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NEURAL REPLAY: A POSSIBLE MECHANISM FOR DIFFERING REHERSAL STRATEGIES ACROSS PARITY

By

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NEURAL REVERSE REPLAY: A POSSIBLE MECHANISM FOR DIFFERING REHERSAL STRATEGIES ACROSS PARITY

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MASTER OF ARTS

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All mammalian females undergo behavioral and neurological changes during pregnancy and motherhood. Many of these changes lead to an enhanced ability to be an effective mother including: increased memory, foraging behaviors, and boldness. Here, we examined the differences in rehearsal strategies between mother and virgin rats. Stops made by rats when exploring their environment have been found to result in reverse replay activity in the hippocampus (Foster & Wilson, 2006). Reverse replay is sequential replay that occurs in the hippocampus immediately after a spatial experience; this replay/activation is in reversed order of the initial spatial episode (Foster & Wilson, 2006). Thus, rats are replaying the steps they have just taken. Here, animals were introduced into a linear track for 3 consecutive days. The number and duration of stops were recorded. We found that parous females made fewer stops compared to virgin females; however, they stopped for the same duration. Therefore, we propose that parous females may utilize reverse replay activation differently than virgin females.

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

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Running head: MATERNAL REHEARSAL STRATAGIES

Neural Reverse Replay: A Possible Mechanism for Differing Rehearsal Strategies Across Parity

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Abstract

All mammalian females undergo behavioral and neurological changes during pregnancy and motherhood. Many of these changes lead to an enhanced ability to be an effective mother including: increased memory, foraging behaviors, and boldness. Here, we examined the differences in rehearsal strategies between mother and virgin rats. Stops made by rats when exploring their environment have been found to result in reverse replay activity in the hippocampus (Foster & Wilson, 2006). Reverse replay is sequential replay that occurs in the hippocampus immediately after a spatial experience; this replay/activation is in reversed order of the initial spatial episode (Foster & Wilson, 2006). Thus, rats are replaying the steps they have just taken. Here, animals were introduced into a linear track for 3 consecutive days. The number and duration of stops were recorded. We found that parous females made fewer stops compared to virgin females; however, they stopped for the same duration. Therefore, we propose that parous females may utilize reverse replay activation differently than virgin females.

Neural Reverse Replay: A Possible Mechanism for Differing Rehearsal Strategies Across Parity

When females make the transition into motherhood many changes occur that may facilitate their ability to care for their offspring. Many of the behavioral and neurological changes serve an evolutionary purpose. First, it ensures the survival of the species, and second, it may increase the mother's likelihood of surviving due to increased cognitive abilities that result from maternity. Such abilities include hormonal, neurological, and physical alterations that take place during pregnancy, and continue thereafter, resulting in changes in post-partum behavior. In various animal species, many of the changes occur to increase the mother's agility, cognitive focus, vision, smell, memory, and overall vigilance. Research in this area has identified a wide variety of differences that take place during motherhood. Of interest here are the neurological and behavioral differences that occur in learning and memory.

The four major components of maternal behavior in rats (retrieval, nursing, pup grooming, and nest building) are all stimulated by fluctuating hormone levels and neurological changes that occur during and after pregnancy. Rosenblatt and Mayer (1995) proposed that mothering behaviors occur because of an approach-avoidance switch that takes place after giving birth, meaning that the tendency to interact with pups is greater than the tendency to avoid or withdrawal from pups. This amplified propensity to interact with pups is greatly increased due to the sensory stimulation that pups provide for the mother. For example, olfactory input from the pups seems to be sought by mother rats (Kinsley & Bridges, 1990), and nursing pups stimulate oxytocin release and activation in the nucleus accumbens, an area of the brain that acts as a reward center (Numan & Insel, 2003).

Oxytocin is a hormone that acts as a neurotransmitter in the brain; its release stimulates further lactation and maternal behaviors such as grooming and in some cases bonding. In turn, lactation has been shown to be associated with increases in exploratory behavior and in aggressiveness, behaviors which are important in the mother's ability to meet the requirements of caring for her young (Neumann, 2001). Mother-offspring interaction plays a pivotal role in facilitating neurological and behavioral changes. The inability to interact with pups has been found to have a detrimental effect on mothers; in some cases they do not show the same cognitive improvements as mothers who allowed to interact with their pups (Lambert et al., 2005). In addition to oscillating hormone levels stimulating maternal behavior, neurological modifications also initiate behavioral changes in a beneficial manner. Numan and Numan (1997) showed that lesions to the medial preoptic area of the hypothalamus severely disrupt a mother's retrieval of pups. Kinsley (2008) suggests that changes occurring in the hippocampus (an area associated with learning and memory) before and after pregnancy catalyze maternal behaviors such as spatial memory. Here, much of our focus is on differences in spatial learning and performance.

Behavioral Differences in Spatial Performance

Spatial memory depends on an organism forming and organizing location cues allowing them to navigate and remember the environment around them (Bures & Fenton, 2000). Rats in particular must be able to remember places of danger, location of food, placement of mates and how to return to their nests. One of the most important behavioral adaptations that takes place during and after pregnancy is the change in foraging ability of mothers, which involves spatial cognition. A mother rat must be able to leave the nest and return in a timely manner without being hurt or endangering her offspring. To do this, she must first overcome her fear of encountering risk, and second she needs to be able to fully utilize her memory for spatial information.

