The Effects of Ovarian Hormones and Reproductive Experience on Multiple Memory System Bias in Female Rats and Women

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A Thesis
In
The Department
Of
Psychology

Presented in Partial Fulfillment of the Requirements
For the Degree Doctorate of Philosophy at
Concordia University
Montréal, Québec, Canada

July 2015

CONCORDIA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

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Entitled: The Effects of Ovarian Hormones and Reproductive Experience on Multiple Memory System Bias in Female Rats and Women

and submitted in partial fulfillment of the requirements for the degree of

Doctorate of Philosophy (Psychology)

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ABSTRACT

The effects of ovarian hormones and reproductive experience on multiple memory system bias in female rats and women

Dema Hussain, Ph.D.
Concordia University, 2015

The present thesis examined the role of ovarian hormones and reproductive experience on multiple memory system bias in female rats and women. First, the effect of 17β-Estradiol (E2) and parity on learning in a dorsal striatum- (DS) mediated response task and a hippocampus- (HPC) mediated place task was investigated. Ovariectomized (OVX) nulliparous and primiparous female rats receiving low or high E2 replacement were trained on both tasks. Nulliparous rats in the low E2 group learned the response task significantly faster than the place task; this facilitatory effect of low E2 on response learning was not observed in primiparous rats, which suggests that the E2-induced effect on response learning disappears with parity. Dopamine (DA) D1 and D2 type receptor (D1R and D2R, respectively) binding was then investigated in the DS and NAcc core and shell of nulliparous and primiparous OVX rats receiving low or high E2 replacement. Primiparous rats had significantly lower D2R binding in the DS than nulliparous rats. These results hint at a possible DS D2R mechanism in altered response learning in reproductively experienced rats.

Second, the effects of E2 and parity on memory bias in humans was investigated. Young, naturally cycling women with and without reproductive experience were tested on the 4-on-8 virtual maze (4/8 VM) task, which can be solved by using response or spatial memory, during the follicular phase (first half of the menstrual cycle) or the luteal phase (second half). Menstrual cycle results revealed predominant use of spatial memory in the luteal phase group, whereas response memory was associated with the follicular phase, showing that memory bias shifts with cyclic changes in ovarian hormones. Moreover, this pattern was also observed and found to be more pronounced in reproductively experienced women. However, mothers and non-mothers differed in terms of learning the 4/8 VM task,

which indicates that there were parity-induced differences despite similar cycle-dependent memory bias. Finally, the role of E2, progesterone (P), and testosterone (T) on this cycle-dependent shift in strategy in mothers and non-mothers was investigated. Results revealed that the follicular phase was associated with low P levels, whereas the luteal phase was linked to a high P state, suggesting that the shift in strategy across the menstrual cycle could be P-mediated in humans. Though not significant, high E2 levels were associated with response memory. Also, the results revealed that P and T both play a significant role in multiple memory system bias; this effect is reversed with parity. Thus, memory bias changes across the menstrual cycle, P and T could have a larger impact than E2 on this shift in humans, and the hormonal profile that underlies this effect is different in reproductively experienced women.

ACKNOWLEDGEMENTS

The process of completing a doctoral degree can be long, frustrating, and difficult. It would not have been possible for me to complete all of the research and work that went into this thesis, the long dissertation writing process, as well as the defence, without the help and support of numerous people.

First and foremost, I would like to thank my supervisor, Dr. Wayne Brake. We have known each other for close to a decade now, since the day that I approached you as a shy undergraduate student looking for an Honours project supervisor. Over the years, you have been supportive and helped me develop my skills as a scientist. Thank you for giving me the freedom to learn new techniques and explore novel research questions. Though many of my experiments didn't work, you always found a way to make me feel better or laugh about those frustrating moments. I'd also like to thank you for helping me learn how to write like a scientist! Even though I am not continuing in research, I have learned so much during my graduate career and it will hopefully allow me to approach future challenges as a well-rounded person. I'm appreciative of the time we've spent working together.

I'd like to thank my committee members, Dr. Jim Pfaus and Dr. Dave Mumby, for their insightful feedback on my thesis and research. I'd also like to thank my external committee members, Dr. Richard Courtemanche and Dr. Dominique Walker, for their helpful feedback on my thesis. The work that went towards chapters 2 and 3 would not have been possible without Dr. Barbara Woodside; thank you for your valuable help and support. I would also like to thank Dr. Véronique D. Bohbot for giving us the opportunity to carry out the research that went towards chapter 4-6. What started as an ambitious idea became a reality thanks to your guidance, generosity, and interest in exploring this idea.

I'd like to thank all of the wonderful people that have worked alongside me in the lab. To my fellow Brake lab graduate students: thank you for all your help and support, and for making the lab environment collaborative and fun. You were not only great to work with, but good friends outside of the lab as well. Anne Almey, you are a great lab and office mate. You always listened to my rants when things in the lab weren't working and helped me troubleshoot problems. I'm going to miss our office chats! Waqqas Shams, thank you for being supportive and always helping me when I struggled with lab issues. Thank you for your input on the review paper and all of those awesome figures you made.

Also, thanks for always helping me when I had Mac related problems! Dan Madularu, it was always fun sharing an office with you! Thank you for all of the help and valuable feedback you gave throughout our PhD years. I'd also like to thank Matthew Quinlan for helping me learn the ropes and learn many techniques and behavioral testing paradigms when I first joined the lab as an undergraduate student. I also could not have gathered all of the data that went towards chapter 2 without the significant help that I got from undergraduate students and volunteers in the lab. Behavioral t-maze testing is time-consuming and at times very frustrating; thank you to all of these awesome students for their help collecting this data.

I'd also like to thank all of the students and research assistants in the Memory and Motion lab, as well as the Douglas Hospital staff, for their help with the work that went towards chapters 4-6. Kyoko Konishi, thank you for your help designing the study, going through the large amount of data that was collected with me, and for teaching me everything I needed to know to work in a human research lab. You made the transition from working with rats to humans smooth and less intimidating. Sarah Hanafi, you were a pleasure to work with! Thank you for your valuable help collecting the data for this study, and for helping me so much with the recruitment process in the beginning. Melissa Berman, we worked together in both labs and I'd like to thank you for your help collecting data for both the t-maze experiment and at the Douglas Hospital.

To all of the graduate students and staff at the CSBN and Psychology departments at Concordia, thank you for all of the help and support you've offered me in the many years that I've been studying and working here. I have made great friends here, and will always cherish the awesome moments I shared with you. A special thank you to Dean Graham for his help with the autoradiography experiment that we collaborated on. Thank you to Shawnna Barrett, Claudia Frate, and Mayte Parada for their help with other experiments that sadly didn't work during my time as a PhD student. I'd like to also thank Heshmat Rajabi for answering my questions and helping me when I had problems in the lab. Thank you to Isabelle Bouvier, Shirley Black, Andrea Jakob, and all of the other staff members at the CSBN and in the Psychology department for always being helpful.

Lastly, I'd like to thank all of the people outside graduate school who were there to listen and support me throughout the years. I couldn't have made through graduate school

without the support of my wonderful family and all my amazing friends. To my parents, thank you for your unconditional love and support. You are always there for me and have always given us the freedom to be whoever we wanted to be. I appreciate everything you have done, and continue to do, for us. To Tamara, thank you for always listening to me rant when I was down, and for always being there for me. I not only consider you an awesome sister, but also one of my best friends. To Hasan, you're an awesome little brother; thank you for listening and joking around with me when I felt stressed out! To Pavel, thank you for being so supportive and for always listening and giving me the constructive criticism and feedback I need. You've been there for me over the last five years and helped keep me sane in my most stressful moments. To Aty, I'm so thankful for your friendship. You have been there for me so many times when I was feeling low or stressed about finishing this PhD. And finally, thank you to my furry companion Simba, for being my audience during many presentation practices!

CONTRIBUTION OF AUTHORS

1. **Reproductive experience modifies the effects of estradiol on learning and memory bias in female rats (Chapter 2)**
   The study was designed by Dema Hussain, Dr. Wayne Brake, and Dr. Barbara Woodside. Dema conducted most of the procedures related to the experiment. Alexandra Hoehne as well as other undergraduate students helped with data collection. This manuscript was written by Dema with the assistance of Dr. Brake and Dr. Woodside.
2. **Reproductive experience decreases dopamine D2 receptor binding in the dorsal striatum of the female rat (Chapter 3)**
   This experiment was designed by Dema Hussain, Dean M. Graham, Dr. Brake, and Dr. Woodside. Dema and Dean conducted all of the procedures related to the experiment. This manuscript was written by Dema with the assistance of Dr. Brake and Dr. Woodside.
3. **Modulation of spatial and response strategies by phase of the menstrual cycle in women tested in a virtual navigation task (Chapter 4)**
   This experiment was designed by Dema Hussain, Sarah Hanafi, Kyoko Konishi, Dr. Véronique D. Bohbot, and Dr. Brake. Dema and Sarah conducted all of the procedures related to the experiment. This manuscript was written by Dema with the assistance of Dr. Brake and Dr. Bohbot.
4. **Learning and memory changes across the menstrual cycle; differences between mothers and non-mothers (Chapter 5)**
   This experiment was designed by Dema Hussain, Sarah Hanafi, Kyoko Konishi, Dr. Bohbot, and Dr. Brake. Dema and Sarah conducted all of the procedures related to the experiment. This chapter was written by Dema with the assistance of Dr. Brake and Dr. Bohbot.
5. **Multiple memory system bias across the menstrual cycle is modulated by progesterone, testosterone, and reproductive experience (Chapter 6)**
   This experiment was designed by Dema Hussain, Sarah Hanafi, Kyoko Konishi, Dr. Bohbot, and Dr. Brake. Dema and Sarah conducted all of the procedures related to

the experiment. This chapter was written by Dema with the assistance of Dr. Brake and Dr. Bohbot.

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LIST OF ABBREVIATIONS

4/8 VM	4-on-8 Virtual Maze
ACh	Acetylcholine
ANOVA	Analysis of Variance
CA1	Cornu Ammonis 1
CEE	Conjugated Equine Estrogen
D1R	D1 Receptor
D2R	D2 Receptor
DA	Dopamine
DAR	Dopamine Receptor
DS	Dorsal Striatum
E	Estrogen
E2	17β-Estradiol
ER	Estrogen Receptor
ERα	Estrogen Receptor α
ERβ	Estrogen Receptor β
GABA	Gamma-aminobutyric Acid
GPER1	G Protein-Coupled Receptor 1
HPC	Hippocampus
HRT	Hormone Replacement Therapy
i.m.	Intramuscular Injection
LSEQ	Leeds Sleep Evaluation Questionnaire
M	Mean
MPA	Medroxyprogesterone Acetate
mPFC	Medial Prefrontal Cortex
NAcc	Nucleus Accumbens
NS	Non-Specific Binding
OVX	Ovariectomized
P	Progesterone
PFC	Prefrontal Cortex
PR	Progesterone Receptor

PSD-95	Post-Synaptic Density 95
PSS	Perceived Stress Score
RAVLT	Rey Auditory Verbal Learning Test
RO	Rey-Osterrieth Complex Figure Task
s.c.	Subcutaneous Injection
SD	Standard Deviation
T	Testosterone
TONI	Test for Non-Verbal Intelligence-3
TL	Total Binding
WHI	Women's Health Initiative

CHAPTER 1

General Introduction

Estrogen and memory system bias in females across the lifespan.

Dema Hussain, Waqqas M. Shams, & Wayne G Brake

Translational Neuroscience, 5(1), 35-50. doi: 10.2478/s13380-014-0209-7

1. 1 Estrogen and cognition

Modern Western society is not only marked by longer life expectancies, but young women are also waiting longer to have children and having fewer of them, therefore having more menstrual cycles in their lifetimes than ever before. Progesterone (P) and 17β-estradiol (E2; the most potent of the estrogens during reproductive years) vary across the menstrual cycle in a consistent and fluctuating manner. During the first half of the cycle, or follicular phase, E2 and P levels are low; E2 levels then start to increase steadily at the end of menstruation, reaching a peak in the middle of the menstrual cycle, right before ovulation occurs. This is followed by the luteal phase, when E2 levels plateau while P levels increase and peak until menstruation begins again (Figure 1.1). Thus, E2 and P work hand in hand across a woman's cycle to orchestrate menstruation, ovulation, and conception. However, these hormones appear to be exerting other effects on the female brain, which could be subject to subtle changes as hormone levels fluctuate over time.

The group of ovarian steroid hormones collectively known as estrogens (estrone, E2, and estriol) have long been implicated in developmental, sexual and reproductive functions through their actions via the hypothalamus. Early studies shed light on the abundance of receptors that respond to this hormone (estrogen receptors; ERs) in the ventromedial hypothalamus and pituitary gland, and how E2's action at these receptors leads to expression of sexual and reproductive behavior via subsequent hormone release (Davis, McEwen, & Pfaff, 1979; Pfaff, 1980).

Although the hypothalamus plays an important role, it's since become clear that E2 affects a wider variety of cortical brain areas, neurotransmitters and neuropeptides (for review, see McEwen, Akama, Spencer-Segal, Milner, & Waters, 2012). Such effects were discovered with the realization that there are sex differences in brain function, and that these differences are likely underpinned in part by E2. Much of the subsequent research was spurred by the finding that ERs are found throughout the brain (Shughrue & Merchenthaler, 2000; 2001), not just in the hypothalamus and, therefore, must be playing some additional functional role in the female brain. Indeed, based on this observation, E2 was thought to be influencing brain functions beyond sexual and reproductive behaviors, such as cognition. However, whether E2 improves or impairs cognition has been a source of debate. For example, E2 has been associated with impaired (Wide, Hanratty, Ting, &

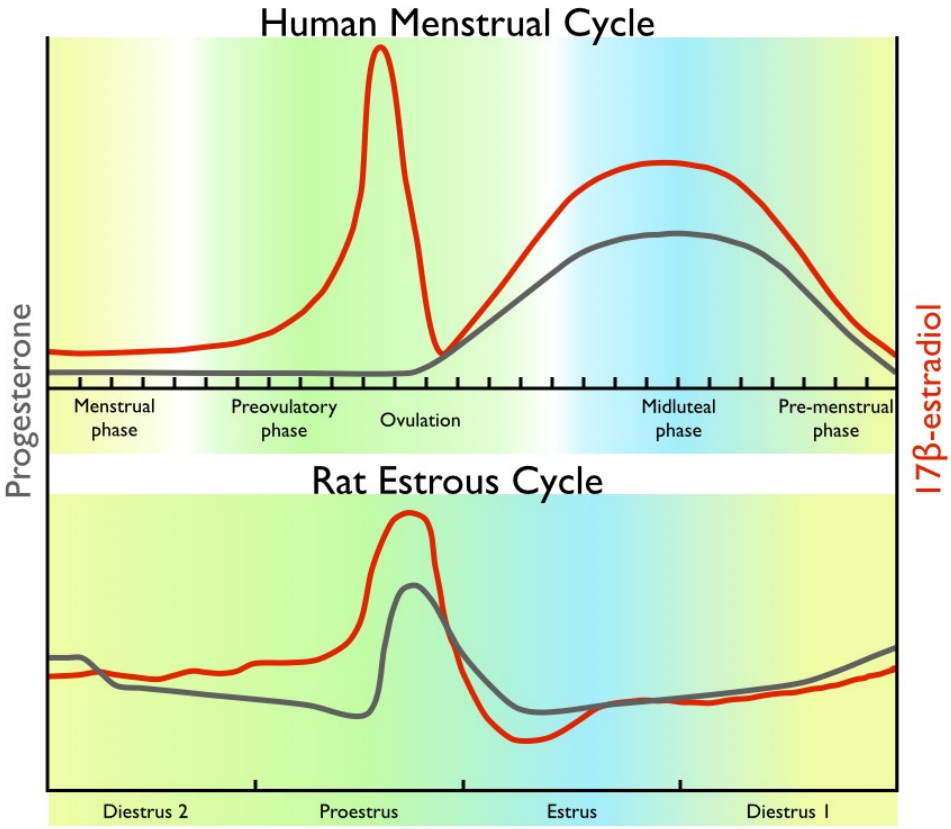


Figure 1.1. Fluctuations of E2 (red line) and P (grey line) over the 28 day human menstrual cycle (top panel) and the 4 day rat estrus cycle (bottom panel).

