THE ROLE OF REPRODUCTIVE EXPERIENCE ON HIPPOCAMPUS-DEPENDENT SPATIAL MEMORY, ADULT HIPPOCAMPAL NEUROGENESIS, AND CORTICOSTERONE IN THE RAT DAM

by

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ABSTRACT

Pregnancy and the postpartum period have remarkable effects on cognition in the mother. Interestingly, motherhood results in enhanced spatial memory in the rodent. This thesis aimed to determine the role of reproductive experience (number of times pregnant and maternal) on enhanced hippocampus-dependent spatial memory in the mother, as well as the neural and hormonal correlates of this effect. Chapter 2 demonstrates that reproductive experience differentially affects spatial memory performance at the time of weaning resulting in enhanced memory performance in primiparous rats compared to nulliparous rats, with a trend toward enhanced memory performance in multiparous compared to nulliparous rats. Chapter 3 demonstrates that the enhanced spatial memory in primiparous rats persists after the time of weaning and is not due to pregnancy or pup-exposure alone. Chapter 4 demonstrates that reproductive experience results in decreased cell proliferation and cell survival in the dentate gyrus during the postpartum in primiparous rats and multiparous rats. Furthermore primiparous rats had decreased levels of cell survival compared to multiparous rats, and pup-exposed nulliparous females had increased levels of cell proliferation and cell death after brief pup-exposure. Chapter 5 demonstrates that primiparous rats have significantly elevated total corticosterone in the early postpartum period as well as lower CBG during the postpartum period. Thus, taken together the results from the experiments suggest that the enhancement in hippocampus-dependent spatial memory seen in primiparous and multiparous rats is coincident with reduced hippocampal neurogenesis, increased corticosterone, and decreased corticosteroid binding globulin (CBG) during the postpartum period in the mother. These findings demonstrate that motherhood is a time of marked neural plasticity in the hippocampus, an area of the brain not traditionally associated with motherhood.
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<td>-bromodeoxyuridine</td>
</tr>
<tr>
<td>CA1</td>
<td>-cornu ammonis 1</td>
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<tr>
<td>CA3</td>
<td>-cornu ammonis 3</td>
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<tr>
<td>CBG</td>
<td>-coritocosteroid binding globulin</td>
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<tr>
<td>Ci</td>
<td>-curie</td>
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<tr>
<td>CORT</td>
<td>-corticosterone</td>
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<tr>
<td>Cv</td>
<td>-coefficient of variation</td>
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<td>Cy3</td>
<td>-indocarbocyanine 3</td>
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<tr>
<td>D</td>
<td>-day</td>
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<tr>
<td>DCC</td>
<td>-dextran-coated charcoal</td>
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<td>DG</td>
<td>-dentate gyrus</td>
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<tr>
<td>DNA</td>
<td>-deoxyribonuceic acid</td>
</tr>
<tr>
<td>E2</td>
<td>-estradiol</td>
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<tr>
<td>ED</td>
<td>-effective dose</td>
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<td>G</td>
<td>-grams</td>
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<td>GCL</td>
<td>-granule cell layer</td>
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<td>GD</td>
<td>-gestation day</td>
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<td>H</td>
<td>-hour</td>
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<td>Hil</td>
<td>-hilus</td>
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<tr>
<td>HPA</td>
<td>-hypothalamic-pituitary adrenal</td>
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<td>HPG</td>
<td>-hypothalamic-pituitary gonadal</td>
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<tr>
<td>i.p.</td>
<td>-intraperitoneal</td>
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<tr>
<td>Ir</td>
<td>-immunoreactive</td>
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<tr>
<td>LG-ABN</td>
<td>-licking/grooming-arched back nursing</td>
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<td>LTP</td>
<td>-long-term potentiation</td>
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<td>Min</td>
<td>-minute</td>
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<tr>
<td>Multip</td>
<td>-multiparous</td>
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<tr>
<td>NDS</td>
<td>-normal donkey serum</td>
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<td>NeuN</td>
<td>-neuronal nuclei</td>
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<td>-phosphate-buffered saline</td>
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<td>Primip</td>
<td>-primiparous</td>
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<td>RIA</td>
<td>-radioimmuno assay</td>
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<td>RME</td>
<td>-reference memory error</td>
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<tr>
<td>SEM</td>
<td>-standard error of the mean</td>
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<td>Sens</td>
<td>-sensitized</td>
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<td>SGZ</td>
<td>-subgranule zone</td>
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<td>SVZ</td>
<td>-subventricular zone</td>
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<tr>
<td>TBS</td>
<td>-tris-buffered saline</td>
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<tr>
<td>WRME</td>
<td>-working/reference memory error</td>
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<td>WME</td>
<td>-working memory error</td>
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Sarah Walker, Brandy Vanderbyl and Kelsey Ragan assisted with data collection in the behavioral studies (Chapters 2 and 3).
CHAPTER 1
GENERAL INTRODUCTION

Approximately 75% of women report short-term memory loss, forgetfulness, disorientation, confusion, lack of concentration, or reading difficulties during late pregnancy and early postpartum (Crawley et al., 2003; Poser et al., 1986; Parsons and Redman, 1991). Anecdotal reports of this “baby brain”, “porridge brain”, or “maternal amnesia” appear to be substantiated with research findings of decreased verbal recall and spatial ability during late pregnancy and the early postpartum period (Buckwalter et al., 1999; De Groot et al., 2006; Galea et al., 2000; Keenan et al., 1998). To date only one study has aimed to determine the long-term implications of pregnancy and the early postpartum period on cognition in women (Buckwalter et al., 2001). Buckwalter et al. (2001) has found that at 2 years postpartum, there is an improvement in cognitive abilities compared to late pregnancy and the early postpartum, suggesting possible enhancing effects of motherhood on memory. Similar findings have been found in animal models; pregnancy is associated with impaired spatial memory in both primates and rodents (Buckwalter et al., 1999; Galea et al., 2000), while motherhood is associated with enhanced spatial memory in rodents (Kinsley et al., 1999).

The role of the steroid hormones estradiol and corticosterone on spatial ability have been well documented (Bowman et al., 2001; Dohanich, 2002) and these hormones are remarkably altered during pregnancy and motherhood (Garland et al., 1987; Rosenblatt et al., 1979; Stern et al., 1973; Yoshinaga et al., 1969): estradiol increases during pregnancy and decreases across the postpartum period (Garland et al., 1987; Hapon et al., 2003; Rosenblatt et al., 1979; Yoshinaga et al., 1969) while basal corticosterone increases during late pregnancy and is elevated during the postpartum period (Atkinson and Waddell, 2005; Fischer et al., 1995; Gala and Westphal, 1964; Stern et al., 1973). Interestingly, spatial memory relies in part on the integrity of the
hippocampus, an area of the brain that shows remarkable plasticity and is very sensitive to the effects of steroid hormones. The hippocampal structure consists of three main regions: cornu ammonis 1 (CA1), cornu ammonis 3 (CA3), and the dentate gyrus (Figure 1.1). The dentate gyrus is the site of continuous neurogenesis in adulthood (Altman, 1962). All three regions exhibit marked changes in response to steroid hormones (Galea et al., 2006; Mirescu and Gould, 2006). For example, estradiol increases dendritic spine density in the CA1 region of the hippocampus (Woolley et al., 1990), enhances adult neurogenesis in the dentate gyrus (Ormerod and Galea, 2001; Tanapat et al., 1999), and differentially regulates both spatial working and reference memory (Dohanich, 2002; Holmes et al., 2002). High levels of corticosterone and/or chronic stress decreases dendritic arbors in the CA3 region of the hippocampus (Galea et al., 1997; Magarinos et al., 1998), decreases adult neurogenesis in the dentate gyrus (Cameron and Gould, 1994; Kuipers et al., 2004; Wong and Herbert, 2004), and paradoxically enhances spatial memory in the female (Bowman et al., 2001). Recently it has been shown that primiparous rats (first time given birth and mothered) had reduced dendritic length and branching in the CA3 and CA1 region of the hippocampus, similar to what is seen in females under chronic stress (Pawluski and Galea, 2006). Given that pregnancy and the postpartum are accompanied by remarkable changes in steroid hormones, including corticosterone (Rosenblatt et al., 1973; Stern et al., 1973), and these hormones have profound effects on structural properties of the hippocampus and behaviors mediated by the hippocampus, it is possible that the changes in spatial memory evident with motherhood (Kinsley et al., 1999) may be due to changes in hippocampus plasticity and altered steroid hormone levels.
Figure 1.1 Representative micrograph of the hippocampal structure. The hippocampus consists of three main regions: cornu ammonis 1 (CA1), cornu ammonis 3 (CA3), and the dentate gyrus.

The first maternal experience (primiparity) has remarkably different effects than subsequent maternal experiences (Bridges et al., 1993; Kinsley and Bridges, 1988; Svare and Gandelman, 1976). Therefore, when considering the effect of pregnancy and motherhood on the brain and behavior of the dam it is important to take into account the role of reproductive experience. Reproductive experience is defined as the number of times pregnant and parturient: primiparity refers to being pregnant once, multiparity refers to being pregnant multiple times, and nulliparity refers to never being pregnant. Maternal behavior can be induced in virgin nulliparous females by continuous exposure to pups, which has been termed sensitization (Rosenblatt, 1967). In the rodent, reproductive experience has considerable effects on the behavior, brain, and hormone levels of the dam. For example: maternal responsiveness (Moltz and Robbins, 1965) and postpartum aggression (Svare and Gandelman, 1976) increase with increasing parity; basal prolactin levels are significantly lower with multiparity compared to primiparity (Bridges et al., 1993); neural sensitivity to opioids is reduced with increased parity in the rat (Kinsley and Bridges, 1988); and the number of astrocytes in the medial preoptic area of
the hypothalamus is increased with multiparity compared to primiparity (Featherstone et al., 2000). In addition, primiparity has been suggested to be remarkably different than multiparity as it is a time when ‘maternal memory’, the retention of maternal responsiveness as a consequence of prior experience with pups, is formed (Orpen and Fleming, 1987). Sensitization or pup exposure given to nulliparous rats also has marked effects on the brain of virgin females. For example, sensitized rats have increased c-Fos immunoreactivity in the medial preoptic area of the hypothalamus compared to controls (Kalinichev et al., 2000). Therefore, when considering the effect of pregnancy and motherhood on the brain and behavior of the dam it is important to take into account the role of reproductive experience. In addition, any effects of motherhood on neurogenesis or learning may be due to the effects of pregnancy or pup-exposure alone. Therefore the present thesis investigates the role of reproductive experience, pregnancy and pup-exposure alone on hippocampus dependent spatial memory, hippocampal neurogenesis, and steroid hormone profiles in the dam.

Apart from the possible effect of reproductive experience, the duration of specific maternal behaviors, such as licking and grooming offspring, also affect hippocampus-dependent learning and memory and hippocampal neurogenesis in offspring during adulthood (Liu et al., 2000; Bredy et al., 2003) and may have considerable effects on the dam. For example, biological and foster male offspring of mothers that spend more time in licking, grooming and arched-back nursing behaviors (high LG-ABN), have enhanced spatial reference memory in adulthood (Liu et al., 2000) and increased neurogenesis in the dentate gyrus in adult male rats (Bredy et al., 2003) compared to offspring of low LG-ABN mothers. In turn, this degree of maternal behavior is stably transmitted from the nursing, and not the biological, mother to the daughter such that a female of a high-licking dam will also be a high-licking dam (Champagne and Meaney, 2001).
Taken together, this suggests that dams that exhibit a high degree of licking and grooming may also exhibit enhanced spatial learning and memory and enhanced hippocampus neurogenesis.

The role of motherhood on hippocampus structure and function in the mother is just beginning to be explored and more research is needed to investigate the role of reproductive experience on these effects. The experiments described in the present thesis examine the influence of reproductive experience on hippocampus-dependent learning and memory, hippocampus neurogenesis, and steroid hormone levels in the mother. The main findings of the experiments within this thesis are that primiparity results in improved hippocampus-dependent spatial learning and memory (Ch 2 and 3; Pawluski et al., 2006 a, b) and decreased hippocampus neurogenesis (Ch 4; Pawluski and Galea, submitted) compared to nulliparity with more subtle effects seen with multiparity. In addition, primiparity resulted in elevated corticosterone levels during the early postpartum period compared to multiparity while both primiparity and multiparity resulted in decreased corticosteroid binding globulin (CBG) levels during the postpartum period (Ch 5: Pawluski et al., submitted). In turn, these effects appear to be due to the combined effects of pregnancy and pup-exposure, and are at times associated with specific maternal behaviors. These findings are discussed in the context of how reproductive experience affects hippocampus-mediated spatial memory and how these changes in spatial memory may be mediated by differences in hippocampal plasticity and steroid hormone profiles between primiparous, multiparous, and nulliparous rats.

1.1 THE HIPPOCAMPUS AND MOTHERHOOD

Pregnancy and the postpartum period are a time of heightened neural plasticity and these changes in neural plasticity may be associated with changes in learning and memory performance in the mother. In women, Oatridge et al. (2002) have shown that total brain size
decreases during pregnancy and returns to preconception size during the postpartum period. In rodents, Galea et al. (2000) have shown that hippocampal volume is decreased during pregnancy compared to nulliparous females, and Hamilton et al. (1977) have shown that cortical thickness is increased immediately after parturition compared to nulliparous females. In addition, the transition to motherhood is associated with the induction of neural circuitry important for maternal responding (Numan, 2007). This ‘maternal circuit’ involves such areas at the hypothalamus, olfactory bulbs, nucleus accumbens and amygdala (Numan, 2007).

The hippocampus has not traditionally been documented as an area important for the onset of motherhood and very little work has focused on this region. One of the only recorded works on the role of the hippocampus in motherhood found that bilateral dorsal hippocampal lesions result in marked alterations in maternal behavior, particularly less time spent nursing, poorer nest building, poorer retrieval of pups, and increased maternal cannibalism than neocortical lesioned dams and sham lesioned controls (Kimble, 1966). In addition, disruption of the main fibre tract projecting to the CA1 and CA3 regions of the hippocampus, via fimbria lesions, results in abnormal nest building and pup retrieval (Terlecki and Sainsbury, 1978). These dams built multiple nest sights and retrieved pups to more than one location (Terlecki and Sainsbury, 1978). Thus, although this early work pointed to a role for the hippocampus in maternal behavior, it was not until the late 1990s that the possible relationship between the hippocampus and motherhood was revisited.

1.2 HIPPOCAMPUS-DEPENDENT SPATIAL LEARNING AND MEMORY PERFORMANCE IN THE MOTHER

It is widely accepted that spatial memory is dependent on the integrity of the hippocampus (Morris et al., 1982; Moscovitch et al., 2005). In rodents, lesions to the
Hippocampal formation result in deficits in spatial working and reference memory (Morris et al., 1982; O'Keefe et al., 1978; Olton et al., 1978). Morris et al. (1982) documented that lesions to the hippocampus, but not the cortex, of female rats caused extensive and lasting impairment of place navigation compared to sham-lesioned rats. However there were no evident impairments in any groups when a cue was provided (Morris et al., 1982), suggesting that place and not cue navigation is dependent on the hippocampus. Following this early work, an extensive amount of research has aimed to document factors that influence spatial learning and memory. This work has demonstrated that spatial ability is differentially affected by a host of different variables including, but not limited to, sex of the subject, steroid hormones, stress, maternal behavior, age and a combination of these variables (Bowman et al., 2001; Galea et al., 1995; Holmes et al., 2002; Liu et al., 2000; Montaron et al., 2006).

Hippocampus-dependent spatial memory relies on allocentric strategies where distant, but not proximal, cues are used for orientation in a given environment (Dohanich, 2002; Olton and Papas, 1979). Spatial memory can consist of both working memory and reference memory. Working memory is defined as the manipulation and retrieval of trial-unique information that is used to guide prospective action (Olton and Papas, 1979), whereas reference memory is defined as a long-term stable memory (Olton and Papas, 1979). The two common tasks used to test spatial memory in rodents are the radial arm maze and the Morris water maze. Both tasks can be used to determine spatial working and reference memory performance, however the working/reference memory version of the radial arm maze is the only within-subject, within-test design that allows for the examination of both spatial working and reference memory (Olton and Papas, 1979). For further details on this task see Chapters 2 and 3.

As mentioned previously, late pregnancy is associated with impaired spatial working memory in both primates and rodents (De Groot et al., 2006; Galea et al., 2000), whereas
motherhood is associated with enhanced spatial working and reference memory in rodents (Kinsley et al., 1999). Kinsley et al. (1999) were the first to document that motherhood enhances spatial learning and memory in the dam. They found that after 16 days of lactation or pup-exposure, primiparous and sensitized rats had shorter latencies to complete the task compared to nulliparous rats when tested on a reference memory version of the dry land version of the water maze (Kinsley et al., 1999). They also found that multiparous dams had enhanced working memory performance on the first 6 days of testing compared to nulliparous rats when tested on the working memory version of the radial arm maze 14 days after weaning (Kinsley et al., 1999). However, there were a number of problems with this work. First, the performance of primiparous and multiparous rats on the same task or at the same time point during the postpartum period was not compared. Second, dams were tested at different time points during lactation and after weaning on the two tasks (weaning occurs 22-28 days after parturition), therefore it is unclear how the removal of pups mid-lactation would affect the dam. Third, the effect of proestrus on spatial memory was not controlled for even though proestrous and high levels of estradiol have well documented effects on spatial memory performance (Holmes et al., 2002; Warren and Juraska, 1997). Finally, latency, and not path length, was used as a measure of performance in one of the tasks and thus it is not clear whether the proposed spatial memory enhancements were due to mnemonic or motor processes.

Recently, further work from Kinsley’s laboratory has demonstrated that parous rats exhibit improvements in spatial memory performance long after weaning (Gatewood et al., 2005; Love et al., 2005). Gatewood et al. (2005) found that both multiparous and primiparous rats had shorter latencies to complete a reference and working version of the dry land maze compared to nulliparous rats. In addition, they found that multiparous rats had shorter latencies to complete the task than primiparous rats (Gatewood et al., 2005). These findings were evident when rats
were repeatedly tested beginning at 6 months of age (3 weeks after weaning) until 24 months of age. Using a similar task to that of Gatewood et al. (2005), Love et al. (2005) found that primiparous and multiparous rats had shorter latencies to complete a reference memory version of the dry land maze compared to nulliparous rats when repeatedly tested at 5 and 13 months of age, but not at 9 months of age. However, after 13 months of age, there were no differences between multiparous, primiparous, and nulliparous rats on spatial memory performance and this was due to improved performance of nulliparous rats (Love et al., 2005). However, it should be noted that these studies (Gatewood et al., 2005; Love et al., 2005) also used latency, and not path length, to determine spatial memory performance, therefore it is possible that motor ability, and not memory ability, accounted for the differences observed between nulliparous, primiparous and multiparous groups. In addition, these studies repeatedly tested rats on the same task and therefore may have measured re-acquisition rather than acquisition of the spatial task.

The findings of Gatewood et al. (2005) and Love et al. (2005) on the prolonged enhancement of spatial reference memory with increased parity are inconsistent. However, this may be due to the effect of estradiol on spatial memory in the female (Holmes et al., 2002; Warren and Juraska, 1997). Neither study controlled for the effect of estradiol or stage of estrous cycle on spatial performance with age and parity. There are lower levels of estradiol during proestrus in parous rats compared to virgin rats (Bridges and Byrnes, 2006), and the cessation of estrous cyclicity occurs later in multiparous rats compared to primiparous rats (Matt et al., 1987). Therefore, it is quite plausible the differences in levels of estradiol or other ovarian hormones may account for the discrepancies between findings of Gatewood et al. (2005) and Love et al. (2005).

Part of the objective of the studies in this thesis was to address problems with the interpretation of these previous studies (Gatewood et al., 2005; Kinsley et al., 1999; Lambert et
al., 2005; Love et al., 2005). Pawluski et al. (2006a, b) demonstrated that primiparous, but not multiparous, rats consistently have enhanced spatial memory performance compared to nulliparous rats both at the time of weaning (Ch 2) and long after the time of weaning (Ch 3). This enhanced spatial memory performance was not due to gross differences in motor ability and was not affected by estrous cyclicity (Ch 2 and 3: Pawluski et al., 2006a, b). These findings were consistent with Lemaire et al. (2007), who found that primiparous rats had improved reference memory performance compared to nulliparous controls shortly after weaning and up to 16 months later (Lemaire et al., 2007).

Enhanced spatial cognition with motherhood, and especially with primiparity in rats, may be needed in order for the mother to efficiently locate and retrieve food. Altered cognition in the dam may be due to the changing hormone levels and neural plasticity with reproduction and lactation. Interestingly, in the rodent, food caching activity in the mother was found to be three times higher during the first 20 days of mothering (Calhoun, 1963) suggesting that effective and efficient foraging behavior may be required in order to optimize the time caring for, and protecting young. However, recent evidence suggests that spatial learning during the early postpartum period is poorer in parous dams than in nulliparous females (Darnaudery et al., 2007). However, spatial memory is enhanced in primiparous females within 14 days of parturition (Darnaudery et al., 2007), demonstrating that long-term memory for landmarks is enhanced with motherhood. Once reproduction is successful and pups have dispersed from the nest, subsequent maternal experience may be less demanding as the mother has learned the essential maternal behaviors needed for reproductive success. Thus, with subsequent mothering, the induction of maternal behaviors may be more rapid, and the mother may not need to spend as much time with her offspring and therefore the need to efficiently forage is not as great a requirement.
Recent work has begun to investigate factors mediating these changes in spatial learning and memory with motherhood by investigating the role of hormones, pregnancy, and pup-exposure (Lambert et al., 2005; Lemaire et al., 2007; Pawluski et al., 2006a, b; Tomizawa et al., 2003). Work by Tomizawa et al. (2003) suggests that oxytocin is important for the improvements in reference memory, but not working memory, seen with motherhood; they found that nulliparous mice administered oxytocin had improved spatial reference memory compared to nulliparous controls and that multiparous mice had improved reference memory compared to multiparous mice administered an oxytocin antagonist (Tomizawa et al., 2003). Although this work points to a possible role for oxytocin on enhanced spatial reference memory in mothers, it failed to compare parous dams with nulliparous females on the same memory task and to account for full maternal behavior as all multiparous mice were tested 3 days after parturition (Tomizawa et al., 2003).

The separate effect of pregnancy and pup-exposure on the improvements in spatial memory with motherhood has also received some investigation (Kinsley et al., 1999; Lambert et al., 2005; Pawluski et al., 2006b: Ch 3). As mentioned previously, pup-exposure to nulliparous females, resulting in sensitization, enhances reference memory performance when compared to nulliparous females (Kinsley et al., 1999). In addition, Lambert et al. (2005) have shown that primiparous and nulliparous females exposed to pups for 21 days have some improvements on reference memory performance. These findings suggest that the enhancement in spatial memory with motherhood is due to cues associated with pup-exposure, and/or pup-directed maternal behaviors, and not pregnancy alone. However, the findings of Lambert et al. (2005) are not consistent across trials, do not take into account changes in hormone levels, and use measures of latency and not path length for memory performance (Lambert et al., 2005). Interestingly, recent
research has shown that both pregnancy and pup-exposure are important for the enhancement in spatial memory performance seen in primiparous dams (Ch 3; Pawluski et al., 2006b).

The next two chapters in this thesis aim to expand research findings on changes in spatial memory in the mother. Chapter 2 describes the role of reproductive experience on spatial reference and working memory performance in the dam at the time of weaning, taking into account the effects of motor ability, proestrus, and maternal behavior. Chapter 3 describes the role of reproductive experience on spatial reference and working memory performance in the dam long after the time of weaning, taking into account the effect of pregnancy and pup-exposure alone, motor ability, proestrus, and maternal behavior.

1.3 HIPPOCAMPUS PLASTICITY IN THE MOTHER

As mentioned previously, there is a large degree of neural plasticity with the onset of motherhood (Numan, 2007; Oatridge et al., 2002). Nonetheless, hippocampus plasticity in the mother has only recently been investigated (Kinsley et al., 2006; Lemaire et al., 2007; Pawluski and Galea, 2006; Tomizawa et al., 2003). Tomizawa et al. (2003) were perhaps the first to show that hippocampal neurotransmission is altered with motherhood. They found that multiparous mice exhibit increased long-lasting long term potentiation (LTP) along the Schaffer collaterals connecting the CA1 region of the hippocampus to the CA3 during the early postpartum period (3 days) compared to nulliparous mice. Interestingly, Tomizawa et al. (2003) suggest that oxytocin may be an important mediator of this effect, as oxytocin enhances LTP in the hippocampus of nulliparous mice (Tomizawa et al., 2003). Further research has shown that this increased LTP with motherhood exists two weeks after weaning and well into aging (Lemaire et al., 2007).

Hippocampus dendritic morphology is also altered with motherhood and reproductive experience (Kinsley et al., 2006; Pawluski and Galea, 2006). Kinsley et al. (2006) have found
that late pregnant and early lactating dams have increased spine density in the CA1 apical region of the hippocampus, and this is due to increased levels of estradiol and progesterone that are evident during late pregnancy. This is not surprising as CA1 apical spine density increases with high levels of estradiol in the virgin female (Woolley et al., 1990; Woolley and McEwen, 1993). Recent work has also shown that primiparous rats, at weaning, exhibit decreased dendritic lengths and number of branch points in the CA1 and CA3 pyramidal neurons of the hippocampus compared to nulliparous and multiparous rats (Pawluski and Galea, 2006). In this same study, multiparous rats were shown to exhibit enhanced spine density in the basal CA1 region of the hippocampus compared to nulliparous and primiparous rats (Pawluski and Galea, 2006). This enhanced spine density correlated with number of male pups in a litter (Pawluski and Galea, 2006). Interestingly, changes in the morphology of CA1 pyramidal neurons with parity are not evident in aged rats, suggesting that these changes are reversible (Love et al., 2005).

