

**THE EFFECTS OF REPRODUCTIVE EXPERIENCE ON PREFRONTAL  
CORTEX DEPENDENT LEARNING AND MEMORY AND PYRAMIDAL CELL  
MORPHOLOGY IN THE RAT DAM**

by

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## **Abstract**

Pregnancy, parturition, and motherhood, collectively known as reproductive experience, bring about profound and enduring changes in the hormonal, neural, and behavioral profile of the female rat. Much of the research to date investigating the effects of reproductive experience on learning and memory and cellular morphology in the rat dam has focused on the hippocampus. These studies revealed enhancements in spatial working and reference memory as well as alterations in pyramidal cell morphology following reproductive experience. Interestingly, it has long been established that other brain regions undergo persistent changes in response to reproductive experience including the prefrontal cortex, yet there remains a paucity of research investigating this area. Thus, the objective of the following experiments was to determine the effects of reproductive experience on prefrontal cortex-dependent learning and memory as well as pyramidal cell morphology in the prelimbic region in nulliparous, primiparous, and multiparous rats. For Experiment 1, age-matched nulli-, primi- and multiparous rats were tested for seventeen consecutive days using the delayed spatial win-shift task. This experiment revealed that multiparous rats committed fewer within-phase and omission errors than nulli- or primiparous rats on Blocks 2, 3, and 4 as well as committing fewer across-phase errors in Blocks 2 and 4 than either the nulli- or primiparous groups. Furthermore, the total number of within-phase errors significantly and negatively correlated with an increase in the total time engaged in nursing behaviors. Using Golgi

impregnation, pyramidal cell morphology in Laminae 2/3 and 5 of the prelimbic region of the prefrontal cortex was examined in Experiment 2. The results of Experiment 2 revealed that multiparous rats have more total branch points in the apical region of Lamina 2/3. In addition, arched-back nursing was found to significantly positively correlate with the number of branch points in apical and basal regions of Lamina 5. Passive nursing significantly correlated with the number of basal branch points in Lamina 5 and apical length in Lamina 2/3. The findings from these studies suggest that multiparity may be necessary in realizing the effects of enhanced learning and memory and morphological changes associated with the prefrontal cortex in female rats.

## **Preface**

Ethical approval for this study was obtained from the University of British Columbia Animal Care Committee on January 18, 2010; Animal Care Committee certificate number: A07-0211.

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## **1. Introduction**

### ***1.1. Reproductive experience and the female rat***

The period of pregnancy, parturition, and motherhood (collectively referred to as reproductive experience) exerts profound neural, hormonal, and behavioral changes in the female mammal that are not experienced at any other time in her lifespan outside of early development (Kinsley et al., 2008). Numerous studies examining the biological, behavioral, and cognitive effects of reproductive experience have been conducted across a variety of mammalian species including humans (Rosenblatt, 1967; Sheckman, 1980; Troisi and D'Amato, 1984; Poindron et al., 1988; Galea et al., 1995). Neuroendocrine regulation of pregnancy and parturition across mammalian species including humans and rodents share some similarities (Numan, 2006).

During pregnancy and parturition, the hormonal profile of the female rat undergoes profound changes (Rosenblatt, 1975; Cohen, 1976). For example, during gestation, progesterone, prolactin, placental lactogens, as well as corticosterone remain elevated (Voogt et al., 1969; Shaikh, 1971; Rosenblatt et al., 1988; Atkinson and Waddell, 1995; Brusco et al., 2008; Pawluski et al., 2009b). In addition, during the last few days prior to parturition, estradiol reaches levels approximately three times those seen during early pregnancy (Garland et al., 1987; Rosenblatt et al., 1988). This pattern is somewhat similar to the human profile of hormonal fluctuations during pregnancy and the postpartum (Said et al., 1973; Garland et al., 1987; Rosenblatt et al., 1988; O'Leary et al., 1991; Atkinson

and Waddell, 1995; Brusco et al., 2008). There have been a number of studies examining the influence of these hormones to promote maternal behaviors in both humans and rodents (Rosenblatt, 1967; Moltz et al., 1970; Fleming and Anderson, 1987). Interestingly, the number of times pregnant appears to exert lasting changes in the hormonal profile of the rat. Multiparity (having given birth and mothered at least twice) is associated with a decrease in sensitivity to opioids (Kinsley and Bridges, 1988; Mann and Bridges, 1992), a decrease in corticosterone expression in the early postpartum (Pawluski et al., 2009a), as well as a decrease in estradiol during proestrus following weaning (Bridges and Byrnes, 2006).

The female rat has proven to be one of the most studied species in order to understand how reproductive experience contributes to the physiological and behavioral changes associated with motherhood (Numan, 1988). It is well known that there are profound and enduring changes in the brain and behavior that are the result of reproductive experience in the rat (Fleming and Korsmit, 1996; Bridges and Byrnes, 2006; Macbeth et al., 2008; Pawluski et al., 2009a). For example, maternal behaviors such as retrieving, nursing, nesting, and licking (Rosenblatt, 1967) occur soon after the first birth in the rat (Fleming and Luebke, 1981) with the onset of some behaviors such as maternal aggression occurring more rapidly with subsequent reproductive experiences (Nephew et al., 2009). However, within the past ten years it has been discovered that the effects of reproductive experience that permanently alter the brain extend beyond maternal

behaviors. Craig Kinsley and colleagues (1999) were the first to show that reproductive experience enhanced hippocampus-dependent learning and memory in the rat dam. Subsequently, most of the recent studies have focused on the effects of reproductive experience on hippocampus-dependent learning and memory and morphology (Kinsley et al., 1999; Galea et al., 2000; Gatewood et al., 2005; Pawluski et al., 2006a; Darnaudery et al., 2007). However, there are good reasons to suspect that other cognitive regions of the brain, such as the prefrontal cortex may also be affected with reproductive experience and the focus of this thesis will in the evaluation of prefrontal cortex-related changes in both learning and memory and morphology following from reproductive experience in the rat dam.

### ***1.2. Brain plasticity and the hormones of reproduction***

One reason for suspecting that other cognitive regions of the brain besides the hippocampus may be affected by reproductive experience is that the hormones produced during pregnancy and parturition are known to alter cognitive regions of the brain. For example, it is well known that gonadal and adrenal hormones produce a number of morphological changes in the female hippocampus and prefrontal cortex (Gould et al., 1990; Woolley et al., 1990b; Shansky et al., 2004; Wallace et al., 2006). Indeed, Woolley et al. (1990b) discovered that the dendritic morphology of pyramidal cells in the CA1 region of the hippocampus undergo significant changes in the twenty-four hour period between late proestrus (peak estradiol and progesterone) and late estrus (basal

estradiol and progesterone). Recently, estrogen and corticosterone have been found to augment pyramidal cell morphology in the prefrontal cortex which is correlated with behavioral performance (Shansky et al., 2006; Wallace et al., 2006; Lin et al., 2008). Furthermore, following periods of chronic stress, female rats show an increase in plasma corticosterone which results in basal dendritic atrophy in CA3 pyramidal cells (Galea et al., 1997). Surprisingly, there are no known studies that have specifically examined the effects of corticosterone on the morphology of pyramidal cells in the female prefrontal cortex. However, a number of studies in male rats have found significant atrophy of pyramidal cell dendritic branches in Lamina 2/3 of the prefrontal cortex following exposure to stress or exogenous administration of corticosterone (Wellman, 2001; Radley et al., 2004; Liston et al., 2006). Furthermore, decrements in estradiol expression following ovariectomy impairs memory performance and alters pyramidal cell morphology in Lamina 2/3 of the medial prefrontal cortex of female rats (Wallace et al., 2006). Thus, it appears that both estradiol and corticosterone levels alter prefrontal cortex pyramidal cell morphology. Both of these hormones are altered with gestation, parturition and during the postpartum and are significantly altered with parity.

### **1.3. Brain changes during pregnancy and postpartum**

During pregnancy the human female brain decreases in size and subsequently returns to its antepartum size within six months following delivery (Oatridge et al., 2002). Similarly, the hippocampi of pregnant rats are slightly smaller than non-pregnant rats (Galea et al., 2000), however, the cortical tissues of pregnant rats are significantly thicker than non-pregnant rats (Hamilton et al., 1977) which may highlight brain region specificity in response to the hormones of pregnancy and the postpartum. Furthermore, the size of the somata of neurons in the medial preoptic area (mPOA)--a region integral to maternal behaviors (Numan et al., 1977)--have been found to be significantly increased during pregnancy and then return to their antepartum size during the postpartum, presumably after maternal behaviors have been well-established (Keyser-Marcus et al., 2001). Neurogenesis in the dentate gyrus of the hippocampus is also influenced by reproductive experience in the rat dam. For example, cell proliferation is decreased during lactation in primiparous rats that is believed to be associated with elevations in corticosterone (Leuner et al., 2007; Pawluski and Galea, 2007). Similarly, the survival of new neurons in the dentate gyrus is reduced in the early postpartum in primiparous rats (Pawluski and Galea, 2007).

A number of brain regions have been identified as being fundamental to maternal behavior. In 1996, using the immediate early gene (IEG) marker Fos, Fleming and Korsmit identified key brain structures that consistently showed enduring differences between reproductively-experienced and -inexperienced



female rats. These structures included the medial preoptic area (mPOA), the basolateral amygdala (BLA), the parietal and prefrontal cortices and form what is referred to as the 'maternal circuit' (Numan, 1988). The mPOA is implicated all aspects of maternal care such as nursing, retrieving, licking, and nest building (Numan, 1974), however it primarily controls the active forms of maternal behavior (i.e.: nest building and retrieving) (Terkel et al., 1979). The amygdala is also implicated as lesions to the amygdala enhance maternal responding (Fleming et al., 1980; Fleming et al., 1983). Furthermore, olfaction is powerful mediator in the initiation of maternal behaviors (Fleming and Rosenblatt, 1974; Fleming et al., 1979) that becomes further enhanced with repeated reproductive experiences (Schwartz and Rowe, 1976). Additionally, the bed nucleus of the stria terminalis (BNST) is involved in maternal behaviors including aggression (Insel, 1992; Meddle et al., 2007; Bosch et al., 2010). The hippocampus also plays an important role in maternal behaviors and has differential effects on learning and memory following reproductive experience (Terkel et al., 1979; Kinsley et al., 1999; Pawluski et al., 2006b; Pawluski et al., 2006a). Surprisingly, despite its early identification as forming part of the maternal circuit (Fleming and Korsmit, 1996), the prefrontal cortex has received very little attention with respect to its role in altering non-maternal behaviors such as learning and memory and cell morphology following reproductive experience.

#### **1.4. Reproductive experience and the hippocampus**

As discussed above, a number of brain structures undergo profound and enduring changes following reproductive experience (Fleming and Korsmit, 1996) many of which are implicated in learning and memory (Numan et al., 1977; Fleming et al., 1983; Kinsley et al., 1999; Pawluski et al., 2006a; Scanlan et al., 2006). Furthermore, it has been well established that the hippocampus is involved in learning and memory (Olton et al., 1978; Walker and Olton, 1979; Aggleton et al., 1986) and although it is not considered part of the maternal circuit, the hippocampus influences maternal behavior (Terlecki and Sainsbury, 1978). Much of the research to date has focused on hippocampus-dependent tasks and cellular morphology in this region following reproductive experience (Kinsley et al., 1999; Galea et al., 2000; Gatewood et al., 2005; Lambert et al., 2005; Love et al., 2005; Pawluski and Galea, 2006; Pawluski et al., 2006b; Pawluski et al., 2006a; Brusco et al., 2008). Interestingly, it appears that the number of times pregnant and having mothered impacts different aspects of hippocampus-dependent learning and memory. For instance, one of the first studies to investigate this phenomenon found that multiparous as well as primiparous rats consistently outperformed virgin rats on different tasks using spatial memory (Kinsley et al., 1999). However, primiparity is also associated with enhanced cue and probe memory (Lambert et al., 2005). Additional investigations confirmed enhanced spatial memory in primiparous rats (Pawluski et al., 2006a), although full mothering experience (pregnancy, parturition,

mothering until weaning) is necessary in order for these effects to be realized (Pawluski et al., 2006b).

Moreover, the effects of reproductive experience on hippocampus-dependent learning and memory extend over the lifespan of the female rat and appears to provide neuroprotective properties (Gatewood et al., 2005; Love et al., 2005). A compelling finding by the Gatewood et al. (2005) study is the fact that spatial memory performance was enhanced in the multiparous rats throughout the study suggesting that repeated reproductive experience may have additive effects that become more apparent with aging. Furthermore, pyramidal cell morphology in the CA1 and CA3 region of the hippocampus is differentially affected by reproductive experience (nulli-, primi-, multiparous) (Pawluski and Galea, 2006). Surprisingly, although primiparous rats in general appear have enhanced hippocampus-dependent learning and memory (Pawluski et al., 2006b; Pawluski et al., 2006a), the pyramidal cells in the CA1 and CA3 region show significantly more 'atrophy' than either nulli- or multiparous rats (Pawluski and Galea, 2006). Thus, it is apparent that reproductive experience induces changes in hippocampus-dependent learning and memory as well in pyramidal cell morphology. Given these findings, the present dissertation aimed to examine whether the function and morphology of another 'cognitive' region of the brain implicated as integral to the maternal circuit and reproductive experience, the prefrontal cortex, is altered with reproductive experience.

### ***1.5. The prefrontal cortex and potential implications in maternal memory and behavior***

The mammalian prefrontal cortex is implicated in complex cognitive functions such as behavioral flexibility, inhibition, decision-making and goal-directed behavior (Ragozzino et al., 1999; De Bruin et al., 2000; Dalley et al., 2004). Furthermore, working memory, in which the information for a given task must held 'on-line' and continuously revised in a trial unique manner, is mediated by the prefrontal cortex (Goldman-Rakic, 1995). The prefrontal cortex is subdivided into three main regions: medial, ventral, and lateral with each sub-serving distinct cognitive functions (Dalley et al., 2004). For example, the medial region is implicated in attentional and prospective functions (Kesner, 1989; Granon et al., 1998) as well as planning and strategy set-shifting (Granon and Poucet, 1995; Floresco et al., 2008) whereas the lateral region primarily functions in inhibitory functions (Dalley et al., 2004). It seems likely that the prefrontal cortex would be involved in the maternal brain as in order to secure the survival of her offspring, the maternal rat must adapt to the ever-changing contingencies of her environment. Thus flexibility as well as inhibition, attentional selection and perhaps 'goals' may prove to be as fundamental as the development of a cognitive map and 'maternal memory' in caring for offspring.