Recent research has shown that pregnant females display fewer anxiety-like behaviors compared to non-pregnant females allowing them to better explore their surroundings. Macbeth, Gautreaux, and Luine (2008) examined the performances of 14 female pregnant and non-pregnant Sprague-Dawley rats on an object placement task and on an elevated plus maze (EPM). These animals were tested during two different time periods of gestation days 7, 9, 16, and 18 (GD7, GD9, GD16, GD18). The object placement task was administered two times, on GD 7 and GD 16. The pregnant females were able to discriminate between the object locations, whereas the non-pregnant females were unable to do so. The pregnant females also showed more exploratory behaviors compared to the non-pregnant females, which may have contributed to their improved spatial learning ability of object location. The EPM (elevated plus maze) was administered during GD 9 and GD 18; the pregnant females displayed decreased anxiety compared to the non-pregnant females on GD 9. These findings point to better spatial learning in mothers even before they give birth, which may be due to increased exploratory behaviors and fewer anxiety-like behaviors. These results are of interest because the mothers could be using reverse replay during exploration of object location during gestation. Reverse replay is sequential replay that occurs in the hippocampus immediately after a spatial experience, this replay/activation is in reversed order of the initial spatial episode. Thus, when a rat pauses during a spatial learning task, the hippocampus is replaying the steps taken to get to the current location in reverse order. This may be a general mechanism used when learning and memorizing spatial information (Foster & Wilson, 2006).

Along with a decrease in anxiety resulting in more exploration, mothers need to be able to increase their ability to remember the spatial information to which they are exposed. Thus, researchers have examined memory errors in differing spatial cognition situations. Bodensteiner, Cain, Ray, and Hamula (2006) studied spatial cognition differences between 7 primiparous and 7 nulliparous (virgin) Long-Evans rats. A Morris water maze was used to test reference memory and working memory differences. Animals were tested during the 3 weeks of pregnancy and during the second week post-partum. Bodensteiner et al. (2006) found that there were no differences between groups in reference memory, however there were significant differences in working memory. The parous females outperformed the nulliparous females, spending less time in the quadrant where the platform had been located. Working memory is utilized for current/situational problem solving. Thus, the researchers concluded that mothers have an enhanced ability to solve differences in spatial locations more quickly than non-mothers. Furthermore, these results show that a mother's ability to remember spatial information better than non-mothers is still present after post-partum. Interestingly, additional studies have revealed that mothers may indeed be better at using reference memory as well (Pawluski, Walker, & Galea, 2006).

Pawluski et al. (2006) observed spatial reference and working memory differences in nulliparous, primiparous, and multiparous female rats. Rats were habituated to a radial arm maze for 3 days, and then shaped for 2 days after that. Training lasted for 24 days, in which the rats were to remain in the maze until all baited arms had been explored or until 10 minutes had elapsed. The number and types of errors were recorded in addition to the number of days to reach criterion (no more than 1 error for two consecutive days). There were three types of errors that could be made: reference memory errors (entry into non-baited arms), working memory errors (repeated entry into baited arms) and working/reference memory errors (repeated entry into non-baited arms). The results showed that primiparous and multiparous females made fewer errors, regardless of the type, than nulliparous females. There were no significant differences between primiparous and multiparous females. The researchers also accounted for licking and nursing behaviors, which was positively correlated with spatial reference memory performance. Pawluski et al. (2006) discovered that more time spent licking and nursing pups was associated with increased reference memory errors. The authors suggest that this association may be due to increased oxytocin levels. In spite of the increased likelihood of committing errors, the mother rats still performed significantly better than the nulliparous rats. These behavioral differences suggest that mothers may use spatial reference and working memory differently than non-mothers.

Oxytocin is not the only chemical to influence maternal spatial memory. One study found that hormones present during and after pregnancy influenced pregnant animals' performance in Morris Water Maze (Galea et al., 2000). Galea et al. (2000) examined differences in spatial working memory and hippocampal size of pregnant and virgin animals. Two experiments were designed to test for differences. Experiment One consisted of testing rats in the maze for three consecutive days with 7 trials a day (during days 7-10 of pregnancy). During all three days, the platform remained in the same location. Experiment Two included a different cohort of females, these animals received training prior to the onset of the experiment, which included testing for 4 days with 4 trials a day over the last three trimesters of pregnancy (weeks 1-3). During Experiment Two, the platform was systematically moved throughout testing. Results showed that pregnant females outperformed non-pregnant females in both Experiment One and Two during their first and second trimester; they were faster in finding the platform compared to the non-pregnant females. The pregnant females' better performance corresponded with lower levels of the steroid hormones progesterone and estradiol that will be discussed in more detail later. It is also important to note that Galea et al. (2000) mention that the pregnant females spent more time exploring target quadrant areas compared to non-pregnant animals. This is of interest here since it implies that the potential mothers may be using different navigational strategies, such as reverse replay and/or more exploration of target areas, to remember spatial information. There is reason to believe that many of the behavioral changes, such as enhancement in spatial learning, are long lasting.