The dentate gyrus of the hippocampus is one of two areas in the brain where adult neurogenesis occurs at a high rate (Altman, 1962; Cameron and McKay, 2001: the other area being the subventricular zone). Adult neurogenesis in the hippocampus was discovered in 1962 (Altman, 1962) and although research is continually investigating the mechanisms responsible for changes in neurogenesis as well as the function of these new neurons, our knowledge of these processes is limited. Neurogenesis consists of at least two processes: cell proliferation, defined as the birth of new cells, and cell survival, defined as new cells that survive to maturity. Factors that affect cell proliferation either suppress or induce mitosis in precursor cells and factors that affect cell survival either promote or prevent the differentiation and/or maturation of labeled cells into mature neurons (Ormerod and Galea, 2001). Therefore, increasing cell proliferation as well as enhancing the survival of new cells can increase the number of new neurons.
The regulation of adult hippocampal neurogenesis is affected by a number of factors including sex of the subject, steroid hormones, stress, age, and environmental enrichment (Cameron and Gould, 1994; Galea and McEwen, 1999; Montaron et al., 2006; Ormerod et al., 2003; van Praag et al., 1999). These factors may differentially affect cell proliferation and cell survival. For example, high estradiol increases cell proliferation (Tanapat et al., 1999), but this effect is time-dependent, such that estradiol increases (within 4h) and then decreases (within 48h) cell proliferation in the dentate gyrus (Ormerod and Galea, 2001). Estradiol also enhances cell survival independently of cell proliferation (Ormerod et al., 2004). Interestingly, the mechanism of estradiol’s suppression of cell proliferation is, at least, partially dependent on the stimulation of adrenal steroids by estradiol (Ormerod et al., 2003). Both exposure to stress and elevated levels of corticosterone, suppress both cell proliferation and survival (Cameron and Gould, 1994; Mirescu and Gould, 2006; Wong and Herbert, 2004).

New neurons produced in the hippocampus of adult rodents are considered functional as they make appropriate synaptic connections and are electrophysiologically active (Zhao et al., 2006). The behavioral function of new neurons in the hippocampus of adult rodents is more controversial but they appear to play a role in spatial learning and memory performance (Winocur et al., 2006). Significantly reducing neurogenesis in the dentate gyrus of the hippocampus using the antimitotic agent methylazomethanol acetate (MAM) or focal irradiation results in deficits in performance on hippocampus-dependent tasks (Shors et al., 2002; Winocur et al., 2006). However, it appears that an optimal amount of hippocampal neurogenesis is needed for improved spatial memory. For example, very low or very high levels of hippocampal neurogenesis in rodents, which are evident after cortical and hippocampal lesions (Cameron et al., 1995; Gould and Tanapat, 1999) and seizures (Scarffman, 2004), are associated with reduced,
not improved, hippocampus-dependent learning and memory (Leung and Shen, 2006; McNamara et al, 1992).

Given the dramatic changes in steroid hormones during pregnancy and the postpartum and altered hippocampus-dependent learning and memory in the mother, it is perhaps not surprising that pregnancy and motherhood may alter adult neurogenesis in the hippocampus of the dam. However, very little research has investigated hippocampal adult neurogenesis with reproductive experience. Pregnancy has been suggested to enhance cell survival in the dentate gyrus (Banasr et al., 2001), but has no significant effect on cell proliferation in the dentate gyrus of the dam on gestation days 7 and 21 (Furato and Bridges, 2005; Shingo et al., 2003). However, increased cell proliferation is evident in the subventricular zone of the dam during pregnancy and the early postpartum period compared to nulliparous controls (Furato and Bridges, 2005; Shingo et al., 2003). The postpartum period has recently been associated with decreased cell proliferation in the hippocampus on postpartum day 2 and 8 compared to diestrus nulliparous controls (Leuner et al., 2007). Leuner et al. (2007) have also shown that the decrease in cell proliferation during the early postpartum period is dependent on pup-exposure and corticosterone levels. However these data did not take into account the role of reproductive experience, changes in cell survival across the postpartum period, the possible effect of pup-exposure, and maternal behavior on adult neurogenesis in the hippocampus of the mother.

Chapter 4 describes how reproductive experience affects adult neurogenesis in the hippocampus by investigating both cell proliferation and the survival of these new cells during the postpartum period in the mother, taking into account any changes resulting from the effects of pregnancy or pup-exposure alone, and maternal behavior.
1.4 CORTICOSTERONE AND CORTICOSTEROID BINDING GLOBULIN IN THE MOTHER

Steroid hormone levels fluctuate remarkably in the female during pregnancy and lactation. In the rodent, estradiol is elevated during late pregnancy (Garland et al., 1987; Rosenblatt et al., 1979; Yoshinaga et al., 1969) and remains low during lactation (Hapon et al., 2003) while progesterone is elevated during pregnancy, decreases prior to parturition, and is elevated during mid-lactation in lactating dams (Gala and Westphal, 1965; Hapon et al., 2003; Nicholas and Hartmann, 1981). Basal corticosterone initially decreases during pregnancy, increases near the time of parturition (Atkinson and Waddell, 2005; Gala and Westphal, 1964), and is elevated during the postpartum period in the early part of the light cycle in lactating dams (Fischer et al., 1995; Stern et al., 1973). As well, the circadian rhythm of corticosterone during early lactation is blunted compared to the nulliparous and pregnant rats, with a 50% decrease in mean corticosterone level from late pregnancy to early lactation (Atkinson and Waddell, 1995).

Corticosteroid binding globulin (CBG), the globulin that binds to corticosteroids and alters their bioavailability to target tissues (Hammond, 2002; Mendel, 1989), is also altered at this time (Gala and Westphal, 1965; Koch, 1969; Pearlman et al., 1981; Raymoure and Kuhn, 1983). CBG is elevated during mid pregnancy, declines markedly at parturition compared to the nulliparous and pregnant rats, and remains low during lactation until pups are weaned (Gala and Westphal, 1965; Koch, 1969; Pearlman et al., 1981; Raymoure and Kuhn, 1983).

Given the marked changes in steroid hormones during pregnancy and the postpartum period, it is not surprising that maternal behavior in the dam is, at least partially, dependent on hormones. It is the rise in estradiol and prolactin, and decrease in progesterone during late gestation that is important for the initiation of maternal behavior in the dam (Mann and Bridges, 2001). However, pup-exposure alone can activate maternal responding in maternally experienced...
dams (Orpen and Fleming, 1987) and virgin females (Rosenblatt, 1967). Interestingly, the modulation of some maternal behaviors in the dam is partially dependent on corticosterone levels during the postpartum period (Hennessy et al., 1977; Rees et al., 2004; Thoman et al., 1970). In the 1970s, Thoman et al. (1970) and Hennessy et al. (1977) found that corticosterone depletion via adrenalectomy resulted in deficits in maternal responding. Recent work from Alison Fleming’s laboratory has shown that the modulation of maternal behavior and the solidification of maternal memory are dependent on the level of corticosterone (Graham et al., 2006; Rees et al., 2004). However, the effect of corticosterone on maternal responding may be less dramatic with increased parity (Thoman et al., 1970).

To date, no work has investigated whether there are changes in corticosterone and CBG profiles of primiparous and multiparous rats during late pregnancy and the postpartum period. It is possible that elevated levels of corticosterone may occur during the first experience with pups in order for the induction of memory for maternal behaviors and these potentially dramatic changes in corticosterone may not be observed in multiparous rats. The remarkable physiological changes in the corticosterone and CBG levels during pregnancy and the postpartum period may also play an important role on hippocampus-dependent spatial memory and hippocampal plasticity in the mother. Chapter 5 describes how reproductive experience affects circulating levels of corticosterone and CBG during late pregnancy, the postpartum period, and after weaning, taking into account how pregnancy, pup-exposure, and maternal behaviors may affect these changes.

1.5 OVERVIEW AND OBJECTIVES

The experiments described in this thesis investigate the effects of motherhood and reproductive experience on hippocampus-dependent learning and memory, hippocampus
neurogenesis, and corticosterone and CBG levels in the dam and determine the potential role of maternal behavior on these effects. The objectives of the present thesis are as follows:

1. To determine whether hippocampus-dependent spatial learning and memory in the dam is affected by reproductive experience (Chapter 2). The experiments described in this chapter will expand the finding that motherhood enhances spatial learning and memory performance (Kinsley et al., 1999). In particular, the effect of reproductive experience (nulliparity, primiparity, and multiparity) on spatial working and reference memory performance in the working/reference memory version of the radial arm maze will be determined at the time of weaning. The specific hypotheses are that working and reference memory will be enhanced with motherhood and these enhancements will correlate with duration of maternal behavior such that dams that have increased maternal behavior will exhibit improved spatial memory performance.

2. To determine whether changes in hippocampus-dependent spatial learning and memory in the dam with reproductive experience persist after weaning, and whether the changes are the result of pregnancy and/or pup-exposure alone (Chapter 3). The experiments described in this chapter will determine whether changes in learning and memory performance in the working/reference memory version of the radial arm maze with reproductive experience (Ch 2) persist after the time of weaning. In addition, the role of pregnancy and pup-exposure alone on these effects will be assessed. The specific hypotheses are that there will be long-lasting effects of motherhood on spatial learning and memory performance and these enhancements will be due, in part, to pup-exposure. It is also expected that degree of maternal behavior during the first postpartum week will be associated with changes in learning and memory performance in the dam.

3. To determine whether adult hippocampus neurogenesis is altered in the dam during the postpartum period, and whether these changes are affected by reproductive experience,
pregnancy alone and/or pup-exposure (Chapter 4). The experiments described in this chapter will determine whether motherhood and reproductive experience alter hippocampus neurogenesis. Cell proliferation during the early postpartum period and the survival of new cells across the postpartum period will be determined. Given the changes in steroid hormones with lactation and the increased learning environment with motherhood, the specific hypotheses are that parous dams will have decreased rates of cell proliferation and possibly cell survival in the postpartum period. In addition, it is expected that reproductive experience will differentially affect hippocampus neurogenesis in the dam.

4. To determine the extent of changes in corticosterone and CBG levels in the dam during late gestation and the postpartum period, and whether these changes are affected by reproductive experience, pregnancy alone and/or pup-exposure (Chapter 5). Previous work has shown that basal corticosterone and CBG are altered in the dam during pregnancy and lactation (Gala and Westphal, 1965; Stern et al., 1973). The experiment described in this chapter will determine whether there are changes in basal corticosterone and CBG during late pregnancy and the postpartum period with reproductive experience. The specific hypotheses are that primiparous, multiparous and possibly sensitized rats will have elevated levels of basal corticosterone during the postpartum period compared to pregnant-only and nulliparous rats. It is also expected that primiparous rats will have elevated levels of corticosterone compared to multiparous rats and that CBG will be reduced during lactation.

Chapters 2-5 will describe experimental data collected to address the objectives. All experimental data have been published or submitted for publication (Pawluski et al., 2006a, b; Pawluski and Galea, submitted; Pawluski et al., submitted) and are in manuscript form. The General Discussion (Ch 6) discusses the effect of reproductive experience on hippocampus plasticity and function, and the possible role of corticosterone on these effects.
1.6 REFERENCES


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CHAPTER 2

REPRODUCTIVE EXPERIENCE DIFFERENTIALLY AFFECTS SPATIAL REFERENCE AND WORKING MEMORY PERFORMANCE IN THE MOTHER

2.1 INTRODUCTION

Successful reproduction in most mammals is contingent upon the mother learning a suite of behaviors necessary to maintain survival of the offspring until they disperse. The initial acquisition of maternal behavior (i.e., licking, grooming, and pup retrieval) appears to rely on the remarkable hormonal changes that occur during the first pregnancy and postpartum period (Garland et al., 1987; Rosenblatt et al., 1979; Rosenblatt et al., 1988; Yoshinaga et al., 1969) and result in permanent neural changes in the maternal circuitry of the new mother (Fleming et al., 1999; Modney and Hatton, 1994). Once these maternal behaviors are acquired, they are retained for future mothering and more efficiently implemented with subsequent reproductive experience (Bridges, 1975; Moltz and Robbins, 1965; Noirot, 1972).

Apart from the dramatic changes in behavior with parity and mothering, evidence suggests that reproduction affects the hippocampus, an area not traditionally thought of as a neural substrate of mothering. Hippocampus-dependent spatial learning and memory (Kinsley et al., 1999) and oxytocin-induced long-term potentiation (Tomizawa et al., 2003) have been shown to be augmented in the mother. Kinsley et al. (1999) showed that multiparous rats have enhanced working memory performance and primiparous rats have enhanced reference memory

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1 A version of this chapter has been published. Pawluski JL, Walker SK, Galea LA. (2006). Reproductive experience differentially affects spatial reference and working memory performance in the mother. Horm Behav. 49(2):143-149.
performance compared to nulliparous rats, but it is not clear whether some of these effects are due to motor or mnemonic processes. In addition, Tomizawa et al. (2003) found that oxytocin significantly enhanced Schaffer collateral long-term potentiation in multiparous mice compared to nulliparous mice. Though these findings are intriguing, the performance of primiparous and multiparous rats on the same spatial task has yet to be compared directly. In addition, neither study fully accounted for the possible effect of reproductive experience with full mothering (in some instances, performance was assessed 3 or 16 days after parturition), proestrus stage (Warren and Juraska, 1997), or degree of maternal behavior on spatial working and reference memory.

When referring to reproductive experience and maternal behavior, it is important to note that the initial reproductive experience (primiparity and mothering) results in behavioral, hormonal, and neural changes in the mother that differ with subsequent reproductive experience (multiparity and mothering). Behaviorally, maternal responsiveness is augmented in multiparous rats (Moltz and Robbins, 1965), and intensity of postpartum aggression is greater in multiparous than primiparous mice (Svare and Gandelman, 1976). Hormonally, basal prolactin levels are significantly higher in primiparous than in multiparous rats (Bridges and Hammer, 1992) and neural sensitivity to opioids is reduced with increased parity in the rat (Kinsley and Bridges, 1988). Neurally, hypothalamic astrocyte number is increased in multiparous rats (Featherstone et al., 2000) and there is increased fos-like immunoreactivity in the maternal circuit of maternally experienced rats exposed to pups compared to inexperienced rats (Fleming and Korsmit, 1996).

Duration of specific maternal behaviors affects spatial working memory in offspring during adulthood (Liu et al., 2000). Biological and foster offspring of mothers that spend more time licking, grooming, and arched-back nursing (high LG-ABN) have enhanced spatial reference memory in adulthood compared to offspring of low LG-ABN mothers (Liu et al.,
2000). In turn, this degree of maternal behavior is transmitted from the nursing, and not the biological, mother to the daughter (Champagne and Meaney, 2001). Given this information, it seems plausible that high LG-ABN mothers may exhibit enhanced spatial reference memory compared to low LG-ABN mothers.

The present study used the spatial working/reference version of the radial arm maze, which enables both working and reference memory to be assessed within the same task (Olton and Papas, 1979). It is expected that motherhood will result in enhanced working and reference memory. Specifically, the present study was undertaken to investigate the effect of reproductive experience (primi- and multiparity and mothering) on performance in the spatial working/reference version of the radial arm maze, controlling for stage of estrous cycle and degree of maternal behavior. Working memory will be defined as the manipulation and retrieval of trial-unique information that is used to guide prospective action and reference memory will be defined as a long-term stable memory (Olton and Papas, 1979).

2.2 METHOD

Animals

Twenty-two female Long–Evans rats (approximately 3 months of age) (Charles River, St.-Constant, Quebec, Canada) were used in the study. Rats were initially housed in pairs in opaque polyurethane bins (48 × 27 × 20 cm) with absorbent bedding and were given Purina rat chow and tap water ad libitum. Rats were maintained in a 12h:12h light/dark cycle (lights on at 7:30 a.m.). All protocols were in accordance with ethical guidelines set by the Canada Council for Animal Care and the University of British Columbia Animal Care Committee.

Rats were randomly assigned to one of three conditions: nulliparous (n = 8), primiparous (n = 7), and multiparous (n = 7). Nulliparous rats were not sexually experienced. Primiparous
rats gave birth once and mothered for 25 days. Multiparous rats birthed twice and mothered twice for 25 days. The births were timed so that primiparous rats birthed when multiparous rats birthed their second litter. All animals were age-matched and nulliparous rats were housed undisturbed in a separate room from pregnant and mothering rats.

**Breeding**

For breeding, two females and one male were housed in large opaque polyurethane bins. The males remained with the females for 14 days. After the male was removed, the females were housed individually in clear polyurethane bins until Day 8 postpartum. The females gave birth 8 to 11 days after the removal of the male and 20–30 h after parturition the size and number of male and female offspring of all litters were recorded and litters were culled to 4 male and 4 female pups. The mother and her pups were housed in large opaque polyurethane bins (51 x 41 x 22 cm) 8 days after parturition.

**Maternal Behavior Testing**

The frequency and duration of the following maternal behaviors were observed during each testing period based on previous work (Champagne et al., 2003; Lovic et al., 2001; Myers et al., 1989): retrieval (carrying a pup to a nest site); licking/grooming (body licking and genital licking whether or not mother was hovering over pups); nest building; arched-back nursing; mouthing (carrying pups in the cage but placing them back at the initial pick up site); self-grooming. For maternal tests, pups were placed in the cage in the diagonally opposite corner of the nest site at each testing period. Observations were made for 10 min once between 9 a.m. and noon on Days 1 to 8 post-parturition, for a total of 8 tests. Data for each behavior were compiled across all test periods. In addition, licking/grooming and arched-back nursing (LG-ABN) behavior was summed to create a single LG-ABN variable. Data from two animals were not available due to equipment failure.
Radial Arm Maze Apparatus and Procedure

Two to 4 days after weaning, all multi-, primi-, and nulli-parous rats (approximately 6 months in age) were habituated on the spatial working/reference memory version of the radial arm maze (Olton and Papas, 1979; Holmes et al., 2002). The eight-arm radial arm maze was elevated 80 cm from the floor, with arms (53 cm long x 10 cm wide) projecting at equal angles from an octagonal center platform (36 cm in diameter). It was located in a room with multiple extra-maze cues that remained in constant positions throughout the duration of the experiment; however, the maze was randomly rotated every 3 days to minimize the use of intra-maze cues. Rats were tested between 1 p.m. and 3 p.m. daily. All rats were introduced to the food reward (Fruit Whirls; Glencourt Distributors, Calgary, Alberta, Canada) in their home cages for 3 days prior to habituation. The maze was cleaned with ethyl alcohol after each rat. Rats were food deprived to 85% their body weight beginning on the first day of habituation.

All rats were habituated to the maze for a total of 25 min over 3 days. Habituation began by placing a rat on the center platform and allowing it to freely explore the maze. After habituation, each rat was randomly assigned a separate pattern of baited and non-baited arms (four baited arms out of eight possible arms). This pattern remained constant for each rat for the duration of the experiment. The rats were shaped for 2 days after habituation. Shaping consisted of the rat being released on the center of the platform with access to all arms. The assigned arms were each baited with four quarters of Fruit Whirl placed at equidistant intervals along the arm, thus shaping the rat to move along the entire length of the arm. Shaping took place until either 10 min had elapsed or the rat had entered all the baited arms. An arm was considered entered when the front limbs of the rat crossed halfway down the distance of the arm. Training sessions, one per day, consisted of the rat being released on the center of the platform and remaining on the maze until all baited arms had been entered or until 10 min had elapsed. During the training
session, the end of the arm was baited with one quarter of a Fruit Whirl. All rats were trained for 24 consecutive days.

Rats could make three types of errors during each training session: (1) reference memory errors (RME) defined as entries into non-baited arms, (2) working memory errors (WME) defined as repeat entries into baited arms, and (3) working/reference memory errors (W/RME) defined as repeat entries into non-baited arms. Total RME, WME, and W/RME, number of days to reach criterion (defined as no more than 1 error per day for 2 consecutive days), and total number of choices to criterion were calculated.

**Estrous Cycle**

Rats in proestrus are more likely to make spatial errors in a Morris Water Maze (Warren and Juraska, 1997). Thus, stage of estrous cycle was determined from daily vaginal swabs beginning on the day of habituation and continuing until the end of testing. Swabs were taken in all rats by placing a cotton swab in the vagina and smearing the contents of the swab on a plain slide. Slides were examined under 10x objective and proestrus was determined when a majority of cells evident in the vaginal mucus (approximately 70%) were nucleated epithelial cells.

**Data Analyses**

A repeated-measures analysis of variance (ANOVA) test was calculated on number of errors per day (RME, WME, and W/RME) with condition (nulliparous, primiparous, multiparous) as the between-subjects variable and error type (RME, WME, W/RME) and number of days (1–24) as the within-subjects variables. Analysis of variance (ANOVA) tests were calculated for the dependent variables: days to reach criterion, total errors to reach criterion, and average latency to reach each arm, with condition (nulliparous, primiparous, multiparous) as the between-subjects variable and number of days (1–24) as the within-subjects variable. To determine effects of estrous cycle, an analysis of covariance was calculated on number of errors.
across days using stage of estrous cycle as the covariate. To determine differences in duration of maternal care between primiparous and multiparous rats, t tests were performed between duration of a maternal behavior (licking, nursing, etc.) and condition (primiparous and multiparous). To determine whether there was a relationship between duration of maternal behavior (LG-ABN) and learning and memory performance, Pearson product–moment correlations were conducted between duration of LG-ABN and RME or WME. Post hoc comparisons utilized the Newman Keul's procedure.

2.3 RESULTS

Primiparous rats made fewer errors, regardless of type, than nulliparous rats, and multiparous had a trend to perform fewer errors than nulliparous rats. Figure 2.1 displays the total number of errors (±SEM). Figure 2.2 displays the total number of (a) reference memory errors (RME), (b) working memory errors (WME), and (c) working/reference memory errors (W/RME). Primiparous rats made significantly fewer errors, regardless of error type, than nulliparous rats (p ≤ 0.04), with a trend for multiparous rats to make fewer errors, regardless of error type, than nulliparous rats (p ≤ 0.07) and no significant difference between primiparous and multiparous rats in total number of errors (p ≤ 0.48; main effect of condition (F(2,19) = 3.95, p ≤ 0.037). There was a significant interaction effect between day and error (F(46,874) = 1.5, p ≤ 0.02), a main effect of day (F(23,437) = 3.90, p ≤ 0.00001), indicating that the number of errors decreased as the number of days increased, and a main effect of error (F(2,38) = 389.87, p ≤ 0.000001) indicating that rats made more RME than both WME (p ≤ 0.00012) and W/RME (p ≤ 0.00012), and more WME than W/RME (p ≤ 0.007). There were no other significant interaction effects (0.28 ≤ p ≥ 0.81).
Figure 2.1 Mean (± SEM) for total number of errors on the working/reference memory version of the radial arm maze. Primiparous rats made significantly fewer errors, regardless of error type, than nulliparous rats (p ≤ .04), with a trend for multiparous rats to make fewer errors, regardless of error type, than nulliparous rats (p ≤ .07) and no significant difference between primiparous and multiparous rats in total number of errors (p ≤ .48). n=7-8. (* denotes significantly different from nulliparous rats.)
Figure 2.2 Mean (± SEM) for total number of (a) reference memory errors (RME), (b) working memory errors (WME), and (c) working/reference memory errors (W/RME). Rats made more RME than WME (p ≤ .00012) and W/RME (p ≤ .00012), and more WME than W/RME (p ≤ .007). n= 7-8.
There was no significant main effect of condition on number of days to reach criterion ($p \leq .51$; Table 2.1) or total choices to criterion ($p \leq .39$; Table 2.1). To evaluate whether there were significant differences between conditions in radial arm maze performance prior to training, an analysis was conducted on the first day of training only. There was no significant effect of condition for the first day of training ($p \leq .51$). For average latency to reach each arm, there was no main effect of condition ($p \leq .47$), nor a significant interaction effect ($p \leq .65$) indicating that there were no motivational/motor differences between conditions. There was, however, a significant main effect of day ($F(23, 437) = 8.47, p < .001$), indicating that the average latency to reach each arm decreased as the number of days increased.

**Table 2.1** Mean (± S.E.M.) number of days to reach criterion and number of total choices to criterion in nulliparous, primiparous, and multiparous rats.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Day to Criterion</th>
<th>Total Choices to Criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulliparous</td>
<td>18.38 ± 2.02</td>
<td>154.38 ± 21.01</td>
</tr>
<tr>
<td>Primiparous</td>
<td>14.57 ± 3.41</td>
<td>111.00 ± 24.59</td>
</tr>
<tr>
<td>Multiparous</td>
<td>19.14 ± 3.18</td>
<td>147.29 ± 23.99</td>
</tr>
</tbody>
</table>

Stage of estrous cycle did not account for differences in working and reference memory performance with reproductive experience. Analysis of covariance on number of errors with proestrus as a covariate across days did not significantly alter the above findings, as primiparous rats made significantly fewer total errors ($p \leq .03$) and multiparous rats tended to make fewer total errors ($p \leq .06$) than nulliparous rats. There was also no significant effect of the covariate. In addition, there was no significant difference in total number of days across testing spent in proestrus between conditions ($p \leq .72$).