Unilateral and unidirectional projections originating from the CA1 region of the hippocampus terminate primarily in the prelimbic and infralimbic regions of the prefrontal cortex (Jay et al., 1989; Gabbott et al., 2002; Cenquizca and Swanson, 2007). Furthermore, enduring synaptic changes in the prefrontal cortex have

been found to originate from long-term potential (LTP) initiated in the CA1 (Jay et al., 1996). Thus, a unique pathway mediating transmission from the hippocampus to the prefrontal cortex may play a fundamental role in learning and memory. It is well-established that one of the primary functions of the hippocampus is in spatial learning and memory (for review see (Squire, 1992). In addition, the prefrontal cortex is fundamental in working memory following a delay and the retrieval of spatial information (Funahashi et al., 1989; Friedman and Goldman-Rakic, 1994; Seamans et al., 1995; Bailey and Mair, 2004), which is mediated, at least in part by the hippocampus (Sutherland et al., 1982). Therefore, it is plausible that neural changes that originate in the hippocampus may therefore induce changes in the prefrontal cortex.

As mentioned earlier, similar to the hippocampus, the prefrontal cortex is also sensitive to the effects of hormones associated with reproduction, particularly estradiol (Keenan et al., 2001; Shansky et al., 2009a) and corticosterone (Wellman, 2001; Radley et al., 2004). Decreases in endogenous estradiol reduce the number of dendritic spines in both rats and monkeys which is correlated with decrements in cognitive performance (Wallace et al., 2006; Hao et al., 2007). Furthermore, elevated corticosterone results in dendritic atrophy in the prefrontal cortex which has also been implicated in cognitive impairments (Wellman, 2001; Radley et al., 2004).

Surprisingly, there remains a relative paucity in the literature with respect to the effects of reproductive hormones, and more specifically, reproductive

experience on the prefrontal cortex of the maternal rat. One of the few studies to specifically examine these issues reported that lesions to the prefrontal cortex disrupted the sequencing, latency, frequency, and duration of sexual and maternal behaviors in female rats (Afonso et al., 2007). However, none of the behaviors were abolished following the lesions (Afonso et al., 2007) suggesting that the prefrontal cortex may function in the execution rather than the acquisition of these behaviors. Furthermore, electroencephalogram (EEG) recordings in the medial prefrontal cortex during performance of active maternal behaviors such as pup licking and retrieving increased whereas recordings decreased when performing similar, yet non-maternal behaviors such as walking or self-grooming (Hernandez-Gonzalez et al., 2005) further substantiating the involvement of the prefrontal cortex in reproductive behaviors. More recently a functional magnetic resonance imaging (fMRI) study conducted on maternal aggression in primiparous lactating rats exposed to male intruder stress revealed that, among other regions, the prelimbic and infralimbic regions of the prefrontal cortex were activated following this type of stressor suggesting prefrontal cortex involvement in mediating aspects of maternal behaviors (Caffrey et al., 2010).

Thus as outlined above; 1) the prefrontal cortex has been identified as forming part of the maternal circuit involved in the onset and display of maternal behavior (Fleming and Korsmit, 1996; Afonso et al., 2007; Afonso et al., 2008); and 2) the prefrontal cortex is a site in which hormones associated with reproduction affect behavior and cellular structure as well as being uniquely

connected and influenced by the hippocampus (Jay et al., 1989; Wellman, 2001; Shansky et al., 2006; Wallace et al., 2006). There remains a dearth in the literature that specifically addresses how reproductive experience may contribute to prefrontal cortex-dependent learning and memory in the maternal rat.

Therefore, the purpose of this thesis is to examine the effects of reproductive experience on prefrontal cortex-dependent learning and memory as well as pyramidal cell morphology in the prelimbic region.

In Experiment 1, I examined performance on the delayed spatial win-shift task in nulliparous, primiparous and multiparous rats. This task specifically examines behavioral flexibility, working memory, and the temporal organization of behaviors (Seamans et al., 1995) and requires the integration of the prefrontal cortex and hippocampus in its execution (Floresco et al., 1997). As prior research has found enhancements in hippocampus-dependent learning and memory following reproductive experience in multiparous, and particularly primiparous rats (Kinsley et al., 1999; Pawluski et al., 2006a) and afferent projections originating in the hippocampus have been found to influence cellular activity in the prefrontal cortex via long-term potentiation (LTP) (Jay et al., 1989; Doyere et al., 1993) I hypothesized that reproductively experienced rats, and more specifically, primiparous rats, would commit the fewest number of errors on the delayed spatial win-shift task.

In Experiment 2 I examined pyramidal cell morphology in Lamina 2/3 and Lamina 5 of the prelimbic region of the prefrontal cortex following Golgi

impregnation. The prelimbic region is involved in a variety of 'higher' order processes such as behavioral flexibility, planning, and inhibition (Ragozzino et al., 1999; De Bruin et al., 2000; Dalley et al., 2004) which may constitute fundamental aspects of maternal care of offspring in the rat. Moreover, afferents from the CA1 region of the hippocampus primarily innervate the prelimbic region (Ferino et al., 1987; Jay et al., 1989; Gabbott et al., 2002) and given that primiparous rats showed significant atrophy in the pyramidal cells of the CA1 region following reproductive experience (Pawluski and Galea, 2006), I hypothesized that these findings would extend into the pyramidal cells of the prefrontal cortex.

Furthermore, Lamina 2/3 of the prelimbic region was selected as it is implicated in mediating communication across multiple brain regions (Gabbott et al., 2005; Goodfellow et al., 2009) and is sensitive to hormones, glucocorticoids and catecholamines (Wellman, 2001; Rossetti and Carboni, 2005; Shimada et al., 2006; Wallace et al., 2006). As such, I felt that this region may be particularly responsive to the effects of reproductive experience. In addition, I chose to examine Lamina 5 pyramidal cells of the prelimbic region as afferent projections are not necessary to induce activation (Hempel et al., 2000). As well, dopaminergic inputs originating in the ventral tegmental area (VTA) synapse in Lamina 5 (Sobel and Corbett, 1984; Berger et al., 1991). As dopamine has been shown to augment motivation and learning, particularly in response to rewards



(Murase et al., 1993; Phillips et al., 2004) I felt that this region may also be specifically affected by reproductive experience.

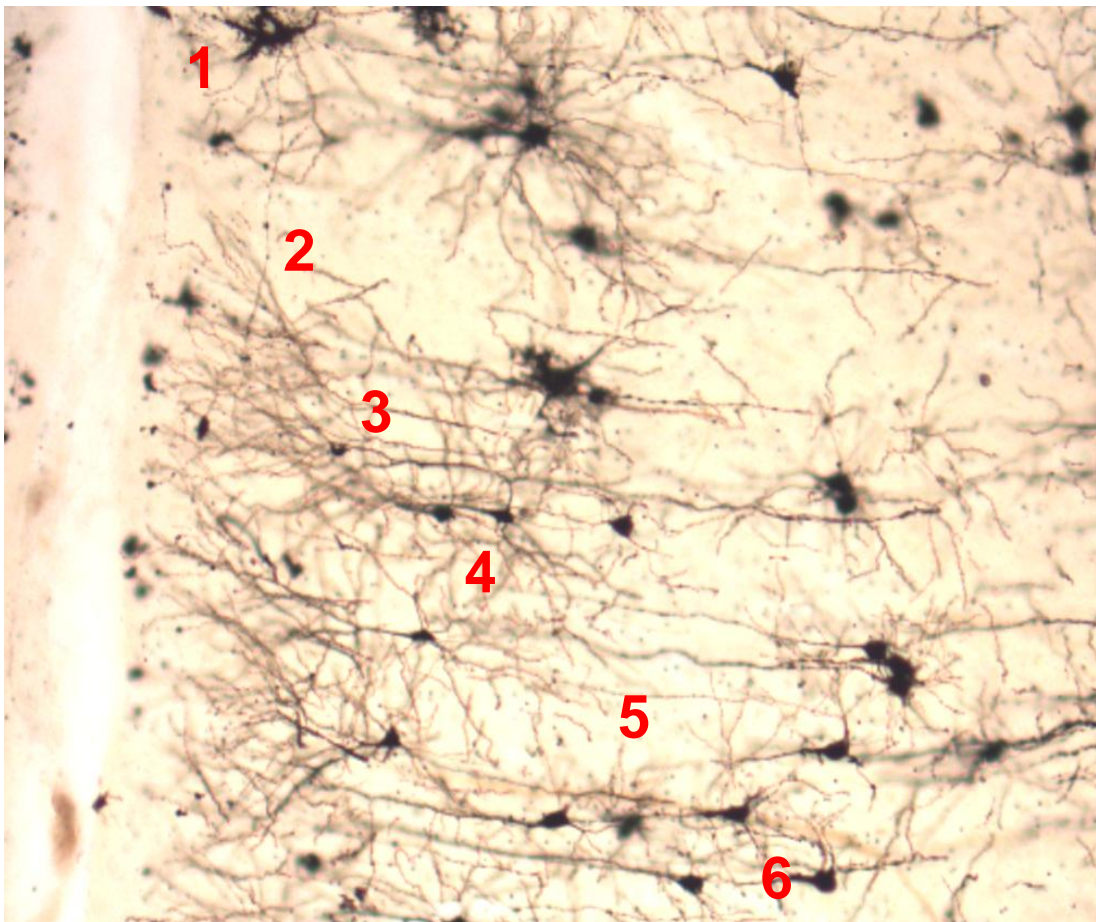


Figure 1. Prelimbic region of the female rat. Cortical laminae 1 - 6 are highlighted.

## **2. General Methods**

### **2.1. Animals**

Forty-eight female Sprague-Dawley rats (approximately 3 months of age at the onset of the experiments) purchased from Charles River (Montreal, QC, Canada) were used in this study. Animals were initially pair-housed in clear, polyurethane cages (48 cm x 27 cm x 20 cm) with absorbent aspen chip bedding (Nepco, Warrensburg, NY) and a solid white polyurethane tube (15 cm x 10 cm in diameter). Rats were provided with rat chow (Lab Diet, Brentwood, MO) and tap water *ad libitum*. Animals were housed in colony rooms with mean temperature 21°C ( $\pm$  2°C) and relative humidity of 42% ( $\pm$  3%) on a 12 hour light/dark cycle (lights on at 7:00 a.m.). All protocols were in accordance with the ethical guidelines established by the Canadian Council for Animal Care and were approved by the University of British Columbia Animal Care Committee. Every effort was made to minimize the number of animals used in these experiments.

Animals were age-matched and randomized to 3 experimental conditions: nulliparous, primiparous and multiparous. Nulliparous animals were not sexually experienced and served as controls. Primiparous animals gave birth and mothered one litter of pups until time of weaning (between twenty-one and twenty-three days postpartum). Multiparous animals gave birth twice and mothered each litter of pups until time of weaning (between twenty-one and twenty-three days postpartum).

## **2.2. Breeding**

Breeding for the multiparous group began when the animals were approximately 3 months of age. Breeding occurred in large opaque cages (51 cm x 41 cm x 22 cm) in which pair-housed breeding females were housed with one male overnight from approximately 1600 hours to 0900 in a separate colony room from the nulliparous and primiparous animals. Each morning, upon removal from the breeding cage, each female was vaginally lavaged with tap water (approx. 36°C). Pregnancy was confirmed with the presence of sperm in the lavage fluid and once confirmed this day was considered Gestational Day 0 (GD0). Upon confirmation of pregnancy the female was individually housed in a clear polyurethane cage (48 cm x 27 cm x 20 cm) until parturition. Non-pregnant breeding females were removed from the breeding cage and housed in the clear polyurethane cages (48 cm x 27 cm x 20) throughout the day and returned to their respective male at approximately 1600 daily. This routine continued until pregnancy was confirmed.

Breeding for the primiparous group and the second breeding of the multiparous group began when the animals were between 130 (Experiment 1) and 150 (Experiment 2) days of age. Breeding was timed so that the primiparous animals gave birth to their first litter at the same time that the multiparous animals gave birth to their second litter. The same procedure as described above was followed. Upon confirmation of pregnancy, yoked nulliparous animals were individually housed in the clear, polyurethane cages for the duration of the

experiment where they remained in a separate colony room from the primiparous and multiparous animals.

Approximately twenty-four hours after parturition on postpartum day 1 (PD1), litters were culled to 5 males and 5 females. The dam and her offspring were housed in the clear, polyurethane cages (48 cm x 27 cm x 20 cm) and remained undisturbed from PD2 through to PD8.

### ***2.3. Maternal behavior***

Maternal behavior was observed and scored at 4 time points each day for Experiment 1 and at two time points for Experiment 2 beginning on postpartum day 2 through to postpartum day 8. Observations were made in the morning (randomized between 0800 and 1200) and in the afternoon (randomized between 1300 and 1800) with no less than 1 hour separating each observation. Cages remained undisturbed during the observation period (Day 2 through 8).

The frequency and duration of maternal behaviors were scored every 5 seconds for a total of ten minutes during each observation as previously described (Myers et al., 1989; Pawluski et al., 2006a). Behaviors recorded were: off pups (off nest/physically separated from pups); nest building; retrieving (carrying pup to nest site); self-grooming (both on and off nest); licking (dam licking body/anogenital region); nursing/licking (pups nursing while dam licking pup's body/anogenital region). Nursing behaviors were further assessed as described by Caldji et al. (1998) and included: arched-back posture; passive (mother either side-lying or on her back); or blanket (mother lying on top of litter)

positions . Data for each behavior was aggregated to determine the total time spent for each scored behavior. The total time included for analysis was compiled from behaviors of the most recent litter prior to behavioral testing (i.e. the second litter of the multiparous group).

### **3. Experiment 1 – Delayed Spatial Win-Shift Task**

#### ***3.1. Apparatus and procedure***

##### ***3.1.1. Maze and testing room***

An eight-arm radial maze was used for this experiment. The maze measured 85 cm from the floor and was centrally placed in a dimly lit room surrounded by extramaze cues that remained constant throughout the experiment. The maze design was comprised of an octagon-shaped centre measuring 34 cm in diameter from which eight arms (56 cm long x 13 cm) radiated. At the end of each arm was a circular food well (4 cm x 1 cm). Removable metal barriers (27 cm x 13 cm ) were used to block entry into their respective arms as outlined below.