Galea, 2004) as well as enhanced (Fader, Johnson, & Dohanich, 1999; Sherwin, 2003) working memory. Furthermore, in humans, E2 has been linked to improved verbal and fine motor skills (Hampson, 1990), higher levels of creativity (Krug, Born, & Rasch, 2006), but worse performance on visual tasks (Sanders, Sjodin, & de Chastelaine, 2002; Sherwin, 2003).

As life expectancies increase in the Western world, so does the proportion of women's lives spent post-menopause. Menopause is marked by a complex series of events that begin with cycle irregularity and eventually lead to the cessation of fertility and menstruation (Burger, Hale, Dennerstein, & Robertson, 2008). Specifically, there is a dramatic decrease in ovarian hormones, follicles stop being released by the ovaries, and the primary source of endogenously synthesized estrogen is estrone (Jensen, Christiansen, & Rødbro, 1985; Wise et al., 1999). Menopause is often accompanied by adverse symptoms such as hot flashes, irritability, insomnia, memory loss, and tiredness (Goldani von Mühlen, Kritz-Silverstein, & Barrett-Connor, 1995). This prompted researchers to develop a therapeutic treatment that would alleviate these symptoms in peri- and postmenopausal women. Numerous studies looking at the benefits of hormone replacement therapy (HRT) in postmenopausal women, in whom E2 levels dramatically decline, have shown that hormone replacement can protect against age-related cognitive decline (Bagger, Tanko, Alexandersen, Qin, & Chritiansen, 2004; Binder, Schectman, Birge, Williams, & Kohrt, 2001; Sherwin, 1988; Yaffe et al., 2007; 2000) while other studies suggest either no or a negative effect (LeBlanc, Neiss, Carello, Samuels, & Janowski, 2007; Rapp et al., 2003; Resnick et al., 2006). These discrepant findings show that there is yet to be a consensus on the specifics of how E2 influences female cognition, and this is possibly due to the age at which HRT is started, hormonal composition of the treatment, route of administration, as well as the time elapsed between menopause and initiation of HRT, as well as other factors (Sherwin & Henry, 2008); this will be discussed in further depth later in this paper. In general, E2 could also exert its effects differentially throughout the brain, specifically, the hippocampus (HPC), striatum, and prefrontal cortex (PFC). These are the three principal brain areas that will be discussed in this review (Figure 1.2).

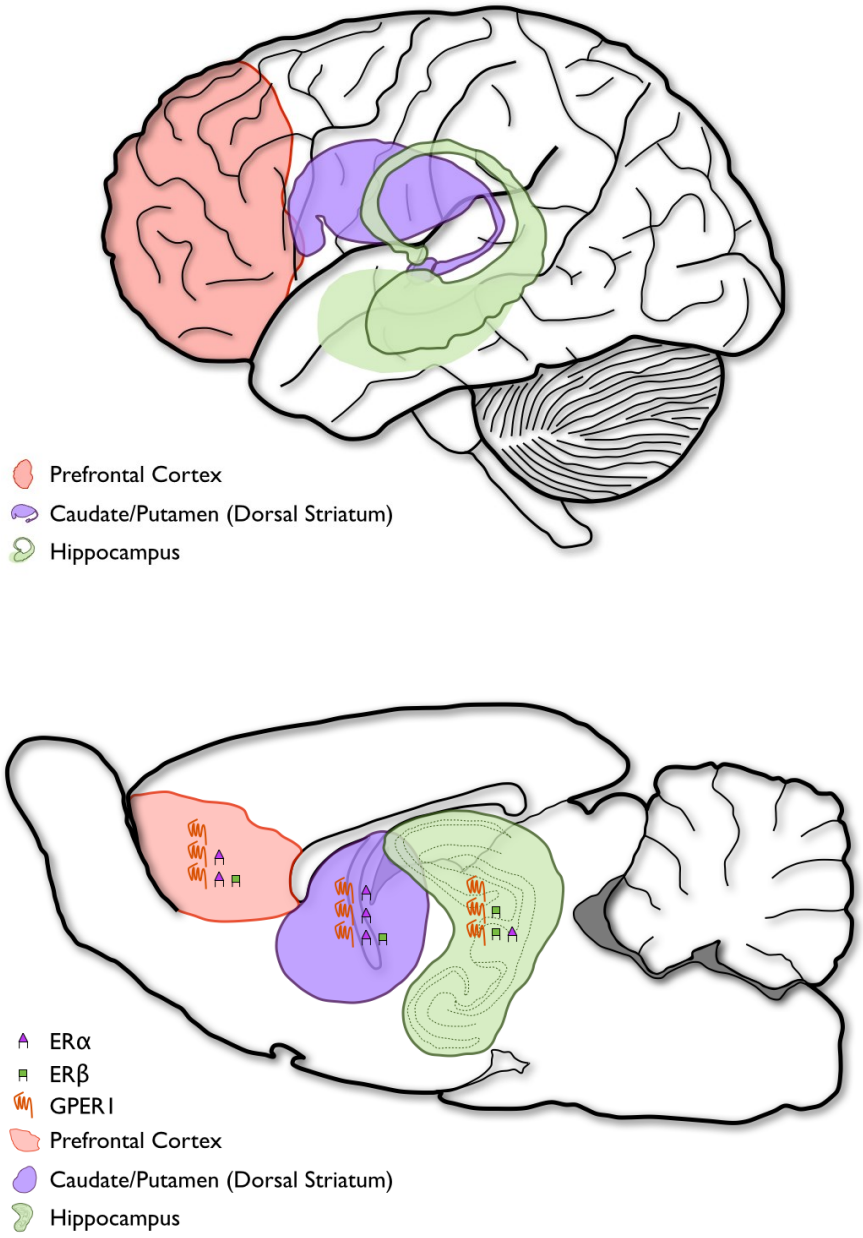


Figure 1.2 The PFC, caudate putamen, and HPC location in the rat brain (top panel; sagittal view) and human brain (bottom panel; lateral view). ER distribution and relative density is shown in the rat brain (top panel).

1.1.1 The hippocampus and cognition

Early studies on the effects of E2 examined the HPC, a brain area important for spatial memory and in which ERs are found. Furthermore, the density of hippocampal ERs fluctuate with age and across the estrus cycle, the ~4 day cycle of sexual receptivity and fertility in rats, akin to the menstrual cycle in women (Adams et al., 2002; Loy, Gerlach, & McEwen, 1988; McEwen et al., 2012; Mitterling et al., 2010). Figure 1.1 shows how the ~4 day estrus cycle in the rodent compares to the profile of the ~28 day menstrual cycle in women. In the HPC, cell synaptogenesis, the formation of new synapses or connections between neurons, also occurs in synchronicity with the ovarian cycle and its varying E2 levels (McEwen, Gould, Orchinik, Weiland, & Woolley, 1995; Woolley, 1999). For instance, it was found that neurons in the CA1 region of the HPC show excitatory synapse density changes in a cyclic manner, with higher E2 levels correlating with more synapses (Gould, Woolley, Frankfurt, & McEwen, 1990; Lewis, McEwen, & Frankfurt, 1995; Woolley & McEwen, 1992). Synaptogenesis also increases in ovariectomized (OVX; with ovaries removed) rats when exposed to high E2 replacement (Woolley, Wenzel, & Schwartzkroin, 1996). Specifically, the proestrus phase, which is marked by an E2 level peak analogous to the late follicular phase in women, is linked with increased synaptic density in the HPC compared to the estrus phase, when the lowest E2 levels are observed, as is seen during menses in women. Such findings suggest that E2 could affect HPC-dependent functions, such as memory.

The HPC plays a key role in spatial memory in rodents. This has been consistently shown in studies in which a damaged or impaired HPC is accompanied by impaired performance on spatial memory-dependent tasks (McDonald & White, 1993; Mao & Robinson, 1998; Packard & McGaugh, 1992; Zurkovsky, Brown, Boyd, Fell, & Korol, 2007). Furthermore, when hippocampal integrity and spatial learning is impaired, rats rely on other learning strategies to complete tasks (Packard & McGaugh, 1992; 1996) . Since high E2 levels are associated with increased excitatory synapses and increased spine density in the HPC (Woolley & McEwen, 1992; 1993; Woolley et al., 1996), it would be expected that E2 enhances spatial memory. Indeed, a large body of research has confirmed this. High E2 levels potentiate the release of acetylcholine (ACh), a neurotransmitter that is heavily implicated in learning, in the HPC during spatial tasks (Gabor, Nagle, Johnson, &

Gibbs, 2003; Marriott & Korol, 2003). Estradiol also enhances performance in rats on HPC-dependent spatial memory tasks, such as a spatial version of a swim task (Packard & Teather, 1997), the radial arm maze (Daniel, Fader, Spencer, & Dohanich, 1997; Luine, Richards, Wu, & Beck, 1998), delayed alternation in a t-maze (Fader, Hendricson, & Dohanich, 1998), and place memory (Korol & Kolo, 2002; Korol, Malin, Borden, Busby, & Couper-Leo, 2004; Quinlan, Hussain, & Brake, 2008). In contrast to this evidence, high E2 levels have also been shown to impair performance on HPC-dependent tasks suggesting that research in this area is yet equivocal (Galea, et al., 2001; Holmes, Wide, & Galea, 2002).

Local synthesis of E2 by the enzyme aromatase, which metabolizes testosterone (T) into E2, also plays a role in affecting cognition. Aromatase inhibition leads to decreased dendritic spines, synapses and axon growth in the HPC (Kretz et al., 2004; Prange-Kiel, 2006; Rune & Frotscher, 2005; Von Schassen et al., 2006), which suggests that local synthesis of E2 is important in hippocampal synaptogenesis. No studies have demonstrated a direct link between local E2 synthesis in the brain and cognitive function, though one study carried out with postmenopausal women revealed no differences in cognition as a result of aromatization of E2 from T (Shah et al., 2006).

In humans, spatial memory and the use of spatial cues to navigate an environment is dependent on hippocampal functioning (Bohbot, Iaria, & Petrides, 2004; Maguire et al., 1998). However, the role of E2 on HPC-dependent tasks is not clear in humans. On the one hand, some studies show that spatial skills, a sexually dimorphic ability in which men typically outperform women (Halpern, 1992), is enhanced in women when they are in the menstrual phase of the cycle (Figure 1.1), when E2 is low (Hampson, 1990; Sherwin, 2012). On the other hand, some studies carried out in postmenopausal women receiving HRT showed an increase in spatial skills with the replacement of E2, compared to its absence (Duff & Hampson, 2000; Wolf et al., 1999). The disparity in these findings may be due to differences in the types of tasks being used to measure spatial memory, the age of participants tested, as well as the influence that other ovarian hormones may have during the menstrual cycle (Figure 1.1).

1.1.2 The striatum and cognition

The striatum is divided into two areas: the ventral and dorsal striatum (DS). While both areas are implicated in learning and memory (Packard & Knowlton, 2002), only the dorsal portion of the striatum will be focused on in this paper, as it is predominantly involved in multiple memory systems. The DS, also known as the caudate nucleus, is implicated in sensorimotor, habit forming, and egocentric stimulus-response learning (Korol, 2004; Packard & Knowlton, 2002). Dorsal striatal lesions are associated with impaired visual discrimination, but not spatial learning, in a water maze swim task (Packard & McGaugh, 1992). The DS is implicated in cue-based habit learning as well, such that when this brain area is damaged in rats, they can no longer utilize this form of learning (McDonald & White, 1994). Another study showed that DS neurons are active while rats are being trained on a habit-forming behavioral task (Jog, Kubota, Connolly, Hillegaart, & Graybiel, 1999) and DS lesions disrupt habit learning in rats (Yin, Knowlton, & Balleine, 2004). Furthermore, the DS has been shown to respond to reward such that striatal neurons are active before and up until the rewarding stimulus is presented (Graybiel, 2005; Hollerman, Tremblay, & Schultz, 1998).

Estradiol has a modulatory effect on the DS by increasing release of the neurotransmitter dopamine (DA; Becker, 1999). It's been consistently shown that E2 increases DA release in the DS (Becker & Rudick, 1999; Xiao & Becker, 1994), enhances amphetamine-induced DA release and potentiates DA-dependent behaviors (Becker, 1999; Becker & Beer, 1986; Becker, Snyder, Miller, Westgate, & Jenuwine, 1987; Van Hartesveldt & Joyce, 1986). In rats, high E2 levels are related to impaired response learning (Hussain, Hoehne, Woodside, & Brake, 2013; Korol, 2004; Korol et al., 2004; Quinlan et al., 2008), but the relationship between E2 and response learning in humans remains unknown as of yet.

In humans, the striatum is associated with stimulus-response, habit learning and procedural memory (Bohbot, Lerch, Thorndycraft, Iaria, & Zijdenbos, 2007; Iaria, Petrides, Dagher, Pike, & Bohbot, 2003; Knowlton, Mangels, & Squire, 1996). As with animals, DS DA transmission is associated with the anticipation of reward (Volkow et al., 2002; Zald et al., 2004). Studies carried out in both primates and humans have shown that the DS is implicated in decision-making through its associations with cortical areas (Balleine,

Delgado, & Hikosaka, 2007). Studies employing decision making tasks show that women are less efficient than men at inhibitory control when in the luteal phase, but this sex difference is no longer present when women are in the follicular phase, suggesting that higher E2 and P levels, which are linked to higher striatal DA levels, weakens their abilities to inhibit impulses (Colzato, Hertsig, van den Wildenberg, & Hommel, 2010). Low E2 levels in women are also associated with reduced sensitivity to loss and gain in a reward based task (Bayer, Bandurski, & Sommer, 2013), though another study found increased activity in the striatum in response to rewarding stimuli during the follicular phase (Dreher et al., 2007). These data suggest that varying E2 levels across the menstrual cycle modulate how rewards are anticipated, decision-making and impulsive behavior.

1.1.3 The PFC and cognition

The PFC is implicated in a myriad of cognitive functions in animals including humans: e.g., attention, working memory, learning and executive function. In rats, loss of E2 is associated with decreased PFC ACh transmission and decreased learning and performance, and this decline is reversed with E2 replacement (Gibbs & Aggrawal, 1998; McEwen & Alves, 1999; Singh, Meyer, Millard, & Simpkins,1994). Also, E2 treatment leads to improved sustained attention in rats, and the opposite is observed in OVX rats that have not been exposed to E2 for a prolonged period (Bohacek & Daniel, 2010). Furthermore, E2 has been linked to improved performance on a working memory task in aging rhesus monkeys, which was worse in OVX monkeys without E2 replacement (Rapp, Morrison, & Roberts, 2003). In rats, low E2 levels have been found to facilitate but high E2 levels impair (Wide et al., 2004; Holmes et al., 2002) or have no effect (Galea et al., 2001) on working memory. It has also been found that E2 exerts different effects on working memory depending on the brain region. High E2 replacement facilitates working memory when infused into the PFC whereas low E2 replacement has this effect in the HPC of OVX rats (Sinopoli, Floresco, & Galea, 2006).