Love et al. (2005) examined the lasting neurological and behavior effects across the life span of nulliparous, primiparous, and multiparous rats, starting at 5 months and ending at 22 months of age. Rats were put through a dry land maze (DLM) with visuospatial cues to test their ability to remember location and retrieve bait. The latency to retrieve bait and the number of successful retrievals were recorded. In addition, rats were placed in an elevated plus maze (EPM) to record their emotional levels. The frequency of entries into open arms, duration of time spent on the open arms, the time spent in closed arms, duration of time spent in center square, number of freezing (stopping) behaviors, and their duration of freezing were all recorded during a 5 minute period on the EPM. The researchers found that parous females performed significantly different across their life span compared to nulliparous females. They spent more time exploring and were faster in their ability find and retrieve bait. However, at 17 months the nulliparous rats outperformed the parous females in their ability to find and retrieve the bait. Love et al. (2005) suggest that this could be due to estrous cycling differences between the parous and nulliparous females. Specifically, the parous females may have still been in transition in their cycling due to their extended estrous cycle period. EPM results showed that the multiparous and primiparous females spent more time in the open arms than the nulliparous females over the 17-month period, suggesting that parous females are bolder and more daring than the nulliparous females. This line of evidence indicates that maternal experience enhances memory performance, which may be facilitated by a willingness to explore dangerous and/or unknown territory. Although Love et al. (2005) did not find significant neurological differences between their parous and virgin animals there is still evidence to suggest that neurological changes do occur as a result of achieving motherhood.

Neurological Evidence

Fluctuating levels of steroid hormones (estradiol and progesterone) throughout pregnancy and afterwards alter brain areas in a way that promotes mothering behavior over avoidance behavior. Both estradiol and progesterone are produced in the ovaries and increase during pregnancy. Estradiol acts as a growth hormone for tissue in the female reproductive organs. Together these hormones act to prepare the female body for egg implantation in the uterus. Estradiol, in particular, changes the mothering brain: it expands the shape and size of neurons in the hypothalamus, enhances production of synaptic proteins, and increases excitatory synapses on hippocampal neurons (Li et al., 2004; Miller & Erskine, 1995; Yankova, Hart, & Woolley 2001). The neurological changes that take place in the hypothalamus may also facilitate more efficient foraging, in that it lowers a mother rat's fear response. Progesterone and estradiol fluctuations also stimulate changes in the hippocampus: there is an increase in dendritic spines that in turn enhances neural plasticity (Kinsley et al., 2006).

Spatial memory, as mentioned above, is an important component of being an efficient mother, since it aids in the ability to retrieve food (forage). At the center of spatial memory and navigational skills is the hippocampus. This area of the brain has the ability to form environmental maps of a rat's surroundings via firing of specific place cells (pyramidal cells) (Bures & Fenton, 2000). Additionally, there is evidence that place cell pairs will show reactivation during slow wave sleep after one environmental exploration session (O'Neill et al., 2008). This reactivation during sleep may contribute to the consolidation of spatial memories for better retrieval in the future. Therefore, any hormonal or behavioral changes that occur during motherhood resulting in altered hippocampal activity or neurophysiology are important to maternal navigational strategies.

Tomizawa et al. (2003) found that oxytocin plays a major role in spatial memory retention by activating the mitogen-activated protein kinease (MAP kinase), which is involved in memory regulation through the N-methyl-$_{\mathrm{D}}$- aspartate (NMDA) receptors in the hippocampus. After looking at the neurological effects of oxytocin, Tomizawa et al. (2003) explored actual behavioral differences in memory as they relate to oxytocin. In one experiment, virgin mice were given injections of oxytocin and placed in a radial arm maze for 10 minutes or until they had retrieved all of the bait. The results revealed that on retention days 8 and 9 the mice with oxytocin administration performed better than controls in their number of reference memory errors, making few errors than the other group. In addition, multiparous animals were given injections of oxytocin receptor antagonist. Results showed that the multiparous animals injected with the antagonist performed significantly worse than the controls. Tomizawa et al.'s (2003) results point to oxytocin as a facilitator in long-term memory formation of spatial information. Certainly, these neurological changes assist in maternal behavior; a mother needs to be able to efficiently forage for food and return to the nest in a timely manner. Thus, these changes in the hippocampus may help in her ability to remember where best to forage and how to get back.

Research has shown that hormones associated with motherhood result in improved navigation skills, learning, memory, predatory behavior, anxiety, and stress regulation among others (Kinsley et al., 1999). More recent research suggests that the changes taking place in the hippocampus are related to neurogenesis inhibition after weaning (Kinsley, 2008; Leuner, Mirescu, Noiman, & Gould, 2007). However, improvements in maternal navigation, memory, etc., can be negated by stressful events during gestation (Lemaire et al., 2006). Thus, we were careful during our investigations to study hippocampal differences under controlled, non-stressful conditions. As aforementioned, there is some evidence that the resulting behavior changes may be due to progesterone and estradiol levels triggering brain reorganization (Galea et al., 2000; Sandstrom & Williams, 2001).