**Duration of maternal behavior (LG-ABN) positively correlates with spatial reference memory performance.** Total licking and nursing behavior (LG-ABN) was compiled and a significant positive correlation was found between duration of LG-ABN and RME ($r = .57, p \leq .05$; Figure 2.3), showing that more time spent licking and nursing offspring was associated with
increased RME in the mother. There was no correlation between duration of LG-ABN and WME ($p \leq .73$).

For total duration of individual maternal behaviors, there was a trend for multiparous rats to spend more time licking their pups than primiparous rats ($p \leq .07$). However, there were no significant differences in nursing ($p \leq .16$), retrieval ($p \leq .81$), mouthing of pups ($p \leq .56$), self grooming ($p \leq .83$), or nest building ($p \leq .43$) between primiparous and multiparous rats.\(^2\)

![Figure 2.3 The association between duration of licking, grooming, and nursing (LG-ABN) and reference memory errors in the mother. There was a significant positive correlation between duration of LG-ABN and RME ($r=.57$, $p \leq .05$). More time licking, grooming, and nursing offspring was coincident with increased RME in the mother. $n=12$.](image)

\(^2\) For information on litter characteristics at birth see Appendix 1.
2.4 DISCUSSION

The present results show that reproductive experience alters both working and reference memory performance. Relative to nulliparous rats, primiparous rats, who gave birth and mothered until weaning showed an enhancement in both working and reference memory performance on the spatial working/reference memory version of the radial arm maze. These group differences in behavior were independent of age, the effects of proestrus, and latency to reach goal arm, indicating that the differences between the groups were likely due to mnemonic, not motor, processes. These findings demonstrate that parity and mothering affect spatial learning and memory long after birth (as training and testing began 25 days after birth and continued for 30 days).

Reproductive experience results in marked changes in learning and memory performance.

Our findings are consistent with and extend previous work that has found that motherhood improves learning and memory performance (Kinsley et al., 1999). Kinsley et al. (1999) report that multiparous rats, with full maternal experience (21 days), had enhanced working memory performance and primiparous rats, with 16 days of maternal experience, had enhanced reference memory performance (using a latency score) compared to nulliparous rats. However, the latency measure for reference memory performance may reflect differences in motor/motivational processes rather than mnemonic processes and, unlike the present study, they did not directly compare learning and memory performance of primiparous and multiparous rats with full maternal experience on the same task. The present study investigated the effect of parity (multi- and primi-parity) and full mothering experience on learning and memory performance in the same task and monitored for the effects of estrous cycle (which can contribute to differences in learning and memory performance; Warren and Juraska, 1997). We found that primiparous
rats exhibit enhanced spatial memory when compared to nulliparous, and multiparous rats showed a trend for enhanced spatial memory performance compared to nulliparous rats. Although multiparous rats did show a reduced number of errors for both working and working/reference memory compared to nulliparous rats, the effect on reference memory was very weak. This partially confirms and further extends the findings of Kinsley et al. (1999) indicating that there is indeed an effect of reproductive experience on learning and memory, with enhanced working and reference memory particularly after the initial reproductive experience.

This enhanced spatial cognition in primiparous mothers may be a result of neural changes that occur with the initial acquisition of maternal behaviors. Improvements in reference memory may aid in remembering food caches in the intricate burrow system and enhanced working memory may be essential for short-term memory of food supplies (Calhoun, 1963). Food caching activity in the mother was found to be three times higher during the first 20 days of mothering than at the time of weaning (days 21–30) (Calhoun, 1963) suggesting the effective foraging may be required to return to the nest and care for and protect young especially in new mothers. Once reproduction is successful and pups have dispersed from the nest, subsequent maternal experience may be less demanding as the mother has learned the essential maternal behaviors needed for reproduction. Thus, with subsequent mothering, the induction of maternal behaviors may be more rapid, and the mother may not need to spend as much time with her offspring and therefore the need to efficiently forage is not as great a requirement.

Possible hormonal correlates of enhanced learning and memory with first reproductive experience.

Among other hormones, estradiol, oxytocin, and corticosterone influence the development of maternal behavior in the rat (Argiolas and Gessa, 1991; Gainer and Wray, 1994;
Moltz et al., 1970; Pedersen et al., 1982; Rees et al., 2004) and have been shown to affect spatial working and memory performance (Bowman et al., 2001; Galea et al., 1995; Holmes et al., 2002; Tomizawa et al., 2003). Oxytocin has been shown to enhance reference, but not working, memory in the nulliparous mouse and administration of an oxytocin antagonist to multiparous mice resulted in deficits in reference, but not working, memory performance compared to controls (Tomizawa et al., 2003). Thus, it may be oxytocin that is mediating the changes we see in reference memory performance of primiparous rats through either increased oxytocin synthesis, or receptor expression in the hippocampus. For the facilitation of working memory seen with first mothering experience, corticosterone may play a role. Chronic stress, which is associated with an elevation in corticosterone, results in facilitated spatial working memory in the adult female rat (Bowman et al., 2001). In addition, basal corticosterone levels are elevated during lactation (Douglas et al., 2003; Stern et al., 1973). Further work is required to understand the intricate relationship between reproductive experience, spatial working and reference memory performance, and oxytocin and corticosterone action in the hippocampus, as well as other brain regions such as the prefrontal cortex (Kolb and Cioe, 1996).

**Degree of maternal behavior and spatial learning and memory performance in the mother**

Degree of maternal behavior, either high or low LG-ABN, has been associated with hippocampus-dependent learning and memory performance in offspring during adulthood such that offspring of high LG-ABN mothers have enhanced spatial reference memory compared to offspring of low LG-ABN mothers (Liu et al., 2000). Recent research has reported that environmental enrichment reverses the decrease in spatial reference memory in male offspring of LG-ABN mothers (Bredy et al., 2004). However, at present, it is unclear whether an enhancement in spatial learning and memory as a function of maternal care is evidenced in adult
female offspring (Bredy et al., 2004; Liu et al., 2000). Degree of maternal behavior has been shown to pass from generation to generation with female offspring in adulthood eliciting the degree of maternal behavior experienced during the neonatal period (Champagne and Meaney, 2001). The results of the present experiment suggest that more time licking and nursing offspring is related to an increase in reference memory errors in the mother. Further work should clarify the effect of degree of maternal behavior on spatial performance of adult female offspring as well as causative factors mediating the relationship between duration of LG-ABN and reference memory performance in the mother.

Conclusion

The present experiments provide new evidence that reproductive experience differentially affects hippocampus-dependent spatial working and reference memory performance. Enhanced learning and memory with the initial reproductive experience (primiparity and mothering) may be mediated by hormonal, behavioral, and neural changes and may be required for the new mother to learn a suite of behaviors necessary to ensure offspring survival and achieve reproductive success with subsequent reproductive experience.
2.5 REFERENCES


CHAPTER 3
FIRST REPRODUCTIVE EXPERIENCE PERSISTENTLY AFFECTS SPATIAL REFERENCE AND WORKING MEMORY IN THE MOTHER AND THESE EFFECTS ARE NOT DUE TO PREGNANCY- OR ‘MOTHERING’- ALONE

3.1 INTRODUCTION

Pregnancy and motherhood are life-altering events that result in hormonal, neural and behavioral changes necessary to ensure reproductive success. Although research has investigated the hormonal, neural, and behavioral alterations related to maternal behaviors such as licking, grooming, and nursing (Numan and Insel, 2003 for review), only recently has research began to thoroughly investigate other hormonal, neural and behavioral changes during motherhood relate to learning and memory including social learning (Fleming et al., 1994) and spatial learning and memory (Kinsley et al., 1999; Pawluski and Galea, 2006).

Hippocampus-dependent spatial learning and memory performance during motherhood is crucial for foraging behavior and as such has been the focus of recent investigation (Gatewood et al., 2005; Kinsley et al, 1999; Lambert et al., 2005; Love et al., 2005; Pawluski et al., 2006; Tomizawa et al., 2003). In order for efficient foraging both working and reference memory are needed. Working memory can be defined as the manipulation and retrieval of trial-unique information used to guide prospective action (Baddeley, 2003) and reference memory can be defined as long-term stable memory (Olton and Papas, 1979). In the natural environment,

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3 A version of this chapter has been published. Pawluski JL, Vanderbyl BL, Ragan K, Galea LA. (2006). First reproductive experience persistently affects spatial reference and working memory in the mother and these effects are not due to pregnancy or ‘mothering’ alone. Behav Brain Res. 175(1):157-65.
improvements in spatial reference memory in the dam may be important for remembering the location of food caches in an intricate burrow system. In addition, improvements in working memory may be essential for shorter-term memory for the location of food that remains to be cached each day.

With the well-known changes in steroid hormones across pregnancy and lactation (Garland et al., 1987; Rosenblatt et al., 1988), and the well-documented effects of these hormones (primarily estradiol and corticosterone) on hippocampus-dependent learning and memory performance in virgin females (Bowman et al., 2001; Holmes et al., 2002), it is not surprising that there are changes in hippocampus function with pregnancy and motherhood. Kinsley et al. (1999) were the first to report that motherhood improves spatial working and reference memory. They found that primiparous rats outperformed nulliparous rats on a reference memory task, while multiparous rats outperformed nulliparous rats on a working memory task two weeks after weaning (Kinsley et al., 1999). Interestingly, Tomizawa et al. (2003) found that oxytocin is one possible mediator of improved hippocampus-dependent reference memory in mothers. They report oxytocin antagonist injections to multiparous mice blocked enhanced reference memory performance 3 days after birth and oxytocin injections to nulliparous mice improved reference, but not working, memory performance (Tomizawa et al., 2003).

Recent research has aimed to determine the impact of reproductive experience (number of times pregnant and mothered) as well as the duration of the enhanced spatial learning and memory performance resulting from pregnancy and motherhood (Gatewood et al., 2005; Love et al., 2005; Pawluski et al., 2006). We were the first to look at both working and reference memory performance concurrently in the multiparous, primiparous, and nulliparous rats after the normal time of weaning (Pawluski et al., 2006). We found that at the time of weaning, primiparous rats
had better spatial reference and working memory performance than nulliparous rats, while multiparous showed a tendency towards better spatial memory performance than nulliparous rats (Pawluski et al., 2006). With regards to reference memory performance the research reports are equivocal. Gatewood et al. (2005) found that multiparous rats were quicker than primiparous and nulliparous rats, while primiparous rats were quicker than nulliparous rats, on a reference memory task when repeatedly tested up to 24 months. It should be noted that these group differences may reflect motoric rather than mnemonic differences. Interestingly, Love et al. (2005) did not replicate this finding using the same task. However, they did report mild enhanced spatial reference memory in primiparous and multiparous rats compared to nulliparous rats when tested at younger ages (Love et al., 2005). Clearly, more work is needed to determine the duration and complexity of pregnancy and mothering on spatial working and reference memory.

Understanding the separate contribution of pregnancy or mothering on reference and working memory has been given little attention. Two studies have investigated the contribution of pregnancy and/or pup-exposure (nulliparous females exposed to pups) on reference memory performance but their findings are inconclusive (Kinsley et al., 1999; Lambert et al., 2005). Kinsley et al. (1999) report that sensitized rats were quicker to perform a reference memory task than nulliparous, but not primiparous rats (with mothering experience) after 16 days of pup-exposure. Contrary to these findings, Lambert et al. (2005) found no significant difference in performance between pup-exposed nulliparous rats, primiparous rats without mothering experience (pregnant-only), and nulliparous rats on the same reference memory task used in Kinsley et al. (1999). However, Lambert et al. (2005) did find that primiparous and nulliparous rats with mothering experience were quicker to perform aspects of a reference memory task compared to nulliparous and primiparous rats without pup exposure, indicating possible motoric rather than mnemonic differences between groups. Further research is needed to determine the
role of pregnancy and/or ‘mothering’ on both working and reference memory performance and the possible duration of these effects. Given that both pregnancy and mothering affect working and reference memory (Kinsley et al., 1999; Pawluski et al., 2006, Ch 2) and environmental enrichment has been shown to enhance working and reference memory (Leggio et al., 2005), it seems plausible that mothering and sensitization, arguably forms of enrichment, may, in part, contribute to enhanced spatial memory performance. There are obvious limitations to simulating ‘mothering’ via sensitization (i.e. lack of lactation), but even with limitations this ‘mothering’ paradigm will aid in determining whether improvements in learning and memory after pregnancy and mothering are due to enrichment effects of pup exposure.

In addition we have recently found that degree of licking and nursing behavior elicited by the mother toward her pups is associated with reference memory performance (Pawluski et al., 2006, Ch 2). We found that dams that spent more time licking and nursing offspring in the first week postpartum had poorer reference memory performance at the time of weaning (Pawluski et al., 2006, Ch 2). Whether or not the relationship between licking/nursing and reference memory performance in the dam persists after pup exposure has yet to be determined.

The present study is the first to determine whether enhanced spatial working and/or reference memory evident shortly after weaning in primiparous and multiparous rats (Pawluski et al., 2006, Ch 2) persists long after weaning and whether these effects are due, in part, to pregnancy and/or ‘mothering’-alone. In addition, this study aimed to investigate the role of maternal care, early in the postpartum, and estrous cycle effects, on memory performance. The present study tested five groups of rats; multiparous, primiparous, nulliparous, pregnant-only, and sensitized rats, approximately one month after weaning, or approximately 55 days after birth, on the spatial working/reference version of the radial arm maze. This task enables both working and reference memory to be assessed simultaneously (Olton and Papas, 1979). It is expected that
long after the time of weaning, primiparity and possibly sensitization, will result in enhanced working and reference memory compared to nulliparity and pregnancy-alone. It is also expected that multiparity will result in enhanced working, but not reference, memory performance compared to nulliparity and pregnancy-alone. It is also expected that reference memory performance may be associated with degree of maternal licking and nursing behavior in primiparous and multiparous dams as previously described (Pawluski et al., 2006, Ch 2).

3.2 METHOD

Animals

Thirty-seven female Sprague-Dawley rats (approximately 65-75 days of age) (UBC Animal Care Facility, Vancouver, Canada) were used in the study. Rats were initially housed in pairs in opaque polyurethane bins (48 x 27 x 20cm) with aspen chip bedding and were given Purina rat chow and tap water ad libitum. Rats were maintained in a 12h:12h light/dark cycle (lights on at 7:30 a.m.). All protocols were in accordance with ethical guidelines set by the Canada Council for Animal Care and the University of British Columbia Animal Care Committee.

Rats were randomly assigned to one of five conditions: nulliparous (n = 9), primiparous (n = 8), multiparous (n = 8), pregnant-only (n = 6) and sensitized (n = 6). All animals were age-matched at the time of testing. Nulliparous rats were not sexually experienced. Primiparous rats gave birth once and mothered once. Multiparous rats birthed twice and mothered twice. For both primiparous and multiparous rats, pups were weaned 21 days after birth. Pregnant-only rats had pups removed within 24 hours of birth. Sensitized rats were not sexually experienced, but were exposed to pups for a total of 21 days (for details see below). Nulliparous rats were housed undisturbed in a separate room from pregnant and mothering/sensitized rats. After weaning, all
animals were housed in the same room. All animals were age-matched: for example primiparous
and pregnant-only rats during their first and only gestational period were approximately the same
age as multiparous rats during their second gestational period. In addition, sensitization began
when nulliparous rats were the same age as primiparous, pregnant-only rats that had just given
birth to their first litter and multiparous rats that had just given birth to their second litter.
Nulliparous rats were age-matched to these animals.

Breeding

For breeding, one female and one male were paired in a wire mesh cage. Upon release of
a vaginal plug females were individually housed in clear polyurethane bins until birth. For
primiparous and multiparous rats, litters were culled to 4 male and 4 female pups approximately
24 hours after parturition. The dam and pups were housed in clear polyurethane bins until day 8
postpartum at which time they were housed in large opaque polyurethane bins (51 x 41 x 22cm)
until weaning (postpartum day 21). Litters were removed from pregnant-only rats within 24
hours of birth and pregnant-only rats were housed in a female-only colony room away from
pregnant and lactating rats.

Sensitization

Sensitization (pup-exposure in nulliparous rats) was modified from previous research
(Stern, 1997) in order to control for duration of mothering. Briefly, virgins were continuously
housed with 3 pups for 21 days. Age of pups at the start of sensitization was 5-8 days. The same
pups were used throughout the 21 days in order to simulate natural mothering. Every 24 hours,
milk replete pups were given to rats being sensitized, and the donor pups were replaced with
their biological mother. Only 3 pups were used as this has been found to be sufficient to elicit
maternal-type behaviors in virgin females (Stern, 1997). To confirm that sensitized rats were
acting maternally, licking of pups, hovering over pups, and pup retrieval were scored from daily
15 minute video-recordings taking place each morning (between 8 and 11am) immediately after new donor pups were introduced to the rats undergoing sensitization. Spot checks were performed approximately 1, 3 and 24 hours after the video-recording. Video recordings and spot checks took place for the first 10 days of exposure to pups or until sensitization occurred (licked and hovered over pups within a 24 hour period). If the rat injured a pup during the video recording the test was terminated for that day and all pups were removed. This occurred three times with three different rats. Sensitization/pup-exposure occurred for 21 consecutive days.

Maternal Behavior Testing

The frequency and duration of the following maternal behaviors were observed during each testing period based on previous work (Myers et al., 1989; Pawluski et al., 2006, Ch2): licking/grooming (body licking and genital licking with the dam off the pups); licking/grooming and nursing; arched-back nursing; “blanket” nursing; passive nursing; and time off pups.

For observation of maternal behavior, dam and pups were left undisturbed in the cage. Observations were made every 5 seconds for 10 minutes once between 9a.m. and noon on Day 1 post parturition and twice a day (between 9-11a.m. and 1-4p.m.) on days 2 to 5 post-parturition. Data for each behavior was compiled across all test periods as percent of total time spent in a scored behavior. In addition licking/grooming and arched-back nursing behavior was summed to create a single LG-ABN variable as previous research has shown that there is a correlation between duration of LG-ABN and number of reference memory errors (Pawluski et al., 2006, Ch 2). Data from one animal is not available due to experimenter error.

Radial Arm Maze Apparatus and Procedure

Thirty-four days after weaning/removal of pups or 55 days after parturition, all multiparous, primiparous, nulliparous, pregnant-only and sensitized rats were habituated on the spatial working/reference memory version of the radial arm maze (Olton and Papas, 1979;
Holmes et al., 2002). All animals were the same age at the habituation. The eight-arm radial arm maze was elevated 80 cm from the floor, with arms (53 cm long x 10 cm wide) projecting at equal angles from an octagonal center platform (36 cm diameter). It was located in a room with multiple extra-maze cues that remained in constant positions throughout the duration of the experiment. The maze was randomly rotated every three to four days to minimize the use of intra-maze cues. Rats were tested between 11 pm and 3 pm daily. All rats were introduced to the food reward (Fruit Whirls; Glencourt Distributors, Calgary, Alberta, Canada) in their home cage starting the day of habituation. The maze was wiped cleaned with 70% ethyl alcohol after each rat. Beginning on the day of habituation, all rats were food deprived to between 80-85% their body weight.

All rats were habituated to the maze for a total of 25 minutes over three days. Habituation began by placing a rat on the center platform and allowing it to freely explore the maze. After habituation, each rat was randomly assigned a separate pattern of baited and non-baited arms (four baited arms out of eight possible arms) that remained constant for each rat for the duration of testing. All rats were shaped for three days after habituation. Shaping consisted of the rat being released on the centre of the platform with access to all arms. The assigned arms were each baited with four quarters of Fruit Whirl placed at equidistant intervals along the arm and therefore shaping the rat to move along the entire length of the arm. Shaping took place until either 10 minutes had elapsed or the rat had entered all the baited arms. An arm was considered entered when the front limbs of the rat crossed midway down the arm. Training sessions occurred once per day and consisted of the rat being released on the centre of the platform, remaining on the maze until all baited arms had been entered or until 10 minutes had elapsed. During the training session, the end of the arm was baited with one quarter of a Fruit Whirl. All rats were trained for 24 consecutive days.
Rats could make at least two types of errors during each training session: 1) reference memory errors (RME) defined as entries into non-baited arms and 2) working memory errors (WME) defined as repeat entries into baited arms. For each rat, total RME and WME, number of days to reach criterion (defined as no more than 2 errors per day for 2 consecutive days), total number of choices to criterion and average latency to reach each arm were also calculated. The number of days the task was completed during testing (completed the task in less than 10 minutes) was also assessed.

**Estrous Cycle**

Rats in proestrus are more likely to make spatial errors in a Morris Water Maze (Warren and Juraska, 1997). Thus, day of proestrus during the estrous cycle was determined from daily vaginal smears beginning on the day of shaping and continuing until the end of testing. Smears were taken after testing was completed each day. Briefly, smears were taken in all rats by placing a cotton swab in the vagina and smearing the contents of the swab on a plain glass slide. Slides were examined under 10x objective and proestrus was determined when a majority of cells evident in the vaginal mucous (approximately 70%) were nucleated epithelial cells.

**Data Analyses**

A repeated-measures analysis of variance (ANOVA) test was calculated on RME and WME, and latency to reach an arm with condition (nulliparous, primiparous, multiparous, pregnant-only and sensitized) as the between subjects variable and error type (RME and WME) or latencies (s) and number of days (1–24) as the within subjects variables. ANOVA tests were calculated for each of the dependent variables: number of days the task was completed, days to reach criterion, total choices to reach criterion, average errors across days completed, errors to criterion, with condition (nulliparous, primiparous, multiparous, pregnant-only and sensitized) as the between subjects variable. Bartlett’s test for homogeneity of variance (HOV) was also
conducted on these variables. To determine effects of estrous cycle, an analysis of covariance (ANCOVA) was calculated on number of errors across test days using proestrus as the covariate. To determine differences in duration of maternal care, t-tests were performed between duration of a maternal behavior (licking, nursing, etc.) and condition (primiparous and multiparous). To determine differences in litter size, number of male, and number of female pups, t-tests were performed between litter size, number of male, and number of female offspring, and condition (pregnant-only, primiparous and multiparous). To determine whether there was a relationship between duration of maternal behavior (LG-ABN) in multi- and primi-parous or onset of ‘maternal behavior’ in sensitized rats and learning and memory performance, Pearson product-moment correlations were conducted between duration of LG-ABN or day of onset of ‘maternal behavior’ and RME or WME. Post hoc comparisons utilized the Fisher's LSD test. A priori tests utilized Dunnett's test.

3.3 RESULTS

Pregnant-only rats failed to complete the task on significantly more days than primiparous, multiparous, nulliparous and sensitized rats. Pregnant-only (gave birth, but did not mother) rats completed the task (i.e. entered the four baited arms) on significantly fewer days than all other groups (primiparous, p ≤ .036; multiparous, p ≤ .026; nulliparous, p ≤ .044; sensitized rats, p ≤ .022; main effect of condition (F(4, 32)=2.89, p=.038; Figure 3.1). There were no other significant differences between conditions (.59 ≤ p ≤ .91).
Figure 3.1 Mean (± SEM) total number of days the task was completed during testing. Pregnant-only rats spent significantly more days not completing the task than primiparous (p ≤ .036), multiparous (p ≤ .026), nulliparous (p ≤ .044), and sensitized rats (p ≤ .022).

Primiparous rats made fewer errors, regardless of type, than nulliparous, multiparous and sensitized rats one month after weaning. Figure 3.2 displays (a) total number of reference memory errors (RME), (b) number of reference memory errors (RME) across 4 blocks of 6 days, (c) total number of working memory errors (WME), and (d) number of working memory errors (WME) across 4 blocks of 6 days. A repeated-measures ANOVA on blocks of RME and WME with condition (nulliparous, primiparous, multiparous, and sensitized), as the between-subjects variable and error type (RME and WME) and blocks (1–4, 4 blocks of 6 days each) as the within subject variable was conducted. The pregnant-only group was excluded from this analysis because we found that these rats did not complete the task as often as the other groups and as a result their error levels were artificially low, reflecting a performance deficit rather than an enhancement. The ANOVA revealed a significant error by block by condition interaction (F(9, 81) = 2.66, p ≤ .009; see Figure 3.2). Post hoc test revealed that primiparous rats made fewer
RME than nulliparous rats (blocks 3 (p ≤ .06) and 4 (p ≤ .009)), multiparous rats (blocks 2 (p ≤ .026), 3 (p ≤ .07) and 4 (p ≤ .07)), and tended to make fewer RME than sensitized rats (blocks 1 (p ≤ .08) and 2 (p ≤ .07)). Furthermore, nulliparous rats tended to make fewer RME than multiparous rats on blocks 1 (p ≤ .08) and 2 (p ≤ .07), while sensitized rats tended to make fewer RME than nulliparous rats on block 4 (p ≤ .08). For WME across blocks, primiparous rats made significantly fewer WME than nulliparous rats (blocks 1 (p ≤ .01), 2 (p ≤ .003) and 3 (p ≤ .03)), multiparous rats (blocks 1 (p ≤ .03) and 3 (p ≤ .01)) and sensitized rats (on block 1, p ≤ .0007). Furthermore, both multiparous and sensitized rats made fewer WME than nulliparous rats on block 2 (.0006 ≤ p ≤ .0009). There were no other differences between conditions (.13 ≤ p ≤ .99; see Figure 3.2). There was also a significant main effect of error type (F(1, 27) = 424.05, p ≤ .0000), indicating that more reference memory errors were made than working memory errors, and of block (F(3, 81) = 3.79, p ≤ .013), indicating that there was a difference in number of total errors across blocks. There was also a significant error by blocks interaction (F(3, 81) = 4.56, p ≤ .005), indicating that there were differences in the number of working memory errors compared to reference memory errors across blocks. There was a tendency for a main effect of group (F(3, 27) = 2.38, p ≤ .09).
Figure 3.2 Mean (± SEM) for (a) total number of reference memory errors (RME), (b) number of reference memory errors (RME) across 4 blocks of 6 days, (c) total number of working memory errors (WME), and (d) number of working memory errors (WME) across 4 blocks of 6 days in primiparous, multiparous, nulliparous and sensitized rats. For RME across blocks (4 blocks of 6 days), primiparous rats made fewer RME than nulliparous rats (Blocks 3 (p < .06) and 4 (p < .009)), and multiparous rats (Blocks 2 (p ≤ .026), 3 (p ≤ .07) and 4 (p ≤ .07)). For WME across blocks, primiparous rats made significantly fewer WME than nulliparous rats ( Blocks 1 (p ≤ .01), 2 (p ≤ .003) and 3 (p ≤ .03)), multiparous rats (Block 1 (p ≤ .03) and 3 (p ≤ .01)) and sensitized rats (on Block 1, p ≤ .0007). Furthermore both multiparous and sensitized rats made fewer WME than nulliparous rats on block 2 (.0006 ≤ p ≤ .0009). * primiparous rats significantly different from nulliparous and multiparous rats, ‘#’ primiparous rats significantly different from multiparous rats, ‘a’ nulliparous rats significantly different from all other conditions rats, ‘b’ primiparous rats significantly different from sensitized rats. It should be noted that pregnant-only rats were not included in Figure 3.2 due to the fact that these rats did not complete the task on average of 16 of 24 test days (Figure 3.1) therefore measure of errors is not indicative of their memory performance on this task.