##### ***3.1.2. Delayed spatial win-shift task***

Following from the experimental design as previously described (Seamans et al., 1995; Sinopoli et al., 2006), twenty-nine animals (nulliparous = 10; primiparous = 10; multiparous = 9) were habituated to the maze for ten minutes once daily for five consecutive days. During this period, animals were placed in the centre of the maze, nose pointed toward the north-facing wall and allowed to freely explore. No food rewards were placed in the food wells during the habituation period. Immediately following each habituation period, animals were returned to their home cages and provided with approximately twenty sugar pellets (BioServ, Frenchtown, NJ, USA) in order to habituate to the food reward. The radial arm maze was rotated randomly every second day to minimize the

effects of intramaze cues in the formation of allocentric memory (Colombo et al., 1989).

Rats were food restricted to approximately ninety percent of their free-feeding weight commencing six days after weaning of the final litter. Age-matched nulliparous rats began food restriction at the same time as their yoked conspecifics. Behavioral testing began approximately two weeks following weaning. Testing started at 0930 each day for seventeen consecutive days. The order of testing was randomized for each rat every day. Daily trials of the delayed win-shift task were comprised of a training phase and a testing phase separated by a fifteen minute delay. Prior to introducing the rat to the maze during the training phase, 4 of the 8 arms were blocked. The pattern of blocked arms was randomized daily for each animal with the same configuration not used for more than 2 consecutive days. The remaining 4 open arms were baited with a single sugar pellet placed in the food well. The rat was placed in the centre of the maze facing toward the north-facing wall and allowed a total of 5 minutes to retrieve all 4 sugar pellets. The training phase was completed once all 4 sugar pellets were retrieved or 5 minutes had lapsed, whichever occurred first. Following this, the rat was immediately returned to its home cage and placed in a dark, quiet room during the fifteen minute delay. The maze was cleaned after each training and testing phase with 10% ethyl alcohol.

During the testing phase, all maze arms were open and the 4 arms that were previously blocked contained the food reward. As in the training phase,

testing was complete once the rat retrieved all 4 sugar pellets or 5 minutes had lapsed. Upon completion of the testing phase, the rat was immediately placed in its home cage and returned to the colony room where it was vaginally lavaged to determine estrous phase, weighed, and fed.

Errors that were committed during the testing phase were scored as across-phase errors (APE), within-phase errors (WPE) and omissions. An across-phase error occurred when the rat initially entered an arm that was baited during the training phase. A within-phase error was re-entry into any arm that was previously entered during the testing phase. An omission was scored if the rat failed to enter a baited arm during the testing phase. In addition, latency to reach the food well of the first arm entered, total time to complete the task, and the number of arm entries were recorded. Criterion performance on this task was reached when the rat made 1 or fewer errors on 2 consecutive days during the testing phase (Seamans et al., 1995; Sinopoli et al., 2006).

### **3.2. Data analyses**

Twenty-nine animals were included in the data analysis. Daily trials were collapsed into totals across all seventeen days of testing in addition to Blocks (i.e. Blocks 1 - 3 were comprised of 4 days each [e.g.: Block 1 = Days 1 -4]; Block 4 was comprised of 5 days; Days 13 - 17) for statistical analyses. Error types (APE, WPE, omissions) were analyzed using a repeated measures analysis of variance (ANOVA) with Condition (nulliparous, primiparous, multiparous) as the between-subjects factor and Error (APE, WPE, omissions) or



Block as the within-subjects factor. A one-way ANOVA was conducted on total number of errors and total days to reach criterion. In addition, to determine if longer latencies resulted in more total errors, the total time to complete each testing phase was divided by the total number of arm choices for each animal and further analyzed using a one-way ANOVA with Condition as the between subjects factor. *Post-hoc* comparisons were conducted using Newman-Keul's procedure whereas planned comparisons employed the Dunnett's test. Pearson product-moment correlations were performed between trials to criterion, error type, maternal behaviors, and litter characteristics.

### **3.3. Results**

#### **3.3.1. Reproductive experience did not significantly affect the total number of errors committed across all days of testing.**

The mean number of total across-phase, within phase, omission errors and trials to reach criterion are displayed in Table 1. A significant main effect of Error was found for all groups [ $F(2, 52) = 20.78, p \leq 0.001$ ] with all groups committing more APE than either WPE or omissions. There was no significant main effect of Condition ( $p \leq 0.32$ ) nor a significant interaction effect between condition and error type, ( $p \leq 0.42$ ).

Table 1. Total number of across-phase, within-phase, omission errors and trials to reach criterion on the delayed spatial win-shift task according to group. Seven animals were excluded from the data for the trials to reach criterion as they did not reach criterion performance over the seventeen days of testing.

		<b>Across-Phase Errors</b>		<b>Within-Phase Errors</b>		<b>Omission Errors</b>		<b>Trials to Reach Criterion</b>	
<b>Group</b>	<b>N</b>	<b>Mean</b>	<b>SEM (±)</b>	<b>Mean</b>	<b>SEM (±)</b>	<b>Mean</b>	<b>SEM (±)</b>	<b>Mean</b>	<b>SEM (±)</b>
nulliparous	10	25.10	3.15	13.30	3.87	15.20	4.68	6.50 (n=6)	2.05
primiparous	10	28.60	2.22	16.40	3.74	7.90	3.29	6.63 (n=8)	1.49
multiparous	9	25.33	1.75	9.89	1.92	8.00	2.66	8.75 (n=8)	1.75

**3.3.2. Multiparous animals showed a tendency to commit fewer across-phase, within-phase, and omission errors across certain blocks compared to nulliparous and primiparous animals.**

In order to determine the effects of Blocks of trials on error type, a repeated measures ANOVA was conducted on APE, WPE, and omissions. A significant main effect of Block [ $F(11, 286) = 14.97, p < 0.001$ ] was found for all groups in which significantly more total errors (APE, WPE, omissions) were committed in Block 1 of trials when compared to subsequent Blocks. There was no significant main effect for Condition ( $p < 0.37$ ) or an interaction effect between Block and Condition ( $p < 0.66$ ). A priori we thought there would be a difference between conditions. Planned comparisons revealed that multiparous animals made significantly fewer omission errors during Block 3 than nulliparous animals ( $p \leq 0.03$ ) (Figure 2A). In addition, analyses revealed a trend for multiparous animals to make fewer APE and WPE than primiparous animals in Block 2 ( $p \leq 0.08$ ) and 4 ( $p \leq 0.06$ ) of trials, respectively (Figure 2B and 2C).

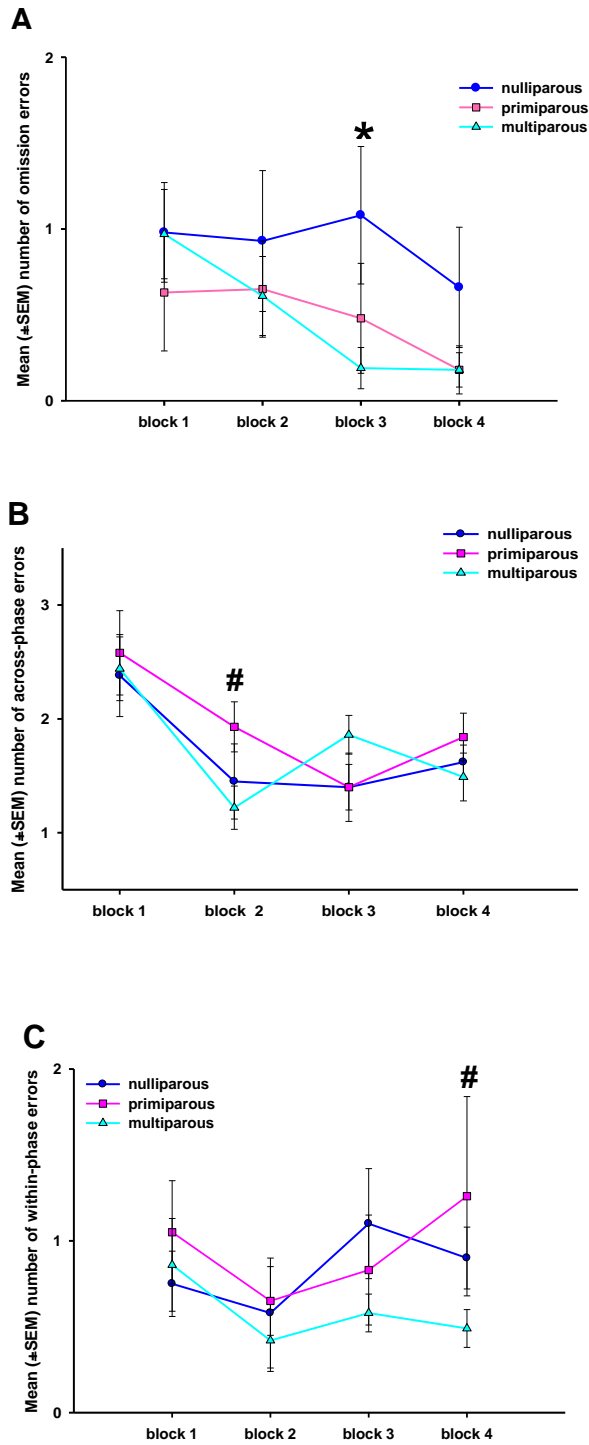


Figure 2. Total number of across-, within-, and omission errors (A) multiparous rats made significantly fewer ( $p \leq 0.03$ ) omission errors than nulliparous rats in Block 3 of trials; (B) a trend ( $p \leq 0.08$ ) was found for multiparous rats to commit fewer across-phase errors than primiparous rats in Block 2; (C) a trend ( $p \leq 0.06$ ) was found for multiparous rats to make fewer within-phase errors in Block 4 than primiparous rats. \* indicates significance from nulliparous rats; # indicates a trend

**3.3.3. Reproductive experience does not influence the total number of days to reach criterion or the latency to initiate or complete trials.**

A one-way ANOVA was conducted to examine the effects of Condition on the total number of days to reach criterion. Analyses revealed that there was no significant difference between condition groups on the total number of days to reach criterion [  $F(2,19) = 0.55, p \leq 0.58$ ]. In addition, there was no significant difference between groups on the latency to reach the first maze arm [  $F(2,26) = 0.53, p \leq 0.59$ ] nor the total time to complete the testing trial [  $F(2, 26) = 0.34, p \leq 0.72$ ]. In addition, Condition did not influence the average time for subsequent maze arm selection during the testing trial [  $F(2, 26) = 1.17, p \leq 0.33$ ] or the total number of errors committed across all days of testing [  $F(2,26) = 0.99, p \leq 0.38$ ] (Table 2).

Table 2. Means and S.E.M.s for the total latency to reach the first maze arm, the total time per trial, and the total number of arm choices per trial.

<b>Condition</b>	<b>Latency to 1st Maze Arm</b>	<b>Total Time per Trial</b>	<b>Total Number of Arm Choices</b>
nulliparous	821.60 ± 308.24	3300.50 ± 416.45	88.20 ± 8.39
primiparous	680.40 ± 231.41	2836.40 ± 425.97	103.70 ± 7.09
multiparous	447.67 ± 203.42	3003.44 ± 386.29	94.56 ± 4.99

### 3.3.4. Within- and across-phase errors negatively correlate with nursing behaviors.

As shown in Figure 3A, a significant negative correlation between passive nursing and the total number of within-phase errors committed was found, as the number of within-phase errors increased as the amount of time passive nursing decreased ( $r = -0.50, p \leq 0.03$ : primiparous:  $r = -0.53, p \leq 0.14$ ; multiparous:  $r = -0.42, p \leq 0.25$ ). One primiparous rat was removed from this correlational analysis as its total number of within-phase errors was 2.83 standard deviations above the mean and positively skewed the data .

A significant negative correlation was found between the total amount of time passive nursing and the total number of across-phase errors. Once again, as the total time passive nursing decreased, the total number of across-phase errors increased ( $r = -0.49, p \leq 0.03$ : primiparous:  $r = -0.57, p \leq 0.08$ ; multiparous:  $r = -0.26, p \leq 0.50$ ; Figure 3B).

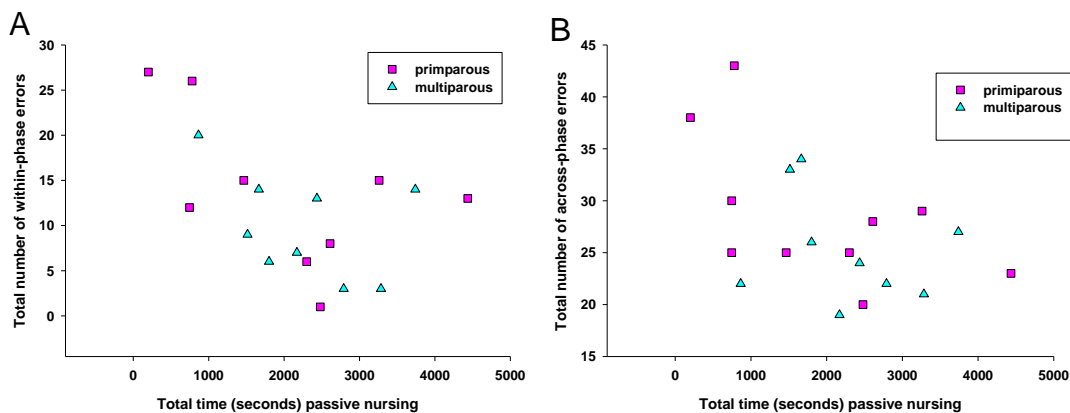


Figure 3. Correlation between within-phase errors and maternal behaviors. A Pearson's product moment correlation was conducted on error type and maternal behaviors. (A) A significant negative correlation ( $r = -0.50, p \leq 0.03$ ) was found between total time spent passive nursing and the total number of within-phase errors; (B) a significant negative correlation ( $r = -0.49, p \leq 0.03$ ) was found such that the total number of across-phase errors decreased as the total time spent passive nursing increased.

### 3.3.5. Litter characteristics correlated with the types of errors dams committed across all days of testing.

A significant negative correlation was found between the total number of male pups born to a dam in the final litter immediately preceding behavioral testing and the number of omission errors with more male pups corresponding to a decrease in the total number of omission errors committed by the dam ( $r = -0.45$ ,  $p \leq 0.05$ : primiparous:  $r = -0.40$ ,  $p \leq 0.26$ ; multiparous:  $r = -0.59$ ,  $p \leq 0.09$ ) (Figure 4).

