J., Collins, J., McLearie, H., & Lambert, K.G. (1999). Motherhood improves learning and memory. *Nature, 402,* 137.

Kruskal, J.B., & Wish, M. (1978). *Multidimensional Scaling*. London: Sage.

Lambert, K.G., Berry, A.E., Griffin, G., Amory-Meyer, E.A., Madonia-Lomas, L.F., Love, G., & Kinsley, C.H. (2005). Pup exposure differentially enhances foraging ability in primiparous and nulliparous rats. *Physiology and Behavior, 85*, 799–806.

Lapiz, M.D.S., Fulford, A., Muchimapura, S., Mason, R., Parker, T., & Marsden, C.A. (2003). Influence of postweaning social isolation in the rat on brain development, conditioned behavior, and neurotransmission. *Neuroscience and Behavioral Physiology, 33*, 13-29.

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., Sharma, S., Pearson, D., Plotsky, P.M., & Meaney, M.J. (1997). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science, 277*, 5322, 1659 – 1662.

Love, G., Torrey, N., McNamara, I., Morgan, M., Banks, M., Hester, N.W., Glasper, E.R., DeVries, A.C., Kinsley, C.H., & Lambert, K.G. (2005). Maternal experience produces long-lasting behavioral modifications in the rat. *Behavioral Neuroscience, 119*, 1084-1096.

Mann, P.E., & Bridges, R.S. (1992). Neural and endocrine sensitivities to opioids decline as a function of multiparity in the rat. *Brain Research, 580*, 241-248.

Mar Sanchez, M., Aguado, F., Sanchez-Toscano, F., & Saphier, D. (1998). Neuroendocrine and immunocytochemical demonstrations of decreased hypothalamo-pituitary-adrenal axis responsiveness to restraint stress after long-term social isolation. *Endocrinology, 139*(2), 579-587.

McCormack, K., Sanchez, M.M., Bardi, M., & Maestripieri, D. (2006). Maternal care patterns and behavioral development of rhesus macaque abused infants in the first six months of life. *Developmental Psychobiology, 48*, 537-550.

Meaney, M.J., Bhatnagar, S., Larocque, S., McCormick, C.M., Shanks, N., Sharma, S., Smythe, J., Viau, V., & Plotsky, P.M. (1996). Early environment and the development of individual differences in the hypothalamic-pituitary-adrenal stress response. In: Pfeffer, C.R. (Ed.) *Severe stress and mental disturbance in children.* (pp. 85-127). Washington, DC, US: American Psychiatric Association.

Mitchell, G.D., Ruppenthal, G.C., Raymond, E.J., & Harlow, H.F. (1966). Long-term effects of multiparous and primiparous monkey mother rearing. *Child Development, 37*(4), 781-791.

Mitchell, G., & Stevens, C.W. (1968). Primiparous and multiparous monkey mothers in a mildly stressful social situation: First three months. *Developmental Psychobiology, 1*(4), 280-286.

Moltz, H., & Wiener, E. (1966). Effects of ovariectomy on maternal behavior of primiparous and multiparous rats. *Journal of Comparative and Physiological Psychology, 62*(3), 382-387.

Moore, C.L., & Morelli, G.A. (1979). Mother rats interact differently with male and female offspring. *Journal of Comparative and Physiological Psychology, 93*(4), 677-684.

Musey, V.C., Collins, D.C., Musey, P.I., Martino-Saltzman, D., & Preedy, J.R. (1987). Long-term effect of a first pregnancy on the secretion of prolactin. *The New England Journal of Medicine, 316*, 229-234.

Neumann, I.D., Wigger, A., Torner, L., Holsboer, F., & Landgraf, R. (2000). Brain oxytocin inhibits basal and stress-induced activity of the hypothalamic-pituitary-adrenal axis in male and female rats: Partial action within the paraventricular nucleus. *Journal of Neuroendocrinology*, *12*, 235-243.

Niesink, R.J.M., & Van Ree, J.M. (1982). Short-term isolation increases social interactions of male rats: A parametric analysis. *Physiology & Behavior, 29*, 819-825.

Panksepp, J. (1981). The ontogeny of play in rats. *Developmental Psychobiology, 14*, 327-332.

Paredes, J., Szeto, A., Levine, J.E., Zaias, J., Gonzales, J.A., Mendez, A.J., Llabre, M.M., Schneiderman, N., & McCabe, P.M. (2006). Social experience influences hypothalamic oxytocin in the WHHL rabbit. *Psychoneuroendocrinology, 31*, 1062-1075.

Paxinos, G. & Watson, C. *The Rat Brain in Stereotaxic Coordinates* (2nd edition), Academic Press, San Diego, 1986.

Pedersen, C.A., Caldwell, J.D., Walker, C., Ayers, G., & Mason, G.A. (1994). Oxytocin activates the postpartum onset of rat maternal behavior in the ventral tegmental and medial preoptic areas. *Behavioral Neuroscience, 108*(6), 1163-71.

Rees, S.L., Lovic, V. & Fleming, A.S. (2004). Maternal Behavior. In Whishaw, I.Q., & Kolb, B. (Eds.). *The Behavior of the Laboratory Rat.* Oxford University Press: New York.

Rima, B., Bardi, M., Friedenberg, J., Christon, L., Karelina, K., Lambert, K.G., Kinsley C.H. (in preparation, 2006). Reproductive experience and the female Sprague-Dawley rat's behavioral and hormonal response to fear and stress. *Physiology and Behavior.*

Schanberg, S.M., & Field, T.M. (1987). Sensory deprivation stress and supplemental stimulation in the rat pup and preterm human neonate. *Child Development, 58*(6), 1431-47.

Spruijt, B.M., Van Hooff, J., & Gispen, W.H. (1992). Ethology and neurobiology of grooming behavior. *Physiological Reviews, 72*(3), 825-851.