Research investigating the role of steroid hormones in causing neurological changes in pregnant and maternal rats has found that these progesterone and estradiol increase dendritic spine density in the hippocampus of mothers (Kinsley et al., 2006). A 2006 study used mother and virgin rats to explore dendritic spine differences related to pregnancy hormones (Kinsley et al., 2006). In experiment one, the brains of nulliparous females in diestrous (DES), proestrous (PRO), and estrous (ES), as well as late pregnant females (LP) and lactating females (LACT) were tested. Results revealed that the LP and LACT females had an increased dendritic spine density compared to the other three groups. In Experiment Two, a group of ovariectomized females with no hormone exposure (OVX-minus) and females exposed to progesterone and estradiol ($P\&E_2$)-treatment implants ($OVX+P\&E_2$) were examined. Upon brain inspection, it was found that OVX+P&E_2 females had increased dendritic spine density similar to that of the LP and LACT females (Kinsley et al., 2006). The increase in dendritic spines is significant since more dendritic spines leads to more connections and greater neurochemical transfer, potentially increasing memory formation and retention. Frye, Duffy and Walf (2007) explored these same parameters but from a behavioral perspective.

The 2007 study investigated DES, PRO, ES, LP, LACT and various combinations of OVX female rats (Frye et al., 2007). Each group of females was subjected to a hippocampal dependent object placement task, results of which supplement some of the associations made by Kinsley et al. (2006) that found that natural fluctuations or administration of estradiol and progesterone result in better performance during testing. Specifically, Frye et al. (2007) found that the females with naturally elevated levels of E_2 and/or P, as well as the female rats it was administered to, spent more time with the object in novel locations. Taken together, these results suggest that increased hormone levels, particularly progesterone and estradiol, that take place during and after pregnancy may alter hippocampal neurons that facilitate maternal behavior in a way that enhances a mother's navigational skills.

Darnaudery et al. (2007) explored changes in hippocampal neurogenesis and spatial memory performance in virgin and parous rats. Using a water maze to test for spatial memory retention, the females were tested over a 2-week period. Neurological evidence showed that cell proliferation decreased during lactation, though at post-partum there was no marked difference found. This implies that the improvements taking place in the hippocampus, which facilitate better spatial memory, may not occur until after weaning when cell proliferation rebounds from the decrease that takes place during lactation. The lack of cell proliferation during lactation also implies that it may be the quality of the cells, not the quantity, that is important in spatial learning during this period, meaning that the cells that are being created are more efficient. Thus, the change in cell proliferation may result in differing performance after lactation because of cell quality not quantity. Behavioral results showed that in early motherhood (before postpartum) there was impairment in performance. However, their ability to retain the spatial information was enhanced. After 10 days, the mothers' performances were significantly different than the virgin females' performance to find the platform; mothers were able to find the platform faster than the virgin females. In addition, the experimenters noticed that the mothers, lactating and primiparous, explored the platform area more than the virgin females (Darnaudery et al., 2007). As with studies of behavioral change during and after pregnancy, there is also exciting research suggesting that neurological modifications to the maternal brain are long lasting.

Far from being a brief alteration in the maternal brain, studies have shown that the increased ability to remember spatial information is present throughout a mother's lifetime (Gatewood et al., 2005). Nulliparous, primiparous and multiparous females were tested in a dry land maze main task and reversal task at ages 6, 12, 18 and 24 months. Gatewood et al. (2005) reported that the multiparous females remembered the location of bait faster than both of the other groups across all age intervals and on both tasks. The primiparous females learned the maze faster than the nulliparous females on the main task at 12, 18 and 24 months, and on the reversal task at 12 and 24 months of age

(Gatewood et al., 2005). These results point to better spatial memory for parous females and especially multiparous females across their lifetime. The authors suggest that these long lasting effects can be attributed to the decreased immunoreactive amyloid precursor protein (APP; a protein associated with age related neurodegeneration and cognitive loss) level in the CA1 area of the hippocampus (area of the brain involved in learning and memory) in the multiparous females. What can be inferred here is that multiparous females will suffer less cognitive deficits and display significantly better memory retention across their life span over both primiparous and nulliparous females.

It is possible that the enhanced exploration described in Gatewood et al. (2005) and in Darnaudery et al.'s (2007) work, and through the possible use of reverse replay mechanisms in the hippocampus, mothers may be able to more efficiently retain spatial cues. To date, no research has been conducted on the neurological mechanisms that result from these changes in the brain and behavior. Specifically, there is little evidence showing the link between neurological changes and how these changes result in more effective performances in learning and memory.

Foster and Wilson (2006) found that rats use rehearsal strategies to remember spatial experiences. In their research, which examined males rats, hippocampal activity was measured using electrodes in rats that were running and stopping through two different linear tracks: a track familiar to the rats and one that was unfamiliar. It was discovered that when a rat paused/stopped running from one end of the track to the other, there were bursts of activity in the hippocampus. This activity was in reverse replay from the activity seen when running, suggesting that the rats were trying to remember what they had just done and the steps they had taken to get to that point. Further, these bursts of activity were found to occur more often when the rats were placed in an unfamiliar track, suggesting that the reverse replay hippocampal activity is tied to learning and remembering new spatial information. A more recent study was able to replicate Foster and Wilson's work, as well as illustrating evidence for prospective and retrospective activation in the CA3 area of the hippocampus (Koene & Hasselmo, 2008). Based on our knowledge of the behavioral and neurological differences between nulliparous and parous females, we proposed to investigate the relationship between reverse replay and the enhanced spatial learning ability in mothers vs. non-mothers. Using behavioral methods, we hoped to discover what association, if any, exists in the number of stops made, their duration, and parity.