Pregnant-only rats took significantly longer to traverse the arms than all other conditions.

A repeated-measures ANOVA on the average latency to reach an arm across testing days
revealed a significant main effect of condition \( (F(4, 32) = 3.78, p < .013; \text{Figure 3.3}) \) with pregnant-only rats taking significantly longer to reach an arm than rats in any other condition \( (.001 < p < .01) \), but no other significant differences between any other groups \( (.40 < p < .90) \). There was a significant effect of day \( (F(23, 736) = 5.78, p < .0001) \) indicating that the average latency to reach an arm decreased across days of testing, and a day by condition interaction \( (F(92, 736) = 1.34, p = .024) \). Post hoc tests revealed that pregnant-only rats took significantly longer to reach an arm than all other conditions on days 3, 8, 9, 22 and 23 \( (p's < .02) \), than primiparous, multiparous and sensitized rats on day 7 \( (p < .002) \), than primiparous, multiparous and sensitized rats on days 10 and 19 \( (p's < .044) \), than primiparous and multiparous rats on days 15 and 18 \( (p's < .008) \), than sensitized and nulliparous rats on day 4 \( (.002 < p < .003) \), than sensitized rats on days 6, 14 and 17 \( (p's < .006) \), and than nulliparous rats on days 11, 16 and 20 \( (p < .0019) \). In addition, primiparous rats took significantly longer to reach an arm than sensitized rats on days 2, 4, 7 and 19 \( (p < .05) \), than multiparous rats on days 4 and 7 \( (p < .01) \), and than nulliparous rats on day 4 \( (p < .005) \). Multiparous rats took significantly less time to reach an arm than nulliparous rats on day 7 \( (p < .04) \) and sensitized rats on day 19 \( (p < .05) \). Nulliparous rats took significantly longer to reach an arm than sensitized rats on days 3 and 19 \( (p < .04) \).
Figure 3.3 Mean (± SEM) for (a) total average latency to reach an arm (seconds) and (b) average latency to each an arm across testing days. On average, pregnant-only rats took significantly more time to reach an arm than all other conditions (.001 ≤ p ≤ .01). Across days, pregnant-only rats took significantly longer to reach an arm than all other conditions on days 3, 8, 9, 22 and 23 (.000007 ≤ p ≤ .02), than primiparous, multiparous and sensitized rats on day 7 (.00000 ≤ p ≤ .002), than primiparous, multiparous and sensitized rats on days 10 and 19 (.00006 ≤ p ≤ .044), than primiparous and multiparous rats on days 15 and 18 (.003 ≤ p ≤ .008), than sensitized and nulliparous rats on day 4 (.002 ≤ p ≤ .003), than sensitized rats on days 6, 14 and 17 (.000001 ≤ p ≤ .006), and than nulliparous rats on days 11, 16 and 20 (.000005 ≤ p ≤ .0019).
There were no significant main effects of condition on number of days to reach criterion (p ≤ .85; Table 3.1), total choices to criterion (p ≤ .76; Table 3.1), number of errors to criterion (p ≤ .72; Table 3.1), and average number of errors across days the task was completed (p ≤ .15; Table 3.1). Bartlett's test for HOV found no significant differences between conditions in HOV (.27 ≤ p ≤ .64).

**Table 3.1** Mean (± S.E.M.) number of days to reach criterion, number of total choices to criterion, and average latency to reach an arm during testing in nulliparous, primiparous, multiparous, and sensitized rats.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Day to Criterion</th>
<th>TC to Criterion</th>
<th>Errors to Criterion</th>
<th>Errors/Completed Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nullip</td>
<td>11.22 ± 3.13</td>
<td>89.00 ± 28.88</td>
<td>39.56 ± 12.95</td>
<td>3.78 ± .21</td>
</tr>
<tr>
<td>Primip</td>
<td>11.75 ± 1.78</td>
<td>83.00 ± 16.50</td>
<td>36.38 ± 8.15</td>
<td>3.12 ± .20</td>
</tr>
<tr>
<td>Multip</td>
<td>12.25 ± 3.09</td>
<td>100.38 ± 27.42</td>
<td>44.88 ± 13.16</td>
<td>3.72 ± .12</td>
</tr>
<tr>
<td>Preg-Only</td>
<td>11.00 ± 4.51</td>
<td>80.67 ± 38.26</td>
<td>36.00 ± 17.07</td>
<td>3.31 ± .23</td>
</tr>
<tr>
<td>Sensitized</td>
<td>15.83 ± 3.24</td>
<td>131.00 ± 26.16</td>
<td>60.00 ± 12.26</td>
<td>3.48 ± .34</td>
</tr>
</tbody>
</table>

Stage of estrous cycle did not account for differences in working and reference memory performance with reproductive experience. Analysis of covariance on number of total errors with proestrus as a covariate by day did not significantly alter the above findings. There was an effect of proestrus on number of total errors by condition on test days 3 and 11, indicating that rats in proestrus rats made more errors on these 2 days (p ≤ .02 for both). There were no significant differences between conditions on number of rats in proestrus for either day (p ≤ .18; day 3 rats in proestrus, nulliparous (n = 1), multiparous (n = 1); day 11 rats in proestrus nulliparous (n = 4), primiparous (n = 2), multiparous (n = 4), pregnant-only (n = 1) and sensitized (n = 2)). In addition, there was no significant difference in total number of test days spent in proestrus between conditions (p ≤ .58).

Duration of maternal behavior in primiparous and multiparous rats does not correlate with spatial learning and memory performance after the time of weaning. Previous work has found that total licking and arched-back nursing inversely correlated with number of RME
(Pawluski et al, 2006) but long after the time of weaning there is no significant correlation between total licking and arched-back nursing behavior (LG-ABN) in primiparous and multiparous rats and RME or WME (RME: \( r = -0.057, p \leq 0.82 \); WME: \( r = 0.058, p \leq 0.84 \)).

For total duration of individual maternal behaviors such as licking/grooming, passive nursing, etc., there was no significant difference between primiparous and multiparous rats on any maternal behaviors measured (.11 \( \leq p \leq .86 \): Table 3.2).

**Table 3.2** Mean (\( \pm \) S.E.M.) total time in seconds (total of 5400 seconds) multiparous and primiparous dams spent in individual maternal behaviors during the first few postpartum days.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Licking/Grooming</th>
<th>Licking and nursing</th>
<th>Arched-back Nursing</th>
<th>Blanket Nursing</th>
<th>Passive Nursing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primip</td>
<td>193.13 ± 82.90</td>
<td>445.63 ± 80.23</td>
<td>1301.25 ± 413.41</td>
<td>1330.00 ± 269.48</td>
<td>986.25 ± 274.59</td>
</tr>
<tr>
<td>Multip</td>
<td>40.71 ± 24.36</td>
<td>512.86 ± 108.84</td>
<td>1690.71 ± 350.36</td>
<td>1397.14 ± 289.75</td>
<td>690.71 ± 238.29</td>
</tr>
</tbody>
</table>

Onset of ‘maternal behavior’ in sensitized rats did not correlate with spatial learning and memory performance. All rats (n=6) became sensitized to pups taking an average of 4.0 ± 2.4 days of pup exposure before being considered maternal (range 1 to 7 days). There was no correlation between day of onset of sensitized maternal behavior and RME or WME in sensitized rats (.14 \( \leq p \leq .16 \)). There was no difference in the size of the litter (p ≤ .85), or the number of male (p ≤ .99) or female pups (p ≤ .74) birthed by primiparous, multiparous and pregnant-only rats prior to testing (data not shown).

### 3.4 DISCUSSION

The present results demonstrate that first reproductive experience alters both working and reference memory performance long after the time of weaning and these effects are not due to the experience of pregnancy or mothering alone. Here we show that primiparous rats tested one month after pups were weaned (all rats were approximately 6 months of age), exhibit enhanced hippocampus-dependent learning and memory performance on the spatial working/reference
memory version of the radial arm maze compared to age-matched multiparous, sensitized, and nulliparous rats. Primiparous rats made significantly fewer total errors (reference and working errors) than nulliparous and multiparous rats and tended to make fewer total errors than sensitized rats (Figure 3.2). Specifically, primiparous rats outperformed nulliparous rats on working memory in 3 blocks of trials and on reference memory in 2 blocks of trials. Primiparous rats also outperformed multiparous rats on working memory in 2 blocks of trials, and on reference memory 1 block of trials. Multiparous rats outperformed nulliparous rats on working memory during 1 block of trials. In addition, pregnant-only rats (animals that did not mother their offspring) completed the task on significantly fewer test days and with a greater latency to reach each arm compared to multiparous, primiparous, nulliparous, and sensitized rats (Figures 3.1 and 3.3). This indicates a motivational/motoric deficit in pregnant-only rats. There was also no significant relationship between duration of maternal behaviors and spatial learning and memory performance long after the time of weaning. These findings clearly demonstrate the enhancement of spatial learning and memory performance with primiparity persists past the time of weaning (with training and testing beginning over one month after weaning and continuing for a month) and is not due to the experience of pregnancy or ‘mothering’ alone.

Primiparity results in marked changes in learning and memory performance in the mother.

Interestingly, it is the first reproductive experience (primiparity), more than subsequent reproductive experiences, that results in significantly improved spatial working and reference memory performance at the time of weaning (Pawluski et al., 2006) and approximately one month after weaning (present study). In the present study we found that subsequent reproductive experience affords some enhancement of working memory but not to as great a degree as first
reproductive experience. The advantage of primiparity on learning does not appear to be solely due to either pregnancy or mothering. This is consistent with previous research showing that primiparous rats are quicker at a reference memory task than nulliparous rats when repeatedly tested between 12 and 24 months of age (Gatewood et al., 2005). In addition, Gatewood et al. (2005) report that multiparous rats were quicker to complete a reference memory task than primiparous rats when tested between 6 and 24 months of age. Our findings, and others (Love et al., 2005), are not consistent with this report of persistent improvement in reference memory performance with multiparity. This may be due to differences in the task used (dry land maze and radial arm maze), the number of times animals were tested, and duration of testing.

In the present study larger differences are evident between primiparous and nulliparous rats in working memory performance compared to reference memory performance (see Figure 2), with effect sizes in working memory larger than for reference memory (0.78 for working memory compared to 0.5 for reference memory). In fact, in the present study we found that primiparous rats had enhanced working memory performance compared to nulliparous, multiparous and sensitized rats long after the time of weaning. This is partially consistent with our previous findings reporting enhanced working memory performance in primiparous rats compared to nulliparous rats at the time of weaning (Pawluski et al., 2006). However, in the present study, with testing beginning 35 days after weaning, we did not find a strong enhancement in working memory performance in multiparous rats (multiparous rats had significantly fewer working memory errors during Block 2 only) as previously reported at other time points after weaning (Kinsley et al., 1999; Pawluski et al., 2006) suggesting that the enhancement in working memory performance with multiparity is not persistent and/or is dependent on recent contact with pups. Indeed, it is clear that first reproductive experience leads to lasting enhancement of spatial learning and memory performance, compared to no
reproductive experience, but more research is needed to determine the role of subsequent reproductive experience on the persistence of this behavior.

**The contribution of pregnancy- and mothering- alone to spatial working and memory performance in the mother.**

Little work has attempted to determine the separate contribution of pregnancy- and/or mothering- alone to the enhancement of learning and memory with reproductive experience in the mother. One study has shown that pregnant-only rats (rats that gave birth but did not mother) do not differ from nulliparous rats in reference memory performance (Lambert et al., 2005). In the present study we found pregnant-only rats, tested 55 days after parturition were less likely to complete the task and took longer to enter an arm than primiparous, multiparous, nulliparous and sensitized rats (Figures 3.1 and 3.3). This indicates that motivation and/or learning strategies may be disrupted in pregnant-only rats. In addition, pregnancy and parturition without mothering may increase the level of anxiety in females and therefore the dams take longer to ‘explore’ the maze. It is not known how removal of pups within 24 hours of birth affects anxiety, but Lonstein (2005) has found that lactating rats on day 7 postpartum exhibit an increase in anxious behavior with increasing amount of time away from pups. In addition preliminary data from our lab suggests pregnant-only rats have decreased exploratory behavior compared to primiparous, nulliparous and sensitized rats (a multiparous group was not included) approximately 55 days after the time of pup removal as measured on the open field test. The fact that we saw very pronounced differences in the motivation of the pregnant-only group is perhaps surprising given that they were removed from their pups approximately 55 days prior to the start of the study.

In addition, the present study did not find a strong significant effect of the ‘enriched’ environment of sensitization (pup-exposure to nulliparous rats) on spatial learning and memory
performance one month after pups were removed from sensitized rats. Sensitized rats performed better than nulliparous rats on reference memory during block 4 only and for working memory during block 3 only. This lack of a difference between sensitized rats and nulliparous rats may be due to the lapse of time between sensitization and testing. Kinsley et al. (1999) have shown that immediately after pup removal, pup-exposed rats perform better than nulliparous rats on a reference memory task. In addition, Lambert et al. (2005) have shown 10 days after pup-exposure reference memory declines (primiparous and nulliparous rats with pup-exposure performed significantly better on 1/9 trials of a reference memory task than pregnant-only rats and nulliparous rats without pup exposure). These findings, coupled with the findings in the present study, suggest that the duration of time between sensitization and testing may be an important consideration when investigating the effect of the pup-enriched environment on learning and memory performance in the female. Indeed, environmental enrichment studies done in adult rodents often test animals while they are being continually enriched (Leggio et al., 2005) and very few studies, if any, have looked at the long term effects of a few weeks enrichment during adulthood on learning and memory performance.

The contribution of hormones during the first reproductive experience on the lasting enhancement in spatial learning and memory performance.

Very little research has investigated the role of hormones on the enhancement of learning and memory performance with primiparity. However, oxytocin and/or corticosterone seem likely candidates mediating the lasting changes in spatial learning and memory performance in primiparous rats. As mentioned previously, Tomizawa et al. (2003) found that oxytocin is a mediator of enhanced reference, but not working, memory performance during lactation in multiparous mice. It has yet to be determined whether oxytocin levels during lactation result in
lasting behavioral changes (ie. behavioral changes at the time of weaning and beyond). In addition, it has not been confirmed that oxytocin mediates changes in spatial reference memory performance in primiparous mice or rats.

With regards to working memory performance in primiparous rats, corticosterone may play a significant role. It has been consistently shown that virgin females exposed to chronic stress (associated with elevated corticosterone levels) have enhanced working memory performance (Bowman et al., 2001; Bowman, 2003; McLaughlin et al., 2005). Basal corticosterone levels are elevated during lactation (Stern et al., 1973) and corticosterone is important for the degree of maternal behavior (Graham et al., 2006; Rees et al., 2005) and consolidation of maternal memory (Graham et al., 2006), it seems plausible that corticosterone plays an active role in spatial learning and memory enhancement with primiparity.

It remains to be determined whether there are differences in hormone profiles of oxytocin and corticosterone, and differences in neural actions of these hormones with primi- and multiparity. It has been shown that it is possible for hormone profiles and/or sensitivity to hormones to differ with reproductive experience. For example, primiparous rats exhibit elevated basal prolactin levels compared to multiparous rats (Bridges and Hammer, 1992) and primiparous rats have elevated neural sensitivity to opioids compared to multiparous rats (Kinsley and Bridges, 1988). Therefore it would not be surprising if differences in hormone actions of oxytocin and/or corticosterone between primiparous compared to multiparous rats, may mediate the lasting enhancement in learning and memory performance found in primiparous rats. It should also be noted that there is significant interaction between oxytocin and corticosterone. However, this is dependent on reproductive experience. For example, oxytocin has been shown to attenuate the corticosterone response to stress in virgin females, but does not appear to significantly affect
corticosterone response to stress during late pregnancy and in lactation (for review see Neumann, 2001).

**Degree of maternal behavior does not persistently correlate with spatial learning and memory performance in the mother.**

Previously, we found that increased nursing and licking by the dam during the first week postpartum was associated with poorer reference memory performance by the dam when testing commenced at the time of weaning in Long-Evans rats (Pawluski et al., 2006, Ch 2). In the present study, we did not find this same association in Sprague-Dawley rats when spatial learning and memory performance was assessed one month after weaning. It is possible that the association between reference memory performance in the dam and the licking/nursing behavior she elicits towards her pups is evident only for a brief period of time after pup exposure. Perhaps a stronger relationship between reference memory and duration of maternal behavior would be evident if testing commenced during lactation as it may be the hormone milieu of lactation that is mediating the relationship between licking/nursing behavior and memory in the dam. This has yet to be determined. In addition, it is possible that this association is less evident in Sprague-Dawley rats as they may exhibit a decreased range of maternal behaviors compared to Long-Evans rats (Pawluski et al., 2006, Ch 2). In addition, most research on the effects of high and low levels of maternal behavior has been done primarily in Long-Evans rats (ie.Bredy et al., 2003; Liu et al., 2000: Liu et al., 1997).

**Conclusion**

The present experiment provides new evidence of the lasting effects of first reproductive experience (primiparity) on hippocampus-dependent spatial working and reference memory...
performance. In addition, these findings suggest that these effects are due to the combination of first pregnancy and mothering experience as pregnancy or 'mothering' alone did not result in an enhancement of spatial working and/or reference memory performance. Therefore, the initial reproductive experience may be a time of maximum plasticity and a prime hormonal environment for learning and memory and neural changes.
3.5 REFERENCES


Lonstein J.S. 2005. Reduced anxiety in postpartum rats requires recent physical interactions with pups, but is independent of suckling and peripheral sources of hormones. Horm Behav, 47, 241-255.


CHAPTER 4

REPRODUCTIVE EXPERIENCE ALTERS HIPPOCAMPAL NEUROGENESIS
DURING THE POSTPARTUM PERIOD IN THE DAM

4.1 INTRODUCTION

There are a number of neural changes that occur in the dam during pregnancy and lactation (Numan, 2007). In women, brain size has been shown to decrease across pregnancy and return to preconception size during the postpartum (Oatridge et al., 2002). In the rodent, pregnancy has been associated with a decrease in hippocampus volume (Galea et al., 2000) while the postpartum has been associated with an increase in cortical thickness in primiparous rats (Hamilton et al., 1977), altered spine density in the oxytocin system of parous rats (Theodosis and Poulain, 2001), and increased astrocyte number in hypothalamus of pup-exposed multiparous rats (Featherstone et al., 2000). In addition, late pregnancy and the early postpartum period involve the activation of the ‘maternal circuit’, a neural circuit which involves brain regions such as the olfactory bulbs, amygdala and hypothalamus and is important for the performance of maternal behavior (Numan and Insel, 2003; Numan, 2007). At the same time, long-term memory of maternal responsiveness is formed in primiparous females (Orpen and Fleming, 1987) and this maternal memory is partially dependent on the nucleus accumbens shell (Li and Fleming, 2003) and the steroid hormone, corticosterone (Graham et al, 2006). Thus, due to this maternal memory, maternal responsiveness is increased with subsequent pregnancy and exposure to pups. Taken together these findings suggest there are a number of brain regions

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undergoing plasticity with reproduction and the extent of these changes may vary with reproductive experience.

Recent work has demonstrated that the hippocampus, an area of the brain not traditionally associated with the 'maternal circuit' and maternal behavior, has been affected by reproduction and reproductive experience. Pawluski and Galea (2006) found that pyramidal neurons of the CA1 and CA3 region of the hippocampus showed significant dendritic pruning at the time of weaning in primiparous, but not multiparous, dams. Kinsley et al. (2006) found increased spine density in the apical region of CA1 pyramidal neurons during late pregnancy and early lactation compared to nulliparous rats, while Pawluski and Galea (2006) found increased spine density in the basal CA1 region of multiparous rats at the time of weaning. Furthermore, Kinsley et al. (1999) and Pawluski et al. (2006a, b, Ch2 and Ch 3) have shown that hippocampus-dependent spatial learning and memory is remarkably altered in the dam and the degree of these alterations depends on the dam's reproductive experience (number of times pregnant and mothered). These effects suggest the hippocampus plasticity may be altered with reproductive experience.

The hippocampus is a brain region that retains the ability to produce new neurons in adulthood. Progenitor cells reside in the subgranular zone of the dentate gyrus and newly produced daughter cells migrate into the granule cell layer and make appropriate connections with CA3 pyramidal cells (Zhao et al., 2006). Adult neurogenesis is comprised of at least two components: cell proliferation and cell survival. Cell proliferation refers to the production of new cells, whereas cell survival refers to the number of new cells that survive to maturity. Factors that affect cell proliferation will modulate mitosis in progenitor cells (Ormerod and Galea, 2001) and factors that affect cell survival will influence the differentiation and/or maturation of cells into mature neurons (Ormerod and Galea, 2001). The number of new neurons can be increased either by enhancing cell proliferation and/or by enhancing the survival of new neurons. Therefore, it is
important to understand how both the proliferation and the survival of new neurons are regulated.

There has been relatively little research on the effects of pregnancy and motherhood on hippocampal neurogenesis in the dam. Late pregnancy has been suggested to enhance cell survival, as the level of PSA-NCAM-ir (polysialated form of the neural cell adhesion molecule), which is associated with migrating new neurons, is increased during this time (Banasr et al., 2001). However during early (GD7) and late (GD21) gestation there is increased cell proliferation in the subventricular zone, but no evidence for changes in cell proliferation in the dentate gyrus during these times (Furuta and Bridges, 2005; Shingo et al., 2003). There is an increase in cell proliferation in the subventricular zone on postpartum day 7 (Shingo et al., 2003), and recent evidence suggests that in the early postpartum cell proliferation is decreased in the hippocampus of primiparous rats (Leuner et al., 2007). However, to date, no work has investigated how hippocampal neurogenesis is altered during the postpartum period with reproductive experience and pup-exposure alone.

Pregnancy, parturition and lactation, are accompanied by dramatic fluctuations in steroid and peptide hormones (Garland et al., 1987; Rosenblatt et al., 1979; Stern et al., 1973; Stern and Levine, 1974). In the rat, estradiol increases dramatically during late pregnancy, and decreases at parturition with the expulsion of the placenta (Rosenblatt et al., 1979; Yoshinaga et al., 1969), while corticosterone levels change dramatically during late pregnancy and lactation (Fischer et al., 1995; Stern et al., 1973). Interestingly, both estradiol and corticosterone are regulators of adult neurogenesis (Cameron and Gould, 1994; Ormerod and Galea, 2001; Tanapat et al., 1999). For example, estradiol has been shown to initially increase (within 4h) but subsequently suppress (within 48h) cell proliferation, via adrenal steroids, in the adult female rodent (Ormerod et al., 2003). Estradiol also alters cell survival independently of cell proliferation (Ormerod et al.,
2004). Conversely, elevated levels of corticosterone suppress both cell proliferation and survival (Cameron and Gould, 1994; Wong and Herbert, 2004). This suggests that late pregnancy and lactation, which are accompanied by changes in estradiol and corticosterone levels, may result in altered hippocampal neurogenesis in the dam. Intriguingly, Leuner et al. (2007) recently showed that the inhibition of cell proliferation in primiparous rats was eliminated with adrenalectomy, suggesting that elevated corticosterone was responsible for the suppression. Motherhood may also be seen as a time of environmental enrichment where the dam is exposed to the ‘enriching effects’ of pups. This form of enrichment may play a role in altering hippocampus neurogenesis during the postpartum, as hippocampus neurogenesis is increased with environmental enrichment in adult rodents (Brown et al, 2003; Olsen et al., 2006 for review; van Praag et al., 1999).