Stalans, L.J. (1995). Multidimensional Scaling. In Grimm, L.G., & Yarnold, P.R. (Eds.) *Reading and Understanding Multivariate Statistics.* Washington, D.C.: APA.

Sulloway, F.J. (1996). *Born to rebel: Birth order, family dynamics, and creative lives.* New York: Pantheon Books.

Tanaka, I. (1989). Variability in the development of mother-infant relationships among free-ranging Japanese macaques. *Primates, 30*(4), 477-491.

Tomizawa, K., Iga, N., Lu, Y.F., Moriwaki, A., Matsushita, M., Li, S.T., Miyamoto, O., Itano, T., & Matsui, H. (2003). Oxytocin improves long-lasting spatial memory during motherhood through MAP kinase cascade. *Nature-Neuroscience, 6*, 384-390.

Tracy, R.L., & Ainsworth, M.D.S. (1981). Maternal affectionate behavior and infant-mother attachment patterns. *Child Development, 52,* 1341-1343.

Van den Berg, C.L. Van Ree, J.M., Spruijt, B.M., & Kitchen, I. (1999). Effects of juvenile isolation and morphine treatment on social interactions and opioid receptors in adult rats: behavioural and autoradiographic studies. *European Journal of Neuroscience, 11*, 3023-3032.

Van den Berg, C.L., Van Ree, J.M., & Spruijt, B.M. (1999). Sequential analysis of juvenile isolation-induced decreased social behavior in the adult rat. *Physiology & Behavior, 67*(4), 483–488.

Varlinskaya, E.I., Spear, L.P., & Spear, N.E. (1999). Social behavior and social motivation in adolescent rats: Role of housing conditions and partner's activity. *Physiology & Behavior, 67*(4), 475–482.

Varty, G.B., Paulus, M.P., Braff, D.L., & Geyer, M.A. (2000). Environmental enrichment and isolation rearing in the rat: Effects on locomotor behavior and startle response plasticity. *Biological Psychiatry, 47*(10), 864-873.

Viveros, M.P., Hernandez, R., Martinez, I., & Gonzalez, P. (1988). Effects of social isolation and crowding upon adrenocortical reactivity and behavior in the rat. *Revista Espanola De Fisiologica, 44*(3), 315-322.

Wang, Z., & Novak, M.A. (1994). Parental care and litter development in primiparous and multiparous prairie voles. *Journal of Mammology, 75*(1), 18-23.

Wartella, J., Amory, E., Macbeth, A.H., McNamara, I., Stevens, L., Lambert, K.G., Kinsley, C.H. (2003). Single or multiple reproductive experiences attenuate neurobehavioral stress and fear responses in the female rat. *Physiology & Behavior, 79*, 373-381.

Weiss, I.C., Pryce, C.R., Jongen-Relo, A.L., Nanz-Bahr, N.I., & Feldon, J. (2003). Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat. *Behavioural Brain Research, 152*, 279–295.

Windle, R.J., Shanks, N., Lightman, S.L., Ingram, C.D. (1997). Central oxytocin administration reduces stress-induced corticosterone release and anxiety behavior in rats. *Endocrinology, 138*, 2829-2834.

Winslow, J.T., & Insel, T.R. (1993). Effects of central vasopressin administration to infant rats. *European Journal of Pharmacology, 233*, 101-107.

Wongwitdecha, N., & Marsden, C.A. (1996). Social isolation increases aggressive behaviour and alters the effects of diazepam in the rat social interaction test. *Behavioural Brain Research, 75*, 27-32.

Young, L.J., & Wang, Z. (2004). The neurobiology of pair bonding. *Nature Neuroscience, 7*, 1048-1054.

Young, L.J., & Insel, T.R. (2002). Hormones and parental behavior. In Becker, J.B., Breedlove, S.M., Crews, D., & McCarthy, M. (Eds.) *Behavioral Endocrinology* (pp. 331-336). Cambridge, MA: MIT Press.

Table 1.

Ethogram for Behavioral Data

Activity	Definition
Affiliation	(Duration in seconds) The subject is actively performing a affiliative behavior on novel rat. Affiliative behavior includes: grooming, sniffing, anogenital sniffing, playing, crawling over novel rat, etc.
Self-grooming	(Duration in seconds) The subject is licking, cleaning, or scratching self. This may include muzzle wipes, self anogenital licking, or soft biting to clean fur.
Exploration	(Duration in seconds) The subject is not engaged in any type of affiliative behavior with the other rat. The subject is moving around cage and exploring environment. Sniffing bedding or walls of cage, rearing, and stretching may all be observed.
Following	(Duration in seconds) One variation on affiliative behavior. Following is different from affiliation in that it involves continual movement. The subject is actively following the novel rat around the test environment.
Social investigation	(Frequency) One variation on affiliative behavior. Social investigation is different from affiliation in that it does not involve physical contact with the other subject. Instead, the subject merely approaches the novel animal and enters into close proximity with him on purpose. Do not include if subject does not actively seek out the other animal. This behavior may immediately precede affiliation or following.
Leaving	(Frequency) Opposite of affiliative behavior. Subject makes conscious effort to move away from a close proximity to the novel animal.
Motionless	(Duration in seconds) Subject is not moving or active and is sitting still. It is the absence of the other scored behaviors.

Table 2.

Intercorrelations Between Behavioral, Neural, and Endocrine Variables

	1	2	3	4	5	6	7	8	9	10
1. Affiliation	—	.00	-.20	.47*	-.72**	.67**	.21	-.01	.13	.16
2. Self-grooming		—	-.13	.02	-.24	-.13	.12	-.15	.00	-.08
3. Exploration			—	-.16	-.20	-.16	-.18	.44*	-.20	-.18
4. Following				—	-.47*	.50**	.33	-.28	.19	.31
5. Motionlessness					—	-.43*	-.30	-.18	-.11	.04
6. Social investigation						—	.52**	-.17	.19	.22
7. Leaving							—	-.12	.23	-.39*
8. OX								—	.23	-.13
9. AVP									—	-.01
10. CORT										—

* = Correlation is significant at the 0.05 level (2-tailed)

** = Correlation is significant at the 0.01 level (2-tailed)

Figure 1. Total duration (in seconds) of affiliation by experimental condition.