The present study tested differences in rehearsal strategies of age matched multiparous (MP), primiparous (PP) and nulliparous (NP) rats. Past research has found that mothers benefit from changes that occur within their brains and bodies; we wanted to find out if one of these benefits includes the ability to remember spatial information faster and more readily than non-mothers by using different navigational strategies. Since there is a correlation between stopping behavior and reverse replay (rehearsal/ remembering), we hypothesized that the mother rats would not have as many of the stopping behaviors as the virgins because they are better able to remember/rehearse the steps they made up to that point. We also hypothesized that the parous animals would stop for a longer duration compared to the virgin animals.

Methods

Animals

Subjects were 24 age matched Sprague-Dawley rats, eight from each parity group. Multiparous animals were about a month older during testing due to their need to mate twice and wean two litters. All rats were housed and tested in reverse light cycle rooms (lights on 4:30pm, off 6:30am) to maximize camera contrast (Noldus system) as well as the animals' agility and visual acuity. Rats were isolated in 20 x 45 x 25cm polypropylene cages with corncob bedding. Food (Purina rat chow) and water was available *ad libitum* for all animals. Animals were maintained according to the guidelines set by the Institutional Animals Care and Use Committee (IACUC) of the University of Richmond (protocol # 06-05-6).

Rats were randomly assigned to one of the three conditions: NP (n=8), PP, (n=8) and MP (n=8). NP rats had no sexual experienced. PP rats gave birth once and mothered for 25 days. MP rats gave birth twice and mothered twice for 25 days. Two animals had to be dropped from our MP group due to not giving birth to a second litter, leaving us with only 6 animals in our MP group. All of the mothers started testing three days after weaning. Thus, Day one of testing for each mother was Day three of post-partum.

Animals' weights were recorded at the time of testing to ensure that it would not serve as a confounding factor. The analysis reveled that there were no significant differences in the weight of the animals in each group during testing ($p = .10$).

Material

Tracks were designed according to Foster and Wilson (2006). A few modifications had to be made based on accessibility to materials and space. An open arena (circular) housed a single linear track measuring: 132.1 cm long, 11.4 cm wide, with a height of 23.7 cm. Plexiglas covers on the track ensured that animals would not be able to escape from the track during testing. The track and covers were cleaned with a 70% ethyl alcohol/water solution before and after each rat was tested.

We used Noldus EthoVision 3.0 behavioral monitoring system. This system includes a computer program that links to a camera installed in the ceiling that looks down on the arena in which the linear track was housed. This system allowed us to choose the parameters we wanted to measure.

Statistical Tests

Two separate 3x3 Mixed Analysis of Variance (ANOVA) with post hoc tests were run to see if significant differences exist between and within the groups for the number of stops made and the durations of stops. Our independent variable was parity, which included the levels: NP, PP, and MP. Our dependent variables were number of stops and duration of each stop in milliseconds for the three consecutive test days. Our within subject variables were the stops and durations of stops on each of the 3 days and our between subject variable was parity (NP, PP, MP). All significance was measured at the .05 alpha level.

Procedure

This study recorded the behavior of the rats while in the linear track. Each rat was placed in the track for 5 minutes for three consecutive days. Using the Noldus

Information Technology- EthoVision 3.0 (behavior monitoring system), we recorded the stopping behaviors and the duration of each stop. The first stop from the starting point was not counted given that Foster and Wilson (2006) found that no reverse replay takes place during this period. All stops after this point were recorded, which is in accordance with Foster and Wilson's (2006) findings that even after the first lap in the tracks reverse replay was evident. Two experimenters using a hand counter also recorded stopping, this was to control for the inclusion of movement velocity with EthoVision 3.0. The first experimenter recorded by hand while the trial was taking place by watching the computer screen. In attempt to make the experiment blind we had a second experimenter count the number of stops while watching the video recorded by the Noldus system. To control for possible variance between the experimenters and the EthoVision 3.0, we check for significant differences between the EthoVisions' count and the two experimenter's counts of the number of stops; no significant differences were found ($p = .122$). The average of the three counts was used for data analysis.

During testing periods in which more than one animal was being tested, the order of subjects was counterbalanced. This was done to ensure that all three animals had an equal opportunity to be tested first, second, third and so on. All animals were tested at the same time on each of the three days.

Results

Analysis of Stops

A 3x3, within subject variable was number of stops for each of the three days and the between subject variable being was parity, Mixed ANOVA was run to see if significant differences exist between and within the groups. As expected analyses revealed that there were no significant differences within-groups across the three days F (2,19) = 1.31, p > .29. However, also as expected, differences were found between all three groups, F (2,19) = 17.25, p < .001. Tukey post-hoc analyses looking at individual group differences showed that the MP animals significantly differed from both the PP (p = .01) and NP (p = .001) females. Specifically, they made fewer stops than the other two groups (see Table 1 & Figure 1 for means and standard deviations). Additionally, the PP females made significantly fewer stops than the NP females (p = .03). The interaction of parity and number of stops was not statistically significant (p = .33).