The present study was designed to determine whether reproductive experience affects neurogenesis in the dentate gyrus via cell proliferation (Experiment 1) and cell survival (Experiment 2), and whether any changes in neurogenesis were the result of pregnancy or pup-exposure alone. In order to investigate the effect of reproductive experience and pup-exposure on neurogenesis, female rats were divided into four groups: multiparous (birthed and mothered twice), primiparous (birthed and mothered once), nulliparous (never pregnant), and sensitized (nulliparous rats that act maternally towards pups) rats for Experiment 1, with an additional primiparous group without pups (primip-no-pups) for Experiment 2. Given the known effect of hormonal (Galea et al., 2006; Tanapat et al., 2005; Wong and Herbert, 2004) and environmental factors (van Praag et al, 1999) on neurogenesis, and the effect of reproductive experience on hippocampus morphology (Kinsley et al., 2006; Pawluski and Galea, 2006) and hippocampus-dependent spatial memory (Kinsley et al., 1999; Pawluski et al., 2006a, b, Ch 2 and Ch 3) it is expected that there will be changes in hippocampal neurogenesis in the dam during the
postpartum period that are dependent on the hormonal environment, pup-exposure, and reproductive experience.

4.2 METHOD

Animals

Forty-six female Sprague-Dawley rats (approximately 65-75 days of age) purchased from the UBC Animal Care Facility (Vancouver, Canada) were used in the study. Rats were initially housed in pairs in opaque polyurethane bins (48 x 27 x 20cm) with aspen chip bedding and were given Purina rat chow and tap water ad libitum. Rats were maintained in a 12h:12h light/dark cycle (lights on at 7:30 a.m.). All protocols were in accordance with ethical guidelines set by the Canada Council for Animal Care and were approved by the University of British Columbia Animal Care Committee.

All animals were age-matched and sexually-naïve prior to random assignment to the following groups: For Experiment 1: nulliparous (n = 5), primiparous (n = 5), multiparous (n = 5), and pup-exposed nulliparous (nullip+pups; n = 5). For Experiment 2: nulliparous (n = 5), primiparous (n = 6), primip-no-pups (n = 5), multiparous (n = 5), and sensitized (n = 5).

Nulliparous rats were not sexually experienced and were not in proestrus at the time of BrdU injection (see below for details), as proestrus has been shown to affect cell proliferation (Tanapat et al., 1999). Primiparous rats gave birth once and mothered once. Multiparous rats birthed and mothered twice. Sensitized rats were not sexually experienced, but were exposed to pups in order to investigate the effects of pup stimulation without prior pregnancy (see below, Sensitization). For Experiment 2 a second primiparous group was added, primip-no-pups, to investigate how pregnancy and brief exposure to pups affected cell survival during the postpartum period. For the primip-no-pups group, pups were removed 24 hours after BrdU injection in order to determine
whether pup-exposure to parous dams had an effect on cell survival across the postpartum period. All animals were age-matched such that primiparous rats during their first and only gestational period were the same age as multiparous rats during their second gestational period.

To test whether reproductive experience and pup-exposure influence hippocampal neurogenesis during the postpartum period, rats were given a single i.p. injection of the cell synthesis marker 5-bromo-2-deoxyuridine (BrdU; Sigma, St. Louis, MO) (200 mg/kg), on the afternoon of postpartum day 1 (day after birth/pup-exposure) and perfused either 24 hours later (Experiment 1: Cell proliferation) or 21 days later (Experiment 2: Cell survival). BrdU is a thymidine analog and a marker of DNA synthesis that labels proliferating cells and their progeny. Given the documented effect of circulating corticosterone on neurogenesis (Cameron and Gould, 1994), BrdU injections were done in the afternoon when circulating corticosterone levels do not differ between parous, lactating and nulliparous dams (Kakihana et al., 1980; Stern et al., 1973). BrdU was dissolved in 0.9% saline one hour prior to injection. For a time line of experiments see Figure 4.1.

Figure 4.1 Time line of BrdU injections and perfusions for Experiments 1 and 2. To test whether reproductive experience and pup-exposure influence hippocampal neurogenesis during the postpartum period, rats were injected with BrdU on the afternoon of postpartum day 1 (day after birth/pup-exposure) and perfused either 24 hours later (Exp 1: Cell proliferation) or 21 days later (Exp 2: Cell survival). Age-matched nulliparous females were also given an injection of BrdU and were perfused either 24 hours or 21 days later. In addition, primip-no-pups (Exp 2) had pups removed on postpartum day 2.
Breeding

For breeding, one male and one female were paired in a wire mesh cage. Following the release of a vaginal plug, impregnated females were individually housed in clear polyurethane bins until birth. For primiparous and multiparous dams, litters were culled to 4 male and 4 female pups 24 hours after parturition. The number of offspring, number of male and female pups, and weight of litter was recorded to ensure consistency between groups. Day 0 was the day a litter was born.

Maternal Behavior Testing

In order to ensure that there was no difference in degree of maternal care between primiparous and multiparous dams and to determine whether the amount of maternal care altered hippocampal neurogenesis, maternal behavior observations were done during the early postpartum. Observations were done for 10 min on Day 2 between 9-11 a.m. for Experiment 1 (Cell Proliferation) and continued twice a day between 9-11 a.m. and 3-5 p.m. on Days 2-5 for Experiment 2 (Cell Survival). For observation of maternal behaviors, dam and pups were left undisturbed in the cage. Observations were made once every 5 seconds for 10 min and data for each behavior were compiled across all test periods. The frequency and duration of the following maternal behaviors were observed during each testing period as previously described (Champagne et al., 2003; Myers et al., 1989; Pawluski et al., 2006b); licking/grooming where the dam is off the pups and licks the body and genital region of the pups; arched-back nursing; blanket nursing where dam lays over the pups; passive nursing where the dam lies either on her back or side while pups nurse; licking/grooming and nursing combined.

Sensitization

Sensitization (pup-exposure to nulliparous rats) was modified from previous research (Stern 1997; Pawluski et al., 2006b) in order to control for the effect of pup-exposure on
neurogenesis. Sensitized rats do not lactate, however they do display maternal behaviors such as licking, retrieval, nest building and hovering over pups. Day 0 was the introduction of pups to virgins (between 2:30 and 4:30 pm). Nullips+pups were continuously housed with 6-8 pups from Day 0 to Day 2 for Experiment 1 (Cell proliferation) and sensitized females were continuously housed with 6-8 pups from Day 0 to Day 22 for Experiment 2 (Cell survival). To ensure that pups could survive 24 hours without food the age of pups at the start of sensitization was 3-5 days. Every 24 hours (between 9:30-11:00 am starting on Day 2), milk-replete pups were given to sensitized females and donor pups were returned to their biological mother. To confirm that sensitized virgins were acting maternally, licking of pups and hovering over pups were scored from daily 10 minute video-recordings taking place between 2:30 and 4:30 pm on Day 0 and Day 1 and between 9:30 and 11:00 am on Day 2 to Day 7, immediately after new donor pups were introduced to the sensitized virgins. Spot checks were performed approximately 1, 3 and 24 hours later. Video recordings and spot checks took place for the first 8 days of exposure to pups or until a virgin rat acted maternally towards pups (licked and hovered over pups within a 24 hour period). If the sensitized virgin injured a pup during the video recording the test was terminated for that day and all pups were removed. This occurred once. It was not expected that full sensitization would occur in rats in Experiment 1 due to the short exposure of to pups. Therefore these rats were referred to as nullip+pups.

**Estrous Cycle**

Proestrus has been known to increase cell proliferation in the dentate gyrus (Tanapat et al., 1999); therefore nulliparous rats were given vaginal smears daily until after BrdU injection. Vaginal smears were taken by placing a cotton swab in the vagina and smearing the contents of the swab on a plain microscope slide. Slides were examined under 10x objective and proestrus
was determined when a majority of cells evident in the vaginal mucus (70%) were nucleated epithelial cells. Only nulliparous rats not in proestrus were injected with BrdU.

**Estradiol Assays**

Serum estradiol levels were measured at time of perfusion to account for possible differences between groups. Blood samples were collected from intracardiac punctures, stored at 4°C overnight and centrifuged at 10 x g for 15 minutes. Serum estradiol was assayed using a Coat-a-Count kit (Diagnostic Products Corporation, Los Angeles, CA) with an intra-assay coefficient of variation averaging ≤ 17% and a sensitivity of 7.4pg/mL. Estradiol was measured by assaying duplicates. One animal was removed from the assay for Experiment 2 due to experimental error.

**Histology**

All histological procedures were based on previous work (Ormerod et al., 2003; Tanapat et al., 2005). Rats were anaesthetized with sodium pentobarbital and then perfused with 4% paraformaldehyde within 24 hours (Experiment 1: Cell proliferation) or 21 days (Experiment 2: Cell survival) following BrdU injection. A 24-hour survival time post-BrdU injection was followed to allow for one mitotic division (Cameron and McKay, 2001). Following extraction, brains were stored at 4°C in 4% paraformaldehyde for 24 hours, and then transferred to 30% sucrose for a minimum of 48 hours. Brains were sliced in 40 μm sections through the entire extent of the dentate gyrus in a bath of TBS (pH 7.4) using a vibratome (Leica VT1000S). The sections were stored at 4°C in sterile culture plates filled with TBS prior to BrdU immunohistochemistry processing. BrdU-ir cells were counted on peroxidase-treated sections.

**BrdU Immunohistochemistry**

Free-floating sections were rinsed repeatedly between steps in TBS (0.1 M tris-phosphate buffer in 0.9 % saline; pH 7.4). Sections were incubated in 0.6% H₂O₂ for 30 minutes at room
temperature. DNA was denatured by applying 2N HCl for 30 min at 37°C. Sections were blocked with 3.0% normal horse serum (NHS) for 30 min and then incubated overnight in mouse monoclonal antibody against BrdU (1:200 + 3% NHS + 10% Triton-X; Boehringer Mannheim, Laval, Quebec, Canada) at 4°C. The following day, sections were incubated in mouse secondary antisera (1:129 + 3% NHS; Vector Laboratories, Burlington, ON, Canada) for 4 hours at room temperature. Sections were incubated in avidin-biotin horseradish peroxidase complex (ABC Elite Kit; 1:50; Vector Laboratories) for 120 min. Sections were reacted for approximately 10 min in 0.02% diaminobenzidine (DAB; Sigma Aldrich Chemicals) with 0.0003% H2O2. The sections were mounted on super frost slides (Sigma Chemicals) and dried overnight. The sections were counterstained with cresyl violet acetate, dehydrated and coverslipped with Permount (Fisher Scientific).

To stereologically estimate cell numbers, total BrdU-ir cells were counted under 100x objective with oil on every 10th section (approx. 11-12 sections per rat) throughout the dentate gyrus separately for the granule cell layer (GCL), which included the subgranular zone, and the hilus. BrdU-ir cells are counted in the hilus and compared to counts in the granule cell region to determine whether any effects are due to generalized effects on blood brain permeability. Progenitor cells in the hilus give rise to a different population of cells that are mainly glial cells compared to progenitor cells in the subgranular zone which give rise to cells that are mainly neurons (Cameron et al., 1993). Furthermore new neurons in the hilus are considered ectopic (McCloskey et al., 2006; Scharfman et al., 2007). Cells were considered BrdU-ir if they were intensely stained and exhibited medium round or oval cell bodies and counted using the same criterion as previous studies (Cameron et al., 1993; Ormerod and Galea, 2001; Figure 4.2).
Figure 4.2 Photomicrograph of representative cells. (a) BrdU-ir cells (black arrows) in the subgranular zone of the dentate gyrus 24 h after BrdU injection (Exp 1: Cell proliferation), (b) BrdU-ir cell in the granular cell layer of the dentate gyrus 21 days after BrdU injection (black arrow) (Exp 2: Cell survival), and (c) pyknotic cell (black arrow) in the granule cell layer. (d) Confocal micrograph with representative double-labeled cell, labeled with both fluorescent antibodies against BrdU (red) and NeuN (green). Scale bar = 10 μm for a, b, c, and 16 μm for d. GCL = granule cell layer.

Separate sets of hippocampus sections were counterstained with cresyl violet acetate, dehydrated and coverslipped with Permount (Fisher Scientific). Pyknotic cells were counted on these sections as a morphological measure of cell death on every 20th section (approx. 6 sections per rat). Cells were considered pyknotic if they lacked a nuclear membrane, had pale or absent cytoplasm and darkly stained spherical chromatin (Ormerod et al., 2003; Figure 4.2).

The area of the GCL and hilus were measured from sections counterstained with cresyl violet acetate using the digitizing program Image J (National Institutes of Health, Bethesda,
Maryland) and estimates of GCL and hilus volumes were made using Cavalieri’s principle (Gundersen et al., 1988).

**Flourescence Immunohistochemistry**

In order to determine whether surviving BrdU-ir cells were neurons, separate sets of sections from Experiment 2 were double-stained with fluorescent probes to assess BrdU-, and NeuN-immunoreactivity (ir) as follows: Free-floating sections were rinsed repeatedly between steps in TBS (pH 7.4). Initially, sections were blocked in TBS+ (3% NDS and 3% Triton-X in TBS) for 30 min and then incubated at 4°C for 36-48 hours in primary antibody rabbit anti-NeuN (1:100; Chemicon) in TBS+ (1% NDS and 3% Triton-X in TBS). Following this, sections were rinsed in TBS and blocked for 30 min in TBS+ and the following steps were carried out under very low light due to the sensitivity of the fluorescent probes. Sections were incubated for 24 hours in the secondary antibody donkey anti-rabbit Alexa 488 (1:200; Invitrogen) in TBS+. Sections were then fixed in 4% paraformaldehyde for 10 min, then rinsed in 0.9% saline and incubated in 2N HCl at 37°C for 30 min in order to denature the DNA. Sections were again rinsed in 0.9% saline, blocked in TBS+ for 30 min and incubated in primary anti-body rat anti-BrdU (1:250; Oxford Biotechnology) for 24 hours at 4°C. After this, sections were rinsed with TBS, blocked in TBS+ for 30 min, and incubated for 24 hours in secondary antibody, donkey anti-rat Cy3 (1:200; Jackson Immunoresearch) in TBS+. Sections were then mounted and coverslipped with 2.5% PVA-DABCO solution and stored in dark and at 4°C.

BrdU-ir cell phenotypes were analyzed on fluorescent probe-treated sections. Approximately, twenty BrdU-ir cells per brain (n = 2-3 per group) were identified on a Nikon fluorescence microscope (Nikon Canada; 100x objective) and a percentage verified by confocal (Zeiss Canada; 63x objective). The percentage of BrdU-ir cells that expressed a neuronal marker (NeuN-ir) was determined (see Figure 4.2 for a representative double-labeled cell).
Data Analyses

Analysis of number or density of BrdU cells, pyknotic cells, and volume in the dentate gyrus was calculated using a repeated-measures analysis of variance (ANOVA) with area (GCL, Hilus) as the within-subjects factor and group (nulliparous, primiparous, multiparous, nullip+pups/sensitized, primip-no-pups) as the between-subjects factor. One-way ANOVA tests were calculated for estradiol levels, and percentage BrdU/NeuN-ir cells as the dependent variables with group (nulliparous, primiparous, primip-no-pups, multiparous, nullip+pups/sensitized) as the between-subjects variable. Differences between primiparous and multiparous rats in maternal behaviors were analysed using a repeated-measures ANOVA with type of behavior (licking, nurse-licking, passive, blanket, arched, time-off nest) as the within-subjects factor and group (primiparous, multiparous) as between-subjects factors. To determine differences in litter size, number of male pups, number of female pups, and weight of the litter, t-tests were performed between litter size/weight, number of male and female offspring, and groups (primiparous, primip-no-pups, multiparous). Pearson product-moment correlations were conducted between litter variable, specific maternal behaviors, estradiol levels, and density of BrdU and pyknotic cells in the GCL and hilus. Post-hoc comparisons utilized Newman-Keuls. A priori tests utilized Fisher LSD. All statistical procedures were set at $\alpha = 0.05$.

4.3 RESULTS

EXPERIMENT 1: Cell proliferation

To ensure that differences between groups in hippocampal neurogenesis are not due to differences in the volume of the dentate gyrus and hilus, repeated measures ANOVAs were conducted. Results demonstrate there were no significant differences between groups on the volume of the granule cell layer (GCL) or the hilus ($p \leq .20$; Table 4.1). Therefore number of
BrdU-cells was reported in Experiment 1. As expected, there was a significant main effect of area with greater hilus volumes than GCL volumes (F(1, 16)=260.4, p ≤ .00001).

Table 4.1 Mean (± S.E.M.) volume of the granule cell layer and the hilus (mm³) in Experiment 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>GCL</th>
<th>Hilus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulliparous</td>
<td>2.48 ± .30</td>
<td>5.20 ± .41</td>
</tr>
<tr>
<td>Primiparous</td>
<td>2.42 ± .17</td>
<td>4.47 ± .45</td>
</tr>
<tr>
<td>Multiparous</td>
<td>2.33 ± .21</td>
<td>4.26 ± .51</td>
</tr>
<tr>
<td>Nullip+pups</td>
<td>2.91 ± .13</td>
<td>5.46 ± .24</td>
</tr>
</tbody>
</table>

Primiparous and multiparous have lower levels of cell proliferation in the GCL than nulliparous and sensitized rats. Pup-exposed nulliparous rats have higher levels of cell proliferation in the GCL than all other groups. Nullip+pups rats had significantly more BrdU-ir cells in the GCL than all other groups (all p's < .004), and nulliparous rats had significantly more BrdU-ir cells in the GCL than both primiparous (p ≤ .0001) and multiparous (p ≤ .0001) rats (significant interaction effect: F(3, 16)=7.2, p ≤ .003; Figure 4.3). There was also a significant main effect of group (F(3, 16)=10.5, p ≤ .0005) and area (F(1, 16)=289.5, p ≤ .00001) on number of BrdU-ir cells in the dentate gyrus.
Figure 4.3 Mean (± SEM) number of BrdU-ir cells in the granule cell layer of the dentate gyrus 24 hours after BrdU injection (Exp 1: Cell proliferation). Nulliparous rats had significantly more BrdU-ir cells in the granule cell layer 24 hours after injection than both primiparous (p ≤ .00012) and multiparous (p ≤ .00014) rats while nullips+pups rats had significantly more BrdU-ir cells in the granule cell layer 24 hours after BrdU injection compared to all other groups (all p’s < .004). * denotes significantly different from all other groups; # denotes significantly different from primiparous, multiparous and nullips+pups.

Pup-exposed nulliparous rats have more pyknotic cells in the GCL than all other groups.

Nullip+pups rats had significantly more pyknotic cells in the GCL than all other groups (all p’s ≤ .006; Figure 4.4; significant interaction effect between group and area F(3, 16)=3.2, p ≤ .05).

There was also a significant main effect of group (F(3, 16)=4.3, p ≤ .02) and area (F(1, 16)=11.7, p ≤ .004). There were no other significant differences (all p’s > .3).
Figure 4.4 Mean (± SEM) number of pyknotic cells in the dentate gyrus 24 hours after BrdU injection (Exp 1:Cell proliferation). Nullip+pups rats had significantly more pyknotic cells in the GCL than all other groups (all p’s < .006). *denotes significantly different from all other groups.

There was a significant positive correlation between serum estradiol levels and number of BrdU-ir cells in the hilus. A one-way ANOVA revealed a significant main effect of group on serum levels of estradiol at time of perfusion (F(3, 16)=3.4, p ≤ .04;Table 4.2). Post-hoc tests revealed a tendency for primiparous and multiparous dams to have lower levels of estradiol than nullip+pups females (all p’s ≤ .07). There were no other differences between groups (p > .1).

Correlations between serum estradiol and number of BrdU-ir cells revealed a significant positive correlation between number of BrdU-ir cells in the hilus and level of serum estradiol (r = .66, p ≤ .001; Figure 4.5). There were no other significant correlations (p > .09). Based on estradiol levels at the time of perfusion, 1 nulliparous female and 1 nullip+pups female were in proestrus (> 40pg/ml).
Table 4.2 Means (± S.E.M.) serum estradiol at time of perfusion in Experiment 1, 24 hours after BrdU injection.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Estradiol (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulliparous</td>
<td>31.83 ± 8.48</td>
</tr>
<tr>
<td>Primiparous</td>
<td>21.00 ± 1.73</td>
</tr>
<tr>
<td>Multiparous</td>
<td>18.77 ± 1.18</td>
</tr>
<tr>
<td>Nullip+pups</td>
<td>36.68 ± 3.31</td>
</tr>
</tbody>
</table>

Figure 4.5 Correlation between serum estradiol levels at perfusion and number of BrdU-ir cells. There was a significant positive correlation between number of BrdU-ir cells in the hilus and level of serum estradiol 24 hours after BrdU injection (r = .66, p ≤ .001; Exp 1).

Primiparous dams spent more time acting maternally towards pups than multiparous dams. All dams spent more time blanket nursing. A repeated-measures ANOVA on the specific maternal behaviours revealed a significant main effect of group (F(1, 8)=5.8, p ≤ .04), with primiparous rats spending significantly more time acting maternally than multiparous rats. All dams spent significantly more time blanket nursing than performing any other maternal
behaviour (p's ≤ .00002; Table 2; main effect of type of behaviour F(3, 24)=18.4, p ≤ .000001).

There were no other significant differences between groups (p ≤ .39), or an interaction effect (p ≤ .3). There were no significant correlations between individual maternal behaviours and number of BrdU-ir or pyknotic cells in the GCL or hilus (all p’s > .1). Nullip+pups rats in Experiment 1 did not act maternally towards pups, and therefore did not become sensitized, due to the short duration of time they spent with pups (2 days).

Table 4.3 Mean (± S.E.M.) percentage of total time during observations that primiparous and multiparous dams spent performing specific maternal behaviors in Experiment 1.

<table>
<thead>
<tr>
<th>Maternal Behavior</th>
<th>Primiparous</th>
<th>Multiparous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lick/groom</td>
<td>-----</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td>Lick and Nurse</td>
<td>1.8 ± .9</td>
<td>10.6 ± 3.8</td>
</tr>
<tr>
<td>Arched Nurse</td>
<td>7.6 ± 7.0</td>
<td>1.9 ± 0.8</td>
</tr>
<tr>
<td>Blanket Nurse</td>
<td>40.6 ± 7.3</td>
<td>29.8 ± 7.1</td>
</tr>
<tr>
<td>Passive Nurse</td>
<td>-----</td>
<td>-----</td>
</tr>
</tbody>
</table>

Multiparous rats gave birth to more female pups than primiparous rats (t = 2.4, p ≤ .04; Table 3). There were no significant differences between primiparous and multiparous rats in any other litter variables (.2 < p ≤ .6) and no significant correlations between number of BrdU-ir cells or pyknotic cells in the GCL or hilus and number of male and female offspring in a litter (.4 < p ≤ .8).

Table 4.4 Mean (± S.E.M.) size of litter, number of male and females pups, and weight of litter for parous rats in Experiment 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Female pups</th>
<th>Male pups</th>
<th>Litter weight(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primiparous</td>
<td>7.25 ± 1.2*</td>
<td>10.0 ± 1.3</td>
<td>109.5 ± 5.9</td>
</tr>
<tr>
<td>Multiparous</td>
<td>10.4 ± 0.7</td>
<td>8.0 ± 0.7</td>
<td>111.6 ± 3.0</td>
</tr>
</tbody>
</table>

* denotes significantly different from multiparous rats

EXPERIMENT 2: Cell survival

To ensure that differences between groups in hippocampal neurogenesis are not due to differences in the volume of the dentate gyrus and hilus, repeated measures ANOVAs were conducted. Results demonstrate, there was a tendency toward a significant difference between
groups in the volume of the GCL and hilus (F(4, 21)=2.4, p ≤ .08; Table 1). A priori comparisons demonstrate that sensitized rats had significantly larger volumes than primiparous (p ≤ .01) and nulliparous females (p ≤ .04), and tended to have larger volumes compared to primip-no-pups (p ≤ .06). Therefore, densities of BrdU-ir cells (number of BrdU-labelled cells/volume of region) were used as the dependent variable in this experiment in order to control for group differences in volume. As expected there was a significant main effect of area with greater hilus volumes than GCL volumes (F(1, 21)=213.8, p ≤ .000001).

Table 4.5 Mean (± S.E.M.) volume of the granule cell layer and the hilus (mm$^3$) in Experiment 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>GCL</th>
<th>Hilus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulliparous</td>
<td>2.28 ± .18</td>
<td>4.61 ± .36</td>
</tr>
<tr>
<td>Primiparous</td>
<td>2.46 ± .12</td>
<td>4.18 ± .19</td>
</tr>
<tr>
<td>Primip-no-pups</td>
<td>2.68 ± .18</td>
<td>4.96 ± .17</td>
</tr>
<tr>
<td>Multiparous</td>
<td>2.77 ± .24</td>
<td>4.31 ± .46</td>
</tr>
<tr>
<td>Sensitized</td>
<td>3.32 ± .14</td>
<td>4.93 ± .41</td>
</tr>
</tbody>
</table>

Primiparous rats, regardless of pup-exposure, had significantly fewer BrdU-ir cells surviving in the GCL compared to multiparous, nulliparous, and sensitized rats. Post-hoc tests revealed that sensitized and nulliparous rats had significantly greater density of BrdU-ir cells in the GCL 21 days after BrdU injection compared to all other groups (all p’s ≤ .04), and multiparous rats had significantly greater density of BrdU-ir cells compared to primiparous rats and primip-no pups (all p’s ≤ .003 interaction of group and area F(4, 21)=6.5, p ≤ .001; Figure 4.6). There were no other significant differences between groups in density of BrdU-ir cells in the GCL or hilus (.4 ≤ p ≤ 1.0). There was also significant main effect of group (F(4, 21) = 9.7, p ≤ .0001) and area (F(1, 21) = 285.7, p ≤ .000001).
Figure 4.6 Mean (± SEM) density of BrdU-ir cells 21 days after BrdU injection (Exp 2: Cell survival). Nulliparous and sensitized rats had significantly greater densities of BrdU-ir cells in the GCL 21 days after BrdU injection compared to all other groups (all p’s < .04), and multiparous rats had significantly greater density of BrdU-ir cells compared to primiparous rats, regardless of pup-exposure (all p’s < .003). * denotes significantly different from nulliparous, multiparous and sensitized; # denotes significantly different from all other groups.