Executive function is traditionally thought to be affected by DA transmission in the PFC of primates (Arnsten, Cai, Steere, & Goldman-Rakic, 1995; Sawaguchi & Goldman-Rakic, 1994) and rats (Busber & Schmidt, 1990; Rios Valentim Jr., Gontijo, Peres, Rodrigues, & Nakamura-Palacios, 2009; Zahrt, Taylor, Mathew, & Arnsten, 1997) although our recent findings suggest this not to be the case in rats (Madularu et al.,

submitted). Administration of E2 increases DA metabolite concentrations in the rat PFC Inagaki, Gautreaux, & Luine, 2010) and enhances PFC DA transmission (Thompson & Moss, 1994). Estradiol is also associated with anatomical changes in the PFC such as increased dendritic spine density. Dendritic spines are thought to be important in the neurobiological mechanisms of learning (Hao et al., 2007; Tang et al., 2004). In humans, E2 has been linked to increased PFC activity during working memory tasks (Dumas, Kutz, Naylor, Johnson, & Newhouse, 2010) and this effect is mediated, in part, by DA activity (Jacobs & D'Esposito, 2011). Another study showed that working memory function fluctuates across the menstrual cycle in young women, with improved working memory observed in the preovulatory phase, when E2 peaks (Rosenberg & Park, 2002).

The most salient observation about the link between E2 and prefrontal cognitive function in humans comes from studies observing postmenopausal women. The postmenopausal decline in E2 is often accompanied by marked cognitive decline, such as memory loss, poor working memory, and worsened attention (Epperson, Amin, Ruparel, Gur, & Lougheah, 2012; Mitchell & Woods, 2011; Weber & Mapstone, 2009). This is emphasized by studies showing that HRT improves or reverses many of these PFC-related problems such as loss of working memory and selective attention (Duff & Hampson, 2000; Keenan, Ezzat, Ginsburg, & Moore, 2001; Krug et al., 2006). In contrast to this are the findings of the Women's Health Initiative (WHI), which did not find any significant improvement on any of the executive function tasks in women taking HRT (Resnick et al., 2006). However, this could be attributed to variations in the time gap between menopause and initiation of HRT, as the length of time elapsed from menopause to hormone administration is important. A possible mechanism for this sensitive time window may be that ERs downregulate after a certain period of time of E2 absence and eventually cannot become active again even if E2 is administered (Lauber, Mobbs, Muramatsu, & Pfaff, 1991; Lauber, Romano, Mobbs, & Pfaff, 1990).

Overall, it has become clear that E2's role in the female brain influences more than just sexual and reproductive behaviors. Estrogen receptors have been discovered in brain areas beyond the hypothalamus, and cognitive function has been found to vary with fluctuating and supplemental E2 levels in both rodents and humans.

1.2 Multiple memory systems

Tolman and colleagues (1946) first discussed different variants of learning strategies in the 1940s, showing that rats can use different types of learning to solve a task (Tolman, Ritchie, & Kalish, 1946). Since then, there has been a large body of evidence showing that learning and memory operate within different types of systems that are distinct and competitive. These memory systems are dissociable and rely on different brain structures, and they each offer a unique approach in solving problems or tasks. These various approaches to solving tasks can be spatial, stimulus-response, or emotional, and they rely on the HPC, striatum, and amygdala, respectively (McDonald & White, 1993; White & McDonald, 2002). Emotional learning and memory depends on forming affective associations with the environment, however it will not be focused on here, as it is beyond the scope of this paper. Spatial, or place, learning and memory refers to making associations between landmarks in the spatial environment to create a cognitive map in order to navigate or remember locations; for example, learning that you have to walk towards the mountain and take a right at the gas station to get to the grocery store. Conversely, response learning is an egocentric strategy that relies on internal cues, such as body turns, to navigate an environment. An individual relying on response learning would ignore where the mountain is and just automatically walk straight ahead for five minutes, and then turn right to get to the store.

Both of these learning strategies are probably recruited in order to navigate one's environment, though they operate in different ways. Spatial strategies are more cognitively demanding and are especially helpful when encountering a novel environment in which it would be beneficial to become familiar with the spatial landscape to orient oneself. A response strategy offers a cognitive short cut and is beneficial when one becomes familiar with an environment. For example, it is easier to learn a series of automatic turns when employing the same daily route to work. Response learners would become lost and disoriented if their usual route is blocked one day, whereas spatial learners would be able to access their cognitive map to think of a detour.

McDonald & White (1993) showed that there is a neurobiological dissociation between these memory systems in rats, such that when the HPC is damaged, spatial learning and memory are impaired. Similarly, when the DS is damaged, subsequent

performance on a response-based task is impaired (McDonald & White, 1993). Furthermore, impairing one memory system leads to rats utilizing a different one: for example, impairing the HPC will lead to rats relying on striatal-mediated response memory to solve a task (Packard & McGaugh, 1996). Similarly, rats with a damaged HPC are not only impaired on a spatial task but also learn a response task faster (White & McDonald, 2002). This suggests that these memory systems are not only independent, but also interact in a competitive manner.

Memory systems are also associated with changes in neurotransmitter levels. Studies have shown that higher ACh levels in the HPC predict place learning, and higher ACh levels in the DS predict response learning (McIntyre, Marriott, & Gold, 2003). The excitatory neurotransmitter glutamate has been shown to enhance place and response learning when it is infused in the HPC or DS, respectively (Packard, 1999). Another aspect of competing multiple memory systems is that, at least in male rats, in a novel setting rats will initially use place memory but switch to response memory with repeated exposure in an appetitively motivated task (Chang & Gold, 2003; Packard & McGaugh, 1996). Acetylcholine levels in these brain areas mirror this pattern, such that they peak in the HPC when rats use spatial memory, and later peak in the DS as learning shifts to more response-based memory (Chang & Gold, 2003). Conversely, it's also been shown that hippocampal cholinergic neuron lesions are associated with facilitated learning in a spatial discrimination task (Cahill & Baxter, 2001). Also, chemical cholinergic blockade in the HPC promotes place learning on the Morris water maze (Bizon, Hans, Hudon, & Gallagher, 2003), and this has been demonstrated in both male and female rats (Jonasson, Cahill, Tobey, & Baxter, 2004) suggesting that the role of hippocampal ACh in spatial learning is not as straightforward as initially thought.

Multiple memory systems are also employed by humans to navigate their environment and solve tasks. On one hand, individuals can use a spatial strategy to locate themselves or a target location by referring to a cognitive map and forming relationships between landmarks to navigate an environment. On the other hand, a stimulus-response strategy would involve using only one landmark as a starting point, then carrying out a series of body turns to navigate an environment, while mostly ignoring spatial landmarks.

Spatial learning offers a more cognitively demanding, albeit more flexible, navigational strategy whereas response learning can be more efficient yet is not as flexible a strategy.

Like in rats, in humans, spatial learning and memory is related to hippocampal activity, and stimulus-response learning and memory with activity in the striatum (Iaria et al., 2003; O'Keefe & Nadel, 1978). Impaired spatial memory has also been observed in individuals with damage to the HPC (Bohbot et al., 1998). One case study showed, for example, that spatial, but not response, memory is impaired in an individual with hippocampal damage (Holdstock et al., 2000). Furthermore, a study conducted with London taxi drivers showed that hippocampal volume is correlated with years of driving experience, which suggests that increased spatial navigational skills are correlated with larger hippocampal volume (Maguire et al., 2000). Some humans preferentially use spatial while others preferentially use response memory when navigating their environments, suggesting that there are inherent biases. Differences have been observed in gray matter, brain tissue that is mostly composed of neuronal cell bodies, between individuals who preferentially use a spatial memory and those who preferentially use a response memory. Bohbot and colleagues (2007) found that spatial learners have more gray matter in the HPC and less gray matter in the caudate nucleus than response learners (Bohbot et al., 2007).

Functional neuroimaging studies have also confirmed that hippocampal activity is associated with spatial learning (Bohbot et al., 2004; Maguire et al., 1998), and striatal activity with response learning (Hartley, Maguire, Spiers, & Burgess, 2003; Iaria et al., 2003). As in rats, the proportion of individuals who use spatial and response strategies is usually equivalent, with half using spatial and the other half using response memory. Upon prolonged training, about 40% of spatial learners shift to using the less cognitively demanding response memory (Iaria et al., 2003; Packard & McGaugh, 1996), whereas rats switch to using response memory after repeated trials in an appetitively motivated task at a rate of 100% (Chang & Gold, 2003). Interestingly enough, in females, the bias toward place or response memory is affected by E2 levels. The question turns to which neurobiological mechanisms could explain such biases. To begin answering this question, we must first understand where and how E2 is playing a role in modulating multiple memory system bias in the female brain. As a start, it is important to understand how E2 interacts with its receptors and where in the brain such receptors are found.

1.3 Estrogen receptor distribution in the female brain

Estrogen receptors fall within two categories: genomic, such as ERα and ERβ, and non-genomic, such as G protein-coupled ER1 (GPER1, formerly known by its orphan receptor name, GPR30; McEwen et al., 2012; McEwen, Krey, & Luine, 1978), a membrane-associated metabotropic receptor. The genomic ERs, sometimes referred to as the "classical" ERs, have a prolonged, delayed effect that involves gene signaling and works in a membrane-independent way via an estrogen-ER dimer that translocates to the cell nucleus and affects gene transcription (Olde & Leeb-Lundberg, 2009). Paradoxically, E2 can have rapid effects on behavior, which cannot be explained by the genomic, or delayed, mechanisms of these receptors. Thus it was hypothesized that there was either some unknown membrane ER, or one or both of the classic ERs (ERα and ERβ) were also acting in a rapid manner at the membrane (Figure 1.3). Recent findings have brought to light how E2 can have rapid effects on behavior.

The classical ERs have been recently shown not to be exclusively genomic, as they have been observed to be located at the cell membrane of neurons in the DS and thus possibly acting in a non-genomic fashion (Almey, Filardo, Milner, & Brake, 2012). The more recently discovered non-genomic ER variety is also membrane-bound and likely operates by activating second messengers in the neuron, and has more rapid and instantaneous actions in the brain (Olde & Leeb-Lundberg, 2009; Figure 1.3).

The first ER that was discovered is ERα; it was later found that ERα knockout mice sometimes show a normal response to E2, which suggested that other ERs must exist in the brain (Korach, 1994; Shughrue, Lane, & Merchenthaler, 1997; Shughrue, Lane, Scrimo, & Merchenthaler, 1998). This second ER to be discovered, ERβ, has since been shown to be involved in fertility and ovarian function in the rodent, whereas ERα is crucial for E2-mediated reproductive and sexual physiology and behaviors (Krege et al., 1998; Ogawa et al., 1998). The role of the most recently identified ER, GPER1, is not yet fully understood, though it is found throughout the nervous system (Olde & Leeb-Lundberg, 2009).

Distribution of ERs in the brain is variable, dynamic, and differs depending on the subtype; ERα has been found in the hypothalamus and amygdala while ERβ has been found throughout the rat brain (Shughrue et al., 1997). As indicated above, a large body of research has shown that E2 plays an important role in hippocampal functioning and in

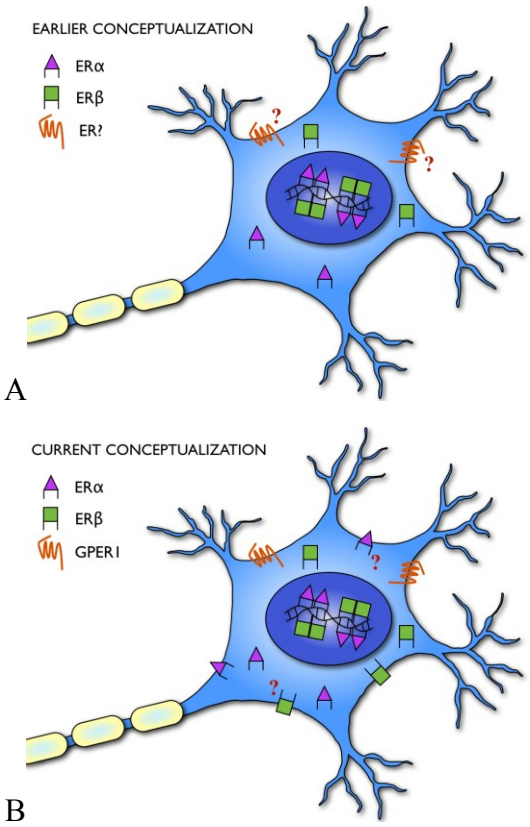


Figure 1.3 Illustration of early and current conceptualization of ER function in the neuron. A. Earlier conceptualization of ER and how they function within the neuron. ERα and ERβ were thought to exert their effects solely via a genomic, long-term mechanism, whereas the more rapid actions of E were attributed to a third, unknown ER (ER?). B. Current conceptualization of ERs and how they function within the neuron. The recently discovered ER, G-coupled estrogen receptor 1 (GPER1), is membrane bound and can be responsible for some of estrogen's rapid actions. The classical ERs, ERα and ERβ, are now known to be also associated with the membrane in certain brain areas, and are additional potential mediators of estrogen's rapid actions. How membrane-associated classical ERs exert such effects in nerve cells is yet unknown.

hippocampal dependent behaviors, like spatial memory. Given these findings, it would be expected that the HPC would contain a high density of ERs. Indeed, ERβ expression has been observed in the HPC of the mouse (Milner et al., 2010), non-human primate (Gundlah et al., 2000), and human (Ōsterlund, Gustafsson, Keller, & Hurd, 2000), which supports E2's involvement in learning and memory. ERα has also been found in the cortex and HPC, but to a lesser extent than ERβ (Milner et al., 2001; 2005). In the mouse HPC, it's been shown that ERα and ERβ levels fluctuate across the estrous cycle, with more ERα- and ERβ-labeled dendritic spines observed in the diestrus phase of the cycle, when E2 is low (Mitterling et al., 2010).

Furthermore, it's been found that the number of ERs in the HPC differs with age. Expression of ERα and ERβ in hippocampal dendritic spines decreases with age (Mehra, Sharma, Nyakas, & Vij, 2005). Additionally, ERβ levels are found to be lower with older age, but this can be reversed with E2 treatment (Waters et al., 2011), which suggests that ERβ responds better to E2 with age and could be a target for HRT to prevent age-related cognitive decline. Not much is known about GPER1 function in the HPC, although there is recent evidence that GPER1 receptors are present in this brain area (Brailoiu et al., 2007). It's been recently shown that GPER1 is found on hippocampal dendritic spines, and administration of a GPER1 agonist leads to an increase in levels of post-synaptic density 95 (PSD-95), a protein important for spine scaffolding (Akama, Thompson, Milner, & McEwen, 2013) and thus thought to be important for the neurobiological basis of learning.

Recent studies have shown that administration of a GPER1 agonist enhances (Hammond, Mauk, Ninaci, Nelson, & Gibbs, 2009) while a GPER1 antagonist impairs (Hammond, Nelson, Kline, & Gibbs, 2012) acquisition of a spatial task in both naturally cycling and OVX rats given E2 replacement, suggesting that E2's enhancement of spatial learning is mediated in part by GPER1 receptor binding. Indeed, chronic administration of ERα, ERβ, and GPER1 agonists restores acquisition of a spatial task in OVX rats that had previously been impaired on the same task (Hammond et al., 2009).

Until recently, the localization of ERs in the DS was not well understood despite the well-established effect of E2 on this brain area. Indeed, E2 acts in the DS to affect response learning (Quinlan et al., 2008; Zurkovsky et al., 2007), and DA transmission (Becker & Rudick, 1999), yet how this occurs is still not fully known. Studies have shown that ERα

and ERβ are present in the DS of mice (Küppers & Beyer, 1999; Mitra et al., 2003). More recently, members of our group, Almey et al. (2012), used electron microscopy to investigate distribution of ERα, ERβ, and GPER1 in the DS. They found that all three ER subtypes were present in the DS at extranuclear sites, and ERα and GPER1 were colocalized with cholinergic, but not dopaminergic, neurons (Almey et al., 2012). This is supported by another study showing that ACh has modulatory effects on DA in this brain area (Threlfell & Cragg, 2011). Furthermore, E2 acts via ERα located on GABA neurons to affect DA release in the DS (Schultz et al., 2009). These studies suggest that E2 may be indirectly influencing DA and its associated behaviors in the DS via neurotransmitter activity (Figure 1.2).