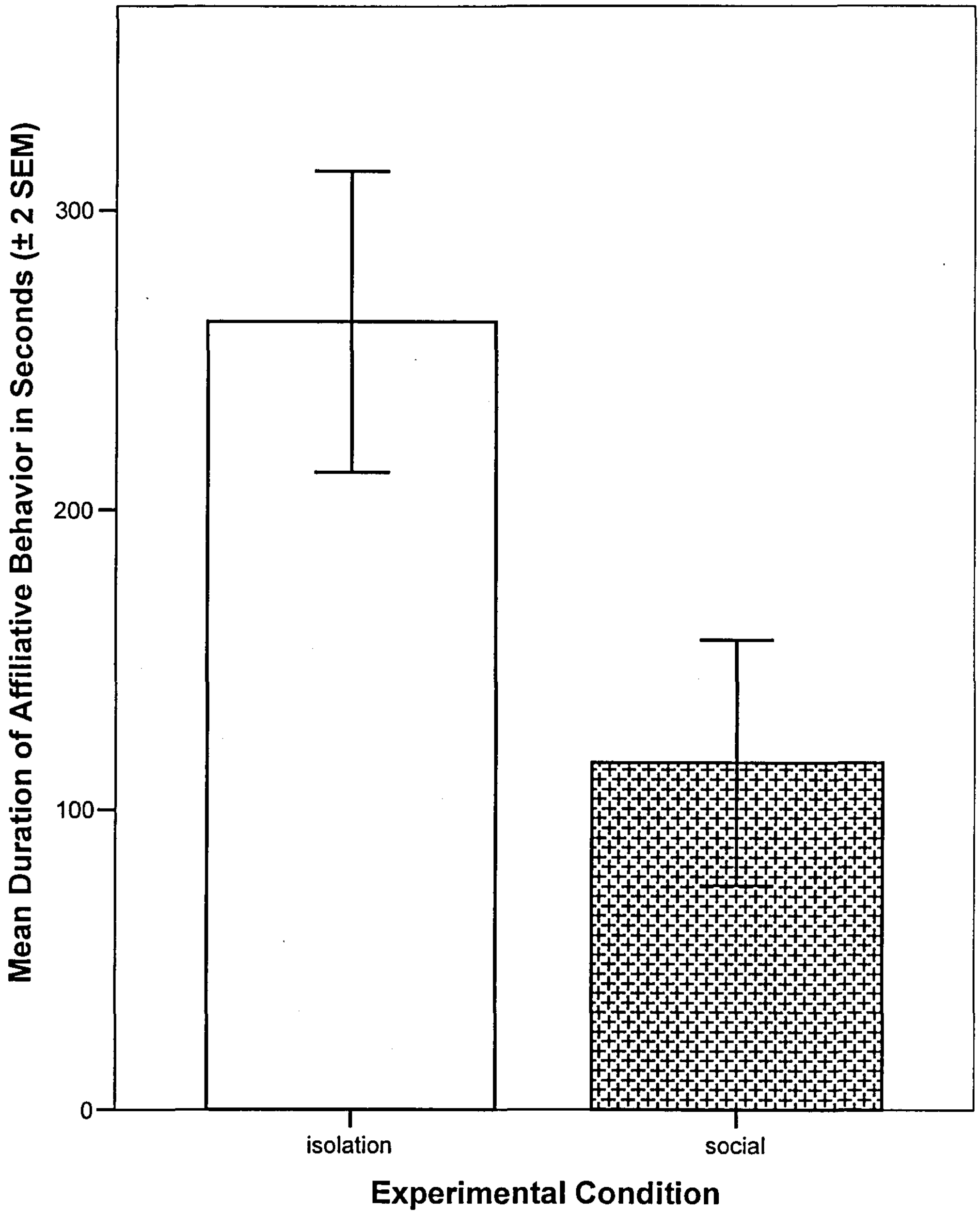


Figure 2. Total duration (in seconds) of motionlessness by experimental condition.