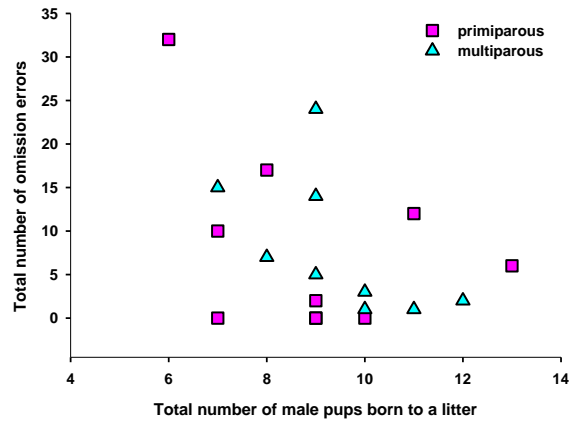


Figure 4. Correlation between total omission errors and total number of male pups. A significant negative trend was found between the number of male pups born to a dam in the litter immediately preceding behavioral testing and the number of omission errors committed across all days of testing ( $r = -0.45$ ,  $p \leq 0.05$ ).

## **4. Experiment 2--Reproductive Experience and Pyramidal Cell Morphology**

### ***4.1. Histological Procedures***

Nineteen animals (nulliparous = 7; primiparous = 6; multiparous = 6) were used for this experiment. At time of weaning (PD21) all maternal animals and their yoked nulliparous counterparts were sacrificed using carbon dioxide immediately followed by decapitation and brain extraction.

Brain tissue was processed for Golgi impregnation using FD Rapid GolgiStain™ (FD NeuroTechnologies, Ellicott City, MD, USA). Briefly, and following from the FD Rapid GolgiStain™ protocol, immediately following extraction the brains were blocked by removal of the cerebellum using a sharp razor, thoroughly rinsed with Milli-Q water and placed in light-impermeable plastic containers filled with 12 millilitres (mL) of the impregnation solution which was changed and replenished approximately twenty-four hours later. The brains remained in the impregnation solution in a dark container for two weeks. Following this, the brains were transferred into 12 mL of FD Rapid GolgiStain™ Solution C for a period of one week. Brains were sliced into coronal sections (100  $\mu\text{m}$  thickness) into a bath of FD Rapid GolgiStain™ Solution C using a Vibratome (Leica VT6000) and directly mounted onto 2% gel-coated slides. The slides were left to dry at room temperature in a dark cabinet for a period of 1 week. The staining procedure was modified from the FD Rapid GolgiStain™ protocol as the initial rinses in Milli-Q solution were omitted in order to maintain adherence of tissue to the slides. Following staining, the slides were dehydrated in incremental



concentrations of ethyl alcohol, cleared with Xylene, and coverslipped with Permount.

Analysis of pyramidal cell dendritic branch length and branch points were in keeping with methods as previously described (Galea et al., 1997; Uylings and van Pelt, 2002; Radley et al., 2004). Sections selected for analysis were located in the prelimbic region (PL) of the medial prefrontal cortex (approximately from Bregma +3.70 to +2.70 mm) (Paxinos, 2004). Five pyramidal cells each from Lamina 2/3 and Lamina 5 were analyzed per rat. Criteria for cell selection were as follows: (1) the soma and primary dendrites were located within the margins of the PL and in their respective laminal layer (2/3, 5); (2) the soma and dendrites were fully impregnated, identified by demarcated tapering tips; (3) the dendritic tree was comprised of intact primary, secondary, tertiary, and quaternary branches from which the apical arbors extended through to Lamina I; (4) the cell was relatively isolated from other neighboring impregnated cells. Every effort was made to analyze an equal number of cells from each hemisphere to reduce the potential effects of lateralization on dendritic complexity (Perez-Cruz et al., 2009). Cell tracings were made using digitalized camera lucida drawings (at 400x magnification) and ImageJ software (Rasband, 2009) from which the total dendritic length and number of branch points were measured for both the apical and basal regions of each cell.

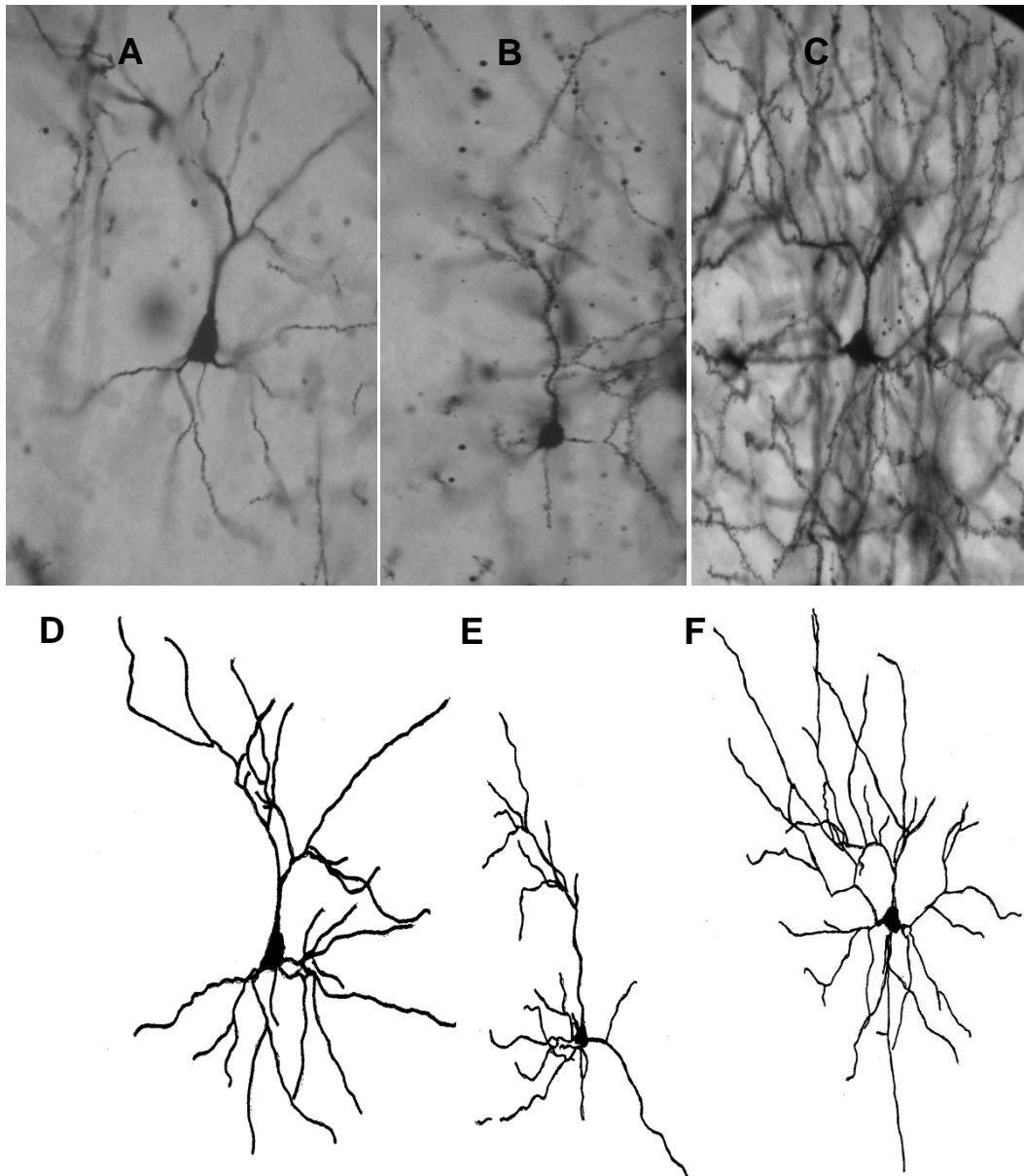


Figure 5. Photo images and their corresponding Golgi tracings of prelimbic pyramidal cells in Lamina 2/3. Pyramidal cells of nulliparous (A,D), primiparous (B,E), and multiparous rats (C,F) are shown above. Original images and drawings were acquired at 400x magnification. Images displayed are smaller than their origin sources.

## **4.2. Data Analyses**

A total of seventeen animals were included in the final analysis (nulliparous = 7; primiparous = 6; multiparous = 4). Two multiparous animals were not included: one was removed from the study during pregnancy and the second due to the loss of prefrontal cortex tissue during staining. A repeated-measures analysis of variance (ANOVA) was performed for each dependent variable of dendritic length and branch point number with Condition (nulliparous, primiparous, multiparous) as the between-subjects factor and region (apical or basal) and Lamina (2/3; 5) as the within-subjects factors. *Post hoc* comparisons were performed using the Newman-Keul's procedure. Planned comparisons were conducted using the Dunnett's test. Additionally, Pearson product-moment correlations were conducted between Lamina 2/3 and Lamina 5 morphology and maternal behaviors scored on the most recent litter prior to sacrifice, litter size, and litter characteristics in the most recent litter.

## **4.3. Results**

### ***4.3.1. In Lamina 2/3, multiparous animals tended to have more apical branch points than either nulliparous or primiparous animals.***

The mean number of apical and basal branch points for Lamina 2/3 is displayed in Figure 6A and for Lamina 5 in Figure 6B. A significant main effect of region was found on total number of branch points for Lamina 2/3 [ $F(1, 14) = 27.40, p \leq 0.001$ ], indicating that there were more basal branch points than apical branch points. There was no significant main effect for Condition ( $p \leq 0.42$ ) or an

interaction effect for Condition and Region ( $p \leq 0.24$ ). A priori we hypothesized that reproductive status would influence the morphology of the prefrontal cortex and analyses revealed a trend for multiparous animals to have more apical branch points than either nulliparous or primiparous animals ( $p \leq 0.09$ ,  $p \leq 0.06$ , respectively).

***4.3.2. In Lamina 5, all groups displayed significantly more branch points in the basal region.***

As depicted in Figure 6B below, a significant main effect for branch points was found for Lamina 5 [ $F(1, 14) = 50.72$ ,  $p \leq 0.001$ ], with all groups showing more total branch points in the basal region compared to the apical region. However, there was no significant main effect for Condition ( $p \leq 0.52$ ) or an interaction effect for Condition and Region ( $p \leq 0.58$ ) found for reproductive status and the number of branch points in this region. Planned comparisons did not reveal any differences between the nulliparous, primiparous, and multiparous groups in either the apical ( $p \leq 0.91$ ;  $0.51$ ) or basal regions ( $p \leq 0.25$ ;  $0.79$ ) for the total number of branch points.

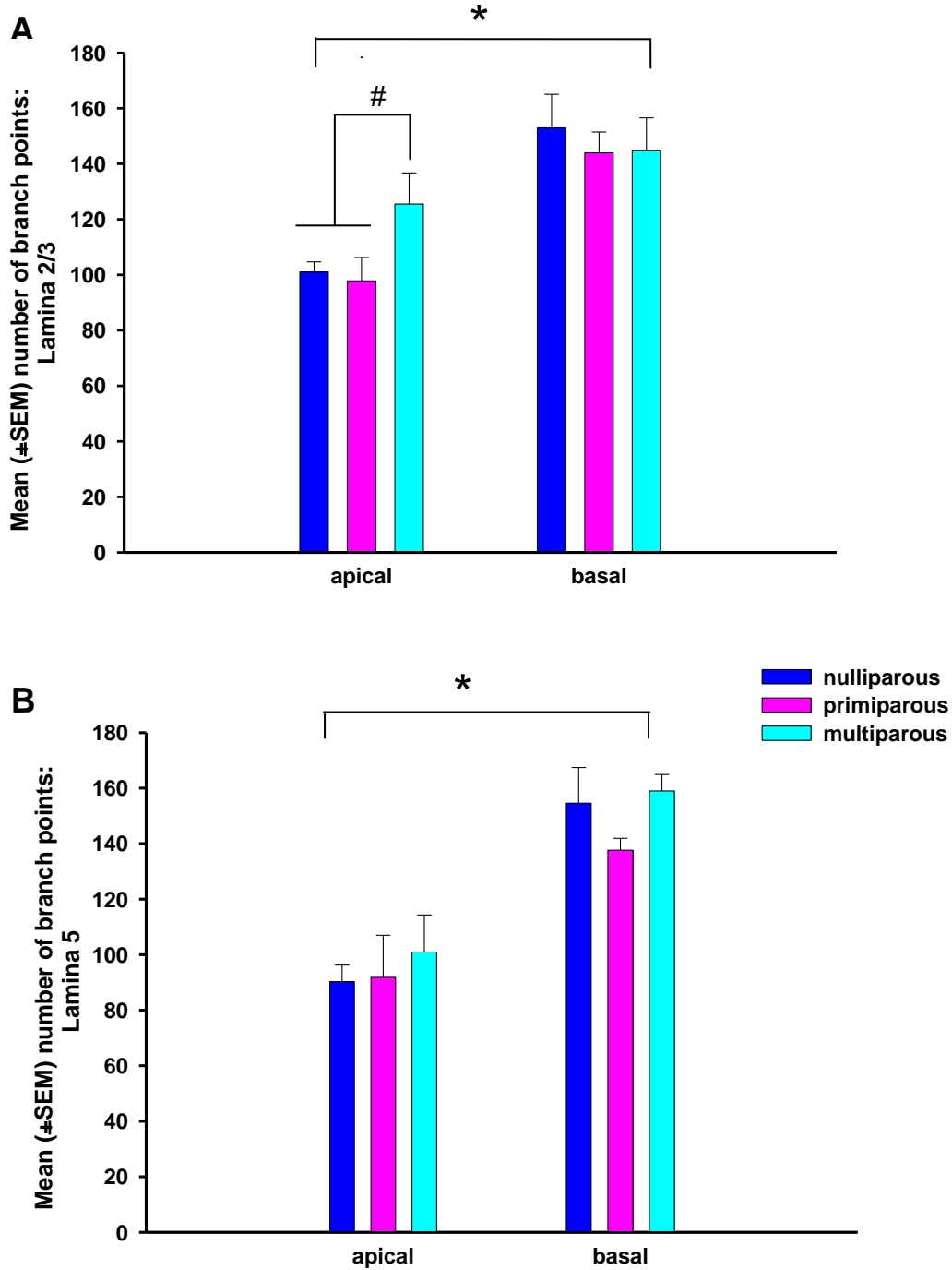


Figure 6. Mean ( $\pm$ -SEM) number of total branch points in apical and basal regions of pyramidal cells in Lamina 2/3 and 5. A significant main effect of area was found in the basal regions of Lamina 2/3 (A) and Lamina 5 (B) each having more total branch points than apical regions ( $p \leq 0.001$  for (A) and (B)). A trend was found in the apical region of Lamina 2/3 of the multiparous rats to have more total branch points than either nulliparous or primiparous rats ( $p \leq 0.09$ ;  $p \leq 0.06$ , respectively).