Estrogen receptors have also been found in the PFC. Studies carried out with nonhuman primates have shown that ERα and ERβ mRNA is present in this brain region (Wang et al., 2004). Another study showed that ERα, but not ERβ mRNA, is expressed in primate PFC (Pau, Pau, & Spies, 1998); this latter pattern was again found in studies looking at ER distribution in rodents and primates, including human PFC. ERα has also been found in rat PFC (Butler, Kalló, Sjöberg, & Coen, 1999), and in human dorsolateral PFC (Perlman et al., 2005). Wang and colleagues (2010) showed that ERα is present in the dorsolateral PFC of both young and aged female rhesus monkeys, and ERα levels correlated with accuracy on a working memory task, which provides further evidence for the link between E2 and cognitive performance (Wang, Hara, Janssen, Rapp, & Morrison, 2010). It was recently found that ERα, ERβ and GPER1 are all present in the rat medial PFC, with GPER1 being the most abundant ER found in this brain region (Almey et al., 2014).

Lastly, ER distribution is not static but changes across the lifespan of the female. It's been shown that female rats that have given birth to and raised a litter of pups are exposed to heightened levels of E2 for a significant period of time (Bridges, 1984), and show increased ERα density in the pituitary gland, medial preoptic area and amygdala (Byrnes, Babb, & Bridges, 2009), brain regions that are important in hormonal, sexual, and emotional regulation, respectively. Furthermore, there is an increase in ERα containing neurons in the DS of primiparous (i.e., having given birth once) middle-aged rats when compared to nulliparous (i.e., having never given birth) females (Byrnes et al., 2009),

which indicates that parity (i.e., the number of times a female has given birth) alters ER distribution in the female brain and, potentially, cognitive function.

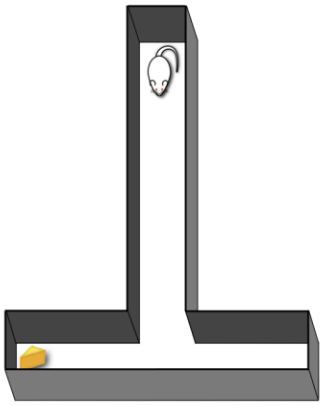
1.4 Hormones, reproductive experience, and multiple memory systems

Although there is evidence that E2 influences cognitive function, the direction of this effect is controversial. It has been suggested that perhaps it isn't so much that E2 is improving or impairing cognition, but rather affecting how a task is solved. This seems to be the case in E2's influence on multiple memory systems. It was the seminal work of Donna Korol and colleagues that established that E2 modulates place and response learning in female rats (Korol, 2004, Korol & Kolo, 2002; Korol et al., 2004; Zurkovsky et al., 2007). As in humans, place learning involves the use of spatial cues in the rat's environment to form a cognitive map of its surroundings. Response learning involves the rat relying on particular body turns to navigate its environment.

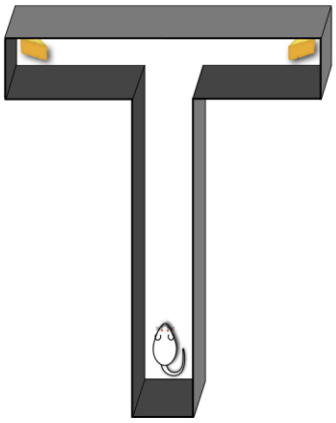
To test multiple memory system bias in rats, the ambiguous t-maze task is often used. This task consists of training rats daily to seek an appetitive reward that is always situated in either the right or left arm of a t-shaped maze (Figure 1.4). Once the rat reaches criterion, that is, finds the reward in eight or nine out of ten trials, a probe test is administered. In the probe test the maze is rotated 180 degrees and the rat is given one trial. If the animal goes towards the same physical location where the reward was located during training, it is considered to be using place memory to locate it. If it carried out the same body turn (e.g., if it had been trained to always turn 90 degrees to the right, it would do so again during the probe trial), it is deemed to be using response memory.

In OVX rats, E2 replacement enhances learning of a place task, but impairs learning on a response task (Korol & Kolo, 2002). In an ambiguous t-maze task in which both learning strategies can be used to solve the task, it's been repeatedly observed that OVX rats given high E2 replacement predominantly use place memory, whereas the opposite pattern is seen in those with low or no E2 replacement (Korol et al., 2004; Quinlan et al., 2008; 2013).

The same effect is observed in naturally cycling females; rats in proestrus use a place strategy more often on a ambiguous t-maze task whereas rats in estrus are more likely to use a response strategy or memory system (Korol et al., 2004). Korol and colleagues (2005) also found that directly inhibiting the HPC leads to rats shifting to striatal response



A



B

Figure 1.4 A. The ambiguous t-maze as used during training to test multiple memory systems. B. Upon criterion, the maze is rotated 180 degrees for the probe test.

learning or memory, with the most dramatic shift observed in proestrus rats (McElroy & Korol, 2005). Additionally, injecting E2 directly into the HPC enhances place memory whereas injecting it directly into the DS impairs response memory (Zurkovsky et al., 2007; Zurkovsky, Serio, & Korol, 2011). These findings indicate that E2 plays a key role in determining which memory system is employed to solve a task. A recent study has shown that hippocampal volume changes across the estrous cycle of mice, with volume increasing in the proestrus phase and decreasing in the estrus phase, and this increase in hippocampal volume is positively correlated with place strategy and negatively correlated with response strategy (Qiu et al., 2013). This supports behavioral findings that show that HPC-dependent spatial learning and memory is enhanced when E2 levels are at their highest and DS-dependent response learning is enhanced when E2 levels decrease. Taken together, these studies show that levels of E2, whether naturally fluctuating across the cycle or administered to OVX rats, modulate the type of memory system employed to learn a task.

We have also demonstrated that DA plays a role in E2's effect on multiple memory system bias. Dopamine antagonists - that is, drugs that block the DA D1 type receptors or D2 type receptors (D1R or D2R, respectively) - were administered to OVX female rats receiving either low or high E2 replacement. These rats were then tested on the ambiguous t-maze task. It was once again confirmed that rats receiving low E2 predominantly rely on response memory whereas those receiving high E2 rely on place memory. Following administration of the DA antagonists, rats with low E2 replacement that had previously used response memory shifted to using place memory whereas rats with high E2 replacement predominantly maintained a bias for place memory (Quinlan et al., 2008). These findings suggest that disrupted global DA transmission impacts how E2 affects strategy use, possibly due to altered striatal DA function. This was confirmed in a follow-up study: it was shown that infusing the same DA antagonists directly into the dorsal, but not the ventral, striatum lead to a different memory bias in OVX rats receiving both low and high E2 replacement (Quinlan et al., 2013). However, this effect was only observed following administration of the D1R antagonist, with no effects observed after the D2R antagonist was infused into the DS, suggesting that another brain region may also be mediating multiple memory system bias and how E2 affects it in particular. It was hypothesized that the PFC maybe playing a role.

Indeed, some studies have shown that the PFC could be playing a central role in modulating multiple memory system bias; specifically, it appears to be important in switching between hippocampal and striatal learning strategies. For instance, medial PFC inactivation was shown to impair strategy switching, but not place or response learning per se, in rats (Ragozzino, Detrick, & Kesner, 1999; Rich & Shapiro, 2007). Rich and Shapiro (2009) confirmed this by showing that neuronal activity in the rat PFC changes when learning to switch between place and response learning, but not when learning within the same strategy. Furthermore, they found that, depending on the specific part of the PFC, some neurons are most active when learning to switch from one memory system to another whereas others are most active when the rat is established in using the new memory system (Rich & Shapiro, 2009). Also, infusing E2 directly into the medial PFC of OVX rats biases them to predominantly use place memory in the ambiguous t-maze task (Almey et al., 2014). This indicates that the PFC orchestrates memory system bias as well as how and when the HPC and DS are activated, and that E2 acts in this brain area to determine, at least in part, which memory system is used at a given instance.

Not only does the cyclic nature of E2 impact memory system bias in females, the experience of pregnancy and pup rearing is related to long-term changes in female rats that are still apparent after the young are weaned. These parity-related organizational effects are wide ranging and include alterations in stress and anxiety behavior (Lambert et al., 2005; Wartella et al., 2003), as well as enhanced immediate and long-term spatial learning (Gatewood et al., 2005). Interestingly, nulliparous females who were induced to show maternal behavior by exposure to foster pups learned a spatial task as quickly as reproductively experienced rats, suggesting that the act of caring for young may be in itself sufficient in inducing these long-term changes (Kinsley et al., 1999).

The dramatic changes that accompany reproduction are not just immediate; long-term changes in sensitivity to E2 following parturition have been reported in reproductively-experienced compared to nulliparous rats. For instance, Bridges and Byrnes (2006) found that reproductively-experienced rats show higher prolactin secretion two days after high doses of E2 were administered, suggesting that these rats are more sensitive to E2 than their nulliparous counterparts (Bridges & Byrnes, 2006). Furthermore, reproductively-experienced rats outperform age-matched nulliparous rats on spatial

learning tasks at up to 22 months of age (equivalent of senescence in humans), indicating that parity-induced differences in learning and memory function are long-lasting, and perhaps even permanent (Love et al., 2005).

The majority of studies examining the effects of parity on cognition in humans look at immediate effects. For example, pregnancy and the post-partum period are associated with both subjectively and objectively rated impaired verbal memory (Sharp, Brindle, Brown, & Turner, 1993), and this is exacerbated with additional pregnancies (Glynn, 2012). One study found that information processing and verbal memory encoding and retrieval are impaired in reproductively experienced women during pregnancy and this lasts into early motherhood (32 weeks post-partum; de Groot, Vuurman, Hornstra, & Jolla, 2006), suggesting that long-term cognitive function may also be affected by parity in humans.

It's been established that E2 affects multiple memory systems in nulliparous rats, but does this relationship between E2, HPC-mediated place learning and DS-mediated response learning change in rats that have given birth to and reared pups? We have shown, Hussain et al. (2013) that OVX nulliparous rats show patterns similar to what's been shown previously. Interestingly, primiparous rats receiving low or high E2 replacement learn a place and a response task at equivalent speeds and are as likely to use either memory system in an ambiguous t-maze task (Hussain et al., 2013). These results suggest that E2's modulatory effect on multiple memory systems disappears with reproductive experience.

Overall, we now understand that E2 plays a key role in modulating multiple memory systems in rats, and this is also affected by DA transmission in the DS, E2 actions in the medial PFC, and reproductive experience. The question turns to how E2 impacts memory system bias in women.

1.5 Estrogen and memory in women across the lifespan

Estrogen plays a role in cognitive function in both rats and humans, and the importance of this effect is especially meaningful in contemporary society, which is marked by an aging population. Also, the average woman now spends a large portion of her lifespan in the postmenopausal period, which is marked by a dramatic decrease in E2 production and exposure. As mentioned above, E2 has been associated with enhanced cognitive functioning in women, especially when it is replaced during the postmenopausal

period with HRT (Sherwin, 2003; Sherwin & Henry, 2008), which suggests that E2 could be playing a key role in modifying brain function.

Several studies have been carried out that have explored cognitive function in young women and how varying E2 levels could affect it across the menstrual cycle. Most of this work has revealed that high E2 levels are associated with increased memory, verbal fluency, verbal declarative memory, articulation and working memory (Hampson, 1990; Phillips & Sherwin, 1992; Protopescu et al., 2008; Rosenberg & Park, 2002). On the other hand, low E2 levels have been linked to increased visuospatial abilities (Hampson, 1990; Hausmann, Slabberkoorn, Van Goozen, Cohen-Kettenis, & Gunturkin, 2000). Taken together, these studies demonstrate that women tend to perform better on male-typical tasks, such as spatial rotation, when their endogenous E2 levels are low. Conversely, they tend to perform better on female-typical tasks, such as verbal memory, when E2 levels are high.

It's been established that multiple memory system bias is affected by E2 both in the naturally cycling rat and OVX rats given replacement E2, with high E2 being associated with hippocampal place and low E2 associated with striatal response memory, and this effect disappears with reproductive experience (Hussan et al., 2013; Korol & Kolo, 2002; Korol et al., 2004; Quinlan et al., 2008). However, it's presently not known if this pattern occurs in young, cycling women, and if it does, whether it would change after they have had children. We are currently in the process of addressing this question: we are testing young women with and without children in a virtual navigation task (developed by Bohbot and colleagues; see Bohbot et al., 1998; 2004; 2007; Iaria et al., 2003) at different points of their menstrual cycles to determine whether E2 and motherhood play a role in multiple memory system bias. Our preliminary yet unpublished findings thus far suggest that they do.

Several comprehensive longitudinal and observational studies have been carried out to investigate the role of estrogen in cognitive function in older women as they reach menopause. In general, it appears that postmenopausal women who are on HRT perform better on tasks measuring verbal fluency, verbal memory, verbal working memory, and visual memory (Espeland et al., 2004; Gibbs & Gabor, 2003; Maki, 2006; Matthews, Cauley, Yaffe, & Zmuda, 1999; Resnick & Henderson, 2002; Sherwin & Henry, 2008). Furthermore, some studies have shown that there is less general cognitive decline over time

in women with higher endogenous E2 levels (Yaffe et al., 2000), and more cognitive decline in older women with lower endogenous E2 levels (Yaffe et al., 2007). Sherwin and colleagues (1988) investigated premenopausal women who had to undergo surgical menopause. These women were tested on a set of neuropsychological tests before and after surgery. It was found that scores on short- and long-term verbal memory were improved in women receiving HRT, but not in those receiving a placebo (Phillips & Sherwin, 1992; Sherwin, 1988).

Where some studies found benefits to HRT in postmenopausal women, others have either found no differences in cognitive function between women on HRT and those not taking any hormones (Binder et al., 2001; LeBlanc et al., 2007), or worse cognitive function and increased risk for Alzheimer's disease (Espeland et al., 2004; Rapp et al., 2003; Resnick et al., 2006). The Women's Health Initiative (WHI) carried out large-scale clinical trials to shed light on these discrepancies in HRT studies. These trials were discontinued earlier than planned, since it was observed that the women receiving estrogen (conjugated equine estrogen; CEE) and P (medroxyprogesterone acetate; MPA) had an increased risk of developing heart disease, breast cancer, pulmonary embolism, and stroke compared to women in the placebo group. The CEE alone group trials were then also discontinued a year later, as these women showed an increased risk for stroke, compared to the placebo group. Overall, the results of the WHI trials seemed to indicate that HRT (at least using the type of hormones employed in the WHI trials) is harmful to postmenopausal women.

It is important to note that the women tested in these trials were 65 years old on average, which means they were many years past menopause before they were exposed to these hormones. Interestingly, it's been found that women who had initiated HRT at an earlier age show less cognitive decline as they age, which suggests that the time at which HRT is initiated after menopause could have an impact on its efficacy (Matthews et al., 1999). In other words, there seems to be a limited number of years after the onset of menopause in which beginning HRT is beneficial; once this window has closed, the benefits of HRT decrease and may even cause harm, as was observed in the WHI trials. This theory, termed the critical period hypothesis, posits that there is an optimal window of time close to and immediately after both natural and surgically-induced menopause during

which HRT will exert its beneficial cognitive effects (Bagger et al., 2004; Gibbs & Gabor, 2003; Henderson et al., 2005; Maki, 2006; Resnick & Henderson, 2002; Sherwin, 2007). This time-dependent benefit of E2 with age was also shown in rats (Adams et al., 2002; Daniel, Hulst, & Berbling, 2006; Gibbs, 2000) and primates (Hao et al., 2003; Rapp et al., 2003). The critical period hypothesis falls in line with studies showing that ERs downregulate and cease to respond to E2 after a period of time has passed without hormone exposure (Lauber et al., 1990;1991).