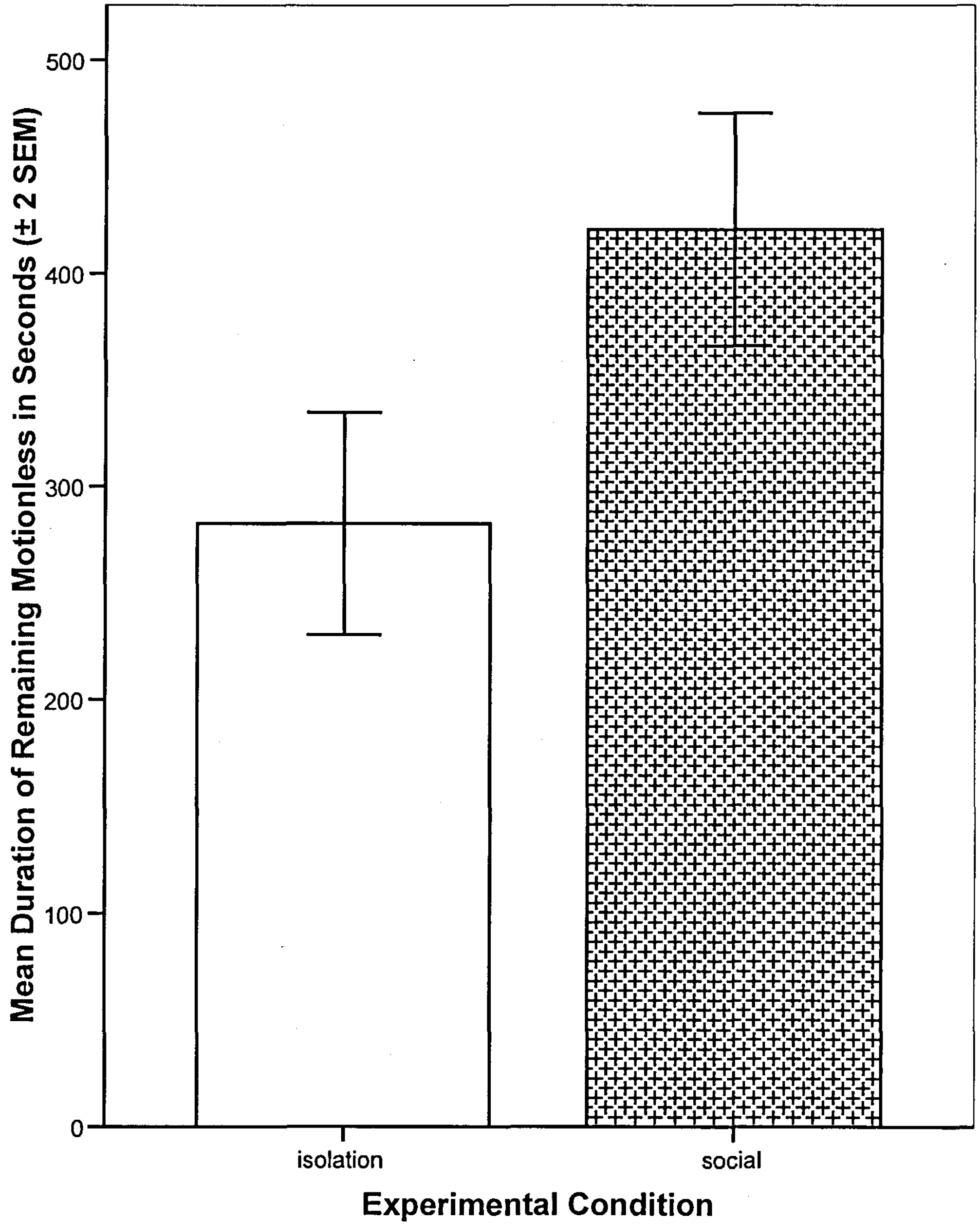


Figure 3. Frequency of social investigations by experimental condition.

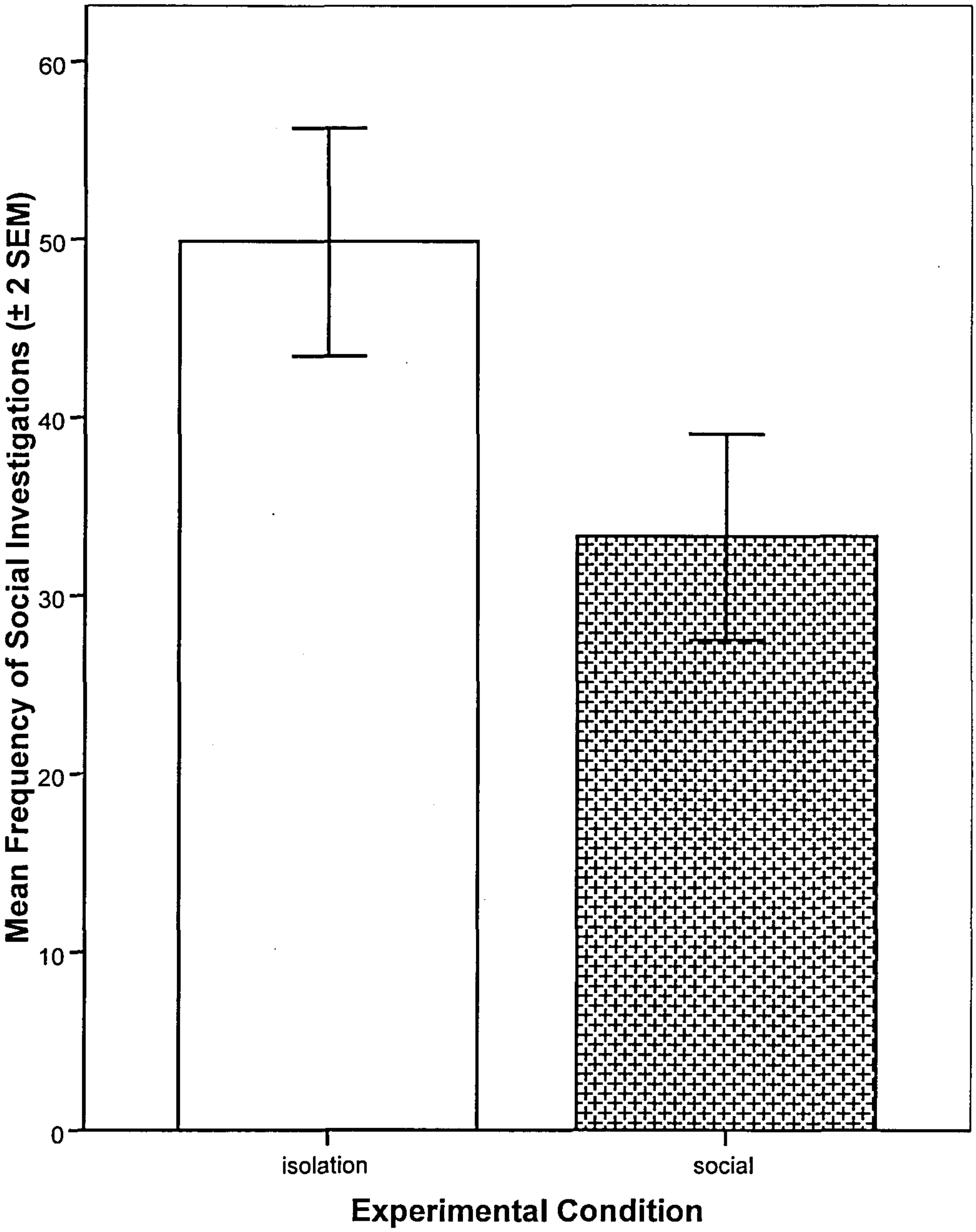


Figure 4. Frequency of social investigations by maternal condition.