Analysis of Duration

A 3x3, within subject variable was the durations of stops for each of the three days and the between subject variable was parity, Mixed ANOVA was run to see if significant differences exist between and within the groups in their duration of stops. Means and standard deviations for duration of stops for each of the three groups can be found in Table 2 and Figure 2. No significant differences were found between groups in their duration of stops over the three days, F (2, 19) = 2.77, p >.088. Neither of our main effects (parity and duration of stops per day) reached statistical significance. However, there was a significant interaction between the duration of stops and parity, F (4, 38) = 3.95, p < .009.

Simple effects tests were run to explore the significant interaction between duration of stops and parity. Results revealed that the PP animals stopped for a significantly shorter period of time on Day 2 compared to the other two groups, F (2, 19)

= 10.14, p < .001. This finding is surprising, in view of the fact it goes against the pattern of both of the other groups. At this time we have no explanation as to why this occurred or if it would continue if more animals were tested. We suggest this be explored more in the future.

Discussion

Reproductive experience, especially for MP females, appears to adjust a mother's ability to remember spatial information in a timely manner. Our results showed that mothers made fewer stops over the three-day period compared to non-mothers. Of interest is the finding that MP females made fewer stops than both PP and NP females. Furthermore, there were no significant differences in the duration of stops between the MP, PP, and NP females. Our results support those of Foster and Wilson (2006) and suggest that mothers may need fewer instances of reverse replay/rehearsal to remember spatial information. Moreover, the lack of difference in stop duration suggests that mothers, specifically MP females, may be able to retain just as much information in each pause compared to non-mothers.

Foster and Wilson (2006) found that when rats stop there are instances of reverse replay in the hippocampus that facilitated their learning of new spatial information. Here, we found that mothers make fewer stops but for the same duration as non-mothers. Foster and Wilson (2006) mention that during each stopping event there are multiple reverse replay episodes. Thus, our research proposes that although the MP females stop significantly fewer times than both NP and PP females for the same duration, there may be more instances of reverse replay that take place in their hippocampus compared to the other two groups. This might explain why, in a spatial learning situation, MP females tend to perform much better on spatial memory tasks than non-mothers and first time mothers.

Gatewood et al. (2005) found that MP animals outperform both PP and NP animals in a DLM task. They also found that MP females have a lack of APP reduction in the CA1 area of the hippocampus, which results in their better spatial memory over time. This could be one of the factors that influences better use of rehearsal strategies (reverse replay) in the MP animals versus the other two groups, including first time mothers. If we connect our results to the neurological findings of Gatewood et al. (2005) and the neurological data from Foster and Wilson (2006), then future studies looking at the differences in stops over time (12 to 24 months after post-partum) should show that the MP animals continue to make fewer stops compared to both NP and PP animals. Furthermore, if the PP animals are mated again we may find that they would begin to show the same trend in decreasing the number of stops they make over a three-day period in an unfamiliar environment. Additionally, this would allow us to see if these differences exist throughout a mother's lifetime.

Research has also shown that parous females tend to spend more time exploring when being tested compared to nulliparous females. Macbeth et al. (2008) attributed the increased exploration during an EPM task to decreased anxiety. Galea et al. (2000) and Bodensteiner et al. (2006), made note of pregnant females exploring target areas in a Morris Water Maze task compared to virgin females. Moreover, Darnaudery et al. (2007) stated that the MP, PP, and lactating females spent more time exploring target areas more compared to the NP females. These findings are of interest here because during exploration the postpartum mothers and pregnant animals could have been stopping/pausing, causing reverse replay, to ensure that the spatial information was stored; resulting in their superior performance compared to the NP animals.

A study looking at pup exposure and spatial memory used to forage found that PP animals exposed to pups outperformed PP and NP animals not exposed to pups and NP animals exposed to pups during Dry Land Maze (DLM) tasks (Lambert et al., 2005). Here the experimenters used spatial, probed and cued DLM tasks to examine foraging ability differences. Results showed that the PP females exposed to pups outperformed the other three groups on all tasks. Primiparous females exposed to pups spent more time exploring previously baited areas as compared to the other three groups during the probe trial (Lambert et al., 2005). Based on our findings, it is possible that during the spatial task the animals had retained the information regarding food location through reverse replay, resulting in them spending more time in this location during the probe task. Additionally, during the cued DLM task, both groups with pup exposure (PP and NP) found the reward faster than those females with no pup exposure on trial 6. This finding can not only be linked to the possible use of reverse replay to find the reward but also to chemical changes that are connected to mothering that may facilitate reverse replay in the hippocampus.

Changes in hormones that occur during and after pregnancy also help explain our results. Kinsley et al. (2006) found that differing levels of progesterone and estradiol result in better spatial memory and increased dendritic spine density in the hippocampus.

Hence, the possible increase in reverse replay events in the hippocampus of MP females may not be that far fetched. With increased spine density comes more neural connections and greater neurochemical transfer, increasing the potential for better memory retention and possibly more reverse replay events with each stop. Moreover, changes in oxytocin levels have been connected to better spatial memory.