It is important to point out that the critical period hypothesis has not always been supported by the evidence at hand. For instance, serotonin receptors are still responsive to E2 in the PFC and striatum in primates that had been OVX for four years (Sánchez, Estrada-Camarena, Bélanger, Morrissette, & Di Paolo, 2011). Furthermore, E2 treatment is associated with improvements in a spatial working memory task in long-term OVX monkeys (Lacreuse, Wilson, & Herndon, 2002). In general, the critical period hypothesis has some support, although some studies show that the timing and initiation of HRT do not, in certain instances, make a difference (for review, see Maki, 2013).

Aside from the age at which HRT was started in the women enrolled in the WHI studies, it should be noted that a significant proportion of them were obese (around 40%), and had preexisting health problems such as hypertension, cardiovascular disease, or diabetes (Sherwin, 2007). Furthermore, the type of hormone compound used, as well as its route of administration can make a difference in whether its effects are beneficial or harmful. When CEE, the compound used in the WHI trials, is metabolized by the body, its major metabolite is estrone sulfate, barely diffuses into the brain and hence may not have effects on cognitive function (Steingold, Cefalu, Pardridge, Judd, & Chaudhuri, 1985). Furthermore, orally administered drugs must be digested within the gastrointestinal tract and undergo first pass metabolism by the liver before becoming biologically active whereas those that are administered intramuscularly or transdermally bypass this process, leading to crucial differences in how effective the estrogen compound will be in the brain Arnsbacher, 2001). Lastly, studies have shown that P may counteract any beneficial effects of E2 (Sherwin & Henry, 2008). However, P administration in HRT is necessary in women with intact uteri and ovaries in order to prevent development of endometrial hyperplasia or

carcinoma (Sherwin & Henry, 2008). This story is complicated and these are still early days of this area of research.

At this time, the available data remains equivocal on whether HRT is beneficial to menopausal women or simply not worth the health risks. The latest analysis of WHI extended trials shows that women treated with CEE & MPA are exposed to a combination of both higher and lower risks for a variety of health problems (Manson et al., 2013). Similar results were found in the CEE alone group, although the risks and benefits were more balanced. It is important to note that fewer risks were observed in the youngest cohort (age 50-59), which lends support to the HRT time window theory.

All in all, despite the wealth of findings generated from animal studies, E2's role in the health and cognitive abilities of both cycling and postmenopausal women remains unclear, as most of the research carried out is limited, sometimes contradictory, and, in the case of the HRT trials, controversial.

1.6 Conclusion

Animal studies have helped us understand that E2 is implicated in learning and memory in a significant way, specifically in whether place or response memory is used to navigate an environment or solve a task. Rodent research on brain ER distribution and how and where E2 acts in the female brain, as well as how it interacts with other neurotransmitters to affect behavior, is providing new insights on the neuronal mechanisms underlying these effects. Conversely, very little is known about E2's actions in the human brain outside of sexual and reproductive function.

With women living longer, particularly following menopause, the impact that E2 may have on female cognition is of great importance. In particular, shedding light on which of the multiple memory systems may be preferentially used as E2 levels vary across the menstrual cycle can help us understand which brain structures and, consequently, memory systems are optimal in different hormonal milieus. Future directions would entail examining this relationship in postmenopausal women to observe whether absence of E2 is correlated with a higher proportion of women relying on striatum-based response memory. It has been posited that the use of spatial memory may be protective against age-related degeneration of the HPC (Bohbot et al., 2007); perhaps menopause and decreasing E2 play a role.

It is clear that more information is needed to understand estrogen's effects on cognitive function in women. Furthering our understanding of how ovarian hormones affect neurobiological function in women hopefully will also inform us about the cognitive health of the increasing population of women who are having more menstrual cycles as well as a population of postmenopausal women who are living longer.

1.7 Rationale and Hypotheses

The purpose of this thesis is to investigate the role of E2 and reproductive experience in multiple memory system bias. First, the effect of varying levels of E2 on performance in a place and response learning task and whether parity modulates this association was observed. Second, since it's been shown that DA is involved in E2's effects on the brain regions that are implicated in place and response memory (Quinlan et al., 2008), DA receptor density was measured in the DS of rats with or without reproductive experience. Third, another purpose of this thesis was to determine if the effect observed in rodents translates to humans; does multiple memory system bias change across the menstrual cycle, and does this differ in women with maternal experience?

Previous work carried out in this laboratory showed that E2 levels modulate whether rats use place or response memory to solve an ambiguous t-maze task and that this effect disappears with parity (Hussain et al., 2013). In the second chapter, nulliparous and primiparous OVX rats were given either low or high E2 replacement and were trained on a place and response maze task in order to observe whether E2 levels affect place and response learning. As observed by Korol's group (Korol & Kolo, 2002), it was hypothesized that nulliparous rats that are high in E2 would learn the place task faster and the response task slower than low E2 rats, because high E2 has been shown to potentiate hippocampal place learning whereas it impairs striatal response learning (Zurkovsky et al., 2007; 2011). Furthermore, as has been previously observed in this laboratory (Hussain et al., 2013), parity leads to observed strategy differences disappearing; therefore, it was expected that primiparous rats show equivalent learning speeds on both tasks, regardless of E2 dose.

Previous research has revealed that the effect of E2 on multiple memory system bias changes with administration of D1R and D2R antagonists, suggesting that DA plays a role in this E2-mediated effect (Quinlan et al., 2013, 2008). In the third chapter, receptor

autoradiography was carried out to quantify D1R & D2R density in the DS of nulliparous and primiparous rats that had been exposed to low or high E2 in order to examine whether there are E2 and parity differences in DA receptor density in the DS. Since it's been shown that E2 is associated with changes in D1R and D2R density in the DS across the cycle (Falardeau & Di Paolo, 1987; Lévesque, Gagnon, & Di Paolo, 1989), it was expected that rats exposed to high E2 would have higher D1R and D2R density when compared to low E2 rats. Since reproductive experience leads to increased striatal DA levels and enhanced sensitivity to DA (Felicio, Florio, Cruz-Casallas, & Bridges, 1996; Byrnes, Byrnes, & Bridges, 2001), it was hypothesized that this could be related to upregulated DA receptors (DAR) in the DS; therefore, it was expected that primiparous rats have higher D1R and D2R density. Finally, D1R and D2R density was also measured in the NAcc core and shell. Though this brain region is not associated with E2's effects on multiple memory system bias, it is a key region that is involved in motivation to solve a task (Grace, Floresco, Goto, & Lodge, 2007; Quinlan et al., 2013), and investigating whether DAR density changes with varying E2 levels and with parity was of interest.

In the fourth chapter, multiple memory system bias was investigated in humans in order to translate these findings to women. Young, healthy, naturally cycling women were tested on a virtual navigation task which can be solved either by using either spatial or response memory (Bohbot et al., 2007; Iaria et al., 2003). Strategy use was compared between the follicular phase, which is characterized by generally low E2 levels, and the luteal phase, which is marked by higher E2 levels. As has been observed in rodents, it was hypothesized that the luteal group (high E2 state) would be associated with spatial memory, whereas the follicular group would use response more often.

Following on from this, in chapter 5, women with and without maternal experience were compared across the menstrual cycle in order to investigate whether reproductive experience changes cycle differences in strategy. As observed in rodents (Hussain et al., 2013), it was hypothesized that the association between E2 and multiple memory bias would disappear in mothers, such that there would be no difference in spatial or response strategy use in mothers across the cycle.

Since there are potential problems with using self-report measures to determine menstrual cycle data, serum levels of E2, P, and T were measured and compared across

cycle phase and strategy in chapter 6. First, it was hypothesized that serum hormone levels would match menstrual cycle data such that the follicular phase would be marked by lower E2 and P levels than the luteal phase, and T would be low and equivalent across phase. Second, we hypothesized that spatial learners will have higher E2 and P levels (consistent with the luteal phase) than response learners.

CHAPTER 2

Reproductive experience modifies the effects of estradiol on learning and memory bias in female rats.

Dema Hussain, Alexandra Hoehne, Barbara Woodside, & Wayne G Brake

Hormones and Behavior, 63, 418-423. doi: 10.1016/j.yhbeh.2012.11.011

Preface

The data from Experiment 1 in this chapter had previously been collected and presented as part of my Master's thesis. The findings from this experiment revealed that OVX nulliparous female rats given low E2 replacement predominantly used response memory to solve a dual solution t-maze task, whereas rats given high E2 replacement used spatial memory more often, though this was not statistically significant. However, this observed effect was not observed in primiparous rats; reproductively experienced rats showed no difference in memory bias, regardless of E2 dose. This was novel evidence that showed that parity alters E2's effect on multiple memory system bias. To extend these findings, a follow-up experiment was carried out at the beginning of my PhD in order to investigate whether parity would similarly affect E2-induced differences in response and spatial learning.

Abstract

Previous studies have shown that estrogen affects whether a hippocampus (HPC)-mediated place (allocentric) or a striatum-mediated response (egocentric) memory system is employed by female rats when searching for a food reward in a maze. Because it has been suggested that reproductive experience alters some of the responses to E in the brain, two experiments were carried out to investigate whether reproductive experience would also alter the effect of E on place and response learning. In experiment 1, 152 ovariectomized (OVX) nulliparous (n = 77; no reproductive experience) and primiparous (n = 74; having had and raised one litter of pups) Wistar rats were trained on an ambiguous t-maze task and tested for memory system bias. In experiment 2, 35 ovariectomized nulliparous (n = 16) and primiparous (n = 19) Wistar rats were trained on place and response plus-maze tasks. All rats were exposed to no, chronic low or chronic low with pulsatile high dose of 17β-estradiol (E2) replacement. Congruent with previous findings, low E2 nulliparous rats showed predominant use of response memory and faster response learning, whereas high E2 nulliparous rats showed a trend towards predominant place memory use. Interestingly, the facilitatory effect of low E2 on response task learning and memory seen in nulliparous rats was not observed in low E2 primiparous rats in either experiment. In conclusion, E2 levels do dictate the rate at which female rats learn a response task and utilize response memory, but only in those with no reproductive experience.

2.1 Introduction

The effects of estrogen (E) on the female brain are not exclusive to reproductive function. Research carried out in rats, for example, suggests that E affects which memory system is used to solve a task. Specifically, in female rats the level of circulating E affects whether a hippocampus- (HPC) mediated place (allocentric) or a striatum-mediated response (egocentric) memory system is employed when searching for a food reward in a maze (Korol, 2004; Quinlan, Hussain, & Brake, 2008). Thus, varying responsivity to E, as has been described in female rats with reproductive experience (e.g., Byrnes, Casey, & Bridges, 2012), would be expected to affect memory bias.

There is evidence in the male rat that place learning and response learning are separate competing memory systems that rely on different brain pathways. When hippocampal integrity is impaired, performance on tasks that rely on spatial cues is diminished (Packard and McGaugh, 1996), and both hippocampal lesions and pharmacological inhibition of the HPC lead to impaired spatial memory and place learning (McDonald and White, 1994; Packard and McGaugh, 1996). For example, when muscimol (a $GABA_A$ receptor agonist) is injected into the dorsal HPC, place memory is impaired (Mao and Robinson, 1998) and when hippocampal function is impaired using lidocaine, rats rely on response memory instead (Packard, Cahill, & McGaugh, 1994).

Conversely, damage to the dorsal striatum (DS) results in impaired response memory, but not place memory (Kesner, 1990). Lesions to the DS result in impaired performance on tasks dependent on response memory (McDonald and White, 1994), and lidocaine infusions into the DS also lead to impaired response memory. Inactivation of the HPC has been shown to result in deficient place memory, while damage to the DS leads to impaired response memory in a cross-maze task (Packard and McGaugh, 1996).

It has been shown that E affects the brain areas underlying these two memory systems in female rats. In ovariectomized (OVX) rats, 17β-estradiol (E2) replacement enhances learning of a place task, but impairs learning of a response task (Korol and Kolo, 2002). In an ambiguous t-maze task, it has been found repeatedly that OVX rats with E2 replacement predominantly use place memory, whereas the opposite pattern is seen in those without E2 (Korol, Malin, Borden, Busby, & Couper-Leo, 2004; Quinlan et al., 2008). The same effect is observed in naturally cycling females. Rats in proestrus, the phase of the

estrous cycle when E is highest, use a place strategy more often on a t-maze task. Conversely, rats in estrus, the phase when E is lowest, are more likely to use a response strategy (Korol et al., 2004). These findings indicate that E plays a key role in memory system bias in females when completing a task.

E has also been linked to a variety of morphological and neurochemical alterations in the HPC, such as increased spine density (Woolley and McEwen, 1992; Woolley, 1998), increased excitatory synapses and synaptic boutons (Woolley, Wenzel, & Schwartzkroin, 1996) and increased synaptic proteins (Brake et al., 2001). Physiologically high (50-90 pg/ml) levels of E are also linked to increased acetylcholine levels in the HPC, especially during place learning (Gabor et al., 2003; Marriott and Korol, 2003). In the DS, on the other hand, it has been suggested that E modulates response learning via potentiation of dopamine release (Quinlan et al., 2008).

Although it is well established that E modulates memory system bias in female rats, these effects have been investigated only in rats with no reproductive experience (viz. nulliparous). There is accumulating evidence that thee experience of pregnancy and pup rearing causes long-term changes in female rats that are still apparent after the young are weaned. These organizational effects are wide ranging and include alterations in stress and anxiety behavior (Wartella et al., 2003; Lambert et al., 2005) and spatial learning (Byrnes and Bridges, 2006). Neither the particular aspects of reproductive experience nor the precise mechanism(s) underlying these effects have been fully elucidated. However, the fact that nulliparous females who were induced to show maternal behavior by exposure to foster pups learned a spatial task as quickly as reproductively experienced rats, suggests that the act of caring for young may by itself be sufficient to induce these long-term changes (Kinsley et al., 1999).

Indeed, long-term changes in sensitivity to E following parturition have been reported in reproductively experienced rats. Bridges and Byrnes (2006) found that reproductively-experienced rats showed higher prolactin secretion two days after high doses of E were administered, suggesting that these rats were more sensitive to E than their nulliparous counterparts (Bridges and Byrnes, 2006). Furthermore, aged parous rats have also been shown to outperform age-matched nulliparous rats on spatial learning tasks at up

to 22 months of age, indicating that parity-induced differences in learning and memory function are long-lasting (Love et al., 2005).

These results raise the possibility that E would have differential effects on learning and memory systems in nulliparous and reproductively experienced rats. As shown previously, nulliparous OVX rats tend to utilize HPC-mediated place memory when given high levels of E2 replacement, whereas they use DS-mediated response memory under low E2 replacement (Korol, 2004; Quinlan et al., 2008). Here we compared the behavior of nulliparous OVX rats with either no, chronic low, or chronic low with pulsatile high E2 replacement with that of OVX females that had previously raised one litter (viz. primiparous) given the same hormone replacements. In experiment 1, rats were tested in an ambiguous t-maze task to determine memory system bias. In experiment 2, rats were trained on either a place or response task to determine if there were learning differences.

2.2 Methods

Experiment 1

2.2.1 Subjects

A total of 152, three- to four-month old female Wistar rats were used in this experiment (Charles River, St-Constant, QC). Seventy-four of these rats were primiparous females, which had given birth one month prior to testing. For breeding, these rats were group-housed (5 females and 1 male per cage) in hanging cages (dimensions: 37.6 cm wide X 56.3 cm long X 22.2 cm high) for a maximum duration of 21 days, after which the pregnant rats were moved to single shoe-box cages (dimensions: 25.5 cm wide X 46.6 cm long X 21.6 cm high). Immediately after birth, the number of pups was culled to 8 per dam. The pups were then kept with the dams for 3 weeks, after which they were all weaned and dams were then housed in pairs until surgery.