In order to investigate the percentage of proliferating cells that survive across the postpartum period, the mean density of cells surviving after 21 days (Exp 2) was divided by the mean density of cells proliferating after 24 hours (Exp 1) and is illustrated in Figure 4.7. The percentage of new cells surviving across the postpartum period is largest in multiparous dams and smallest in primiparous rats.
Figure 4.7 Percentage of BrdU-ir cells surviving across lactation. This was calculated by dividing the mean density of cells surviving after 21 days (Exp 2) by the mean density of cells proliferating after 24 hours (Exp 1).

The majority of 21-day old BrdU-ir cells expressed the neuronal protein NeuN (approximately 60%) and this percentage was not significantly different between groups (p > .9; Mean ± SEM per group: nulliparous 60.0 ± 7.6, primiparous 60.7 ± 4.7, primip-no-pups 61.7 ± 4.4, multiparous 55.0 ± 5.0, sensitized 58.3 ± 4.4).

There was no significant difference between groups in the density of pyknotic cells in the dentate gyrus. There was a greater density of pyknotic cells in the GCL than in the hilus,
regardless of group (main effect of area (F(1, 21)=38.3, p ≤ .000001; Figure 4.8). There were no other significant differences (all p’s > .3).

Figure 4.8 Mean (± SEM) number of pyknotic cells in the dentate gyrus 21 days after BrdU injection (Exp 2:Cell survival). There was a significant main effect of area (p ≤ .000001) with a greater density of pyknotic cells in the GCL than in the hilus. There were no other significant differences (all p’s > .3).

There was no significant difference in estradiol levels between groups. A one-way ANOVA revealed no significant main effect of group on serum levels of estradiol at time of perfusion (F(4, 19)=.3, p ≤ .9; Table 4.6). There were no significant correlations between estradiol levels at perfusion and density of BrdU-ir and pyknotic cells in the dentate gyrus (.09 ≤ p ≤ .9).
Table 4.6 Mean (± S.E.M.) serum estradiol (pg/ml) at time of perfusion in Experiment 2, 21 days after BrdU injection.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Estradiol (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulliparous</td>
<td>24.74 ± 2.06</td>
</tr>
<tr>
<td>Primiparous</td>
<td>21.84 ± 2.04</td>
</tr>
<tr>
<td>Primip-no-pups</td>
<td>25.45 ± 5.19</td>
</tr>
<tr>
<td>Multiparous</td>
<td>21.43 ± 3.08</td>
</tr>
<tr>
<td>Sensitized</td>
<td>21.70 ± 3.78</td>
</tr>
</tbody>
</table>

Dams spent significantly more time blanket and arched-back nursing. Sensitized rats took an average of 5.2 ± 1.1 days of pup exposure before being considered maternal. There was no significant difference between primiparous, primip-no-pups, and multiparous rats in number of offspring, number of male or female offspring, and litter weight (.41 ≤ p ≤ .9; Table 4.7). All dams spent significantly more time blanket and arched-back nursing than performing any other maternal behaviour (all p's ≤ .0002; Table 4.8: main effect of type of behaviour F(4, 36)=22.3, p ≤ .000001). There was a tendency toward an interaction effect (p ≤ .07) and no other significant differences between groups (p > .7). There was also a tendency toward a significant positive correlation between blanket nursing and density of pyknotic cells in the GCL (r = .57, p ≤ .07). There were no other significant correlations between maternal behaviors in parous dams and the onset of maternal behaviour in sensitized females (.1 ≤ p ≤ 1.0).

Table 4.7 Mean (± S.E.M.) size of litter, number of male and females pups, and weight of litter for parous rats in Experiment 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>female pups</th>
<th>male pups</th>
<th>litter weight(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primiparous</td>
<td>8.3 ± 1.0</td>
<td>8.7 ± .7</td>
<td>112.0 ± 6.3</td>
</tr>
<tr>
<td>Primip-no-pups</td>
<td>6.8 ± .7</td>
<td>9.2 ± .6</td>
<td>115.6 ± 5.5</td>
</tr>
<tr>
<td>Multiparous</td>
<td>6.8 ± 1.2</td>
<td>10.4 ± 1.4</td>
<td>110.4 ± 8.3</td>
</tr>
</tbody>
</table>

Table 4.8 Mean (± S.E.M.) percentage of total time during observations that primiparous and multiparous dams spent performing specific maternal behaviors in Experiment 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>Primiparous</th>
<th>Multiparous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lick/groom</td>
<td>0.1 ± .1</td>
<td>0.1 ± .1</td>
</tr>
<tr>
<td>Lick and Nurse</td>
<td>8.7 ± 1.1</td>
<td>7.4 ± 1.1</td>
</tr>
<tr>
<td>Arched Nurse</td>
<td>31.5 ± 5.5</td>
<td>50.7 ± 10.0</td>
</tr>
<tr>
<td>Blanket Nurse</td>
<td>34.5 ± 7.0</td>
<td>33.1 ± 5.9</td>
</tr>
<tr>
<td>Passive Nurse</td>
<td>14.8 ± 5.2</td>
<td>1.0 ± 0.6</td>
</tr>
</tbody>
</table>
4.4 DISCUSSION

The results of the present study demonstrate that hippocampal neurogenesis is affected by reproductive experience. The present research found that during the early postpartum period (24 h after parturition) primiparous and multiparous rats had lower levels of cell proliferation in the granule cell layer compared to nulliparous rats with or without pup-exposure. Furthermore twenty-two days after giving birth primiparous rats, regardless of whether they were exposed to pups, had fewer new neurons surviving in the granule cell layer compared to all other groups, and multiparous rats had fewer new neurons surviving in the granule cell layer compared to sensitized and nulliparous rats. Both cell proliferation and cell death were elevated in the granule cell layer of pup-exposed nulliparous rats in Experiment 1, during the early postpartum period, compared to all other groups. These effects were independent of age, degree of maternal behavior, and litter characteristics.

Hippocampal neurogenesis is decreased in the dam during the early postpartum period.

To date there has been very little investigation on hippocampal neurogenesis in the dam during the postpartum period. Shingo et al. (2003) found increased cell proliferation in the subventricular zone (SVZ) during the early postpartum period (PD 7); while in the present study we show a decrease in cell proliferation in the granule cell layer of parous dams early in the postpartum period (PD 2) consistent with Leuner et al., (2007). Leuner et al. (2007) further found a decrease in hippocampal cell proliferation on postpartum day 8 but not postpartum day 28. The opposing findings of cell proliferation rates during the postpartum in the dentate gyrus and the SVZ are likely due to the fact that the olfactory bulb (the destination of cells migrating from the SVZ) and the hippocampus mediate very different processes in the dam during the early postpartum period. The olfactory bulbs play a major role in the neural circuit that mediates
maternal behaviors (‘maternal circuit’) and are vital for appropriate maternal responding to offspring (Fleming and Rosenblatt, 1974; Kinsley and Bridges, 1990; Numan, 2007), while the hippocampus, an area not traditionally considered as part of the ‘maternal circuit’, is required for spatial navigation (Morris et al., 1982; Moser et al., 1995). Furthermore, late gestation and the early postpartum period are associated with the induction of maternal behaviours that are dependent on neural regions of the ‘maternal circuit’ such as the olfactory bulbs and the medial preoptic area but not the hippocampus (Numan and Insel, 2003; Numan, 2007).

**Reproductive experience affects cell survival during the postpartum period.**

The present study demonstrated that the survival of new neurons in the dam during the postpartum period is dependent on previous reproductive experience. For example, when the ratio of surviving new cells to the number of cells that proliferated (Figure 4.7) was examined, we found that multiparous rats have more new cells surviving across the postpartum period than primiparous rats. At first glance, this finding is somewhat curious given that both primiparous and multiparous dams are exposed to essentially the same experience of motherhood and have relatively the same number of cells proliferating in early lactation. However, these differences may be due to known behavioural, neural and/or hormonal changes between primiparous and multiparous rats (Moltz and Robbins, 1965; Svare and Gandelman, 1976; Kinsley and Bridges, 1988; Bridges and Hammer, 1992; Featherstone et al., 2000; Pawluski and Galea, 2006; Byrnes and Bridges, 2006). For example, previous maternal experience results in a reduced latency to act maternally toward offspring (Orpen and Fleming, 1987; Scanlan et al., 2006), decreased basal prolactin levels during lactation (Bridges and Hammer, 1992), and increased GFAP-ir cells in the hypothalamus (Featherstone et al., 2000).
Pup-exposure differentially affects cell proliferation and cell survival in parous dams and nulliparous rats.

The present study found that primiparous dams, regardless of pup-exposure during the postpartum period, have significantly lower levels of cell survival compared to nulliparous, multiparous, and sensitized rats. This suggests that the suppression in neurogenesis across the postpartum period in primiparous rats, regardless of pup exposure, is affected by pregnancy, parturition, and pup-exposure during the first few postpartum days, as primip-no-pups rats had pups removed on postpartum day 2, 24h after BrdU injection. However, these new surviving neurons in primiparous rats with and without pup-exposure may be integrated differently in the hippocampus and potentially mediate different behaviors. In support of this, previous research has shown that primiparous females with limited exposure to pups failed to complete a radial arm maze task on more days than parous dams, sensitized, and nulliparous rats (Pawluski et al., 2006b), exhibit increased anxiety-like behavior at least 30 days after birth (J.L. Pawluski and L.A.M. Galea, unpublished data), and exhibit increased ‘depressive-like’ behavior at the time of weaning (Boccia et al., 2007). However primiparous dams exposed to pups throughout the postpartum period do not exhibit similar behavioral deficits and in fact show improved spatial memory performance (Pawluski et al., 2006b) and decreased anxiety (Love et al., 2006; Byrnes and Bridges, 2006) compared to controls.

Pup-exposure to nulliparous rats (sensitization in Experiment 2), was also found to have a remarkably different effect on hippocampal neurogenesis in the present study. Pup-exposed nulliparous rats showed increased cell proliferation and cell survival compared to parous dams. These results point to the role of pup-exposure, or factors associated with exposure such as pup odor and maternal responding, as a form of ‘enrichment’ for nulliparous females. However, as mentioned previously, we did not find similar effects of pup-exposure on cell proliferation or
survival in parous females. This may be due to different physiological demands accompanying lactation. For example, parous rats feed their offspring to ensure that they survive; whereas pup-exposed nulliparous rats do not. Lactation itself results in neural, behavioral and hormonal changes that are unique to the lactating dam and may counteract the pup-enriching effects on hippocampal neurogenesis. As well, changes in hormone levels during pregnancy and parturition may be important in mediating the differences in cell proliferation, cell survival and cell death between parous dams and sensitized rats. Finally, it is also possible that increased neurogenesis in pup-exposed nulliparous rats is offset by increased cell death as pup-exposed nulliparous rats had significantly greater levels of cell death compared to all other groups in the early postpartum period.

The possible role of steroid hormones on hippocampal neurogenesis during the postpartum period in parous dams.

The immediate decrease in cell proliferation in the dentate gyrus during the early postpartum period in primiparous and multiparous rats compared to nulliparous rats points not only the marked neural plasticity during the onset of motherhood, but also to the possible role of estradiol and corticosterone, on hippocampal neurogenesis at this time. During late pregnancy and lactation there are a number of changes in steroid and peptide hormones (Stern and Levine, 1973; Rosenblatt et al., 1979). The steroid hormones estradiol and corticosterone are known mediators of hippocampal neurogenesis (Tanapat et al., 1999; Ormerod et al., 2003; Ormerod et al., 2004; Wong and Herbert, 2004), whereas the peptide hormone prolactin has been shown to be a mediator of olfactory bulb neurogenesis (Shingo et al., 2003).

With regards to estradiol, we found a positive association between estradiol levels and the number of proliferating cells in the hilus in Experiment 1 (Figure 4.5). Interestingly, this effect
appears to be due to variability in estradiol levels and cell proliferation in nulliparous females with and without pup-exposure, and not parous dams. Therefore, this association between estradiol and cell proliferation may be due to changes in estradiol levels across the estrous cycle and not primarily associated with changes in estradiol during the postpartum period. In addition, it should be noted that we only found this association with estradiol level in proliferating and not surviving new cells in the hilus.

In parous dams, it seems plausible that a combination of the decrease in estradiol from late pregnancy to lactation (Yoshinaga et al., 1969; Rosenblatt et al., 1979), the increase in corticosterone and decreased corticosteroid binding globulin (CBG) during lactation (Gala and Westphal, 1965; Stern et al., 1973; Pawluski et al., submitted) are responsible, in part, for the decrease in adult hippocampal neurogenesis in the dam. In support of this, Leuner et al. (2007) have shown that adrenalectomy and low level of corticosterone replacement reverses the suppression of cell proliferation during the early postpartum period (PD 8) in the hippocampus of primiparous dams.

It may also be argued that decreased cell proliferation and cell survival in parous dams is due to the stress of motherhood and pup-exposure. However, pup-exposure did not appear to have stress-related effects on nulliparous rats, as nulliparous rats exposed to pups and sensitized did not show the suppression in either hippocampal cell proliferation or survival. Furthermore, very few studies have investigated the role of stress or elevated levels of corticosterone on adult neurogenesis in the hippocampus of the female and it is not evident that hippocampal neurogenesis is consistently decreased in the female in response to stress (Falconer and Galea, 2003; Kuipers et al., 2006; Westenbroek et al., 2004). Falconer and Galea (2003) found that exposure to an acute stressor, which elevated corticosterone levels in both male and female rats, decreased cell proliferation in the dentate gyrus of male, but not female rats. While Westenbroek
et al. (2004) found that chronic stress decreased cell proliferation in the dentate gyrus of male, but increased cell proliferation in female, rats. More recently, Kuipers et al. (2006) found that chronically stressed females rats, which had elevated corticosterone levels, had decreased cell survival (6-10 days after BrdU injection) compared to non-stressed females. These findings, together with the findings from the current study and those of Leuner et al. (2007), suggest that the action of stress or elevated corticosterone on adult hippocampal neurogenesis may vary depending on the sex of the subject or reproductive status (i.e., whether a dam is lactating).

The functional role of new neurons in the hippocampus of the mother.

Although adult hippocampal neurogenesis was discovered in 1962 by Altman the functional relevance is still not understood. However, new neurons produced in the hippocampus of adult rodents are considered functional as they make appropriate synaptic connections and are electrophysiologically active (Zhao et al., 2006). The behavioral function of new neurons in the hippocampus of adult rodents is more controversial but they appear to play a role in hippocampus-dependent learning and memory performance and depression (for review see Leuner et al., 2006; Winocur et al., 2006; Santarelli et al., 2003). Significantly reducing neurogenesis in the dentate gyrus of the hippocampus using the antimitotic agent methylazomethanol acetate (MAM) or focal irradiation results in deficits in performance on hippocampus-dependent tasks and in anxiety-related learning (Santarelli et al., 2003; Shors et al., 2002; Winocur et al., 2006). Thus it may appear that the suppression in hippocampal neurogenesis seen in the primiparous rats is counterintuitive given that primiparous rats also show enhanced hippocampus-dependent learning and memory after weaning (Kinsley et al., 1999; Pawluski et al., 2006). However, it appears that an optimal amount of hippocampal neurogenesis is needed for improved spatial memory. For example, very low or very high levels
of hippocampal neurogenesis in rodents, which are evident after cortical and hippocampal lesions (Cameron et al., 1995; Gould and Tanapat, 1999) and seizures (Scarfman, 2004), are associated with reduced, not improved, hippocampus-dependent learning and memory (Leung and Shen, 2006; McNamara et al., 1992). In addition, females typically have greater levels of hippocampal cell proliferation than males (Galea and McEwen, 1999; Tanapat et al., 1999) and females typically do not perform as well as males on hippocampus-dependent tasks (Galea et al., 1996). This suggests that lower levels of hippocampal neurogenesis may be optimal in females for enhanced hippocampus-dependent memory. Therefore, the rate of neurogenesis in the hippocampus does not necessarily reflect the use of new neurons.

Given that an optimal level of neurogenesis is important for hippocampus-dependent learning and memory, it is perhaps not surprising that lower levels of hippocampal neurogenesis in primiparous rats across the postpartum period are coincident with improved hippocampus-dependent learning and memory at the time of weaning (Pawluski et al., 2006a). In addition, the less extensive decrease in cell survival with multiparity is coincident with slight (and not significant) improvements in spatial memory performance at weaning (Pawluski et al., 2006a). Additionally, it may be possible that there are differences in adult neurogenesis in the hippocampus of the dam at other time periods during the postpartum and these new cells may be differentially involved in hippocampus-dependent spatial learning and memory.

**Conclusions**

The present study demonstrates that hippocampal neurogenesis is altered during the postpartum period and differentially affected by reproductive experience. Future research is needed to determine the mechanisms behind these changes as well as the role of these new
hippocampal neurons on behavior in the mother. Clearly the hippocampus, although not
traditionally considered part of the maternal circuitry, is significantly altered with motherhood.
4.5 REFERENCES


Pawluski J.L., Vanderbyl B.L., Ragan K., Galea L.A. 2006b. First reproductive experience persistently affects spatial reference and working memory in the mother and these effects are not due to pregnancy or 'mothering' alone. Behav Brain Res. 175, 157-165.


CHAPTER 5

REPRODUCTIVE EXPERIENCE AFFECTS CORTICOSTERONE AND CORTICOSTEROID BINDING GLOBULIN IN THE POSTPARTUM RAT DAM

5.1 INTRODUCTION

Pregnancy and motherhood have marked effects on neural, hormonal and behavioral correlates in the mother. Recently we have investigated the role of pregnancy and motherhood on neural and behavioral outcomes that are dependent on the hippocampus and have found that primiparous rats (first time mothers) exhibit more remarkable neural and behavioral changes with motherhood than multiparous rats (second time mothers). For example, primiparous rats exhibit decreased dendritic arbors (Pawluski and Galea, 2006) and paradoxically, demonstrate enhancements in spatial learning and memory performance (Pawluski et al., 2006a) at the time of weaning. Interestingly, enhanced spatial learning coupled with dendritic atrophy in the hippocampus is similar to the pattern seen in chronically stressed nulliparous rats (Galea et al., 1997; Bowman et al., 2001). These chronically stressed female rats also exhibit elevated levels of corticosterone and decreased corticosteroid binding globulin (CBG) compared to non-stressed females (Galea et al., 1997). Therefore, given our past findings, primiparity may result in high levels of corticosterone and/or low levels of CBG during the postpartum period.

Reproductive experience, the number of times pregnant and mothered, has been shown to affect hormone levels. For example, prolactin levels are reduced in multiparous rats during

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5 A version of this chapter is submitted. Pawluski, JL, Charlier, TD, Hammond, GL, and Galea, LAM. Reproductive experience affects corticosterone and corticosteroid binding globulin during the postpartum period in the dam. J Endo.
pregnancy compared to primiparous rats (Bridges et al., 1993), and previous parity results in reduced estradiol and prolactin levels during proestrus (Bridges and Byrnes, 2006). In addition, work has investigated the role of glucocorticoid depletion and administration on maternal behavior. Hennessy et al. (1977) have shown the pup retrieval is deficient in adrenalectomized lactating rats, and it has been shown that corticosterone is important for the modulation of maternal behavior in the primiparous rat (Rees et al., 2004; Graham et al., 2006) and the solidification of maternal memory (Graham et al., 2006). Cortisol, the primary glucocorticoid in humans, has been shown to be important for a mother’s attraction to her infant in primiparous but not multiparous human mothers (Fleming et al., 1997). To our knowledge, there is no information about changes in corticosterone level in primiparous versus multiparous rats. It is possible that elevated levels of corticosterone may only occur during the first experience with pups in order for the induction and memory of maternal behaviors.

Corticosterone plays an important role in both milk production and the solidification of maternal memory (Nicholas and Hartmann, 1981; Pearlman et al., 1981; Graham et al., 2006), and it has been well documented that corticosterone levels change dramatically during late pregnancy and lactation (Gala and Westphal, 1965; Koch, 1969; Stern et al., 1973; Nicholas and Hartmann, 1981; Garland et al., 1986; Walker et al., 1992; Atkinson and Waddell, 1995; Fischer et al., 1995). Stern et al. (1973) have shown that parous rats exposed to pups have elevated levels of basal corticosterone during the early part of the light cycle, as compared to parous rats not exposed to pups. In addition, Fischer et al. (1995) have shown that lactating dams have elevated levels of basal corticosterone when compared to nulliparous rats. Others have found an overall decrease in corticosterone levels during lactation compared to nulliparous rats (Atkinson and Waddell, 1995; Hapon et al., 2003). Possible reasons for these discrepancies may be time of
According to the free hormone hypothesis, globulins are needed to transport steroid hormones to their target tissues but only free steroids (steroids not bound to globulins) are accessible to target tissues (Mendel, 1989). Measurement of plasma corticosterone levels are therefore of limited value if one does not also estimate the binding capacity of corticosteroid binding globulin (CBG) for corticosterone. Corticosteroid binding globulin binds corticosteroids and other steroids and alters their bioavailability to target tissues (Hammond, 2002). There is little information about changes in CBG levels during late pregnancy and lactation. Throughout pregnancy in rats, CBG levels are slightly increased compared to the virgin state (Gala and Westphal, 1965; Koch, 1969), peak during mid pregnancy, and decline markedly at parturition (Pearlman et al., 1981; Raymoure and Kuhn, 1983). During lactation, CBG levels decrease in primiparous rats compared to the nulliparous and pregnant rats (Gala and Westphal, 1965; Koch, 1969) and this difference is no longer evident a few days after weaning (Gala and Westphal, 1965). Similar changes in CBG levels during late pregnancy and lactation have been documented in other species such as the mouse, guinea pig and rabbit (Gala and Westphal, 1967).

The present work therefore aimed to determine the role of reproductive experience, pregnancy, and pup-exposure on corticosterone and CBG levels during late pregnancy, the postpartum period, and post-weaning. In order to investigate these effects, female rats were divided into five age-matched groups: multiparous, primiparous, nulliparous, sensitized and primip-no-pups. Given the effect of primiparity and chronic stress in female nulliparous rats to be associated with both dendritic remodeling in the hippocampus and better hippocampus-dependent learning (Galea et al., 1997; Bowman et al., 2001; Pawluski and Galea, 2006; Pawluski et al., 2006), we expect an increase in corticosterone and/or a decrease of CBG levels
across lactation in primiparous rats compared to multiparous and nulliparous rats. The contribution of pregnancy or pup-exposure alone was assessed. In addition, we aimed to determine whether increased corticosterone levels and/or decreased CBG levels were associated with maternal behavior during the first week postpartum.

5.2 METHOD

Animals

Twenty-nine female Sprague-Dawley rats (*Rattus norvegicus*), approximately 70-80 days of age, obtained from the UBC Animal Care Facility (Vancouver, Canada) were used in the present study. Rats were initially pair housed in opaque polyurethane bins (48 x 27 x 20cm) with aspen chip bedding with Purina rat chow and tap water ad libitum. Rats were maintained in a 12h:12h light/dark cycle (lights on at 7:20 a.m.). All protocols were in accordance with ethical guidelines set by the University of British Columbia Animal Care Committee and the Canada Council for Animal Care.

Rats were randomly assigned to one of five groups: nulliparous (n = 6), primiparous (n = 6), multiparous (n = 6), primips-no-pups (n = 6) and sensitized (n = 5). All animals were age-matched at the time of testing. Two primiparous and one multiparous dam did not get pregnant therefore n = 4 for the primiparous group and n = 5 for the multiparous group. Nulliparous rats were virgins. Primiparous rats birthed and mothered once. Multiparous rats birthed twice and mothered twice. Day of parturition/pup-exposure was considered Day 0. For both primiparous and multiparous rats, pups were weaned on Day 21. Primip-no-pups rats were primiparous dams that had pups removed on Day 0 (within 24 hours of giving birth). Sensitized rats were not sexually experienced, but were exposed to pups for a total of 22 days (for details see below). Nulliparous rats were housed in a separate colony room from pregnant and mothering/sensitized
rats in order to eliminate the possible effects of exposure to pup odor and vocalisations. After weaning, all animals were singly housed in the same colony room.

All animals were age‐matched such that multiparous rats during their second gestational period were the same age as primiparous and primip‐no‐pups rats during their first and only gestational period. In addition, pup‐exposure to females in the sensitized group began when rats were the same age as primiparous and primip‐no‐pups rats that had just given birth to their first litter and multiparous rats that had just given birth to their second litter. Nulliparous rats were age‐matched to these animals.

Breeding

For breeding, one female and one male were housed together in a wire mesh cage. Upon release of a vaginal plug females were individually housed in clear polyurethane bins until birth. For primiparous and multiparous rats, litters were culled to 4 male and 4 female pups approximately 24 hours after parturition. The dam and pups were housed in clear polyurethane bins until day 8 postpartum at which time they were housed in large opaque polyurethane bins (51 x 41 x 22cm) until weaning (postpartum day 21). Primip‐no‐pups rats and nulliparous rats were housed in a female‐only colony room away from pregnant and lactating rats.