**4.3.3. The dendrites in the basal region in Lamina 2/3 are longer than in the apical region in Lamina 2/3 for all groups.**

Analysis of Lamina 2/3 branch length revealed a significant main effect of length [ $F(1, 14) = 16.77, p \leq 0.001$ ] with total basal branch length being longer than total apical branch length (Figure 7A). There was no significant main effect for Condition ( $p \leq 0.58$ ) or an interaction effect for Condition and Region ( $p \leq 0.75$ ). *A priori* analyses revealed no significant differences between nulliparous, primiparous, and multiparous rats on total branch length in Lamina 2/3 in either the apical ( $p \leq 0.72, p \leq 0.35$ , respectively) or basal regions ( $p \leq 0.98, p \leq 0.55$ ).

**4.3.4. In Lamina 5, there are no significant differences in total branch length in either the apical or basal regions.**

Analysis of total branch length in Lamina 5 revealed no significant main effect for Region ( $p \leq 0.13$ ) or a main effect for Condition ( $p \leq 0.31$ ) (Figure 7B). Additionally, there was no interaction effect found for Condition and Region with respect to total branch length ( $p \leq 0.98$ ). *A priori* results revealed no significant differences in branch length in Lamina 5 between nulliparous, primiparous, and multiparous animals in either the apical ( $p \leq 0.75, p \leq 0.35$ , respectively) or basal regions ( $p \leq 0.67, p \leq 0.30$ ).

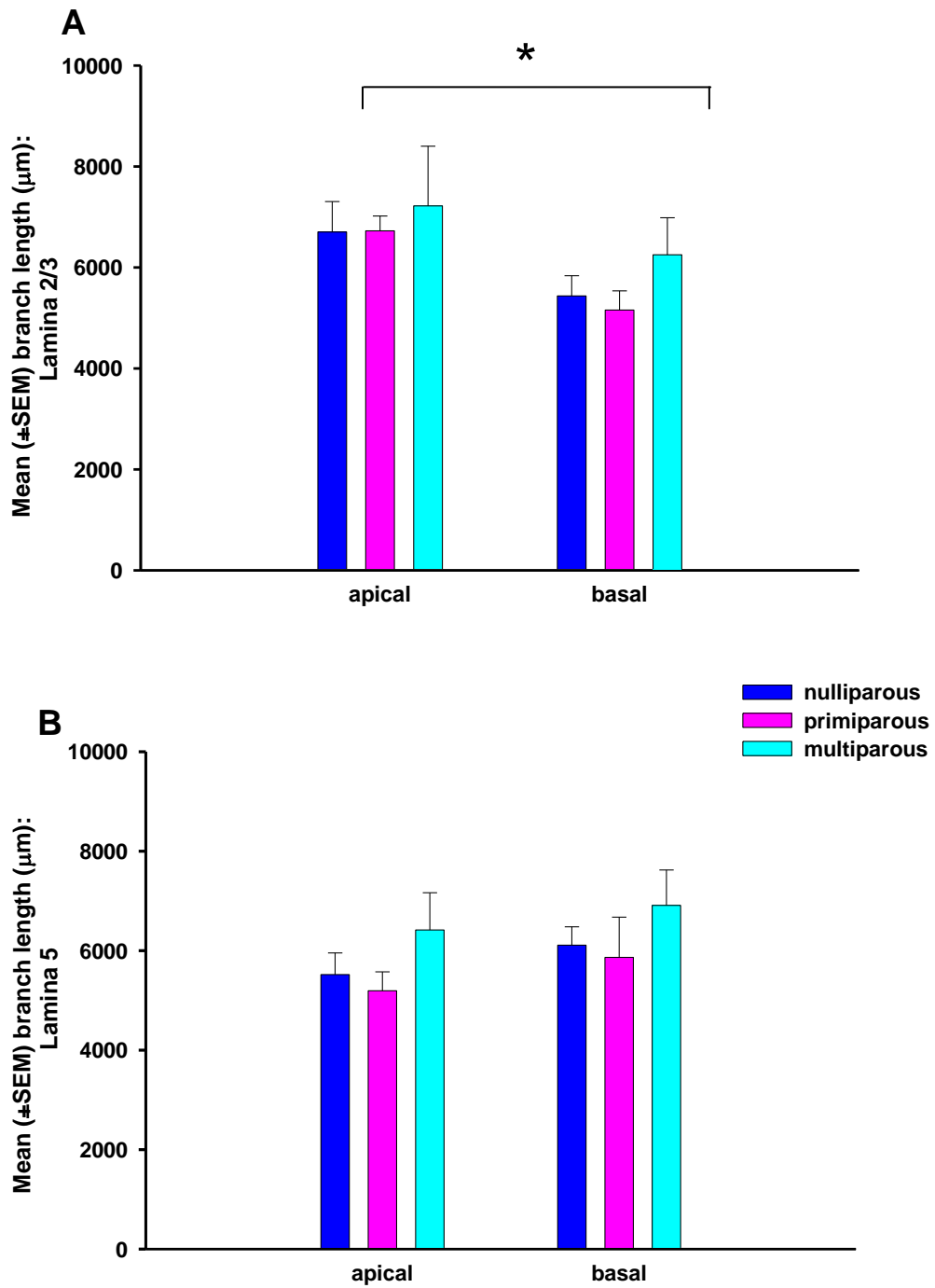


Figure 7. Mean ( $\pm$ SEM) total branch length for Lamina 2/3 and Lamina 5. A significant main effect of region was found in Lamina 2/3 (A) with apical dendrites showing significantly longer total lengths than basal dendrites ( $p \leq 0.001$ ). No significant differences were found between groups in either apical or basal regions in both Lamina 2/3 and Lamina 5 (B).

**4.3.5. The total time dams spent blanket nursing negatively correlated with branch points in Lamina 5.**

There was a significant negative correlation between the total duration of blanket nursing and the total number of branch points in the basal region of Lamina 5 ( $r = -0.67$ ,  $p \leq 0.03$ ; multiparous:  $r = -0.26$ ,  $p \leq 0.74$ ; primiparous:  $r = -0.73$ ,  $p \leq 0.09$ ; Figure 8A). Further analyses revealed a significant positive correlation with the total time retrieving pups and the total basal branch length in the Lamina 2/3 region ( $r = 0.81$ ,  $p \leq 0.005$ ; multiparous:  $r = 0.93$ ,  $p \leq 0.07$ ; primiparous:  $r = 0.22$ ,  $p \leq 0.68$ ; Figure 8B).

In addition, multiparous rats showed a significant positive correlation between the total time spent off pups and the total apical branch length in Lamina 2/3. However, this was not observed for group effect nor in the primiparous rats ( $r = 0.39$ ,  $p \leq 0.27$ ; primiparous:  $r = -0.64$ ,  $p \leq 0.17$ ; multiparous:  $r = 1.00$ ,  $p \leq 0.002$ ; Figures 9A). Further analyses revealed a positive trend between the total time the dams spent off of the pups and the total number of basal branch points in Lamina 5 such that the more time the dams spent off of the pups corresponded with an increase in the total number of basal branch points in Lamina 5 ( $r = 0.59$ ,  $p \leq 0.08$ ; primiparous:  $r = -0.07$ ,  $p \leq 0.89$ ; multiparous:  $r = 1.00$ ,  $p \leq 0.004$ ; Figure 9B).



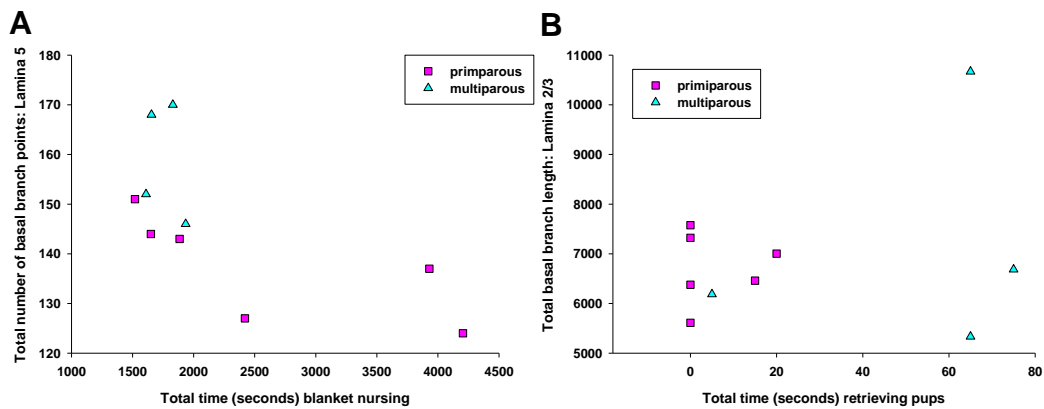


Figure 8. Correlation between blanket nursing and Lamina 5 basal branch points. A Pearson's product-moment correlation was conducted between the total time primiparous and multiparous rats spent blanket nursing and the total number of basal branch points in Lamina 5 (A); and total time retrieving pups and total basal branch length in Lamina 2/3 (B), respectively. Results revealed that the more time dams spent blanket nursing significantly correlated with a decrease in basal branch points in Lamina 5 basal branch in Lamina 2/3 ( $r = 0.81, p \leq 0.005$ ).

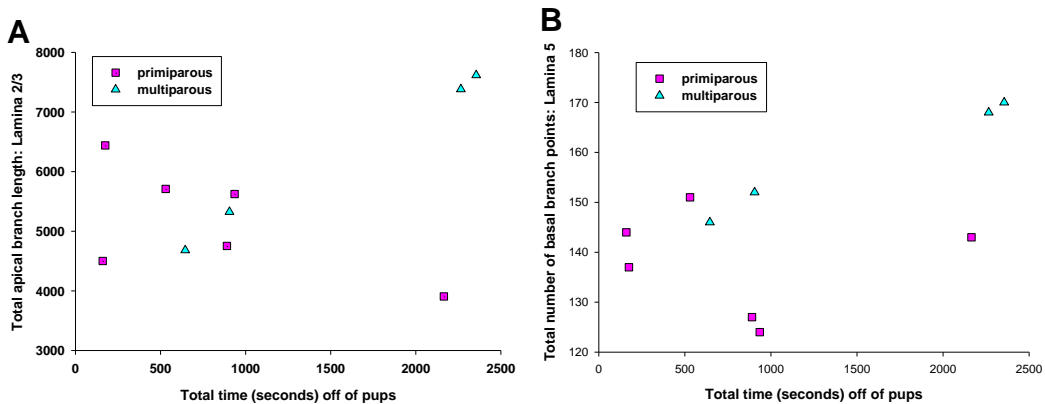


Figure 9. Correlation between total time spent off pups and pyramidal cell morphology. A significant positive correlation was found between the total time multiparous rats spent off of their pups and an increase in the total apical branch length in Lamina 2/3 ( $r = 1.00, p \leq 0.002$  (A)); again a positive trend was found between the total time multiparous rats spent off of their pups and an increase in the total number of branch points in Lamina 5 ( $r = 0.59, p \leq 0.08$ )(B).

#### **4.3.6. Arched-back nursing positively correlated with the total number of branch points in the apical and basal regions of Lamina 5**

There were significant positive correlations found between the total time the dams spent arched-back nursing and the total number of apical and basal branch points in Lamina 5. Interestingly, although no group correlation was found between the total time arched-back nursing and the total number of *apical* branch points on Lamina 5 pyramidal cells, multiparous rats showed a significant positive correlation on this measure ( $r = 0.13, p \leq 0.71$ : primiparous:  $r = 0.57, p \leq 0.24$ ; multiparous:  $r = 0.98, p \leq 0.02$ ). Primiparous rats showed a similar effect such that the more time primiparous rats spent arched-back nursing significantly positively corresponded with an increase in Lamina 5 *basal* branch points ( $r = 0.43, p \leq 0.22$ : primiparous:  $r = 0.86, p \leq 0.03$ ; multiparous:  $r = 0.41, p \leq 0.60$ ).

#### **4.3.7. Nest building was found to correlate with the total number of branch points in Lamina 5**

Further, a negative trend was found such that the total time spent nest building corresponded with a decrease in the total number of branch points in the basal region of Lamina 5 ( $r = -0.61, p \leq 0.06$ : primiparous:  $r = -0.87, p \leq 0.02$ ; multiparous:  $r = 0.49, p \leq 0.51$ ).

**4.3.8. Multiparous rats show significant correlations between the total time spent passive nursing and lamina 2/3 and 5 morphology.**

There was a significant negative correlation seen in the multiparous rats between the total time spent passive nursing and the total number of Lamina 5 basal branch points ( $r = -0.26$ ,  $p \leq 0.47$ : primiparous:  $-0.01$ ,  $p \leq 0.98$ ; multiparous:  $r = -0.96$ ,  $p \leq 0.04$ ). A similar correlation was found in Lamina 2/3 for the multiparous group in which more time spent passive nursing significantly negatively corresponded with the total apical branch length in Lamina 2/3 ( $r = -0.49$ ,  $p \leq 0.15$ : primiparous:  $r = -0.23$ ,  $p \leq 0.67$ ; multiparous:  $r = -0.97$ ,  $p \leq 0.03$ ).

**4.3.9. The total number of pups born in the most recent litter positively correlated with an increase in the number of apical branch points in Lamina 2/3.**

Analyses revealed a significant positive correlation between the total number of pups born in the most recent litter and the total number of apical branch points in Lamina 2/3 ( $r = 0.66$ ,  $p \leq 0.04$ ; primiparous =  $0.34$ ,  $p \leq 0.052$ ; multiparous:  $r = 0.82$ ,  $p \leq 0.018$ ) (Figure 10).

For the multiparous group, Lamina 5 appeared to correlate most significantly with litter characteristics. For example, a significant *negative* correlation was found between the total number of apical branch points in Lamina 5 and the total number of *male* pups in the most recent litter ( $r = -0.95$ ,  $p \leq 0.05$ ). In addition, a significant *positive* correlation was found with the total number of *female* pups born to the most recent litter and the total number of apical branch points in this region ( $r = 0.98$ ,  $p \leq 0.02$ ).

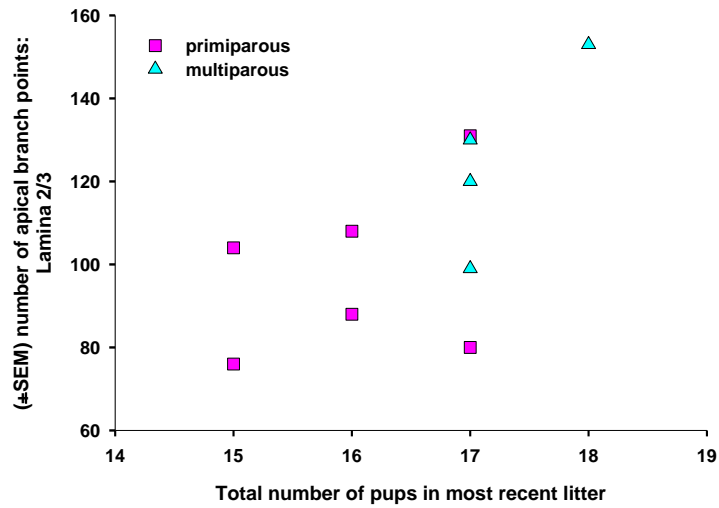


Figure 10. Correlation between total branch points and total number of pups. A Pearson's product-moment correlation was conducted on the total number of pups born in the most recent litter for primiparous and multiparous rats and the total number of apical branch points in Lamina 2/3. A significant positive correlation was found such that a larger total litter number corresponded with more apical branch points in Lamina 2/3 ( $r = 0.66$ ,  $p \leq 0.04$ ).