For instance, Tomisawa et al. (2003) found that increased oxytocin levels enhance memory through activation of the MAP kinase may be one explanation as to why the stopping and reverse replay activation in the hippocampus is more efficient in MP females versus PP and NP females. Moreover, since significant differences were found between the two motherhood groups (MP and PP), this could indicate that there is a significant difference in oxytocin levels present at post-partum due to an accumulation effect for the MP females. As previously stated, the MAP kinase facilitates memory retention through activation of the NMDA receptors. These receptors are said to play a role in the synaptic activity in the CA1 region of the hippocampus, which is the same area Foster and Wilson (2006) observed reverse replay activation in their animals. The connection between Tomisawa et al. (2003), Foster and Wilson (2006) and our current study can be found in the differences observed in stopping behaviors between our parous and non-parous females. Since the activation of the MAP kinase results in differing memory retention through activation of the NMDA receptors, there may be a link between this neurological process and reverse replay. We found that our parous females made fewer stops for a similar duration compared to our NP females. These results may be due to increased oxytocin levels in the parous females activating the MAP kinase and

NMDA receptors, which in turn may activate the CA1 area allowing for more instances of reverse replay activation in each stop. This line of evidence may explain why the parous females made fewer stops, but stopped for a similar duration compared to the NP females.

Darnaudery et al.'s (2007) finding that the marked decrease in cell proliferation seen during lactation becomes absent at post-partum also helps in explaining our results. The lack of cell proliferation at post-partum means that some of the neurological changes that take place during motherhood do not occur until after pup weaning. This may not only help explain the differences between the MP and NP females, but also the significant differences between the MP and PP females. Since MP females gave birth to two litters, it may be the accumulation of several neurophysiological events that result in their increased propensity to have multiple reverse replay events during each stopping behavior, and thus led MP females to make fewer stops for about the same duration as the NP females.

Future studies should focus on the differences found here and explore the exact amount of differentiation in neuron activity between MP, PP, and NP females during each stop while exploring a new environment. The best course of action would be to repeat Foster and Wilson's (2006) study using parous and virgin females to examine the exact amount of reverse replay taking place and the differences between the MP females and the other two groups. Examining oxytocin levels and MAP kinase activation after track exploration would also be beneficial in connecting our work to Tomisawa et al.'s (2003) findings. Examining cell proliferation and including a group of lactating females would allow connections to be made to Darnaudery et al.'s (2007) work. Moreover, we suggest that other researchers performing behavioral experiments looking at spatial memory differences in motherhood record instances of stopping behaviors during each trial to use as supplementary data.

Also, having MP females tested after their first litter is weaned and then again after their second litter, using two different tracks (unfamiliar environments) would provide valuable data showing the changes in behavior of the same animals. It may also help to explain the large drop in mean duration of stops on day two of testing found in our PP females. In addition to expanding the current study there is also a need to avoid its limitations.

There are several limitations to the current work. To begin with, we did not actually collect any neurological data; we simply relied on the neurological evidence presented by Foster and Wilson in their 2006 study. Another possible limitation is the difference in time between when the PP and MP mothers were tested. However, unpublished findings from Dr. Craig Kinsley's lab have shown that there are no differences in PP females and MP females even when tested months later; thus, this may not have affected our outcomes. It must be noted that we are only proposing a mechanism through which differences in spatial performance across parity may be explained. We understand that there are many other explanations for our findings. For instance, it may be a natural occurrence for females to differ in stopping behavior because of estrous cycling, agility, and other maternal factors that have nothing to do with reverse replay in the hippocampus.

In conclusion, parous females made fewer stops compared to our non-parous females. However, there were no significant differences between the duration of theses stops. The performance differences may be due to hormonal changes that take place resulting in changes in the hippocampus. If our proposed rehearsal mechanism (reverse replay) holds true, multiparous females, in particular, may have some added benefit from giving birth to a second litter, as shown by the significant differences in the number of stops made between multiparous group and the primiparous and nulliparous groups. Being an effective mother requires the ability to leave the nest and return as quickly as possible to ensure the safety of her own being and her offspring. The ability of a mother to do this includes navigational skills; here, we have presented a possible navigational strategy that ensures that a mother can do this more effectively. Distinctively, the main implication of this work is that it may provide another piece to the puzzle in explaining why parous females perform differently than non-parous females when confronted with a spatial learning task.

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Table Captions

Table 1. Means and Standard Deviations for the number of stops the nulliparous, primiparous, and multiparous females made for each of the three testing days.

Table 2. Means and Standard Deviations for the duration (in milliseconds) of stops the nulliparous, primiparous, and multiparous females made for each of the three testing days.

Figure Captions

Figure 1. The mean number of stops each group made during the three-day testing period.

Figure 2. The mean duration of stops, in milliseconds, each group made over the three-day testing period.

Table 1.

Parity	Day 1	Day 2	Day 3
Nulliparous	21.83	21.37^{b}	16.25^{b}
	(1.78)	*(3.43)*	*(3.43)*
Primiparous	19.79	15.92^{a}	16.29^{b}
	(2.61)	*(6.82)*	*(3.73)*
Multiparous	17.96	14.42^{a}	10.39^{a}
	(4.47)	*(2.06)*	*(3.10)*

Note. For the number of stops made for each day, means with a common subscript (a) differed at the $p < .05$ level by Tukey post hoc test from means with an alternative subscript (b). The number in parentheses is the standard deviation.

Table 2.