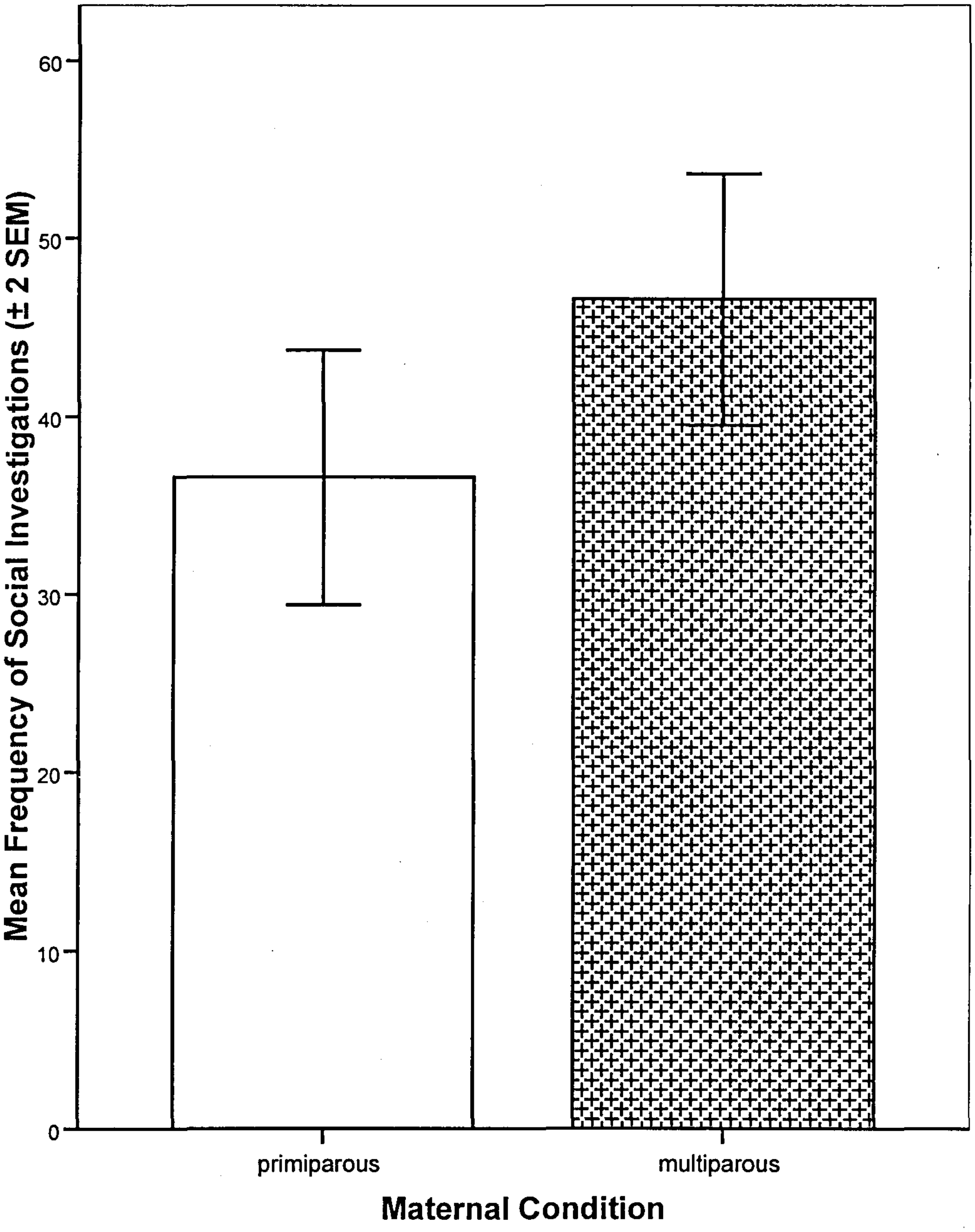


Figure 5. Frequency of leaving by maternal condition.