## 5. Discussion

The current studies were performed to a) investigate the effects of reproductive experience on learning and memory involving the prefrontal cortex, and b) to examine the effects of reproductive experience on the morphology of pyramidal cells in the prelimbic region of the prefrontal cortex. In Experiment 1 reproductive experience did not significantly affect the total number days necessary to reach criterion performance or the total number of errors committed on the delayed win-shift task. However, multiparous rats tended to make fewer errors than both primiparous and nulliparous rats across Blocks 2, 3, and 4 of trials. Furthermore, passive nursing behaviors were negatively correlated with within-phase errors as increased within-phase errors were associated with less time the dams spent passive nursing. The results from Experiment 2 found that multiparous rats had more apical branch points than either nulliparous or primiparous rats in the prelimbic region of Lamina 2/3 in the prefrontal cortex. In addition, there were significant correlations between maternal nursing behaviors (arched-back, passive, and blanket nursing) and Lamina 2/3 and Lamina 5 pyramidal cell morphology. For example, the more time dams spent either arched-back nursing or passive nursing corresponded with the total number of apical branch points and total branch length in Lamina 2/3 and Lamina 5. Thus, the findings from these studies suggest that both working memory and pyramidal cell morphology in the prelimbic region of the prefrontal cortex are influenced by maternal behaviors and reproductive experience.

### **5.1. Multiparous rats committed fewer across-, within-, and omission errors**

The results of Experiment 1 modestly support the initial hypotheses that reproductive experience may influence prefrontal cortex-dependent learning and memory. In Experiment 1, a strong trend was found for multiparous animals to commit fewer within-phase errors than primiparous animals in Block 4 of trials and fewer mean within-phase errors than either nulliparous or primiparous animals in Blocks 2 and 3 (Figure 2C). These findings suggest that repeated reproductive experience (multiparity) may enhance working memory processes dependent on the prefrontal cortex. Similar findings have been observed in earlier studies that found multiparous rats performed significantly better than either nulliparous or primiparous rats on working memory tasks involving the hippocampus (Kinsley et al., 1999; Gatewood et al., 2005; Love et al., 2005). For example, using an eight-arm radial arm maze, Kinsley et al. (1999) found that multiparous rats made significantly more correct arm choices than nulliparous animals over the first 6 days of testing. Furthermore, using a different task of spatial memory, the dry land maze, Gatewood et al. (2005) found that multiparous rats showed significantly enhanced spatial memory than either the nulliparous or primiparous rats. In addition, the Gatewood et al. study found that the multiparous rats had significantly fewer amyloid precursor protein immunoreactive (APP-ir) cells than either the nulliparous or primiparous animals. Thus, as suggested by the Gatewood group, repeated reproductive experience may result in enhanced neuroprotection. Importantly, the fewer number of APP-ir

positive cells significantly and positively correlated with latency performance on the dry land maze at twenty-four months. As such, these potential neuroprotective effects of multiparity may be expressed through enhanced cognitive performance throughout the lifespan. Curiously, studies using a hippocampus-dependent task such as the radial arm maze showed a distinct advantage for primiparous but not multiparous rats (Pawluski et al., 2006b; Pawluski et al., 2006a) suggesting there may be differences in how reproductive experience modulates hippocampus versus prefrontal cortex-dependent cognition.

### ***5.2. Reproductive experience influences hippocampus and prefrontal cortex–dependent tasks***

Previous research has utilized predominately hippocampus-dependent tasks as spatial memory performance is sensitive to the effects of reproductive experience (Galea et al., 2000; Tomizawa et al., 2003; Gatewood et al., 2005; Lemaire et al., 2006; Pawluski et al., 2006b; Pawluski et al., 2006a; Kinsley et al., 2008). However, akin to the findings of Experiment 1, multiparity has also been found to enhance learning and memory performance that depends on the integrity of other brain regions besides the hippocampus (Numan, 1974; Li and Fleming, 2003; Macbeth et al., 2008; Paris and Frye, 2008). Recent studies using object placement (predominately hippocampus-dependent; (Ennaceur et al., 1997; Mumby, 2001) and object recognition (predominately cortical-dependent; (Ennaceur et al., 1997; Mumby, 2001) tasks have found that multiparous rats outperform both nulliparous and primiparous rats on each of these measures

(Macbeth et al., 2008; Paris and Frye, 2008). Of significance is the fact that the Paris & Frye (2008) and Macbeth et al. (2008) studies constitute some of the first to use animal models to demonstrate enhanced cortical-dependent learning and memory following reproductive experience. It is important to note that not unlike the delayed spatial win-shift task, the object recognition task also involves limbic as well as diencephalic structures (Floresco et al., 1997; Mitchell and Dalrymple-Alford, 2005) and as such, the involvement of other brain structures such as the hippocampus cannot be excluded from these findings. Nevertheless, it appears from previous research and the findings of Experiment 1 that multiparous rats consistently outperform nulliparous and primiparous rats on a number of cortical-dependent cognitive tasks.

Interestingly, in Experiment 1 we did not find any significant enhancement in performance on the delayed win-shift task in the primiparous group. These findings differ from those of previous reports that found primiparous rats outperformed both nulliparous and multiparous rats on hippocampus-dependent tasks (Pawluski et al., 2006b; Pawluski et al., 2006a). For example, Pawluski et al. (2006a) found that although multiparous rats performed better than nulliparous rats on the radial arm maze, the primiparous rats demonstrated significantly enhanced spatial working and reference memory than either the nulli- or multiparous rats. Furthermore, Pawluski et al. (2006b) determined that both pregnancy and the full mothering experience (birth through to weaning) were necessary for the cognitive performance seen in the primiparous group.



### ***5.3. The differential hormonal profile of multiparous rats may contribute to fewer errors on the delayed spatial win-shift task***

The hormonal profile of the multiparous rat differs from that of the nulli- and primiparous rat. For instance, multiparous rats showed less sensitivity to opioids (both endogenous and exogenous) than either nulli- or primiparous rats (Kinsley and Bridges, 1988; Mann and Bridges, 1992). However, following lactation and the resumption of the estrus cycle, circulating estradiol and prolactin levels as well as sensitivity to estradiol are decreased in the multiparous rat (Bridges and Byrnes, 2006). As estradiol has been implicated in facilitating learning and memory as well as synaptic structure and associated protein expression, (Weiland, 1992; Woolley and McEwen, 1992; McEwen and Woolley, 1994; Luine et al., 1998; Galea et al., 2001; Jelks et al., 2007; Zadran et al., 2009; Pawluski et al., 2010) it appears counterintuitive that the multiparous rats in the present study appeared to learn the task better than either the nulli- or primiparous rats. Curiously, estrogen demonstrates a dose-dependent sensitivity threshold with respect to learning and memory. Lower levels of estradiol facilitate, while high levels of estradiol impair both hippocampus and prefrontal cortex-dependent learning and memory (Holmes et al., 2002; El-Bakri et al., 2004; Wide et al., 2004; Sinopoli et al., 2006; Barha et al., 2010). Fluctuations in estrogen receptor(ER)-alpha occur during proestrus in primiparous rats in brain regions associated with maternal behaviors such as the MPOA and medial amygdala and the bed nucleus of the stria terminalis (BNST) (Shughrue et al., 1997;

Helena et al., 2006; Byrnes et al., 2009). ER-alpha is also found in the hippocampus and prefrontal cortex (Mazzucco et al., 2006; Ottowitz et al., 2008) where it mediates glutamatergic transmission (El-Bakri et al., 2004; Jelks et al., 2007). Recently, estrogen has been implicated in enhancing prefrontal cortex-hippocampus connectivity (Ottowitz et al., 2008). It may be that a reduction in sensitivity to estrogen vis-à-vis reduced ER-alpha expression or circulating estrogens in the multiparous rats enhanced group task performance in this study, particularly within-phase errors. In fact, in a recent study examining ER-alpha expression in the CA3 of the hippocampus, Pawluski et al. (2010) found that primiparous and multiparous rats showed fewer ER-alpha positive cells than nulliparous rats. Future studies examining the effects of reproductive experience and ER-alpha would benefit from the inclusion of behavioral testing to determine how ER-alpha may contribute to learning and memory. The addition of a behavioral paradigm may also reveal ER-alpha expression in other brain regions involved in cognition in the maternal rat.

In addition, multiparity has been found to reduce anxiety (Macbeth et al., 2008; Rima et al., 2009) as well as the behavioral effects of stress in both rodents and humans (Wartella et al., 2003; Tu et al., 2006). Thus the reduction of stress and anxiety responses in the multiparous rat may account, at least in part, to the enhanced cognitive performance in this group seen in Experiment 1 (Herrero et al., 2006; Muigg et al., 2008). With respect to Experiment 1, perhaps the first reproductive process of pregnancy, birth, and mothering serves as a

stressor, therefore impeding working memory performance for the primiparous group on the delayed spatial win-shift task. As the prefrontal cortex is particularly sensitive to the effects of stress as well as to fluctuations in hormonal profiles (Shansky et al., 2004; Cerqueira et al., 2005; Shansky et al., 2006; Wallace et al., 2006), the higher number of within-phase errors seen in the primiparous group may represent the effects of the stress of first-time motherhood on prefrontal cortex-dependent learning and memory. Indeed primiparity is associated with increased corticosterone postpartum when compared to multiparity (Pawluski et al., 2009a). Conversely, with repeated reproductive experience, the multiparous group may have developed 'maternal memory' (Li and Fleming, 2003) that provided for adaptive mechanisms in response to chronic stress which may account for the fewer number of working memory errors committed by this group. Thus, the effects of repeated reproductive experience may provide for enhancements in learning and memory processes that are dependent upon the prefrontal cortex.

Perhaps as has been suggested previously, the hormonal and neural mechanisms associated with primiparity may prime the female rat for enhanced spatial memory that is predominately reliant upon the hippocampus following the first reproductive experience (Kinsley et al., 1999; Pawluski et al., 2006b; Pawluski et al., 2006a). Subsequently, during the first reproductive experience in which efficient foraging and providing safety for offspring may be novel for the primiparous rat, hippocampus-dependent spatial learning and memory may play

a more fundamental role (Pawluski et al., 2006b), representing the current literature surrounding the enduring hormonal, neural and behavioral changes associated with motherhood in the rat (Fleming and Korsmit, 1996; Kinsley et al., 2008). Conversely, during subsequent pregnancies and mothering experiences, these neural and hormonal mechanisms may play less of a predominate role as the spatial map has been established or the neural and hormonal processes that contribute to this mapping process have been altered in order to allow for more rapid acquisition of spatial information. For example, Pawluski et al. (2009a) found that free circulating corticosterone is significantly more elevated in primiparous rats than in multiparous rats. Previous research has found that elevations in corticosterone in rodents may contribute to enhancement in hippocampus-dependent learning (Catalani et al., 2000; Quirarte et al., 2009) although this effect may be context dependent. For example, chronic restraint stress in females enhances spatial memory performance (Bowman et al., 2001). As suggested by Pawluski et al. (2006a), the first reproductive experience may represent a period of chronic stress that, when coupled with elevated levels of free corticosterone (Pawluski et al., 2009a), may account for their findings of enhanced hippocampus-dependent spatial working and reference memory in the primiparous rats. However, acute stress, particularly during acquisition, has been implicated in augmenting prefrontal cortex learning and memory in males and females in both human and rodent populations (Shors et al., 1998; Wolf, 2003; Smeets et al., 2006; Yuen et al., 2009). As such, through the development

of 'maternal memory' (Li and Fleming, 2003) and the neural and hormonal changes associated with the first reproductive experience, repeated mothering experiences may not be experienced as stressful to the multiparous rat. Feasibly, initial introduction to the delayed spatial win-shift task may have served as an acute stressor, however, it is probable that the animals in the current study habituated to the task and thus the 'stressful' effects of behavioral testing likely do not fully account for the findings reported here. However, enrichment effects following from exposure to pups may have contributed to the reduction in omission errors on Blocks 2, 3, and 4, across-phase errors on Block 2, and within-phase errors on Blocks 2, 3, and 4 in the maternal groups. Van Wass and Soffie (1995) found that four month old male rats showed enhanced spatial acquisition if housed in an enriched environment. Thus, if pup exposure is considered an enrichment as has been argued elsewhere (Sparling et al., 2010) the modest enhancement in performance on the delayed spatial win-shift task by the maternal groups may be a reflection of continued interaction with offspring. Furthermore, as the multiparous rats had mothered two litters of pups until time of weaning, the putative additive effects of repeated maternal experience may account for the reduction in the number of errors performed by the multiparous group.

In addition, the modestly enhanced spatial working memory seen in the multiparous rats is not accounted for by litter characteristics. Previous research has found that certain species (i.e.: mountain goats) are more likely to give birth

to male offspring as they age (Cote, 2001). However, all of the rats in the current studies were age-matched and gave birth to their first (or final in the case of the multiparous rats) when they were approximately six to seven months old. Furthermore, carrying male fetuses (at least in humans) has been found to augment spatial performance and working memory in the mother that extends well beyond birth (Vanston and Watson, 2005). However, there were no statistically significant differences between the primiparous and multiparous rats on the total number of male pups born to the most recent litter prior to behavioral testing [ $t(17) = -0.65, p \leq 0.53$ ]. Single doses of testosterone have been found to significantly enhance visuospatial performance in women (Aleman et al., 2004) thus dams with a larger number of male pups may develop enhanced spatial abilities as a result of continual, exogenous testosterone exposure. In addition, rat dams preferentially anogenital lick male offspring (Moore and Morelli, 1979; Moore, 1982). As the litters in the current studies were culled to five males and five females, the differential effects of mothering, if any, in response to male versus female pups would have been controlled for across the maternal groups. The nulliparous rats in the current experiments did not benefit from the physical or putative hormonal exposure from pups. Interestingly, in Blocks 1 through 4 on omission errors, the nulliparous rats consistently made more errors than either the primiparous or multiparous rats. Interpreting these findings remains speculative at present, but perhaps the environmentally 'impoverished' housing conditions impaired behavioral flexibility in the nulliparous rats and therefore they

consistently committed more omission errors than either the primiparous or multiparous rats. In order to assess the effects of single-housing on behavioral performance following reproductive experience, pair-housing the nulliparous controls may provide social/environmental stimulation that may enhance performance similar to that of the maternal groups.