The remaining 77 rats were age-matched nulliparous females with no prior reproductive experience. Both nulliparous and primiparous groups were randomly assigned to 3 treatment conditions: no E2 (n = 39), chronic low E2 (n = 58), and chronic low with pulsatile high E2 (n = 55) dose. All rats were housed in plastic shoe-box cages, under a 12 h reverse light-dark cycle (2100 to 0900). Rats were housed in pairs prior to OVX surgery, after which they were housed singly. All rats had *ad libitum* access to rat chow and water, except for the training and testing periods, during which they were food restricted. Starting

three days prior to the beginning of training, food was restricted and weight was maintained at 90% of free-feeding levels. The rats were handled daily prior to and throughout the experiment. All procedures involving rats were in accordance with guidelines established by the Canadian Council on Animal Care and approved by the Concordia Animal Research Ethics Committee.

2.2.2 Apparatus

All training and testing was carried out in a black Plexiglas t-maze situated on a table one meter above the floor (see Figure 2.1). The t-maze was comprised of black walls (28 cm high), grid floors, a start arm (130 cm long) and two goal arms (75 cm long), which were each positioned at a 90 degree angle to the start arm. The start arm contained a removable door, which created a start chamber. Each goal arm contained a white ceramic bowl placed at its end. Froot Loops (Kellogg's) were placed underneath the maze at each bowl to later avoid odor cues during testing. Two guillotine doors separated the goal arms from the choice point of the start arm and could be closed to prevent animals from leaving the goal arm once they were inside. An additional arm (identical in dimensions to the start arm) was added to the t-maze to form a plus-shaped maze. Another guillotine door closed off access to this part of the maze at all times, except during probe testing. The t-maze was situated in a room dimly lit with overhead red fluorescent lamps, a lamp facing the wall (40W light bulb), and a small 15W light bulb mounted on one of the walls. Other spatial cues included cupboards and posters on the walls.

2.2.3 Surgeries and estrogen replacement

2.2.3.1 Ovariectomy surgery

Approximately 2 to 3 days following weaning of pups from the primiparous group, surgeries were conducted on all rats. Prior to surgery, rats were anaesthethized using Halothane gas (4% for induction, 2% for maintenance) and the ovaries were removed bilaterally through a dorsal incision using standard aseptic procedures, and the incision was then sutured using 9 mm stainless steel surgical staples (EZ clips; Stoelting Co., Wood Dale, Illinois). Following surgery, rats were administered the analgesic Anafen (0.1 ml/animal, s.c.) and the antibiotic Penicillin G (0.1 ml/animal, i.m.). Saline was also administered to rehydrate the animals (1.5 ml/animal, s.c., bilaterally). Each rat's health was monitored daily following surgery.

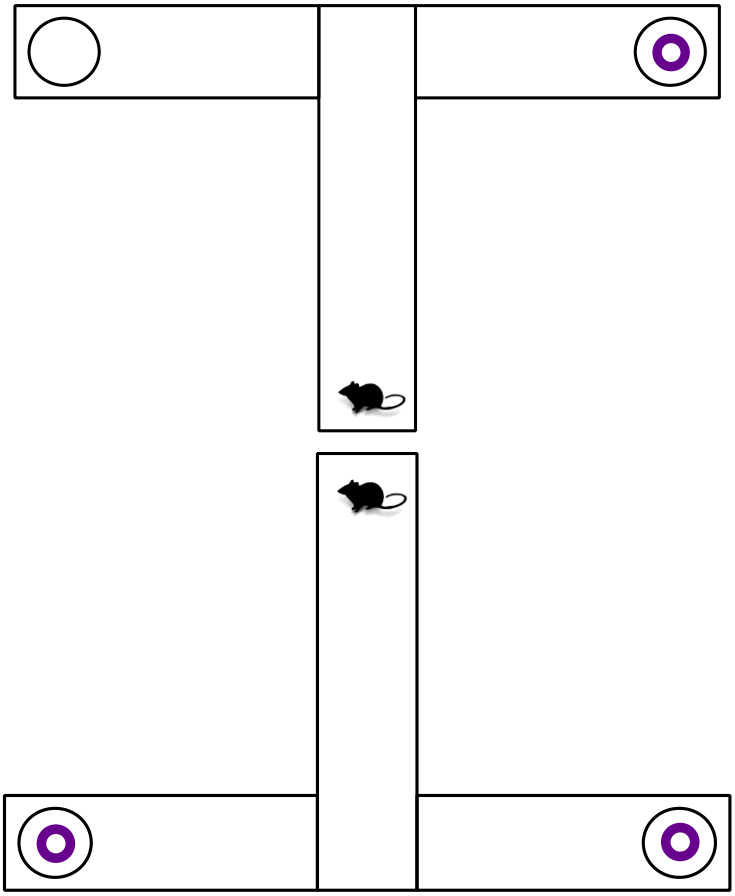


Figure 2.1. The t-maze and modified plus-maze designs used were adapted from Korol et al. (2004). Top panel. Rats were initially trained to receive a reward in either the left or right goal arm. Bottom panel. Upon reaching criterion, rats were then placed in the opposite arm for the probe trial. The dark ring represents the reward.

2.2.3.2 Hormone administration

At the same time as the ovariectomy surgery, a small incision was made in the nape of the neck and a Silastic tube was inserted (1 cm long). The implant releases a low, chronic, steady dose of 5% E2 in cholesterol resulting in a 20 pg/ml serum concentration (Mannino, South, Inturrisi, Quinones-Jenab, & 2005). This procedure was carried out to provide all rats with a chronic low basal rate of E2 similar to that seen in the estrus phase of the rat estrous cycle (Quinlan et al., 2008). After an initial peak and decline of serum E2 levels, a steady dose has been shown to last approximately 24 days post-surgery (Mannino et al., 2005).

Rats in the pulsatile high E2 condition were administered daily injections of E2 (10 μg/kg, SC; Sigma Chemical Co.). This dose has been previously shown to result in circulating E levels of approximately (75-90 pg/ml; Quinlan et al., 2008). Rats in the chronic low E2 and no E2 replacement conditions were given daily injections of sesame oil vehicle (0.1 ml, s.c.). All injections took place daily between 1600-1800 hrs. Thus, there were three conditions, OVX with no, chronic low, or chronic low with pulsatile high E2 replacement. The rationale for having chronic low dose E2 in one condition and chronic low dose E2 plus daily acute injections of high E2 in another condition was to mimic the pulsatile nature of E2 during proestrus in the high E2 condition.

2.2.4 Behavioral testing

2.2.4.1 Training

Training began one week following surgery. On the first three days of training, all rats were exposed to a daily 10 min habituation session in the t-maze to familiarize them with the environment. All guillotine doors were open and food rewards (Kellogg's Froot Loops) were distributed around the maze. Following habituation, training was started. Rats were paired and each pair randomly assigned a direction (right or left arm), which they would subsequently be trained to enter in order to receive the reward. The reward arm remained constant relative to spatial cues around the room. All training and testing was started at approximately 1400-1500 hrs.

Each rat was given 10 daily choice trials (alternating with its paired partner), in which the rat was placed in the start arm and all doors removed to allow the animal to choose a directional arm. Once the rat had entered either one of the two goal arms, the

guillotine doors were closed and the rat removed after having eaten the food reward at the end of the arm. If the incorrect arm was chosen, the rat was permitted enough time to explore the empty bowl before being removed from the maze. The rats were considered to have successfully learned this task once they reached a criterion of 80% correct trials, that is, 8 out of the 10 daily trials, for three days in a row. Based on previous findings (Korol, 2004), the number of days needed to reach this criterion is approximately 15-20 days, thus, rats that did not reach criterion by 20 days were omitted from the study and additional rats were tested to compensate for the reduced *n*.

2.2.4.2 Probe tests

On the third day of criterion, the rats were exposed to a probe test immediately after their tenth trial. Once the rat had completed the last trial, it was placed in the probe arm situated 180 degrees relative to the start arm (see Figure 2.1). The start arm was closed and all other doors opened to allow the rat to enter a goal arm. If the rat entered the same arm where it received a reward during training (e.g., if the rat was trained to go to the right arm from the start arm to receive the reward, then entered the same arm during the probe test), it was deemed as using place memory; the rat turned to the same spatial location where the reward was found during training. If the rat entered the opposite arm to that used during training (e.g., the rat previously trained to receive the reward in the right arm during training now enters the left arm during the probe trial), it was deemed to be using response memory; the rat turned in the same direction as it did during training.

2.2.5 Statistical Analysis

The dependent variable of interest in this experiment was the proportion of rats using a particular strategy. Thus, non-parametric statistics were used to analyze the results. To compare strategy use within each of the 6 experimental conditions, Chi square (χ^2) tests were carried out. Kruskal-Wallis tests were used to determine whether the E2 replacement condition altered memory bias in either nulliparous or primiparous rats. To determine whether there was a different rate of learning the task, days to criterion were compared across all groups using a 2-way analysis of variance (ANOVA).

Experiment 2

2.2.6 Subjects

A mixed design in which each rat was tested on both a place and a response task was used in this experiment. A total of 35 five to six-month old Wistar rats were used; nineteen of these were primiparous and 16 were nulliparous (all animals were housed as described in experiment 1). All rats were randomly assigned to either chronic low E2 or pulsatile high E2 replacement and trained on either a place or response task. They were then switched to the other E2 condition and trained on the second task in a counterbalanced manner. All surgeries, implantations, hormone replacement, and timing of training and testing was carried out as in experiment 1.

2.2.7 Apparatus

All training and testing was carried out in a black Plexiglas plus-maze situated on a table one meter above the floor. The plus-maze was comprised of four arms (75 cm long) with black walls (28 cm high), grid floors and transparent Plexiglas ceiling panels. Each goal arm contained a white ceramic bowl placed at its end. Froot Loops (Kellogg's) were placed underneath the maze at each bowl to avoid odor cues during testing. The plus-maze was situated in a room dimly lit with overhead red fluorescent lamps, a lamp facing the wall (40W light bulb). Other spatial cues included cupboards and posters on the walls.

2.2.8 Behavioral testing

2.2.8.1 Place task

All rats were habituated as described in experiment 1. Rats were randomly assigned to one of the four arms of the plus-maze, which they would subsequently be trained to enter in order to receive the reward. The reward arm remained constant relative to spatial cues around the room.

Each rat was exposed to 10 daily choice trials. The rat was placed in a pseudo-randomly chosen, non-baited arm such that a rat never started in the same arm more than three times in a row (see Figure 2.2). Rats were tested as described in experiment 1. Rats were given at least a 30 sec interval between trials. The rats were considered to have successfully learned this task once they reached a criterion of 80% correct trials in a single day. Based upon our pilot study, most rats learn this task within a week; rats that did not learn the task within 15 days were omitted from the study.

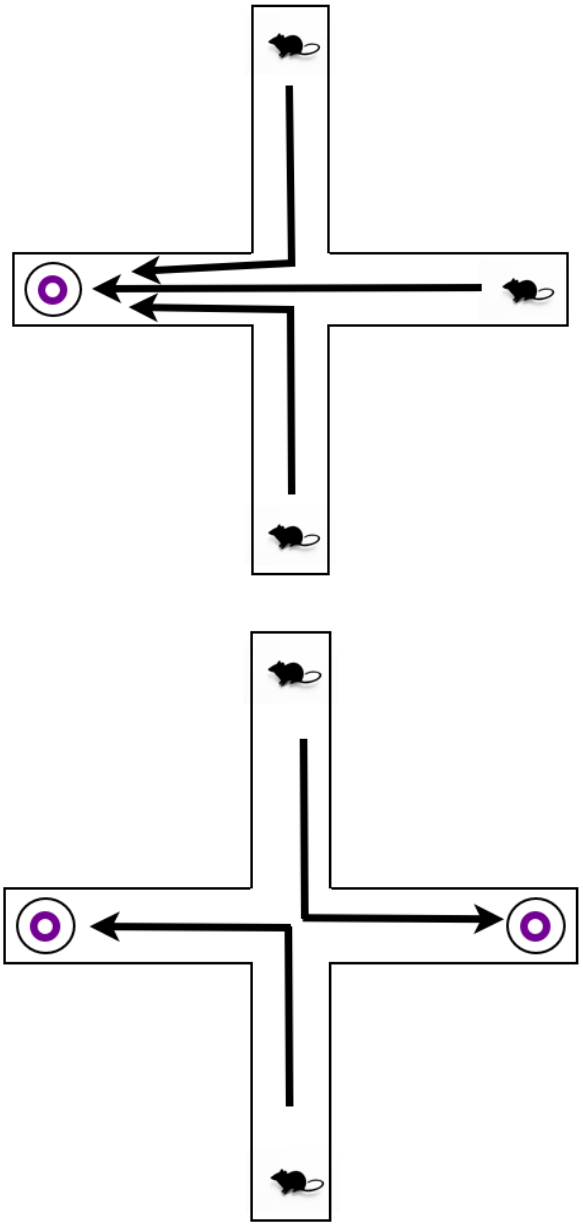


Figure 2.2. Illustration of the place and response plus-maze tasks. Top panel. On the place task, rats were trained to receive a reward in the same spatial location in the maze. The start arm varied across trials. Bottom panel. On the response task, rats were trained to receive a reward by either turning 90 degrees to the right or left relative to the start arm.

2.2.8.2 Response task

All rats were habituated as described for experiment 1. Rats were randomly assigned a direction (turn right or left), which they would subsequently be trained to enter in order to receive the reward. Each rat was given 10 daily choice trials. The rat was placed in a pseudo-randomly chosen, non-baited arm such that a rat never started in the same arm more than three times in a row (see Figure 2.2). The doors were opened to allow the animal to choose an arm. The arm that was situated at 180 degrees relative to where the rat was placed was always closed, to give the rat the choice to turn right or left only. Rats were tested exactly as described above in experiment 1.

A 3-way mixed ANOVA was carried out with parity as a between factor and E2 level and task as within factors. Furthermore, post-hoc t tests using a Bonferroni correction were used to determine whether there were any differences between E2 and parity groups with respect to place and response learning.

2.3 Results

2.3.1 Experiment 1

Groups learned the task at the same rate as revealed by no significant interaction or main effects from the ANOVA. Mean number of days to criterion +/- SEM for each group are: nulliparous; no E2 (6.0 +/-0.47), chronic low E2 (6.6 +/-0.43), pulsatile high E2 (6.1 +/-0.34): primiparous; no E2 (5.7 +/-0.47), chronic low E2 (5.6 +/-0.47), pulsatile high E2 (5.7+/-0.40).

A significantly higher proportion of nulliparous rats given chronic low E2 replacement used response memory ($\chi^2 = 3.903$, $p = .048$; see Figure 2.3), supporting previous findings (Korol, 2004a; Quinlan et al., 2008). There was no evidence for a significant memory bias in any of the other groups, although both nulliparous and primiparous rats given pulstaile high E2 replacement showed a trend towards using place memory.