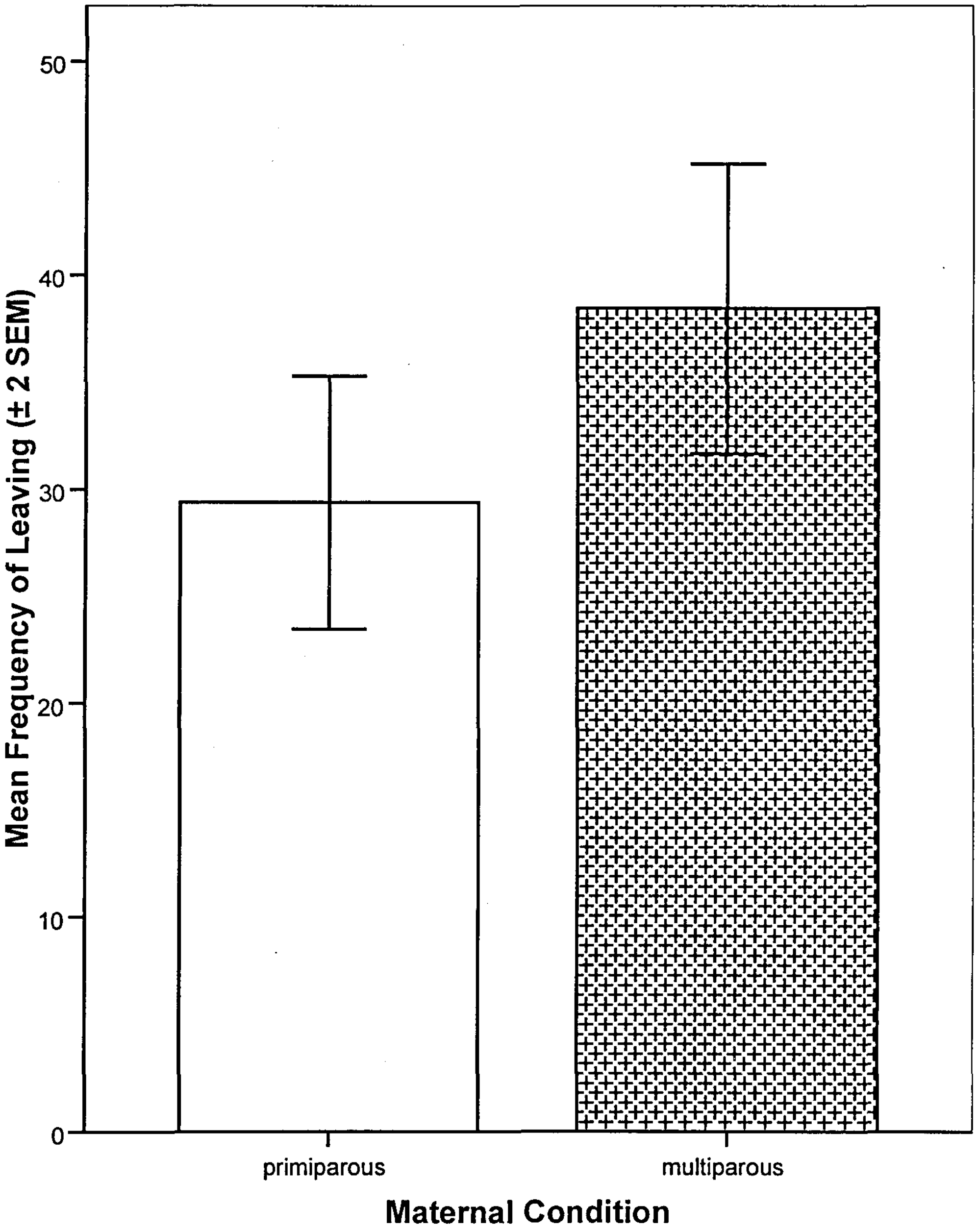


Figure 6. Interaction between corticosterone levels and time.

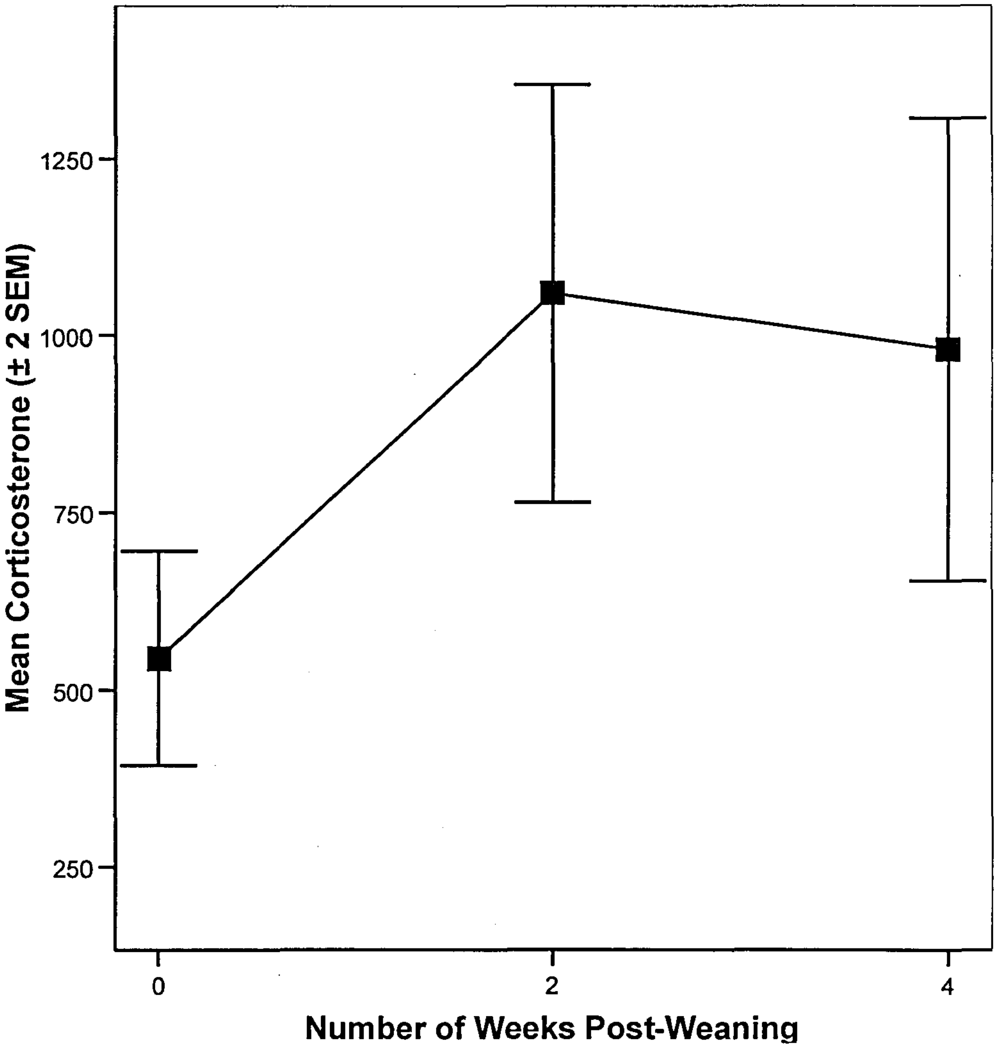


Figure 7. Interaction between corticosterone levels and maternal experience over time.