#### ***5.4. Heightened neural plasticity in multiparous rats may contribute to fewer within-, across, and omission errors***

In the present thesis I found some support for the fact that multiparity was associated with better performance on the delayed spatial win-shift task--this improved performance may be due to changes in neural cell adhesion molecule (NCAM), BDNF or changes in pyramidal cell morphology. It is well established that NCAM functions in synaptic plasticity, cell migration, cell to cell interaction and in learning and memory (Butler et al., 1997; Rouillet et al., 1997; Sandi and Loscertales, 1999; Uryu et al., 1999; Diestel et al., 2005). Furthermore, NCAM expression has been found to increase on day eighteen of gestation in the hippocampus (Banasr et al., 2001) and in response to acute elevations in corticosterone in the frontal cortex (Sandi and Loscertales, 1999). Perhaps the performance on the delayed spatial win-shift task was facilitative for the multiparous group during the both the training and test phases resulting in fewer across-, within- and omission errors. There are no known studies that have investigated NCAM expression following reproductive experience so it is possible that NCAM expression is enhanced in multiparous rats and may augment learning and memory following reproductive experience. In order to determine

the contribution of NCAM, if any, to the findings in Experiment 1, future studies are required that would specifically evaluate NCAM expression immediately following weaning as well as following behavioral tasks. In this way, determination can be made surrounding 'basal' NCAM expression in response to reproductive experience as well as the putative enhanced effects following behavioral testing and mothering experience.

In addition, brain-derived neurotrophic factor (BDNF) is thought to be fundamental in synaptic plasticity, long-term potentiation, and learning and memory (Kiprianova et al., 1999; Mu et al., 1999). Multiparity is associated with increases in BDNF expression in the hippocampus and is correlated with enhanced performance on spatial and non-spatial tasks (Macbeth et al., 2008). Furthermore, novel learning contexts enhances BDNF expression in the prefrontal cortex of rats (Rapanelli et al., 2010). Perhaps the combination of novel learning during the delayed spatial win-shift task coupled with repeated reproductive experience may account for the fewer errors committed by the multiparous group in Experiment 1. Unfortunately, the MacBeth et al. (2008) study did not include a primiparous group, therefore limiting conclusions surrounding the specific contributions of multiparity in BDNF expression, *per se*. However, it is apparent that reproductive experience influences BDNF expression, and subsequently, learning and memory. Inclusion of additional groups such as pregnant-only, sensitized, as well as primiparous rats in the analysis of BDNF expression and behavioral correlates may reveal time points



and/or the reproductive status that initiates BDNF augmentation. Thus, both NCAM and BDNF may contribute to the moderate enhancement in learning that was demonstrated in Experiment 1 – another possible contributing factor could be the effect of reproductive experience to influence pyramidal cell morphology in the prefrontal cortex as was examined in Experiment 2.

### ***5.5. Multiparity influences pyramidal cell morphology in the prelimbic region***

As well as behavioral differences, the multiparous group showed a trend towards having more total apical branch points in Lamina 2/3 pyramidal cells of the medial prefrontal cortex than either the nulli- or primiparous groups (Figure 6A). These findings extend those previously reported on dendritic morphology in the CA1 and CA3 regions of the hippocampus at time of weaning (Pawluski and Galea, 2006). The Pawluski and Galea (2006) study found that primiparous rats had significantly shorter branch lengths and fewer branch points than either the nulliparous or multiparous groups in both the apical and basal regions of CA1 and CA3 pyramidal cells. Furthermore, there were no discernable differences between the nulliparous and multiparous rats in branch length or branch points in any of the areas examined (Pawluski and Galea, 2006). However, the multiparous rats in the Pawluski and Galea (2006) study had greater dendritic spine density on the basal region of CA1 pyramidal cells than either the nulliparous (significant difference) or primiparous (trend) rats.

Dendritic length and branch points are considered indices of cellular complexity (Scholl, 1953; Ireland et al., 1985; Carriquiry et al., 1991). The

purpose of this complexity remains under investigation, however a recent study suggests that neuronal complexity is based upon the 'cost' of maintaining its arbors in relation to the cell's function (Wen and Chklovskii, 2008). Therefore, with respect to the Pawluski et al. studies (Pawluski and Galea, 2006; Pawluski et al., 2006b; Pawluski et al., 2006a), the atrophy observed in the primiparous rats may function as a neuroprotective measure in response to enhanced glutamatergic activity in the hippocampus (Monaghan et al., 1985; Mattson and Kater, 1989; Prendergast et al., 2004; Butler et al., 2010). The first reproductive experience is time of intense learning following from which dramatic increases in glutamate are seen, particularly in the hippocampus (Monaghan and Cotman, 1985; Nicoletti et al., 1988). Subsequently, it may be that pyramidal cell remodeling in the hippocampus occurs in a manner that provides optimal synaptic input in the context of novel learning while simultaneously preserving the cell as has been suggested to occur in the prefrontal cortex (Radley et al., 2006). Thus, the atrophy seen in the primiparous rats in the Pawluski and Galea (2006) study may be reflective of a homeostatic process in response to learning. In contrast, the increase in dendritic spines seen in the multiparous rats in the hippocampi in the Pawluski and Galea (2006) study may be reflective of prior learning, or 'maternal memory' in which there may be a lesser influx of glutamate allowing for enhanced pyramidal cell complexity in response to novel learning conditions.

What is of importance to note is that primiparity was associated with hippocampal pyramidal cell changes (Pawluski & Galea, 2006) but that multiparity was associated with prefrontal cortex changes in pyramidal cell morphology (Experiment 2, present thesis). Furthermore, primiparity caused atrophy of branch length and points but multiparity caused an increase in branch points suggesting that reproductive experience can have differential effects on morphology dependent upon the region and the amount of experience.

#### ***5.6. Putative hormonal contributions of multiparity in pyramidal cell complexity***

Hippocampal pyramidal cell morphology fluctuates in concert with hormonal vacillations of the estrus cycle (Woolley et al., 1990b). Within the twenty-four hour period between proestrus and estrus, apical dendritic spines decrease as much as thirty-two percent in CA1 neurons (Woolley et al., 1990b). Estradiol has been implicated in augmenting synapses via dendritic spines in the apical and basal regions of CA1 pyramidal cells (Woolley et al., 1990a; Wallace et al., 2006). Similar effects have been found in the prefrontal cortex where estradiol has been found to enhance dendritic spine density in the apical and basal regions of Lamina 2/3 pyramidal cells in the medial prefrontal cortex (Wallace et al., 2006; Garrett and Wellman, 2009). With respect to the findings of Experiment 2, it appears that multiparity may augment apical complexity in Lamina 2/3 pyramidal cells. As multiparous rats have greater circulating estradiol throughout gestation than primiparous rats (Paris and Frye, 2008), it may be that pyramidal cell morphology is affected during gestation and sustained throughout lactation.

Evidence for this comes from the fact that differences in circulating estradiol are not observed between age-matched nulli- primi- and multiparous rats at time of weaning (Pawluski and Galea, 2006). As many of the changes in the maternal rat brain are enduring (Fleming and Korsmit, 1996), it is plausible that neuronal alterations occur earlier than at the time of weaning when the Pawluski and Galea (2006) and animals in Experiment 2 were sacrificed. Further research is required to examine pyramidal cell morphology at different time points throughout gestation and lactation in order to identify the onset of dendritic remodeling and its putative maintenance following reproductive experience.

Corticosterone is also implicated in learning and memory, pyramidal cell morphology, as well as in maternal behavior (Luine et al., 1996; Akirav and Richter-Levin, 2002; Conrad et al., 2004; Graham et al., 2006; Rees et al., 2006). Exposure to corticosterone via acute stress has been shown to facilitate learning and memory in the hippocampus and prefrontal cortex (Akirav and Richter-Levin, 2002; Yuen et al., 2010) whereas chronic elevations to corticosterone impede these processes (Bodnoff et al., 1995; Cerqueira et al., 2005). The first reproductive experience may be considered a stressful period for the primiparous rat which may account for the hormonal and morphological differences observed in the current and previous studies (Pawluski and Galea, 2006; Pawluski et al., 2009a). The effects of stress and its subsequent increase in corticosterone appear to have differential effects on learning and memory following reproductive experience. As previously observed, the apparent prolonged stress of first time

motherhood appears to enhance hippocampus-dependent learning and memory (Kinsley et al., 1999; Pawluski et al., 2006b; Pawluski et al., 2006a) but perhaps counterintuitively reduce dendritic arborizations in the hippocampus (Pawluski and Galea, 2006) although these effects on morphology are not enduring (Love et al., 2005). Corticosterone has been implicated as fundamental in the development of maternal memory (Li and Fleming, 2003; Graham et al., 2006). Thus the dendritic 'atrophy' seen in the hippocampus of primiparous rats (Pawluski and Galea, 2006) may be representative of this.

The morphology of prefrontal cortex pyramidal cells, as well as behaviors dependent upon the prefrontal cortex are particularly sensitive to the effects of gonadal hormones and corticosterone (Sullivan and Gratton, 2002; Cook and Wellman, 2004; Brown et al., 2005; Radley et al., 2006; Shansky et al., 2006; Wallace et al., 2006; Radley et al., 2008; Garrett and Wellman, 2009; Yuen et al., 2009). Acute and chronic exposures to stress result in dendritic atrophy and remodeling of dendritic spines of pyramidal cells in the prefrontal cortex (Cook and Wellman, 2004; Radley et al., 2004; Brown et al., 2005; Radley et al., 2006). In addition, exposure to stress has been found to both enhance prefrontal cortex dependent learning and memory in males and females (Shansky et al., 2004; Porcelli et al., 2008; Chauveau et al., 2009; Yuen et al., 2009) as well as impede it (Radley et al., 2004; Liston et al., 2006; Oei et al., 2006). However sex of subject, hormonal status, as well as the environment have all been found to influence the prefrontal cortex (Beck and Luine, 2002; Shansky et al., 2006; Del

Arco et al., 2007; Park et al., 2008; Segovia et al., 2008; Garrett and Wellman, 2009; Lin et al., 2009). Some studies have found enhanced performance on prefrontal cortex-dependent tasks in male populations of humans and rodents following periods of stress (Oei et al., 2006; Porcelli et al., 2008; Yuen et al., 2009). However, research examining the effects stress on female performance on prefrontal cortex dependent learning and memory is less definitive. For example, Qin et al. (2009) found that following acute psychological stress, women showed poorer performance on prefrontal cortex-dependent working memory tasks than non-stressed women. Similar findings were found in rodent populations following pharmacological induction of stress particularly when estradiol levels were elevated during proestrus (Shansky et al., 2004). Conversely, Bowman et al (2009) reported males and females performed similarly on the object recognition task--a task primarily independent of the hippocampus (Ennaceur et al., 1997; Mumby, 2001). However, it should be noted that the Bowman et al. (2009) study did not report estrous phase in their findings. Furthermore, the object recognition task utilized in the Bowman et al. study is completed in one day. Therefore, it may be that the female rodents in the Bowman et al. (2009) study were tested during estrus, and subsequently their performance reflected lower estradiol levels.

The multiparous animals in Experiment 2 showed a strong trend toward having more apical branch points on Lamina 2/3 pyramidal cells than either the nulli- or primiparous rats (Figure 6A). These findings may be the result of

reduced stress, as multiparous rats have been shown to have significantly reduced anxiety than either nulli- or primiparous rats (Wartella et al., 2003; Rima et al., 2009). Moreover, multiparous rats have reduced circulating corticosterone (Pawluski et al., 2009a), which could conceivably contribute to the enhanced dendritic arborizations of the pyramidal cells seen in this group in Experiment 2 (Cook and Wellman, 2004; Brown et al., 2005; Perez-Cruz et al., 2009) as well as that in the hippocampus (Pawluski and Galea, 2006). Furthermore, this reduction of stress may be the result prior learning. As 'maternal memory' may be a product of the first reproductive experience there may be a reduction in the demand in the brain regions associated with learning and memory in response to motherhood. Feasibly reducing the 'emotional' demand of new motherhood may result in a further reduction of circulating corticosterone which may have contributed to the findings of Experiment 2.

In addition, circulating estradiol is reduced in multiparous rats following weaning (Bridges and Byrnes, 2006). However, exposure to acute and chronic stress elevates estradiol levels (Goldman and Vogel, 1985; Shors et al., 1999) and recent studies suggest that it may enhance the stress response in the prefrontal cortex in females (Shansky et al., 2004; Shansky et al., 2006; Shansky et al., 2010) which may account for the findings in Experiment 1.

Interestingly, only the total number of branch points in Lamina 2/3 showed group differences in which the multiparous animals had a greater number than either the nulli- or primiparous rats (Figure 6A). It may be that a reduction in

circulating estradiol in the multiparous rats contributed to the dendritic organization seen in Experiment 2 through an attenuation of the stress response. Recently, estradiol via the ER-alpha in the paraventricular nucleus (PVN), has been found to impede the negative feedback loop of the hypothalamic-pituitary-adrenal (HPA) axis resulting in an increase in both daily as well as stress mediated corticosterone expression (Weiser and Handa, 2009). The prefrontal cortex is fundamental in the regulation of the HPA axis (Diorio et al., 1993) as well as being a site for ER-alpha expression (Montague et al., 2008). Furthermore, ER-alpha expression is augmented in response to gonadal hormones. Therefore, it may be that the increased number of apical branch points seen in the multiparous rats in Experiment 2 are a product of a dampened HPA axis response via a reduction in estradiol and ER-alpha expression. Further research investigating HPA axis activation and its associated neuroendocrine and catecholamine expression throughout lactation may begin to determine how repeated reproductive experience influences dendritic morphology in the prefrontal cortex. Additionally, this line of research would provide further evidence surrounding the activation of the HPA axis and its associated neurochemical release throughout lactation in the maternal rat.