Kruskal-Wallis analyses revealed a significant difference in memory bias between chronic low and pulsatile high E2 nulliparous rats ($\chi^2 = 5.374$, $p = .020$; see Figure 2.3), indicating that the proportion of rats using response or place memory was reversed in these two conditions. Again, these data support previous findings showing that low E2 rats

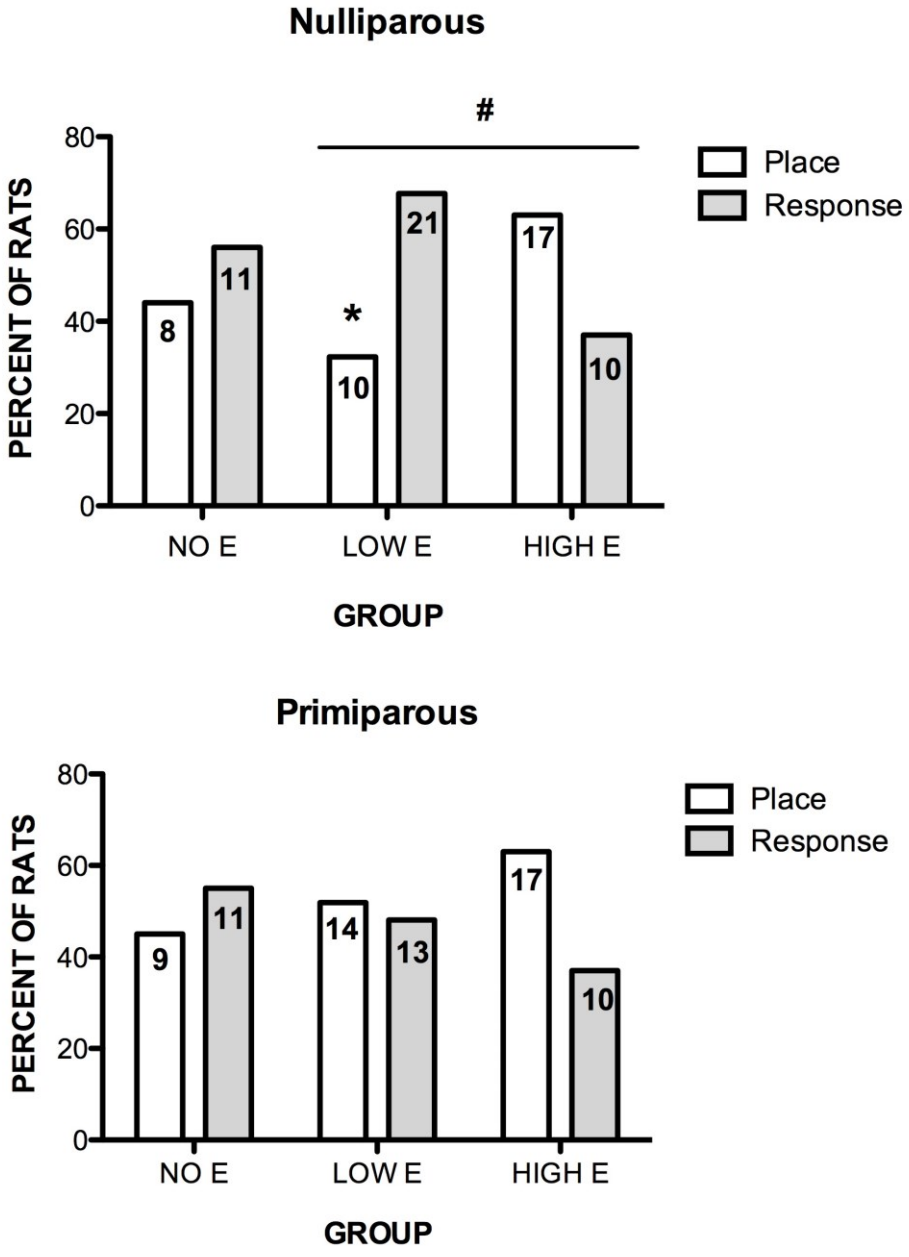


Figure 2.3. Proportion of nulliparous (top panel) and primiparous (bottom panel) rats using either place or response learning in no, low, and high E2 conditions. * indicates significant difference between place and response learning in low E2, nulliparous rats; $p = .048$. # indicates significant difference in proportion of low and high E2 nulliparous rats using place and response learning; $p = .02$.

predominantly use response memory, and high E2 rats place memory (Korol, 2004; Quinlan et al., 2008). Notably, no such effect was observed in the primiparous groups.

2.3.2 Experiment 2

Performance on a place or response task was dependent on both reproductive experience and E2 level resulting in a significant task by parity by E2 level interaction ($F_{(1,27)} = 6.797$, $p = .015$; see Figure 2.4). Findings also revealed a significant task by parity interaction ($F_{(1,27)} = 6.797$, $p = .015$) and a significant effect for task ($F_{(1,27)} = 4.665$, $p = .040$).

Paired samples t-tests showed that nulliparous rats given chronic low E2 replacement were significantly faster at learning the response task than the place task ($t_{(1,6)} = 6.844$, $p < 0.0001$; see Figure 2.4). Although there was a trend, nulliparous rats given pulsatile high E2 replacement were not significantly slower than low E2 nulliparous rats on learning the response task ($t_{(1,7)} = 2.253$, $p = 0.059$). Post-hoc, independent samples t-tests revealed that primiparous rats were slower to learn the response task than the nulliparous group when administered chronic low E2 replacement ($t_{(1,13)} = 3.598$, $p = 0.003$; see Figure 2.4). No other significant differences were observed between primiparous and nulliparous groups.

2.4 Discussion

The results of experiment 1 showed that E2 dose did not affect use of response memory observed in primiparous females, although, as expected, nulliparous females in the chronic low E2 condition used this strategy (see Figure 2.3). Furthermore, primiparous OVX rats without E2 replacement did not differ in memory type, indicating that OVX, itself, abolishes memory system bias in both reproductively naïve and experienced females. The findings from experiment 1 support previous research carried out on nulliparous rats (Korol, 2004; Quinlan et al., 2008): OVX rats receiving chronic low E2 replacement predominantly used striatally-mediated response memory, and those receiving pulsatile

high E2 replacement showed a strong trend towards using hippocampally-mediated place memory, and both of these groups significantly differ from each other (see Figure 2.3). It was expected that OVX rats without E2 replacement would behave like the low E2 group, as previous studies have shown that oil-treated OVX rats tend to use response

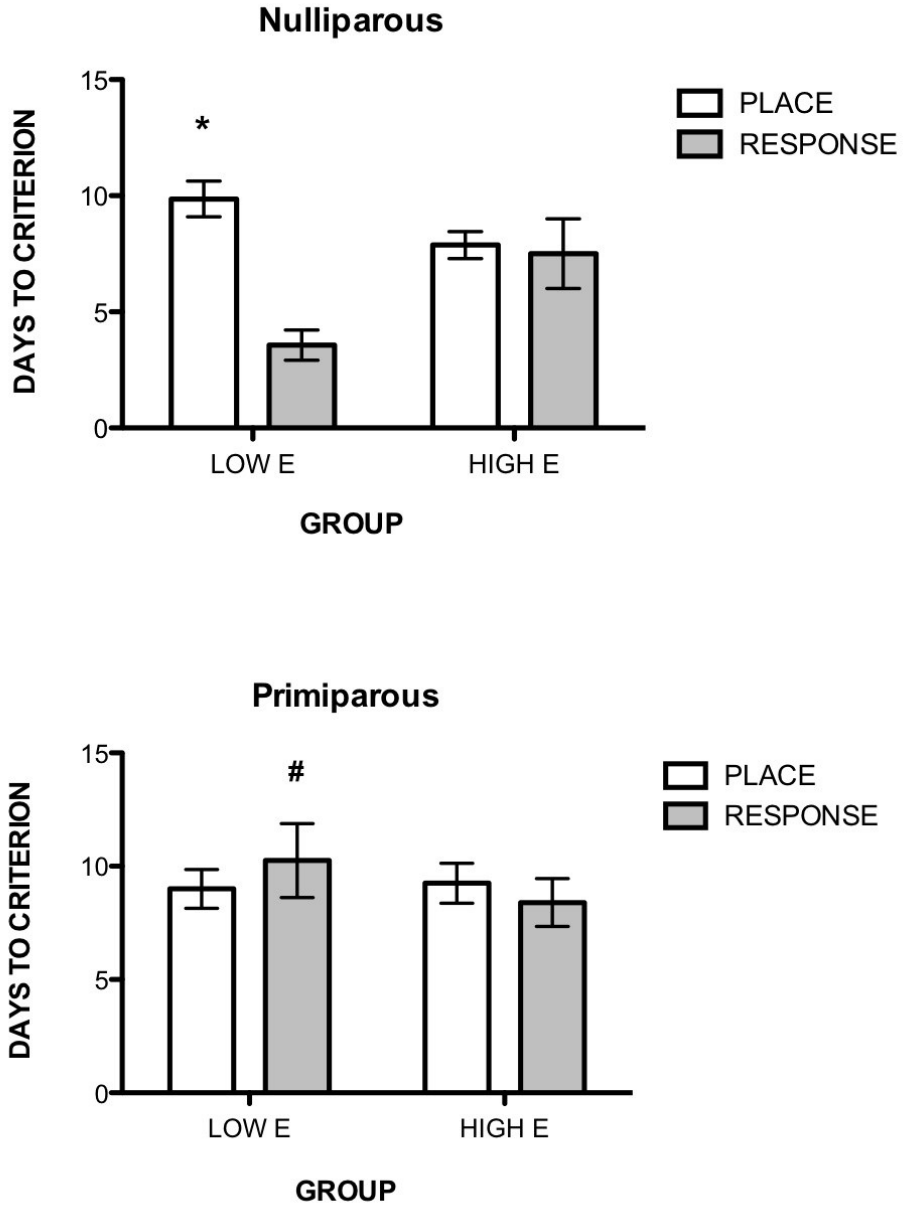


Figure 2.4. Numbers of days to criterion for nulliparous (top panel) and primiparous (bottom panel) rats across E2 group and task type. * indicates a significant difference between number of days to criterion on place and response tasks in low E2 nulliparous rats; $p < .0001$. # indicates a significant difference between nulliparous and primiparous low E2 rats on a response task; $p = .00$.

memory, as well as learn a response task faster (Korol and Kolo, 2002; Korol et al., 2004). However,the rats without E2 replacement in this study showed an equal propensity to use either memory system, and thus did not behave like the low E2 rats. One difference here from previous experiments is that Wistar rats were used whereas Sprague Dawley rats were used in previous experiments examining OVX rats without E2 replacement.

Finally, the high E2 primiparous group showed the same pattern as the high E2 nulliparous groups; all rats exposed to a pulsatile high E2 dose showed a strong, but non-significant, trend towards use of place memory (see Figure 2.3). One possible source of the discrepancy between these and past findings (Korol, 2004; Quinlan et al., 2008) is that the room was dimly lit to reduce anxiety. Such lighting could reduce the number of extra-maze cues available to the rat and this could potentially reduce place learning (McNamara, Long, & Wike, 1956). Overall, the effects of E2 observed in nulliparous rats seem to disappear with parity, especially with the chronic low E2 dose.

Primiparous rats in experiment 2 showed no learning differences across E2 dose; the number of days taken to learn either the response or place task was similar, which suggests that reproductive experience modified E2's modulatory effects on response and place learning. Furthermore, the faster response learning observed in low E2 nulliparous rats was no longer seen in their primiparous counterparts. This is again consistent with the results of experiment 1 insofar as in that experiment the changes in response memory in response to a chronic low E2 dose in nulliparous rats were not observed in the primiparous group (see Figure 2.4). In general, the results of both experiments show that chronic low E2 replacement is linked with potentiated response learning and memory, and that this effect disappears in reproductively experienced rats, rendering them equally proficient at either memory system across E2 dose and administration.

As expected, nulliparous rats receiving chronic low E2 replacement learned a response task significantly faster than the place task, mirroring the low E2 nulliparous rats' predominant use of response memory in the first experiment (see Figure 2.4) and supporting previous findings (Korol and Kolo, 2002; Korol, 2004; Quinlan et al., 2008). However, the expected facilitation of place learning in nulliparous rats receiving pulsatile high E2 replacement was not observed here; both tasks were learned with equal speed. It is possible that this high E2 facilitation of place learning was not observed here because of the

age of the rats used in this experiment. Whereas Korol and Kolo (2002) used three- to four-month old rats in their study, the rats used here were older, with some being tested at almost 6 months of age by the time the experiments started. Indeed, it has been shown that the task-dependent effects of E2 on hippocampal and striatal activation appear with age (Pleil, Glenn, & Williams, 2011), which indicates that place and response learning are accompanied by different brain changes depending not only on E2 level, but also on the age of the rats. However, the number of days to reach criterion observed in Pleil et al.'s four-month old rats were similar to what was found in this study, with oil-treated OVX rats learning the response task significantly faster than E2-treated OVX rats whereas there were no differences in learning speed in the place task.

Enhanced sensitivity to E in reproductively experienced rats could be playing a role in the disruption of the E-induced memory system bias that is observed in nulliparous females. As parous females are exposed to heightened levels of E for a significant period of time (Bridges, 1984), and show increased E receptor α (ERα) density in the pituitary gland, medial preoptic area and amygdala (Byrnes, Babb, & Bridges, 2009), females could potentially become more sensitive to E. Recent work has shown that there is an increase in ERα positive cells in the DS of primiparous middle-aged rats, when compared to nulliparous females (Byrnes et al., 2009), which indicates that parity may also affect long-term striatal ER expression and sensitivity to E.

Furthermore, primiparous rats receiving chronic low E2 replacement might no longer rely on a response strategy due to disrupted DA transmission in the DS, as reproductive experience has been shown to alter striatal DAergic function (Felicio, Florio, Cruz-Casallas, & Bridges, 1996) thus response learning could be compromised. This would explain why, in both experiments, low E2 primiparous rats seem to no longer predominantly use response memory or learn a response task faster, and instead rely on both memory systems equally.

Another factor that could play a role in the behavioral differences observed here between low and high E2 rats is the way E2 was administered. Steady, tonic E2 release via Silastic capsules has been associated with reinstated reproductive and sexual behavior in OVX rats after 7 days, providing an advantageous, stable testing alternative to studying hormonal effects on behavior in naturally cycling females (Mannino et al., 2005). However,

others have pointed out that female rats exposed to a continuous low dose of E2 express maintained estrus phase-like sexual receptivity, which is not ecologically valid in terms of normal endocrine functioning (Albert et al., 1991). Conversely, acute E2 administration results in increased female receptivity 48 hours after hormone injection (Brandling-Bennett, Blasberg, & Clark, 1999). It is therefore possible that these differences in female receptivity are underscored by differential ER priming in response to chronic and pulsatile E2 administration. In the present study, rats in the low E2 condition received a tonic, constant dose of E2, whereas those in the high E2 condition received both the chronic implant with the addition of daily acute high E2 injections. Thus, the pulsatile nature of the high dose of E2 may contribute to the differences observed here.

It is apparent that E's effects could be occurring through a variety of mechanisms, and more research is needed to further elucidate exactly how E and reproductive experience are causing these key changes in the female brain. In the present study, it is unclear why the E2-modulated effects in response learning and memory were no longer observed in the primiparous rats, though perhaps such E-sensitive behaviors are dampened in the female brain once maternity and pup-rearing become a priority. It is possible that other behaviors, types of learning and memory take precedence over memory system bias. Increasing our understanding of how E and reproductive experience alter the female brain can help shed light on the intricate changes that occur across hormonal cycles, pregnancy, and maternal experience in women.

CHAPTER 3

Reproductive experience decreases dopamine D2 receptor binding in the dorsal striatum of the female rat

Dema Hussain, Dean M. Graham, Barbara Woodside, Wayne G. Brake

Preface

The previous chapter provides evidence that low E2 levels are associated with improved learning on a response-based task, but only in rats with no reproductive experience. Parity is associated with no differences in learning, regardless of E2 levels. Since response memory and learning relies on the interaction between E2 action and DA transmission in the DS, it would be predicted that parity is altering the relationship between low E2 levels on DA function in this brain area. It has previously been observed that E2 changes both D1R and D2R density in the DS. Moreover, E2 affects DS DA-mediated behaviors and DA levels in this region. Furthermore, it has been demonstrated that D1R and D2R antagonists modify memory bias. Finally, parity is associated with long-term changes in DA levels and sensitivity in the DS. Thus, one possible mechanism for the parity-induced changes in response memory observed in the previous chapter would be D1R and D2R density in the DS. Thus, in the present study, receptor autoradiography was carried out in order to quantify D1R and D2R binding in the DS and in the core and shell of the NAcc, an area where DA is thought to be important for motivation to complete a task, in nulliparous and primiparous rats that had previously been exposed to low and high E2 levels.