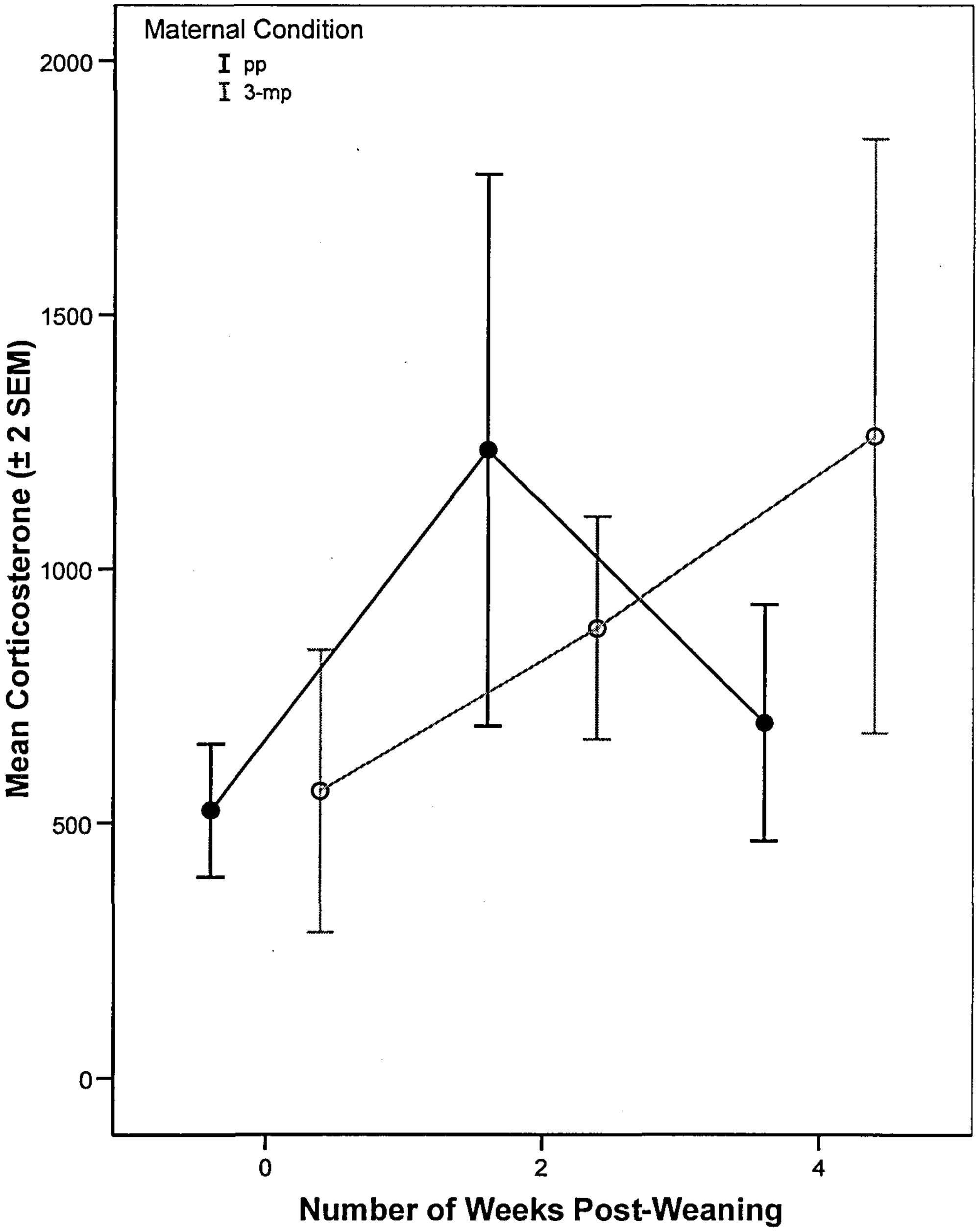


Figure 8. Multidimensional map of ten variables and subjects.