Interestingly, following stress and/or increases in corticosterone, it appears that only the apical region of pyramidal cells in Lamina 2/3 of the medial prefrontal cortex undergo morphological changes in both male and female rats (Wellman, 2001; Radley et al., 2004; Brown et al., 2005; Garrett and Wellman,



2009; Shansky et al., 2009b). Furthermore, noradrenaline, a catecholamine associated with stress (Glavin, 1985) and maternal behaviors (Calamandrei et al., 1992) is significantly elevated during pregnancy and early postpartum (Parvez et al., 1973). In addition, noradrenaline expression increases following further exposure to stressors (Thierry et al., 1968). Shimada et al. (2006) suggest that excess catecholamine expression may be a contributing factor in the atrophy of Lamina 2/3 dendrites as the afferents from the brain stem that form part of the dopaminergic and noradrenergic pathways terminate in this region. Thus, multiparity may mediate this process as both the nulli- and primiparous rats had fewer total number of branch points in Lamina 2/3 (Figure 6A). Moreover, Lamina 2/3 of the prelimbic region is implicated in mediating communication across multiple brain regions (Gabbott et al., 2005; Goodfellow et al., 2009). Therefore, pyramidal cell complexity in this region may be indicative of enhanced intracerebral communication. Conversely, atrophy in this region may result in impairment, and as suggested by Goodfellow et al. (2009) may contribute to a maladaptive response to learning and stress. Furthermore, afferents from the CA1 region of the hippocampus primarily innervate the prelimbic region of the medial prefrontal cortex with most synapsing in Laminae 3 and 5 (Ferino et al., 1987; Jay et al., 1989; Gabbott et al., 2002). Pawluski and Galea (2006) found that both nulliparous and multiparous rats had significantly longer branch lengths and significantly more branch points in both the apical and basal regions of pyramidal cells in the CA1 and CA3 regions of the hippocampus

than primiparous animals. However, spine density in the basal region of CA1 pyramidal cells in the multiparous rats only was significantly increased in the Pawluski and Galea (2006) study. Perhaps, the increased total number of branch points found in the multiparous rats in Experiment 2 may be reflective of the hippocampus CA1 innervations as dendritic spines are the primary terminal endpoint for CA1 afferents (Gabbott et al., 2002). Prefrontal excitability has been found to originate from the CA1 region (Ferino et al., 1987; Laroche et al., 1990). Thus, it may be that in order to accommodate the influx of glutamatergic input from the CA1, the total number of branch points in Lamina 2/3 must increase as was observed in the multiparous rats in Experiment 2. Furthermore, the enhancement in dendritic spine density in the basal region of CA1 pyramidal cells in the multiparous rats in the Pawluski and Galea (2006) study may reflect a form of enhanced connectivity between the hippocampus and the prefrontal cortex. In the CA1, dendritic branching and dendritic spine density is greater in the basal region in control and spatial learning conditions (Moser et al., 1997). Perhaps these afferents originating in the CA1 synapse with the apical region of Lamina 2/3 pyramidal cells and contribute to the findings in Experiment 2.

### ***5.7. Potential correlates between dendritic complexity and performance on the delayed spatial win-shift task***

Additionally, the increase in Lamina 2/3 branch points in the multiparous rat may account for the reduction in the number of within-, across-, and omission errors observed in Experiment 1. As the pyramidal cells of Lamina 2/3 are believed fundamental in intracerebral communication (Gabbott et al., 2005;

Goodfellow et al., 2009), perhaps the increases in the total number of branch points observed in the multiparous rats in Experiment 2 reflect cognitive changes as behavior has been correlated with prefrontal pyramidal cell morphology (Liston et al., 2006; Wallace et al., 2006). However, different groups of rats were used for Experiment 1 and 2, therefore these correlations remain speculative at present.

Furthermore, the results of Experiments 1 and 2 may help explain the findings from the Pawluski et al. studies (Pawluski and Galea, 2006; Pawluski et al., 2006b; Pawluski et al., 2006a) with respect to the morphological and behavioral differences following from reproductive experience. The first reproductive experience may constitute a period of learning in which spatial memory is predominant in locating food caches, shelter, and the retrieval of offspring (Pawluski et al., 2006b). Thus, enhanced glutamatergic input to the hippocampus results in pruning of dendritic processes in the CA1 and CA3 as seen in the primiparous rats of the Pawluski and Galea (2006) study. However, it may be that once this cognitive map has been developed, more complex cognitive tasks are integrated into the maternal rat's behaviors that require greater involvement of the prefrontal cortex.

Spine density in the prefrontal cortex and hippocampus has been implicated in learning and memory as dendritic spines serve as the primary excitatory sites for glutamatergic activity and the initiation of long-term potential (LTP) (Toni et al., 1999; Elston, 2000; Oray et al., 2006; Matsuo et al., 2008). Furthermore,

spine density and morphology in both the prefrontal cortex and hippocampus are influenced by gonadal hormones and corticosterone (Gould et al., 1990; Woolley et al., 1990b; Radley et al., 2006; Chen et al., 2009). For example, Wallace et al. (2006) found that ovariectomized female rats performed significantly worse on memory tasks when dendritic spine density was reduced in the medial prefrontal cortex as well as in the hippocampus than did controls. Furthermore, repeated corticosterone administration or exposure to stressors results in a significant decrease in spine density in the prefrontal cortex (Seib and Wellman, 2003; Radley et al., 2008; Morales-Medina et al., 2009). However, Pawluski and Galea (2006) revealed that it was multiparous rats that had significantly greater spine density (at time of weaning) in the hippocampus than primiparous rats although primiparous rats were found to perform significantly better than either nulli- or multiparous rats on a task of working and reference memory (Pawluski et al., 2006a). As estradiol is implicated in enhancing dendritic spine density (Gould et al., 1990; Woolley et al., 1990b), this may account, in part, for the morphological findings in the Pawluski and Galea (2006) study. However, an increase in dendritic spines in the hippocampus may not correlate with enhanced learning and memory as female rats in proestrus--a period in which dendritic spine density is greatest--consistently show poorer performance on behavioral tasks (Diaz-Veliz et al., 1989; Warren and Juraska, 1997). Furthermore, a reduction in dendritic spine density in the hippocampus of aging rats was not found to correlate with spatial memory performance (Calhoun et al., 2008). Thus, it is

likely that the morphology and function of pyramidal cells in the hippocampus and prefrontal cortex differ in response to learning and memory.

### ***5.8. Passive nursing was associated with performance on the delayed spatial win-shift task***

There was a significant negative correlation with the total time spent passive nursing and the total number of within-phase errors committed on the delayed spatial win shift task (Figure 3A). As the total time passive nursing increased the total number of within-phase errors decreased ( $r = -0.50$ ,  $p \leq 0.03$ ). Passive nursing has been implicated as a pup-initiated behavior that requires little physical and (hypothetically) cognitive resources from the dam (Galler and Propert, 1982). Furthermore, passive nursing is considered the least effective means for milk let down which may lessen oxytocin expression via the minimalized physical stimulation necessary for oxytocin release from the supraoptic nucleus (SON) (Clinton et al., 2010). Oxytocin is implicated in impairing memory consolidation as well as the maintenance of maternal behaviors (Kovacs et al., 1979; Pedersen et al., 2006). Therefore the reduction in within-phase errors seen in the multiparous rats may be the result of less cognitive load coupled with a decrease in circulating oxytocin (Figure 3A).

Interestingly, the number of across-phase errors also significantly negatively correlated with passive nursing behaviors with an increase in passive nursing corresponding with less total within-phase errors (Figure 3B). The reduction in within-phase errors seen in Experiment 1 may represent maternal memory (Li and Fleming, 2003) that was acquired during the first reproductive experience.

Interestingly, oxytocin has also been shown to enhance long-term potentiation (LTP) in the hippocampus of multiparous mice--a region rich with oxytocin receptors--resulting in a decrease in spatial reference, but not working memory errors (Tomizawa et al., 2003). Oxytocin is also implicated in promoting upright nursing behaviors and the licking of offspring (Pedersen and Boccia, 2003).

Perhaps the multiparous rats committed fewer within-phase errors on the delayed spatial win-shift task due to enhanced spatial memory. As the prefrontal cortex has been identified as fundamental in the retrieval of spatial information following a delay (Funahashi et al., 1989; Friedman and Goldman-Rakic, 1994; Seamans et al., 1995; Bailey and Mair, 2004) and contains oxytocin receptors (Febo et al., 2005), it may be that enhanced LTP originating in either the hippocampus or putatively the prefrontal cortex, contributed to the performance of the multiparous rats in Experiment 1.

### ***5.9. Arched-back and passive nursing is correlated with dendritic morphology in the prelimbic region***

Arched-back nursing is an active process that is initiated by the dam although it has been posited to be a reflexive behavior (Galler and Probert, 1982; Hansen et al., 1991; Slamberova et al., 2005). An increase in the total number of branch points in the apical and basal regions of Lamina 5 was found to correlate with an increase in the total time the dams in Experiment 2 spent arched-backed nursing. In particular, multiparous rats showed a significant positive correlation between the total time spent arched-back nursing and the total number of *apical* branch points in Lamina 5 ( $r = 0.98$ ,  $p \leq 0.02$ ). However, primiparous rats also revealed

a significant positive correlation with the total time spent arched-back nursing corresponding to the total number of *basal* branch points in Lamina 5 ( $r = 0.86, p \leq 0.03$ ).

Reproductive behaviors are thought to influence dendritic morphology in regions such as the MPOA, SON, and perhaps the hippocampus (Salm et al., 1988; Keyser-Marcus et al., 2001; Pawluski and Galea, 2006). The multiparous rats in Experiment 2 showed the strongest correlations in each of the regions examined, specifically with respect to total apical branch length in Lamina 2/3 ( $r = 0.97, p \leq 0.03$ ; Figure 9A) and the total number of apical branch points in Lamina 5. Pyramidal cells in Lamina 5 are unique in that afferent projections or local networks are not necessary in altering their morphology (Hempel et al., 2000). Rather, Hempel et al. (2000) found that single connections between Lamina 5 pyramidal cells were sufficient in augmenting dendritic morphology and speculated that this may serve to enhance network efficacy in response to brief excitability. Additionally, pyramidal cells in Lamina 5 are also believed to be influenced by dopamine innervations (Van Eden et al., 1987). Dopamine regulation by the prefrontal cortex is believed to facilitate motivation and learning, particularly in response to rewards (Murase et al., 1993). The dopaminergic inputs originating in the VTA synapse preferentially in Lamina 5 of the prefrontal cortex of the rat, however innervations also occurs in Lamina 2/3 (Sobel and Corbett, 1984; Berger et al., 1991). Interestingly, multiparity augments sensitivity to dopamine during pregnancy and in the postpartum in rats

that persists for several weeks into the postpartum period (Felicio et al., 1996; Byrnes et al., 2001). The dendritic complexities observed in Experiment 2 may reflect morphological alterations necessary to accommodate the repertoire of behaviors that are specific to the maternal rat. The stronger correlations observed in the multiparous rats may be the product of repeated reproductive experience in which the hormonal fluctuations of pregnancy coupled with the environmental enrichment of pup exposure and subsequent maternal behaviors may have provided an additive effect on prefrontal cortex pyramidal cell complexity. Furthermore, the increased sensitivity to dopamine in the multiparous rat may have further enhanced 'maternal memory' and the motivation to engage in maternal behaviors. This may have been expressed through arched-back behavior as the multiparous rats spent significantly more time ( $p \leq 0.002$ ) engaged in this behavior than the primiparous rats. Unlike passive nursing, arched-back nursing, akin to nursing and licking behaviors, may require active initiation and participation by the dam (Galler and Propert, 1982; Clinton et al., 2010). Perhaps the multiparous rats experienced the process of engaging in maternal behaviors as more rewarding which may have contributed to the increase in dendritic complexity in the prefrontal cortex. Further research is required to determine the contributions of maternal behaviors on dopamine expression in the prefrontal cortex and how this may influence learning and motivation in the maternal rat.



## 6. Conclusion

Collectively, these studies provide novel evidence that reproductive experience, and particularly multiparity, may influence the behavior and cellular morphology of the prefrontal cortex. The multiparous rats in Experiment 1 committed significantly fewer omission errors on Block 3 of trials than the nulliparous rats on the delayed spatial win-shift task. Similarly, the multiparous group committed fewer within-phase errors than the primiparous group on Block 4 and fewer across-phase errors than primiparous rats on Block 2 of trials. Thus, the findings from Experiment 1 suggest that multiparity may enhance learning and memory that requires integration of both the hippocampus and prefrontal cortex. Furthermore, the apical region of pyramidal cells in Lamina 2/3 of the prelimbic region may be influenced by multiparity as the multiparous rats approached significance in the total number of apical branch points in this region when compared to the nulli- or primiparous rats. As the pyramidal cells in this region are implicated in mediating communication across multiple brain regions (Gabbott et al., 2005; Goodfellow et al., 2009), it may be that repeated reproductive experiences augments dendritic complexity in this region. This finding may account for the reduction in the number of errors committed by the multiparous rats in Experiment 1. Prior research may provide support for this claim as enhanced cognitive performance has been correlated with increased dendritic complexity in Lamina 2/3 (Liston et al., 2006; Wallace et al., 2006).

Obviously, more research is required in order to draw definitive conclusions about the contributions of dendritic complexity in the prelimbic region to learning and memory following reproductive experience, but the findings from the current experiments suggest that reproductive experience, and more specifically, multiparity, influences pyramidal cell morphology and behaviors involving the prefrontal cortex.

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## Appendix: UBC animal care certificate



THE UNIVERSITY OF BRITISH COLUMBIA

### ANIMAL CARE CERTIFICATE

**Application Number:** A07-0211

**Investigator or Course Director:** Liisa Galea

**Department:** Psychology, Department of

**Animals:**

Rats Sprague dawley 125
Rats Sprague dawley 340
Rats Sprague Dawley 450
Rats Sprague dawley 176
Rats Sprague dawley 220

**Start Date:** May 25, 2007

**Approval Date:** January 18, 2010

**Funding Sources:**

**Funding Agency:** Canadian Institutes of Health Research (CIHR)  
**Funding Title:** Stress, corticosterone and postpartum depression: effects on depressive-like behaviors and neural ma...

**Funding Agency:** Canadian Institutes of Health Research (CIHR)  
**Funding Title:** Parity effects on brain morphology and function

**Funding Agency:** National Alliance for Research (US)  
**Funding Title:** Models of post-partum depression: Effects on behavior, stress reactivity and hippocampal neurogenesis in both mother and offspring

<b>Funding Agency:</b>	British Columbia Ministry of Children and Family Development
<b>Funding Title:</b>	Effects of social and enriched rearing conditionings on development of offspring from depressed dams
<b>Unfunded title:</b>	N/A

The Animal Care Committee has examined and approved the use of animals for the above experimental project.

This certificate is valid for one year from the above start or approval date (whichever is later) provided there is no change in the experimental procedures. Annual review is required by the CCAC and some granting agencies.