Maternal Behavior Testing

For maternal behavior observations, the dam and pups were left undisturbed in the home cage. Observations were made every 5 seconds for 10 minutes twice a day (between 8:30-10:00a.m. and 12:00-1:00p.m.) on days 2 to 5 post‐parturition. The frequency and duration of the following maternal behaviors were observed during each testing period as previously described (Myers et al., 1989; Champagne et al., 2003; Pawluski et al., 2006b): licking/grooming (body licking and genital licking with the dam off the pups); arched‐back nursing; blanket nursing where the dam lays over the pups; passive nursing where the dam lies either on her back or side
while pups nurse; licking/grooming and nursing; and time off pups. Data for each behavior was compiled across all test periods and presented as total time spent (seconds) in a scored behavior. Scores from two multiparous rats are not available.

**Sensitization**

Sensitization (pup-exposure in nulliparous rats) was modified from previous research (Stern, 1997) and carried out as previously described (Pawlusi et al., 2006b). Briefly, nulliparous rats were continuously housed with 3 donor pups for 22 days. Three donor pups were used to induce sensitization as this has been found to be sufficient to elicit maternal-type behaviors in nulliparous females (Stern, 1997). The age of donor pups at the start of sensitization was 5-8 days. The same pups were used throughout the 22 days. This was done in order to simulate natural mothering. Milk-replete pups were given to sensitized rats every 24 hours and the donor pups were returned to their biological mother. Sensitization was confirmed from daily 15 minute video-recordings that took place each morning (between 8am and 11am) immediately after new donor pups were introduced to sensitized rats. Licking of pups, hovering over pups, and pup retrieval were scored from the tapes. Spot checks were performed approximately 1, 3 and 24 hours after the video recording to ensure that pups had not been attacked. All video recordings and spot checks took place for the first 10 days of exposure to pups or until sensitization occurred (licked and hovered over pups within a 24 hour period). Pups were removed for that day if a female injured a pup. No female injured pups more than once.

**Blood Collection**

Blood was collected from tail nicks; on gestational days 14 and 19, postpartum days 1, 5, 14, 21 (weaning), 35, and 45 in primiparous, multiparous, primip-no-pups rats; at similar time points prior to and during pup-exposure (ie ‘postpartum period’) in sensitized rats; and at similar time points in nulliparous controls. However, for simplicity, blood collection days in the
nulliparous and sensitized rats will be designated using the same day designation as those for parous dams (ie GD14, GD19, PD 1 etc). All blood samples were collected between 7:30-9:00am. Corticosterone levels in the rats have been shown to be elevated within 3 minutes of handling (Vahl et al., 2005), therefore samples were taken within 3 minutes of disturbing the animal (to max 100 μl). After collection, samples were stored overnight at 4°C and centrifuged at 10xg for 10 min. Serum was removed from the samples and stored at -20°C.

Corticosterone Assays

All samples were run in duplicate using commercially available RIA kits with slight procedural modifications. 125I-labeled ligands were used as tracer in all cases. Serum corticosterone was measured using the RIA kit for rat corticosterone from MP Biomedicals (Orangeburg, NY, USA). The average intra-assay coefficient of variation for all assays was below 10 %. The standard curve ED80 was between 22.5 and 32.3 ng/ml, ED50 was between 114.5 and 161.8 ng/ml, and for ED20 was between 731.4 and 927.0 ng/ml, with a detection limit of 7.7 ng/ml.⁶

Corticosteroid binding capacity

Corticosteroid binding capacity, which reflects the level of CBG, was determined following procedures developed by Hammond and Lähteenmäki (1983). Briefly, plasma samples were diluted 1/1000 and incubated 30 minutes at room temperature with dextran-coated charcoal (DCC) suspension to remove endogenous steroids. The dextran-coated charcoal was then precipitated by centrifugation and an aliquot of the samples was added into duplicate tubes containing 1 picomole of [1,2-³H] corticosterone (specific activity: 50Ci/mmol; ARC, St Louis, MO, USA) and to 1 tube containing 0.5 μM of cold corticosterone for the evaluation of the non-

⁶ For information on estradiol levels see Appendix 2.
specific binding. After incubation for 1 hour at room temperature, tubes were placed into an ice-water bath for 30 minutes. Ice-cold DCC was then added to remove unbound steroids and the reaction was incubated for another 10 minutes and centrifuged at 2800 rpm at 4°C for 10 minutes. The incubation with the cold DCC requires a correction factor (off rate) in the calculation of the binding capacity to account for the dissociation during DCC separation. This correction was determined experimentally and estimated at 80%. Supernatants were transferred into scintillation vials and 4 ml of Aqueous Counting Scintillant (Amersham Bioscience, UK) was added. The samples were counted in a scintillation spectrophotometer Beckman Coulter LS6000K. Specifically-bound counts were obtained by subtracting the non-specific background counts to the average of the total bound counts and converted in pmol/ml of serum.

Data Analyses

Separate repeated-measures analysis of variance (ANOVA) tests were calculated on basal corticosterone and CBG with group (nulliparous, primiparous, multiparous, primip-no-pups, sensitized) as the between-subjects variable and day (GD14, 19, PD1, 5, 14, 21, 35, 45) as the within-subjects variables. To determine differences in duration of maternal care, t-tests were performed between duration of a maternal behavior (licking, nursing, etc) and group (primiparous and multiparous). To determine differences in litter characteristics, ANOVAs were performed on litter size, number of male, and number of female pups and group (primip-no-pups, primiparous and multiparous). Pearson product-moment correlations were conducted between duration of specific maternal behaviors, day of onset of ‘maternal behavior’ in sensitized rats, with corticosterone levels and CBG levels from GD14 to PD5. Post-hoc comparisons utilized the Neumann Keuls test. \( P \) set at 0.05.

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\(^7\) For information on the estimation of free, unbound corticosterone see Appendix 3.
5.3 RESULTS

Litter size, sex of offspring and duration of maternal care was not significantly different between parous rats. A one-way ANOVA revealed no significant difference between primiparous, multiparous, and primip-no-pups rats in the size of the litter at birth, the number of male or female pups (all p's ≤ .4; Table 5.1).

Table 5.1 Mean (± SEM) number of female and male pups birthed by primiparous, multiparous, and primip-no-pups rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>female pups</th>
<th>male pups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primiparous</td>
<td>6.0 ± .9</td>
<td>10.8 ± .6</td>
</tr>
<tr>
<td>Primip-no-pups</td>
<td>6.2 ± 1.3</td>
<td>8.7 ± 1.3</td>
</tr>
<tr>
<td>Multiparous</td>
<td>8.0 ± 0.8</td>
<td>9.8 ± 0.7</td>
</tr>
</tbody>
</table>

A repeated-measures ANOVA revealed no significant main effect of group (primiparous vs. multiparous) (p ≤ .5) or an interaction effect between group and type of maternal behavior (p ≤ .1). There was a significant main effect of type of maternal behavior (F(5, 25)=14.331, p ≤ .000001; Table 5.2). Post-hoc tests revealed that dams spent significantly more time blanket nursing than all other maternal behaviors (.0001 ≤ p ≤ .0003), significantly more time arched-back nursing than licking (p ≤ .04), and tended to spend more time arched-back nursing than off pups (p ≤ .06). There were no other significant differences between behaviors (.2 ≤ p ≤ .7).

Table 5.2 Mean (± SEM) total time (seconds) during observations that primiparous and multiparous dams spent performing specific maternal behaviors during the first few postpartum days.

<table>
<thead>
<tr>
<th>Behavior (sec)</th>
<th>Primiparous</th>
<th>Multiparous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lick/groom</td>
<td>105.0 ± 47.3</td>
<td>61.7 ± 10.1</td>
</tr>
<tr>
<td>Lick and Nurse</td>
<td>411.3 ± 141.1</td>
<td>541.7 ± 157.7</td>
</tr>
<tr>
<td>Arched Nurse</td>
<td>1403.8 ± 334.7</td>
<td>543.3 ± 402.8</td>
</tr>
<tr>
<td>Blanket Nurse</td>
<td>2563.8 ± 560.2</td>
<td>2181.7 ± 413.0</td>
</tr>
<tr>
<td>Passive Nurse</td>
<td>206.3 ± 57.5</td>
<td>1176.7 ± 329.1</td>
</tr>
</tbody>
</table>

Total corticosterone was significantly elevated on postnatal day 1 in primiparous rats. A repeated-measures ANOVA on total corticosterone level revealed a significant interaction effect of group by day (F(28, 147) = 2.3, p ≤ .00098; Figure 5.1). Post-hoc tests revealed that primiparous rats had significantly elevated levels of corticosterone on PD1 compared to...
multiparous, primip-no-pups and sensitized rats (.001 ≤ p ≤ .05) and nulliparous rats had significantly elevated levels of corticosterone on GD19 (p ≤ .03) and PD35 (all p’s ≤ .00004) compared to all other groups. In addition, nulliparous rats had significantly elevated corticosterone levels on PD1 compared to primip-no-pups rats (p ≤ .002). There were no other significant differences between groups (.08 ≤ p ≤ 1.0). There was also a significant main effect of group (F(4, 21) = 5.9, p < .002) and day (F(7, 147) = 6.7, p ≤ .000001).

![Figure 5.1 Mean (± SEM) total serum corticosterone (nM). Primiparous rats had significantly elevated levels of corticosterone on PD1 compared to multiparous, primip-no-pups and sensitized rats (.001 ≤ p ≤ .05) and nulliparous rats had significantly elevated levels of corticosterone on GD19 (.02 ≤ p ≤ .03) and PD35 (.00002 ≤ p ≤ .00004) compared to all other groups. In addition, nulliparous rats had significantly elevated corticosterone levels on PD1 compared to primip-no-pups rats (p ≤ .002) and tended to have lower levels of corticosterone on PD 1 compared to sensitized and multiparous rats (.08 ≤ p ≤ .09). There were no other significant differences between groups (.25 ≤ p ≤ 1.0). *denotes primiparous significantly different from multiparous, primip-no-pups and sensitized rats.

CBG was significantly decreased in lactating dams and was significantly lower in primiparous dams than multiparous dams mid lactation. A repeated-measures ANOVA on
CBG revealed a significant interaction effect of group by day (F(28, 147) = 5.33, p ≤ .00001; Figure 5.2). Post-hoc tests revealed that primiparous and multiparous rats had significantly lower CBG than nulliparous and sensitized rats on PD1, PD14, and PD21 (all p’s ≤ .01), and primip-no-pups rats on PD14 and PD21 (.00002 ≤ p ≤ .01). Primiparous rats also had significantly lower CBG than sensitized rats on GD19 and PD5 (.01 ≤ p ≤ .02), primip-no-pups rats and nulliparous rats on PD5 (.004 ≤ p ≤ .0009), and multiparous rats on PD14 (p ≤ .04). There were no other significant differences between groups (all p’s > .2). There was also a significant main effect of group (F(4, 21) = 3.2, p ≤ .03) and day (F(7, 147) = 18.8, p ≤ .00001).
Figure 5.2 Mean (± SEM) corticosteroid binding capacity (CBG, nM). Primiparous and multiparous rats had significantly lower CBG than sensitized and nulliparous rats on PD1, PD14, and PD21 (all p’s ≤ .01) and primip-no-pups rats on PD14 and PD21 (.00002 ≤ p ≤ .01). Primiparous rats also had significantly lower CBG than sensitized rats on GD19 and PD5 (.01 ≤ p ≤ .02), and then primip-no-pups rats and nulliparous rats on PD5 (.004 ≤ p ≤ .0009), and than multiparous rats on PD14 (p ≤ .04). There were no other significant differences between groups (.2 < p < 1.0). # denotes primiparous and multiparous rats significantly different from all other groups; + denotes primiparous and multiparous significantly different from sensitized and nulliparous rats; ‘a’ denotes primiparous rat significantly different from sensitized rats; ‘b’ denotes primiparous rats significantly different from sensitized, primip-no-pups and nulliparous rat; * denotes primiparous rats significantly different from multiparous rats.

Corticosterone and CBG did not correlate with maternal behaviors. Sensitized rats took an average of 4.4 ± 1.2 days of pup exposure before being considered maternal (range 2 to 8 days). There was no correlation between day of onset of sensitized maternal behavior with corticosterone levels or CBG (.2 ≤ p ≤ 1.0). There were no significant correlations between specific maternal behaviors and CBG or corticosterone levels (p > .1).
5.4 DISCUSSION

The results of the present study demonstrate that reproductive experience affects corticosterone levels and CBG levels particularly during the postpartum period. Here we show that primiparous rats have significantly elevated total corticosterone on postnatal day 1 compared to multiparous, primip-no-pups, and sensitized rats. In addition, CBG levels decreased in lactating dams (both primiparous and multiparous rats) and this decrease in CBG was dependent on reproductive experience at mid-lactation with primiparous rats having lower levels than multiparous rats. These results are not due to differences in degree of maternal care or particular litter characteristics.

Our finding of elevated corticosterone in primiparous rats on the morning of the first postpartum day is in agreement with work in humans demonstrating that primiparous women have elevated levels of cortisol during the early postpartum period compared to multiparous women (Grajeda and Perez-Escamilla, 2002). In addition, this finding partially agrees with previous work demonstrating that corticosterone levels are elevated during the early postpartum in primiparous rats compared to controls, and this elevation appears to decline during lactation (Fischer et al., 1995). Our results also show that there are no group differences in total corticosterone levels, apart from on postpartum day 1, throughout lactation when tested in the early part of the light cycle. Interestingly, Stern et al. (1973) found that during the early part of the light cycle, lactating dams have elevated levels of corticosterone compared to non-lactating parous females when tested during mid lactation (days 8-15: Stern et al., 1973). Discrepancies between our findings and that of Stern et al. (1973) are unclear but may be due to the method of blood collection. Stern et al. (1973) collected blood from decapitation at various time points throughout the day where as we took blood samples from the tail. It is possible that these different techniques and different areas of blood collection could result in differences in the level
of circulating corticosterone measured. Previous research in other species has found the level of steroids in the blood is dependent on the blood withdrawal site (carotid artery versus jugular vein; Schlinger and Arnold, 1993). In addition, it has been found that peripherally circulating levels of corticosterone may be very different than neural levels. One study has found that plasma levels of corticosterone did not differ between lactating and non-lactating females, while levels of corticosterone in the hippocampus, cerebral cortex, and hypothalamus were significantly higher in lactating rats (Kakihana et al., 1980).

**The role of corticosterone in maternal behavior.**

Our finding of increased corticosterone levels on postpartum day 1 in primiparous rats is in agreement with recent research that has pointed to the importance of glucocorticoids in the induction and maintenance of maternal behavior, particularly in the first-time mother. In the human, Fleming et al. (1997) have shown that first time mothers with high levels of cortisol were more attracted to their infant. In the rodent, recent observations have shown that corticosterone is important for the onset and maintenance of maternal behaviors in the rat (Graham et al., 2006; Rees et al., 2004). Rees et al. (2004) have shown that corticosterone administration to adrenalectomised primiparous rats reverses deficits in maternal licking and time spent in their nest, thus suggesting a role for corticosterone in the modulation of ongoing maternal behavior. In addition, Graham et al. (2006) have shown that corticosterone administration enhanced maternal memory, defined as the retention of maternal responsiveness as a consequence of prior experience with pups (Li and Fleming, 2003) in primiparous rats. Interestingly, corticosterone does not have the same effect on maternal responding and maternal behavior in sensitized rats (Rees et al., 2006), and appears to be less important for maternal responding in multiparous dams (Thoman et al., 1970). Given the results of these studies, it appears that glucocorticoids are
important for the initial induction of maternal behavior in primiparous dams. Therefore, it is possible that a significant elevation in corticosterone during the early postpartum period in primiparous dams is needed to permanently alter neural circuitry, maintain adequate maternal behavior, and develop long-term maternal memory. With the establishment of maternal memory the increase in corticosterone levels during the early postpartum period may no longer be necessary in multiparous dams.

CBG is decreased during the postpartum period in parous rats and is significantly lower in primiparous dams during mid-lactation.

Previous research has documented a decrease in plasma CBG levels during the postpartum in primiparous rats (Gala and Westphal, 1965; Koch, 1969; Nicholas and Hartmann, 1981; Pearlman et al., 1981). In the present study, we found that CBG is not only decreased during lactation, but this decrease is more pronounced in primiparous rats than multiparous rats and is significantly lower on postpartum day 14. The differences in CBG levels between primiparous and multiparous dams in the present study further support the idea of the predominant role of corticosterone for the onset of motherhood and the induction of maternal memory with primiparity. This is important because a decrease in circulating CBG levels will cause transient increase in free corticosterone levels.

Our results also indicate that the decrease in CBG is dependent on processes associated with lactation. We found that parous females that had pups removed within 24 hours of birth (primip-no-pups) and pup-exposed virgin females (sensitized) did not show a decrease in CBG levels. In addition, parous dams did not have a decrease in CBG levels once pups were weaned. Previous work has demonstrated the larger litters result in a greater decrease in CBG in dams (Gala and Westphal, 1965), thus providing more evidence that lactation and suckling is
important for the decrease in CBG levels in the dam. In the present study it should be noted that
effects were not due to litter size as all litters were culled during the early postpartum period.
Taken together these data confirm that lactation and suckling is involved in the alteration of
circulating CBG levels.

Although the mechanism behind the decrease in CBG during lactation has yet to be
determined, it seems plausible that the thyroid hormone, thyroxine, and glucorticoids may play a role. Thyroxine has been shown to significantly increase CBG biosynthesis in the male rat, probably through an increase of mRNA stability (Smith and Hammond, 1992). Interestingly, thyroxine shows a dramatic decrease during lactation in rat dams which is associated with increased lactation intensity or suckling burden (Jack et al., 1994; Kahl et al., 1987). Therefore a decrease in thyroxine levels may contribute to decreased CBG levels during lactation. Furthermore, high levels of glucocorticoids have been shown to feedback and decrease CBG levels in female rats (Gala and Westphal, 1966). Therefore high levels of corticosterone, as seen in the present study in primiparous rats, may result in a further decrease in CBG during lactation. Prolactin and oxytocin also play a major role during lactation suggesting that these peptides might be involved in the control of CBG.

Conclusions

Results from the present study demonstrate that reproductive experience differentially affects circulating corticosterone and CBG levels in the dam. Here we show that primiparous rats have significantly elevated levels of corticosterone on postpartum day one and significantly lower levels of CBG on postpartum day 14. In addition, both primiparous and multiparous dams have lower CBG during the postpartum period compared to all other groups suggesting an increase in circulating levels of free corticosterone during the early postpartum period.
Interestingly, this work suggests that the decreased dendritic arbors and increased spatial memory at the time of weaning in primiparous dams (Pawluski and Galea, 2006; Pawluski et al., 2006a) may be due to increased levels of free corticosterone across the early postpartum period. Indeed, recent work has shown that increased levels of corticosterone during early lactation mediate the decrease in hippocampal cell proliferation in the dam (Leuner et al., 2007). Further research is needed to confirm the role of elevated corticosterone and decreased CBG during the postpartum period on hippocampal morphology and hippocampus-dependent behavior with primiparity. In addition, the mechanisms behind the changes in corticosterone and CBG with reproductive experience have yet to be determined.
5.5 REFERENCES


Pawluski J.L., Vanderbyl B.L., Ragan K., Galea L.A. 2006b. First reproductive experience persistently affects spatial reference and working memory in the mother and these effects are not due to pregnancy or 'mothering' alone. Behav. Brain Res., 175, 157-165.


CHAPTER 6

GENERAL DISCUSSION

It has not been until recently that research has focused on the effect of motherhood on hippocampus structure, function, and plasticity. The experiments in this thesis aimed to thoroughly investigate the role of motherhood and reproductive experience on hippocampus-dependent spatial memory, adult neurogenesis in the hippocampus, and corticosterone levels throughout late gestation and the postpartum. Interestingly, the main findings from the present thesis demonstrate that: 1) primiparity results in significant improvements in hippocampus-dependent spatial memory compared to nulliparity (Ch 2: Pawluski et al., 2006a); 2) primiparity results in enhanced spatial memory that persists long past the time of weaning, and is not due to the effect of pregnancy or pup-exposure alone (Ch 3: Pawluski et al., 2006b); 3) primiparity and multiparity result in decreased hippocampal neurogenesis via decreased cell proliferation during the early postpartum period compared to nulliparity, and primiparity with or without pup-exposure results in decreased cell survival across the postpartum period compared to nulliparity, multiparity, and pup-exposure alone (Ch 4: Pawluski and Galea, in press); and 4) primiparity results in increased levels of corticosterone during the early postpartum period compared to multiparity and both primiparity and multiparity result in decreased CBG throughout the postpartum period (Ch 5: Pawluski et al., submitted).

Multiparity also affects hippocampus-dependent spatial memory (Ch 2: Pawluski et al., 2006a; Ch 3: Pawluski et al., 2006b), hippocampal neurogenesis (Ch 4: Pawluski and Galea, in press), corticosterone and CBG in the mother (Ch 5: Pawluski et al., submitted), but to a lesser extent than primiparity. Multiparity results in a tendency toward enhanced spatial working, but not reference, memory performance compared to nulliparity (Ch 2: Pawluski et al., 2006a; Figure 2.1 and Figure 2.2). However, the enhancement of spatial working memory with multiparity does
not persistent as it does with primiparity (Ch 3: Pawluski et al., 2006b). In addition, across the postpartum period, multiparity results in decreased cell survival compared to nulliparity but increased cell survival compared to primiparity (Ch 6: Pawluski and Galea, in press; Figures 4.5 and 4.6). Therefore, the data in the present thesis suggest that while motherhood, regardless of parity, has an effect on hippocampus function and plasticity, the greatest effects on hippocampal dependent memory and hippocampal neurogenesis are seen with primiparity.

6.1 ENHANCED SPATIAL MEMORY IN THE MOTHER

The transition to motherhood is associated with increased learning and memory as a new mother must learn a host of pup-directed behaviors, such as licking and nursing, to ensure that offspring survive. In addition, the primiparous dam forms maternal memory (defined as the retention of maternal responsiveness as a consequence of prior experience with pups; Orpen and Fleming, 1987) for these behaviors so they are quickly implemented with subsequent exposure to pups. Parturient females also demonstrate improved social learning during the postpartum period compared to nulliparous females (Fleming et al., 1994). The work of the present thesis demonstrates that parous dams also have enhanced spatial memory and this enhancement is greater in primiparous females compared to multiparous females (Ch 2 & 3: Pawluski et al., 2006a, b). Improved spatial memory with motherhood has been suggested to be important for efficient foraging in order for the mother to return to her pups to care for and protect them (Kinsley et al., 1999; Pawluski et al., 2006a). In addition, enhanced spatial ability in the dam may be needed to quickly find a mate during post-parturient estrous before ovulation is suppressed during lactation, and to remember the location of the nest site to properly retrieve pups to the nest. However, recent evidence suggests that early motherhood is associated with poorer spatial learning but enhanced memory mid-lactation in primiparous days (Darnaudery et al., 2007). The greater enhancement of spatial memory in primiparous dams compared to
multiparous dams at weaning may be the result of increased demands on primiparous dams as they need learn a host of pup-directed behaviors during early motherhood. The changing hormone levels and neural plasticity during pregnancy, parturition and the postpartum period likely play a critical role in altered hippocampus-dependent spatial memory in the new mother.

6.2 HIPPOCAMPAL PLASTICITY AND REPRODUCTIVE EXPERIENCE

The research in this thesis and previous research demonstrates that hippocampus morphology and neurogenesis is altered with reproductive experience (Pawluski and Galea, 2006; Ch4 Pawluski and Galea, in press). Previously, I have found a decrease in dendritic arbourizations in the CA1 and CA3 regions of the hippocampus in primiparous dams at weaning compared to both multiparous and nulliparous females (Pawluski and Galea, 2006). Present work in this thesis demonstrates that primiparous dams have significantly lower levels of hippocampal neurogenesis via cell survival at weaning compared to multiparous and nulliparous females (Ch 4: Pawluski and Galea, in press). It is interesting to note that there is more pronounced changes in hippocampal plasticity in primiparous dams compared to multiparous dams. This may be due to an overall increase in neural plasticity in the new mother with the induction of the maternal circuit (Numan, 2007) and the formation of maternal memory (Orpen and Fleming, 1981).

The relationship between dendritic arbourizations in the CA1 and CA3 regions and neurogenesis in the dentate gyrus has yet to be determined. One possibility is that decreased dendritic arbourizations in primiparous dams may lead to decreased ability for new cells to integrate into hippocampal circuitry by forming connections with CA3 neurons, and therefore reduce the number of new cells that survive during the postpartum period. The opposite may also be true, fewer cells survive therefore fewer connections are made with CA3 pyramidal neurons and these neurons contract. In addition, further work is need to determine whether decreased
neurogenesis in primiparous females, and to a lesser extent in multiparous females, at weaning is
coupled with altered dendritic lengths and spine densities of mature granule cells in the dentate
gyrus of the hippocampus.