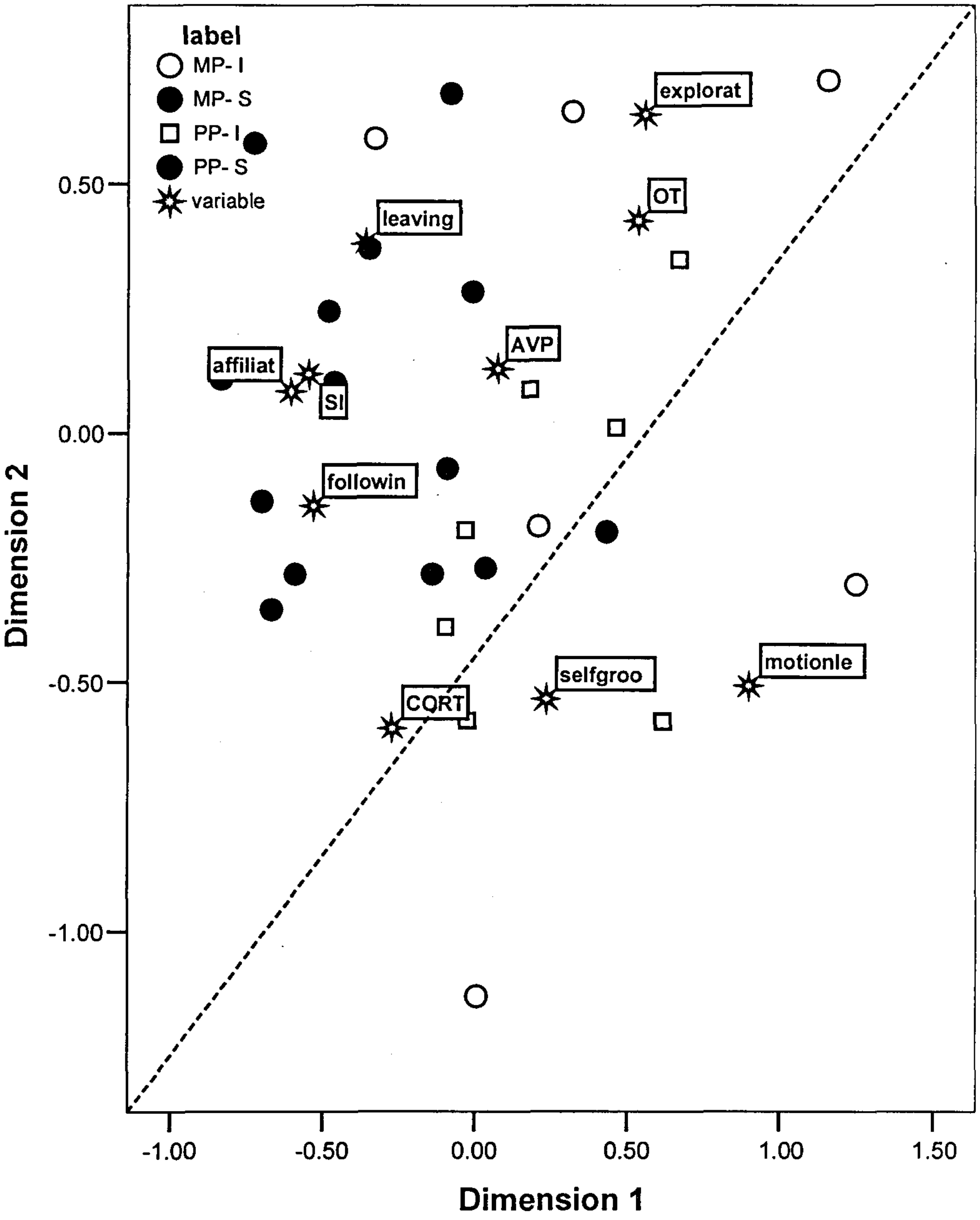
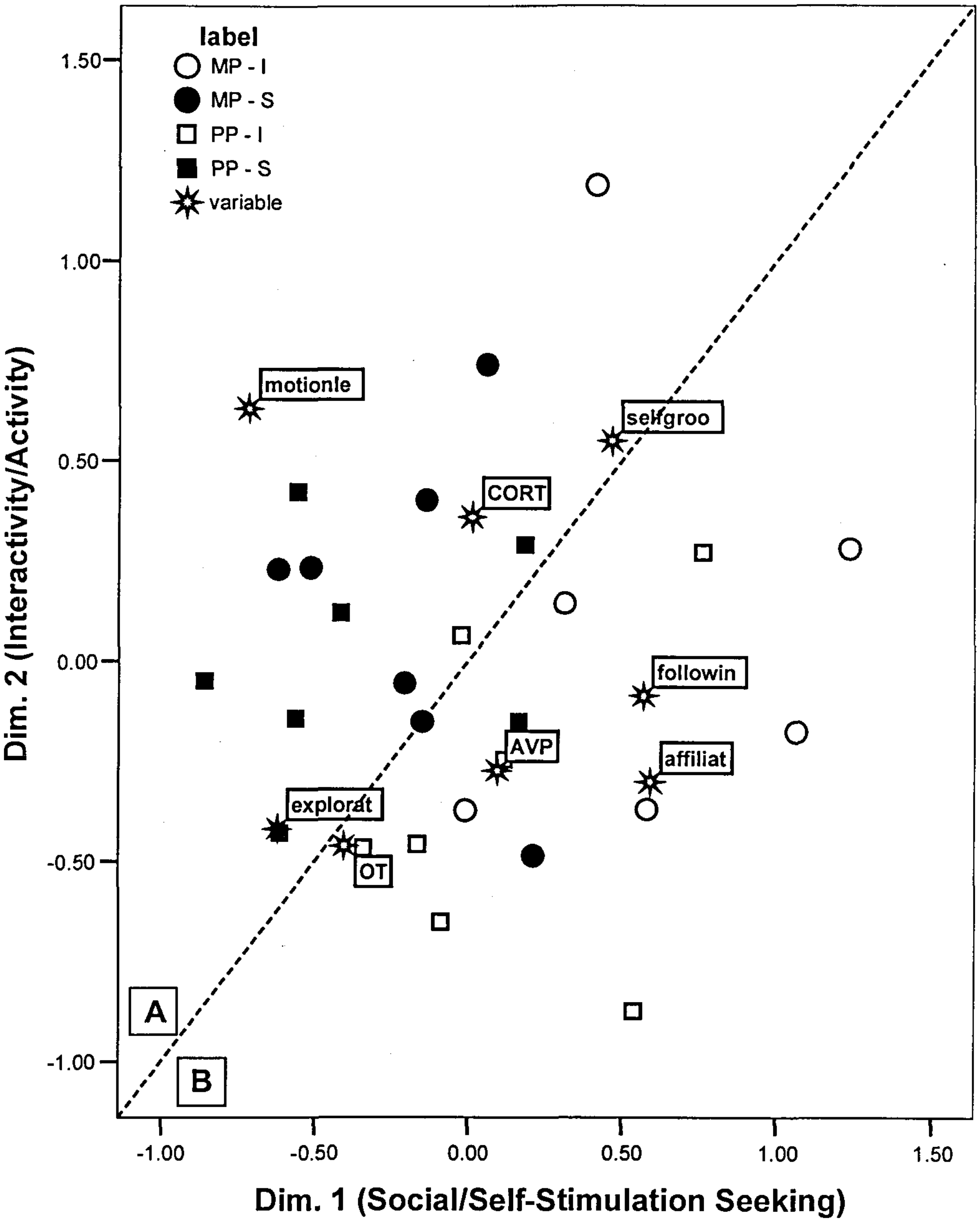


Figure 9. Final multidimensional map of eight variables and subjects.



Vita

Lillian (Lily) Maria Christon received a Bachelor of Arts degree from Wake Forest University in 2005. She graduated with a major in psychology and a minor in international studies. She received her Master of Arts in psychology with a concentration in neuroscience from the University of Richmond in August 2007. In the fall of 2007, Lily will begin her doctorate work at Virginia Commonwealth University. She will pursue a dual-track Ph.D. in applied developmental psychology and child clinical psychology. In the future Lily hopes to pursue research on childhood disabilities, especially autism, and to work in both applied and clinical settings.