Parity	Day 1	Day 2	Day 2
Nulliparous	628.75	767.50^b	625.00
	(125.74)	*(168.84)*	*(198.42)*
Primiparous	793.75	532.50^a	743.75
	(267.63)	*(91.45)*	*(145.39)*
Multiparous	841.66	810.00^b	691.66
	(150.25)	*(103.34)*	*(100.87)*

Note. For the duration of stops on each day, means with a common subscript (a) differed at the $p < .05$ level by Tukey post hoc tests from means with an alternative subscript (b). The italicized number in parentheses is the standard deviation.

Figure 1.

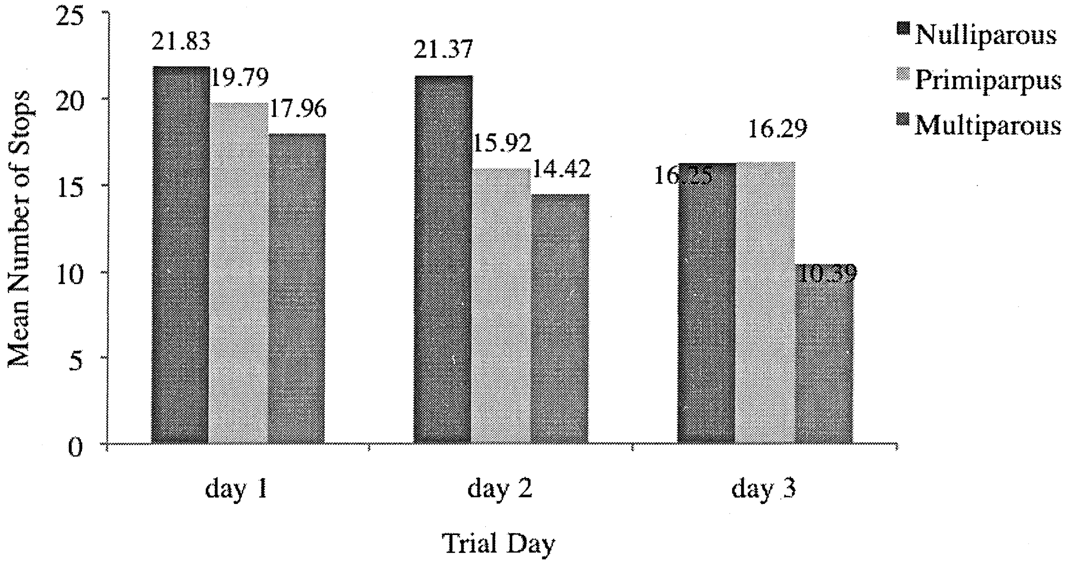
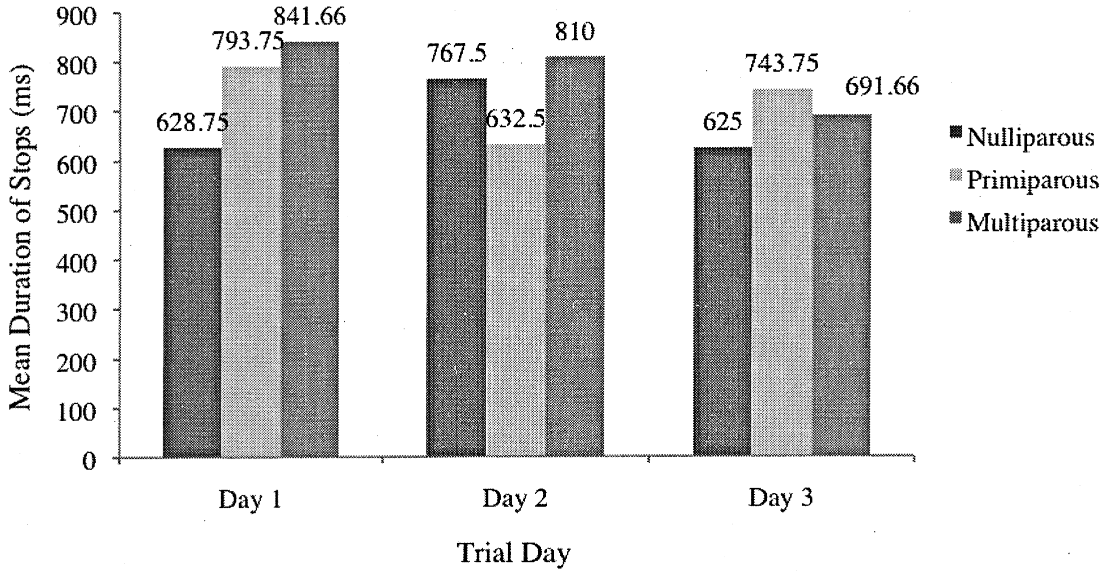

25
20
15
10
5
0
Mean Number of Stops
21.83
19.79
17.96
21.37
15.92
14.42
16.25
16.29
10.39
day 1
day 2
day 3
Trial Day
Nulliparous
Primiparpus
Multiparous

Figure 2.


900
800
700
600
500
400
300
200
100
0
Mean Duration of Stops (ms)
628.75
793.75
841.66
767.5
632.5
810
625
743.75
691.66
Day 1
Day 2
Day 3
Trial Day
Nulliparous
Primiparous
Multiparous

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6. "How to Survive Begin a Psychology Major: Questions about Graduate schools (getting in and staying in)." Mary Baldwin College, Staunton Virginia 2009 (Graduate Student Panel Member)

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