6.3 ENHANCED SPATIAL MEMORY AND DECREASED HIPPOCAMPAL
DENDRITIC MORPHOLOGY IN THE MOTHER

It may seem paradoxical that there is improved spatial memory in primiparous dams (CH 2: Pawluski et al., 2006a; Kinsley et al., 1999; Lemaire et al., 2006) at a time when there is
decreased dendritic arbourizations on CA1 and CA3 pyramidal neurons (Pawluski and Galea, 2006) and decreased hippocampal neurogenesis (Ch 4: Pawluski and Galea, in press; Leuner et al., 2007). However, a similar pattern of enhanced spatial learning and memory and decreased
dendritic arbors in the hippocampus is seen in nulliparous female rats exposed to chronic stress
(Bowman et al., 2001; Galea et al., 1997). For example, Galea et al. (1997) found that
chronically stressed female rats have reduced dendritic arbors on the basal dendrites of CA3
pyramidal cells in the hippocampus. Using the same stress paradigm, Bowman et al. (2001)
found that chronically stressed female rats exhibit improved spatial memory compared to non-
stressed females. Therefore enhanced spatial memory with primiparity (Ch 2: Pawluski et al.,
2006a), at a time when there is hippocampal dendritic atrophy (Pawluski and Galea, 2006), is
similar to what is seen in chronically stressed females (Bowman et al., 2001; Galea et al., 1997).
Furthermore, the effects of chronic stress on hippocampal structure and function in the females
are likely due to elevated corticosterone levels and decreased CBG (Galea et al., 1997;
Magarinos et al., 1998). Because primiparity also results in increased corticosterone and
decreased CBG levels during the postpartum period (Ch 5: Pawluski et al., submitted), this
suggests a similar mechanism may be behind the enhanced spatial memory and altered
hippocampal morphology. It should be noted that the similar pattern of improved hippocampal spatial memory and decreased dendritic arbors in chronically stressed females and primiparous dams (Bowman et al., 2001; Galea et al., 1997; Pawluski and Galea, 2006; Ch 2: Pawluski et al., 2006a) does not indicate that the initial experience of motherhood (primiparity) is analogous to the experience of chronic stress. However, the first maternal experience may be considered a type of stress that is required for the induction of neural and behavioral processes needed for the onset of maternal behavior and offspring survival.

Furthermore, multiparous rats do not show the dendritic atrophy in the CA1 and CA3 region of the hippocampus (Pawluski and Galea, 2006), while at the same time only exhibit a slight improvements in spatial memory at weaning compared to nulliparous rats (Pawluski et al., 2006, Ch 2). Thus, it is possible that different aspects of hippocampal plasticity that are altered with reproductive experience differentially affect hippocampus-dependent spatial memory. For example, multiparous rats have increased spine density in CA1 pyramidal neurons of the hippocampus at weaning compared to primiparous and nulliparous rats (Pawluski and Galea, 2006). Therefore, increased synaptic transmission and not altered dendritic structure per se may be responsible for these changes in learning and memory performance with multiparity. In accordance with this, Tomizawa et al. (2003) report greater long-lasting LTP along the Schaffer collateral fibers, which synapse onto the CA1 pyramidal cells, in multiparous mice during the early postpartum period (3-6 days) compared to nulliparous females (primiparous mice were not tested). Using a similar paradigm to Tomizawa et al. (2003), Lemaire et al. (2006) report increased LTP, but not increased long-lasting LTP, along the Schaffer collaterals in primiparous rats two weeks after weaning and 16 months later compared to nulliparous females. Taken together the findings of Tomizawa et al. (2003) and Lemaire et al. (2006) demonstrate that LTP is differentially affected at different timepoints during motherhood. However, to date no work
has investigated whether there are differences between multiparous, primiparous, and nulliparous rats on measures of LTP during the postpartum period, at the time of weaning, or long past the time of weaning. Given the data on altered hippocampus-dependent spatial learning and memory with reproductive experience, it is expected that motherhood, in particular primiparity, will result in enhancements in LTP that will persist past weaning.

6.4 ENHANCED SPATIAL MEMORY AND DECREASED HIPPOCAMPAL NEUROGENESIS IN THE MOTHER

The data from Chapter 2 and 3 suggest that increased spatial memory performance in parous dams is coincident with decreased cell survival in the hippocampus, which may also seem paradoxical. However, it has been suggested that there is an optimal amount of hippocampal neurogenesis that may be important for spatial memory performance. For example, females typically have greater levels of hippocampal cell proliferation than males (Galea and McEwen, 1999; Tanapat et al., 1999) and females typically do not perform as well as males on hippocampus-dependent tasks (Galea et al., 1996). This suggests that lower levels of hippocampal neurogenesis may be optimal in females for enhanced hippocampus-dependent memory. Thus, it is perhaps not surprising that lower levels of hippocampal neurogenesis in primiparous rats across the postpartum period (Ch 4: Pawluski and Galea, in press) are coincident with improved hippocampus-dependent learning and memory at the time of weaning (Ch 2: Pawluski et al., 2006a). In addition, the less extensive decrease in cell survival with multiparity is coincident with slight (and not significant) improvements in spatial memory performance at weaning (Ch 2: Pawluski et al., 2006a; Ch 4: Pawluski and Galea, in press). This provides further evidence for an optimal rate of neurogenesis for spatial memory performance. Additionally, it may be possible that there are differences in adult neurogenesis in the
hippocampus of the dam at other time periods during the postpartum period and these new cells may be differentially involved in spatial learning and memory. However, Leuner et al. (2007) have recently demonstrated that there is no significant difference in hippocampal cell proliferation in parous dams at weaning or two weeks after weaning (during diestrus) and diestrus virgins. Therefore, there may be a minimal role for neurogenesis in enhanced spatial memory past the time of weaning.

It is also important to understand the functional role of new neurons in the hippocampus of the mother, as an optimal amount of neurogenesis may not always correlate with optimal spatial memory performance. For example, the work in Chapter 4 demonstrates that primiparous rats with pups removed 24 hours after the BrdU injection exhibit the same density of new cells surviving in the hippocampus across the postpartum period as primiparous rats with pup-exposure (Pawluski and Galea, in press; Figure 4.5); however findings in Chapter 3 demonstrate that pregnant-only females were remarkably slower to complete a spatial memory task when tested 8.5 weeks after parturition and pup removal (Pawluski et al., 2006b; Figure 3.3). Therefore, it seems likely that the beneficial incorporation of the new neurons into the hippocampus of primiparous dam is dependent on aspects related to pup-exposure and lactation. It is also possible that the rate of hippocampal neurogenesis in the dam is not related to the improved spatial learning seen with motherhood. Further work is needed to determine the relationship between neurogenesis and hippocampus-dependent spatial learning and memory in the dam.

6.5 PERSISTENCE OF IMPROVED SPATIAL MEMORY WITH PRIMIPARITY

The findings of the present thesis demonstrate that enhanced spatial memory in motherhood persists past the time of weaning in primiparous rats alone (Ch 3: Pawluski et al.,
2006b) and this is consistent with past research (Gatewood et al., 2005; Lemaire et al., 2006; Love et al., 2005). However, the mechanisms behind this improvement in spatial memory with primiparity have yet to be fully determined. As mentioned previously, it is also possible that the enhanced LTP may be important for improved spatial memory in the dam after the time of weaning: Lemaire et al. (2006) have demonstrated that LTP along the Schaffer collaterals is enhanced in primiparous dams compared to nulliparous females at the same time points that dams have improved spatial reference memory performance compared to nulliparous females (two weeks and 16 months after weaning). Furthermore aging-related deficits may be reduced with reproductive experience as Gatewood et al. (2005) have demonstrated that reproductive experience results in decreased amyloid precursor protein immunoreactivity, a marker of neurodegeneration, in the hippocampus of aged (18 month old) primiparous and multiparous dams compared to nulliparous females. They also found that enhanced spatial memory performance was associated with lower amyloid precursor protein immunoreactivity in the hippocampus (Gatewood et al., 2005). Taken together these findings suggest that the persistent effect of motherhood on spatial memory is associated with changes in hippocampus structure and plasticity.

6.6 ALTERED SPATIAL MEMORY AND HIPPOCAMPAL NEUROGENESIS WITH PREGNANCY OR PUP-EXPOSURE ALONE

The work of the present thesis demonstrates that the cognitive (Ch 3: Pawluski et al., 2006b), neural (Ch 4: Pawluski and Galea, in press), and hormonal (Ch 5: Pawluski et al., submitted) effects seen with primiparity are due to the combined effect of pregnancy and motherhood, and not pregnancy or mothering alone. Indeed, the effects of pregnancy alone or pup-exposure alone often resulted in dramatically different responses than those seen with
primiparity. For example, pup-exposure to nulliparous rats resulted in increased cell proliferation and increased cell death, with no significant difference in cell survival, while primiparity resulted in decreased cell proliferation and decreased cell survival across the postpartum period, regardless of pup-exposure (Ch 4: Pawluski and Galea, in press). This increased cell proliferation with pup-exposure in nulliparous, but not primiparous, rats suggests that pup-exposure acted as a type of enriched environment for nulliparous females (Ch 4: Pawluski and Galea, in press). In addition, pregnancy-alone resulted in dramatically different hippocampus-dependent spatial memory performance, with the pregnant-only females failing to complete the task on significantly more of the days than all other groups (Ch 3: Pawluski et al., 2006b, Figure 3.3). This finding suggests that pregnant-only females (parous females with pups removed within 24 hours of birth) are affected by the loss of pups more than 60 days later. Preliminary evidence suggests that pregnant-only females display heightened levels of anxiety-like behaviors at the normal time of weaning (but long after the pups have been removed from these parous rats; Pawluski and Galea, in prep), and current studies are underway to determine whether these rats also show depressive-like behavior. Furthermore, dams with pups removed for long periods of time throughout the postpartum period exhibit depressive-like behavior compared to dams that did not have pups removed (Boccia et al., 2007). Thus, it appears that parous dams that are not given minimal exposure to pups throughout the postpartum period may exhibit a variety of cognitive and behavioral deficits.

6.7 POSSIBLE ROLE OF CORTICOSTERONE IN SPATIAL MEMORY AND HIPPOCAMPAL NEUROGENESIS IN THE MOTHER

Pregnancy and motherhood are accompanied by a host of changes in steroid hormones (Gala and Westphal, 1965; Nicholas and Hartmann, 1981; Rosenblatt et al., 1979; Stern et al.,
1973; Yoshinaga et al., 1969) and it is likely that these hormones play an important role in mediating changes in hippocampus function and plasticity in the mother. Certainly the induction of maternal behavior is dependent on changing levels of estrogens, progesterone and prolactin during pregnancy (Mann and Bridges, 2001). As previously mentioned, the data from the present thesis found changes in the circulating levels of basal corticosterone and CBG during the postpartum period with reproductive experience. Given that there is increased levels of corticosterone and decreased levels of CBG with reproductive experience that precede the cognitive and neural effects of motherhood it is possible that corticosterone may play a role in these effects in the dam (Ch 2: Pawluski et al., 2006a; Ch 4: Pawluski and Galea, in press). The relationship between increased corticosterone, decreased CBG, and hippocampus function and plasticity is perhaps not surprising, as previous research have found a similar relationship in chronically stressed female rats. For example, the work of Bowman et al. (2001) suggest that prolonged periods of high levels of corticosterone and/or low CBG (Galea et al., 1997) as a result of chronic stress are associated with improved spatial memory in the nulliparous female. This enhanced spatial memory after twenty-one days of chronic stress in the virgin female appears to be due to a peak in corticosterone during the early days of the chronic stress, and decreased CBG levels a few days later (Bowman et al., 2001; Galea et al., 1997). This same profile of an initial elevation in corticosterone followed by decreased CBG is evident in primiparous and, to a lesser extent, multiparous rats across the postpartum period (Ch 5: Pawluski et al., submitted: Figure 5.1). Taken together the work of this dissertation suggests that corticosterone and the availability of corticosterone to target tissues via decreased CBG levels, may play an important role in improved spatial memory at the time of weaning (Ch 2: Pawluski et al., 2006a).

However, the findings in Chapter 5 suggest there is no direct role for corticosterone in mediating the persistent enhancement in spatial memory after the time of weaning in primiparous
The work of Chapter 3 demonstrates that when testing begins 35 days after weaning, primiparous rats had enhanced hippocampus-dependent spatial memory compared to multiparous and nulliparous rats (Pawluski et al., 2006b), however, as evident from Chapter 5, there is no significant persistent difference found between groups in corticosterone and CBG levels after weaning (Figure 5.3). This does not rule out the possibility that pregnancy and lactation have permanent effects on the neural circuitry in the dam. In accordance with this idea, recent work by Bridges and Byrnes (2006) have shown that there are permanent changes in the hypothalamic-pituitary gonadal (HPG) axis after weaning which result in decreased levels of estradiol at the peak of proestrus in primiparous rats compared to cycling virgin females.

It also seems plausible that increased corticosterone and decreased CBG levels during the postpartum period in the mother are responsible for the decreased adult neurogenesis in the hippocampus of the dam. In support of this, a recent study demonstrates that adrenalectomy and low level of corticosterone replacement reverses the suppression of cell proliferation during the early postpartum period in the hippocampus of the primiparous dam (Leuner et al., 2007). Interestingly, a similar relationship between elevated corticosterone and suppressed hippocampal neurogenesis via cell proliferation in nulliparous females has not been reported. To date very few studies have investigated the role of stress or elevated levels of corticosterone on adult neurogenesis in the hippocampus of the female (Falconer and Galea, 2003; Kuipers et al., 2006; Westenbroek et al., 2004). Falconer and Galea (2003) found that exposure to an acute stressor, which elevated corticosterone levels in both male and female rats, decreased cell proliferation in the dentate gyrus of male, but not female rats. While Westenbroek et al. (2004) found that chronic stress decreased cell proliferation in the dentate gyrus of male, but increased cell proliferation in female, rats. More recently, Kuipers et al. (2006) found that chronically stressed
female rats, which had elevated corticosterone levels, have decreased cell survival (6-10 days after BrdU injection) compared to non-stressed females. These findings suggest that the action of corticosterone on adult hippocampal cell proliferation may vary depending on the sex of the subject or reproductive status (i.e. whether a dam is lactating). However, more work is needed to determine the role of corticosterone on adult hippocampal cell proliferation and cell survival in the female.

The action of corticosterone on hippocampal function and plasticity in the mother may be due to differences in the density and binding capacity of corticosteroid receptors in the hippocampal formation. For example, improved spatial memory performance in chronically stressed nulliparous female rats results in increased glucocorticoid receptor immunoreactivity in CA1 area of the hippocampus and increased mineralocorticoid receptor immunoreactivity in the CA3 area of the hippocampus compared with non-stressed females (Kitraki et al., 2004). In addition, the action of corticosterone on the suppression of cell proliferation in the hippocampus of adult male rats is dependent on both mineralocorticoid and glucocorticoid receptors (Montaron et al., 2003) but to date no work has investigated these effects in females. However, in the lactating dam, the binding capacity of glucocorticoid receptors in the hippocampus is decreased (Meaney et al., 1989) suggesting that changes in corticosteroid receptor density and binding capacity in the hippocampus may play a role in corticosterone action on hippocampus-dependent spatial memory and hippocampal neurogenesis in the dam.

6.8 POSSIBLE ROLE OF ESTRADIOL AND PROGESTERONE ON HIPPOCAMPAL NEUROGENESIS IN THE MOTHER

In addition to the potential role of corticosterone on hippocampal neurogenesis in the dam during the early postpartum period, the steroid hormones estradiol and progesterone may
also play a role. Estradiol levels are elevated during late pregnancy, decrease during parturition and remain low during the postpartum period (Garland et al., 1987; Rosenblatt et al., 1979; Yoshinaga et al., 1969). Progesterone levels are elevated during pregnancy, decrease prior to parturition, and are elevated during mid-lactation in lactating dams (Gala and Westphal, 1965; Hapon et al., 2003; Nicholas and Hartmann, 1981). In the nulliparous female the role of estradiol and progesterone on cell proliferation has been documented. For example, estradiol initially increases cell proliferation within 4 hours of administration and subsequently decrease cell proliferation, 48 hours after administration (Ormerod et al., ). An elevation in progesterone following an elevation in estradiol decreases estradiol’s enhancing effects on cell proliferation (Tanapat et al., 2005). Unpublished work by Falconer and Galea has found that ovariectomized rats administered a low level of estradiol and progesterone for 3 days followed by a high dose of estradiol have significantly suppressed cell proliferation in the dentate gyrus. This data suggests that the fluctuation of estradiol and progesterone during late pregnancy, parturition and the early postpartum period may also contribute to the decreased cell proliferation in parous dams during the early postpartum period (Ch 4: Pawluski and Galea, in press; Leuner et al., 2007; Darnaudery et al., 2007). Taken together, it seems plausible that the elevation in estradiol during late pregnancy followed by an elevation in progesterone during the early postpartum period results in decreased cell proliferation in the hippocampus of the dam. Further work is needed to determine the relationship between circulating levels of the steroid hormones estradiol, progesterone and corticosterone hippocampal neurogenesis in the dam during the postpartum period.
6.9 POSSIBLE ROLE OF PEPTIDE HORMONES ON THE HIPPOCAMPUS OF THE MOTHER

Apart from the role steroid hormones may play in improved hippocampus-dependent spatial memory and decreased hippocampus neurogenesis in the dam, recent work has demonstrated that the actions of the peptide hormones, oxytocin and prolactin, may be important in these processes. Oxytocin, a hormone which increases in response to milk ejection (Buhimschi, 2004), is associated with enhanced reference memory and increased long-lasting LTP in the dam shortly after parturition (PD 3-6: Tomizawa et al., 2003). In addition, prolactin, which increases near the end of pregnancy and during the postpartum period (Grattan, 2001) and is essential for lactation (Buhimschi, 2004), has been suggested as a mediator of increased cell proliferation in the subventricular zone of the dam during the early postpartum period (Shingo et al., 2003). However, prolactin has not been shown to affect cell proliferation in the hippocampus of the nulliparous female (Shingo et al., 2003). To date no work has investigated the role of oxytocin on adult hippocampal neurogenesis and hippocampal cell morphology or the role of prolactin on spatial learning and memory performance. More importantly, how these peptide hormones may interact with basal corticosterone and CBG levels to alter hippocampus-dependent behavior and hippocampal neuroplasticity in the dam has yet to be considered.

6.10 POTENTIAL LIMITATIONS OF PRESENT WORK AND AIMS FOR FUTURE RESEARCH

The experiments in the present thesis provide insight into how hippocampus function and plasticity are altered in the parous female and how these alterations vary with increased parity. In addition to these experiments it should be noted that different time points during the postpartum period may be interesting to investigate. It would also be beneficial to look at
coincident changes in spatial memory, hippocampal neurogenesis, hippocampal morphology, and hormones profiles. The experiments in this thesis investigated different time points throughout the postpartum period. For example, spatial learning and memory performance in the dam was investigated at the time pups are weaned (Ch 2: Pawluski et al., 2006a), and long after pups were weaned (Ch 3: Pawluski et al., 2006b), whereas hippocampal neurogenesis in the dam was investigated during the postpartum period when the dam was with pups and lactating (Ch 4: Pawluski and Galea, in press). It is possible that investigating changes in spatial learning and memory and hippocampus neurogenesis in the dam at the same time points would lead to a greater understanding of how these processes are related. Interestingly, Darnaudery et al. (2007) have recently investigated both spatial reference memory and hippocampal neurogenesis in primiparous females compared to nulliparous females at the same points during the postpartum period. They report that decreased cell proliferation in the dam during the early postpartum period is coincident with decreased learning on a reference memory task (Darnaudery et al., 2007). Interestingly, ten days later, when primiparous dams have lower levels of cell survival (though not significant), reference memory is enhanced compared to nulliparous females (Darnaudery et al., 2007). Further work is needed to understand the relationship between spatial memory performance, hippocampal neurogenesis, hippocampal morphology and steroid hormones levels at other time points during the postpartum period and post-weaning in the dam as well as the role of reproductive experience on these effects.

6.11 GENERAL CONCLUSIONS

This work demonstrates that reproductive experience differentially enhances hippocampus-dependent spatial memory and decreases hippocampal neurogenesis in the dam, resulting in more pronounced effects with primiparity than with multiparity. In addition, these
effects in the mother are coincident with high levels of corticosterone and low levels of CBG during the postpartum period. In conclusion, the findings of this thesis provide further evidence that reproductive experience, which is accompanied by heightened behavioral and neural plasticity, remarkably affects the hippocampus.
6.12 REFERENCES


Pawluski J.L., Galea L.A.M. Reproductive experience alters hippocampal neurogenesis during the postpartum period in the dam. in press.

Pawluski J.L., Vanderbyl B.L., Ragan K., Galea L.A. 2006b. First reproductive experience persistently affects spatial reference and working memory in the mother and these effects are not due to pregnancy or 'mothering' alone. Behav Brain Res. 175, 157-165.


APPENDIX 1

Results

In the litter prior to testing, multiparous rats gave birth to significantly more pups than primiparous rats ($p \leq .03$) and had significantly more male ($p \leq .04$), but not female ($p \leq .55$) pups (Table A.1).

Table A1.1 Mean (+ S.E.M.) for litter size and sex of pups born to primiparous and multiparous rats.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Litter size</th>
<th>Male pups</th>
<th>Female pups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primiparous</td>
<td>14.9 ± 0.61</td>
<td>7.7 ± 0.84</td>
<td>7.1 ± 0.34</td>
</tr>
<tr>
<td>Multiparous (litter 2)</td>
<td>17.0 ± 0.72*</td>
<td>10.4 ± 0.90*</td>
<td>6.6 ± 0.87</td>
</tr>
<tr>
<td>Multiparous (litter 1)</td>
<td>15.4 ± 0.72</td>
<td>8.3 ± 0.71</td>
<td>7.1 ± 1.06</td>
</tr>
</tbody>
</table>

* denotes significantly different than primiparous rats.
APPENDIX 2

Estradiol Assays

All samples were run in duplicate using a commercially available double antibody estradiol RIA kit from MP Biomedicals (Orangeburg, NY). Samples were ether extracted and re-suspended in zero standard prior to the assay. Additional standard points were added to the low end of the estradiol RIA standard curve. For estradiol RIAs, the standard curve ED80 was between 25.84 and 30.95 pg/ml, ED50 was between 125.6 and 155.7 pg/ml, and ED20 was between 578.7 and 782.7 pg/ml, with a detection limit of 10 pg/ml. Group means per day were used for missing cells.

Data Analysis

Separate repeated-measures analysis of variance (ANOVA) tests were calculated on estradiol with group (nulliparous, primiparous, multiparous, primip-no-pups, sensitized) as the between-subject variable and day (GD14, 19, PD1, 5, 14, 21, 35, 45) as the within-subjects variables. Post-hoc comparisons utilized the Neumann Keuls test.

Results

A repeated-measures ANOVA on total estradiol level revealed a significant interaction effect of group by day (F(28, 147) = 3.6, p ≤ .000001; Figure 5.3). Post-hoc tests revealed that estradiol levels were significantly elevated on GD19 in multiparous and primip-no-pups rats compared to sensitized and nulliparous rats (all p’s ≤ .03). Primip-no-pups rats had significantly elevated levels of estradiol on GD19 compared to primiparous rats ( p ≤ .01) and significantly lower levels of estradiol than sensitized and primiparous rats on PD35 (all p’s ≤ .005). There were no other significant differences between groups (.2 ≤ p ≤ 1.0). There was also a significant main effect of day ( F(7, 84) = 8.2, p ≤ .000001) and there was no significant main effect of group ( p ≤ .2).
Figure A2.1 Mean (± SEM) total serum estradiol (nM). Estradiol levels were significantly elevated on GD19 in multiparous and primip-no-pups rats compared to sensitized and nulliparous rats (.00002 ≤ p ≤ .03). Primip-no-pups rats had significantly elevated levels of estradiol on GD19 compared to primiparous rats ( p ≤ .01) and significantly lower levels of estradiol than sensitized and primiparous rats on PD35 ( p’s ≤ .005). There were no other significant differences between groups (.2 ≤ p ≤ 1.0). * denotes multiparous and primip-no-pups significantly different from sensitized and nulliparous; # denotes primip-no-pups rats significantly different from primiparous rats; @ denotes primip-no-pups rats significantly different from sensitized and primiparous rats.
APPENDIX 3

We chose not to report free corticosterone as this estimation does not take into account binding of CBG to other steroids and albumin binding of steroids. Also the assays for total corticosterone and CBG are not performed at physiological temperature; therefore these are also relative estimations of corticosterone and CBG levels.

Free Corticosterone Estimation

A variation of a mass action equation (Plymate et al., 1987) was used to calculate the free or non-CBG-bound fraction of total corticosterone from the total molar concentrations of corticosterone and CBG. This equation has been previously described (Viau et al., 1996; Weaver et al., 2000).

Data Analysis

Separate repeated-measures analysis of variance (ANOVA) tests were calculated on estimated free corticosterone with group (nulliparous, primiparous, multiparous, primip-no-pups, sensitized) as the between-subjects variable and day (GD14, 19, PD1, 5, 14, 21, 35, 45) as the within-subjects variables. Post-hoc comparisons utilized the Neumann Keuls test.

Results

A repeated-measures ANOVA on estimated free corticosterone revealed a significant interaction effect of group by day (F(28, 140)=1.66, p < .030; Figure A). Post-hoc tests revealed that primiparous rats had significantly elevated level of estimated free corticosterone on PD 1 compared to sensitized, primip-no-pups and nulliparous rats (all p’s < .02). In addition, nulliparous rats had significantly elevated levels of estimated free corticosterone on PD 35 compared to all other groups (all p’s < .04). There were no other significant differences between groups (all p’s > .10). There was also a significant main effect of group (F(4, 20)=3.57, p < .02) and day (F(7, 140)=6.16, p < .000001).
Figure A3.1 Mean (± SEM) total free corticosterone (nM). Primiparous rats had significantly elevated levels of estimated free corticosterone on PD 1 compared to sensitized, primip-no-pups and nulliparous rats (all p’s ≤ .02). In addition, nulliparous rats had significantly elevated levels of estimated free corticosterone on PD 35 compared to all other groups (all p’s ≤ .04). ‘a’ denotes significantly different from sensitized, pregnancy-only, and nulliparous rats. * denotes significantly different from all other groups.

References