Abstract

Rats use multiple memory systems to find a reward in a familiar maze. Circulating estrogen levels bias which memory system will be used by female rats. Parity also affects memory system bias because the effects of estrogen dissipate after a female has given birth to and raised a litter of pups. A possible mechanism underlying this behavioral effect is estrogen's ability to alter dopamine receptors in the striatum. Here dopamine D1 and D2 receptor binding was measured in both the dorsal and ventral (nucleus accumbens) striatum of ovariectomized female rats with either chronic low or chronic low plus phasic high estradiol replacement. Female rats had either no reproductive experience (nulliparous) or had given birth and raised one litter of pups (primiparous). No differences in dopamine receptor levels were observed across estradiol levels used here. There was an effect of parity, however. Contrary to expectation, nulliparous rats had significantly more D2 receptor binding in the dorsal striatum than primiparous rats. No other differences were observed across groups. These results suggest that D2 receptor binding decreases with reproductive experience, and that this could be a potential mechanism to previously observed changes in multiple memory system bias in reproductively experienced rats.

3.1 Introduction

The effects of the steroid hormone, estrogen, in the female brain extend beyond sexual and reproductive function. For instance, in rodents, circulating levels of 17β-estradiol (E2) affect which memory system is used to find a reward in a location where it was previously found. That is, higher E2 levels bias female rats to use hippocampus (HPC)-mediated place memory rather than dorsal striatum (DS)-mediated response memory to navigate a maze (Hussain, Hoehne, Woodside, & Brake, 2013; Korol & Kolo, 2002; Korol et al., 2004; Quinlan, Hussain, & Brake, 2008; Zurkovsky, Brown, Boyd, Fell, & Korol, 2006). With lower E2 levels, the opposite is true. This association between E2 and memory system use changes with reproductive experience such that primiparous rats use both memory systems equally, regardless of E2 levels (Hussain et al., 2013).

Response memory is also dependent upon dopamine (DA) transmission in the DS (Packard, Cahill, & McGaugh, 1994; Packard & White, 1991), and striatal DA receptors increase following E2 replacement in ovariectomized (OVX) rats (Dupont, Paolo, Gagné, & Barden, 1981; Falardeau & Paolo, 1987). Furthermore, sensitivity of the striatal DA system increases with reproductive experience (Byrnes et al., 2001). It can therefore be posited that striatal DA receptor binding differs depending on circulating estrogen levels as well as reproductive experience, and such differences could be one mechanism whereby either circulating E2 levels and/or parity affect memory system bias in female rats.

The DS is implicated in a variety of learning and memory functions, such as cue-based learning, habit forming and the formation of stimulus-response associations (Packard & Knowlton, 2002; Yin et al., 2004; Zurkovsky et al., 2011). It is also important for response memory, in which egocentric, internal body cues are used in order to learn how to navigate an environment. Indeed, it has been consistently shown that damage to the DS leads to impaired performance on response-based tasks and in response memory (McDonald & White, 1994; Packard, Hirsh, & White, 1989; Packard & McGaugh, 1996; White & McDonald, 2002). There is also evidence that the underlying mechanism of this link between response memory and the DS seems to be, at least partially, DA mediated. For instance, Packard and colleagues have demonstrated that direct administration of DA agonists targeting either D1 or D2 receptors (D1R and D2R, respectively) into the DS leads to enhanced performance on a response task. On the other hand, they have no effect on

performance in a hippocampally-dependent place, or spatial, task (Packard, Cahill, & McGaugh, 1994; Packard & White, 1991). This suggests that DA function within the striatum is one of the mechanisms responsible for response learning and memory.

In females, it has been consistently observed that E2 plays a role in the likelihood that response memory will be employed to solve a maze as well as in performance on response-based tasks. Several studies have shown that naturally cycling rats tested during the estrus phase, when E2 is low, perform better in response learning tasks and tend to use response memory more often, whereas the opposite is seen in proestrus, when E2 levels are high (Korol et al., 2004). In addition, the same pattern is observed in ovariectomized (OVX) female rats with no or low levels of E2 replacement (Davis et al., 2005; Galea et al., 2001; Hussain et al., 2013; Korol & Kolo, 2002; Quinlan et al., 2008; Zurkovsky et al., 2011). This has also been observed directly following E2 infusion into the DS, which suggests that E2 impairs response learning and memory by acting rapidly within the DS (Zurkovsky et al., 2007). It is believed that this is due to an interaction between E2 and DA function in the DS; indeed, it has been shown that E2 enhances DA release and transmission in this brain region (Becker & Beer, 1986; Becker & Cha, 1989; Becker, Robinson, & Lorenz, 1982; Becker, Snyder, Miller, Westgate, & Jenuwine, 1987; Becker, 1990, 1999; Becker & Rudick, 1999; Castner, Xiao, & Becker, 1993). Also, E2 replacement is related to higher density and sensitivity of DA receptors in the DS in OVX rats. That is to say, ovariectomy reduces DA receptors in the DS and E2 replacement restores these levels (Bossé et al., 1997; Dupont et al., 1981; Falardeau & Di Paolo, 1987; Le Saux, Morissette, & Di Paolo, 2006; Lévesque & Di Paolo, 1993; Lévesque & Di Paolo, 1991; Morissette & Di Paolo, 1993; Di Paolo et al., 1982, 1981).

D2R antagonist administration disrupts response learning in OVX rats receiving high E2 replacement, which could be due to DS D2Rs being more sensitive when exposed to high levels of E2 (Daniel, Sulzer, & Hulst, 2006). Similarly, research carried out in our laboratory in which D1R and D2R antagonists were systemically administered to OVX rats given low or high E2 replacement demonstrated that low E2 rats shifted from a striatal response strategy to a hippocampal place strategy following both drugs. However, high E2 rats were not affected, which suggests that E2 and DA function interact to affect multiple memory system bias (Quinlan, Hussain, & Brake, 2008). In a follow-up study, the same

antagonists were microinfused directly into either the DS or in the ventral portion of the striatum, also known as the nucleus accumbens (NAcc). This was done to verify whether it is DA activity in the DS that is implicated in E2's effect on response memory, and not the NAcc, which is adjacent to the DS and strongly affected by DA neurons. It was found that strategy shifted in both low and high E2 rats that received the drugs in the DS, but not in the NAcc, which further supports the idea that DA function in the DS is specifically implicated in multiple memory system bias (Quinlan et al., 2013).

It was recently observed that these E2-mediated changes in DS-sensitive response memory change with reproductive experience, in the rat. Specifically, nulliparous (not having borne offspring) OVX rats receiving low E2 replacement were more likely to rely on response memory to solve an ambiguous t-maze task and faster in learning a response task compared to the high E2 group. Conversely, primiparous (previously gave birth to and raised one litter of pups) rats given low E2 showed no difference in memory system bias or learning speed on a response task, which suggests that the E2-mediated effect is no longer present in reproductively experienced rats (Hussain et al., 2013). This could possibly be due to a long-term parity-induced disruption of the interaction between E2 and striatal DAergic function. Indeed, studies have shown that reproductive experience is related to higher sensitivity to DA and increased levels of striatal DA in rats (Felicio, Florio, Sider, Cruz-Casallas, & Bridges, 1996; Byrnes, Byrnes, & Bridges, 2001).

It has been established that E2 modulates striatal DA function and this interactive effect impacts response learning and memory, and this changes with parity. In the present study, the underlying mechanism of these behavioral findings was investigated: D1R and D2R binding was measured in the DS and NAcc using quantitative receptor autoradiography in nulliparous and primiparous OVX rats receiving either chronic low or chronic low plus intermittent high E2 replacement. Since it has been previously shown that E2 is related to higher D1R and D2R binding in the DS (Falardeau & Di Paolo, 1987; Le Saux et al., 2006; Lévesque, Gagnon, & Di Paolo, 1989), it was hypothesized that the high E2 group would have higher striatal DA receptor binding. It was also hypothesized that D1R and D2R binding would be higher in reproductively experienced rats since parity has been linked to increased sensitivity to DA in the DS. Finally, the NAcc (core and shell) was

also investigated since it is in close proximity to the DS and is important in motivation to solve a task (Grace, Floresco, Goto, & Lodge, 2007).

3.2 Methods

3.2.1 Subjects

A total of 16 five- to six-month old OVX female Wistar rats were used in this experiment (Charles River, St-Constant, QC). Eight of these rats were nulliparous (viz. had no reproductive experience) and nine were primiparous (viz. were pregnant and gave birth to and raised one litter of pups), and all rats were age-matched. Additionally, rats were given either low or high E2 replacement (nulliparous group: high E2 = 4, low E2 = 4; primiparous group: high E2 = 5, low E2 = 4). The primiparous rats were group-housed (5 females and 1 male per cage) in hanging cages (dimensions: 37.6 cm wide X 56.3 cm long X 22.2 cm high) for a maximum duration of 21 days, after which the pregnant rats were moved to single shoe-box cages (dimensions: 25.5 cm wide X 46.6 cm long X 21.6 cm high). Immediately after birth, the number of pups was culled to 8 per dam. The pups were then kept with the dams for 3 weeks, after which they were all weaned and dams were then housed in pairs until the start of the experiment. All rats were housed in plastic shoe-box cages, under a 12 h reverse light-dark cycle (2100 to 0900) and were handled regularly. All procedures involving rats were in accordance with guidelines established by the Canadian Council on Animal Care and approved by the Concordia University Animal Research Ethics Committee.

All rats had previously been trained and tested in a behavioral experiment (see Hussain et al., 2013). Rats were ovariectomized and randomly assigned to either chronic low E2 or pulsatile high E2 replacement and trained on either a place or response task. They were then switched to the other E2 condition and trained on the second task in counterbalanced manner. During OVX surgery, a small incision was made in the nape of the neck and a 1 cm long Silastic tube was inserted. This implant releases a low, chronic, steady dose of 5% E2 in cholesterol, which results in a 20 pg/ml serum concentration (Mannino, South, Inturrisi, & Quinones-Jenab, 2005) and provides all rats with a chronic low basal rate of E2 similar to that seen in the estrus phase of the rat estrous cycle (Quinlan et al., 2008). Rats in the high E2 condition were administered daily injections of E2 (10 μg/kg, s.c.; Sigma Chemical Co.). This dose has been previously shown to result in

circulating E2 levels of approximately 75–90 pg/ml (Quinlan et al., 2008). Rats in the low E2 replacement group were given daily injections of sesame oil vehicle (0.1 ml, s.c.).

Following the end of that experiment, rats were anaesthetized using CO_2 and decapitated. The brains were then extracted and rapidly frozen by submerging them in isopentane (-40C) for several seconds. Once the brains were frozen, they were stored at -80C. Brains were then sliced coronally at 20 micron thickness using a cryostat. The slices were mounted onto slides, desiccated overnight at 4C and stored at -80C until receptor autoradiography began.

3.2.2 Receptor Autoradiography

Slides were moved to room temperature and left to defrost for at least one hour. Meanwhile, brain slices were outlined with a grease pencil to ensure that any solution added to the slides would coat the tissue. For each rat, a total of two slides were needed: a set for the nonspecific (NS) binding and one for total (T) binding. Receptor binding in the NAcc core and shell was also measured from the DS slides.

For D1R binding, a 50mM Tris-HCl buffer solution (pH7.4, 25ºC) containing 120mM NaCl, 5mM $CaCl_2$ $2H_2O$, 1mM $MgCl_2$ $6H_2O$, 5mM KCl, and 30nM ketanserine dissolved in 50% acetic acid was made. Slides were incubated for 90 min in the buffer solution with added 2nM of [^{3}H]SCH23390 (specific activity 81.9 Ci/mmol; PerkinElmer, Waltham, MA). For the NS slides, 1µM DA hydrochloride (Sigma-Aldrich, St. Louis, MO) was added as a competitor. Once the incubation time had passed, all slides underwent a series of rinses in buffer solution (4ºC) and distilled water (dH_2O). Slides were then left to dry overnight at room temperature. The following day, slides were placed in x-ray cassettes along with microscale-calibrated tritium standards (American Radiolabeled Chemicals, Inc) and apposed to film (Amersham Hyperfilm MP, GE Healthcare Limited) in a dark room. All cassettes were inserted in opaque black bags and placed in an area that was ensured not to be exposed to any light. Films were exposed for four weeks.

For D2R binding, the 50mM Tris-HCl buffer solution (pH 7.4, 25ºC) containing 120mM NaCl, 2mM $CaCl_2$ $2H_2O$, 2mM $MgCl_2$ $6H_2O$, and 5mM KCl was made. Slides were incubated for 120 min in the buffer solution with added 10nM [^{3}H]Sulpiride (specific activity 83.4Ci/mmol; PerkinElmer, Waltham, MA). For the NS slides, 30µM of Haloperidol (Sandoz Canada Inc., Boucherville, QC) was also added as a competitor.

Slides were exposed for a period of 12 weeks for D2R binding. Films were then developed and fixed for image capture.

3.2.3 Image Capture

The films were placed on a Kaiser Fototechnik RS1 dual lamp light table from which pictures were captured using a QICAM High Performance IEEE 1394 Firewire digital camera (Quantitative Imaging Corp.) connected to a 50mm 1:2.8 macro lens (Sigma) mounted on a Kaiser Fototechnik RA1 camera stand. The camera was connected to a desktop computer and MCID Core InterFocus Imaging software (GE Healthcare Niagara Inc.) was used to capture pictures from the autoradiograms. Binding densities were converted to fmol/mg protein based on the tritium standard calibrations and the specific activity of each respective ligand in order to quantify receptor binding. All receptor binding values were recorded and compared across groups. For each brain region, the NS values (mean n = 6 per rat) were subtracted from T values (mean n = 6 per rat) in order to determine specific binding. A sample image showing NS and TL binding for D2R in nulliparous and primiparous DS can be seen in Figure 3.1.

3.2.4 Statistical Analysis

During optimization experiments of this procedure, some brain sections were depleted and none were left for the final experiment, therefore the final number of rats used for D2R binding was 12. A series of 2 (parity) X 2 (E2) factorial analysis of variance (ANOVA) analyses were carried out to compare receptor binding in each brain area for each E2 level and parity group. This was carried out for both D1R and D2R binding in the DS, NAcc core, and NAcc shell. Levene's tests were carried out for each ANOVA to ensure that the assumption of equal variances was not violated. Effect sizes were also computed for these data; for each comparison, a partial eta squared value (η_p^2) was computed to determine the robustness of the effect.

3.3 Results

3.3.1 D1R binding

Neither parity nor E2 level affected striatal D1R binding (see Figure 3.2) and no significant interaction was found between reproductive experience and E2 level on this

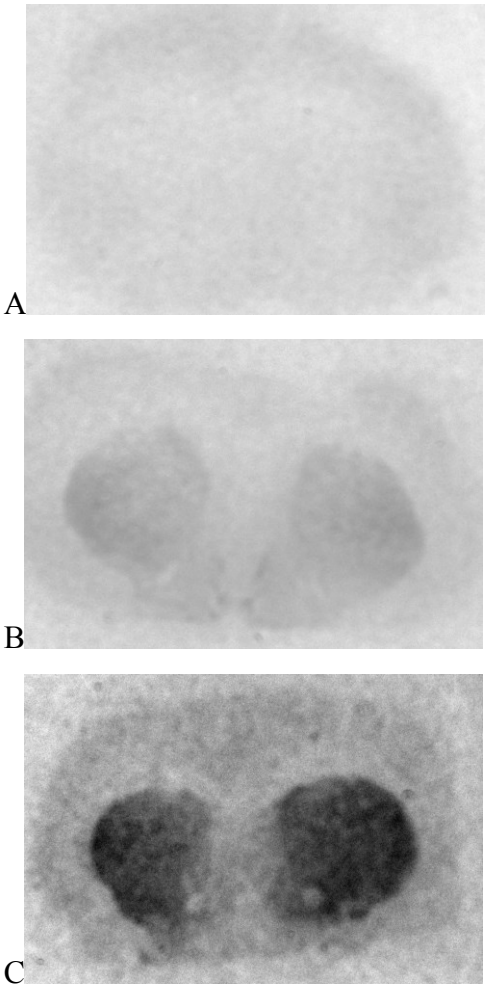


Figure 3.1. Sample DS D2R binding radiogram images. A. NS binding. B. TL binding from a nulliparous subject. C. TL binding from a primiparous subject.

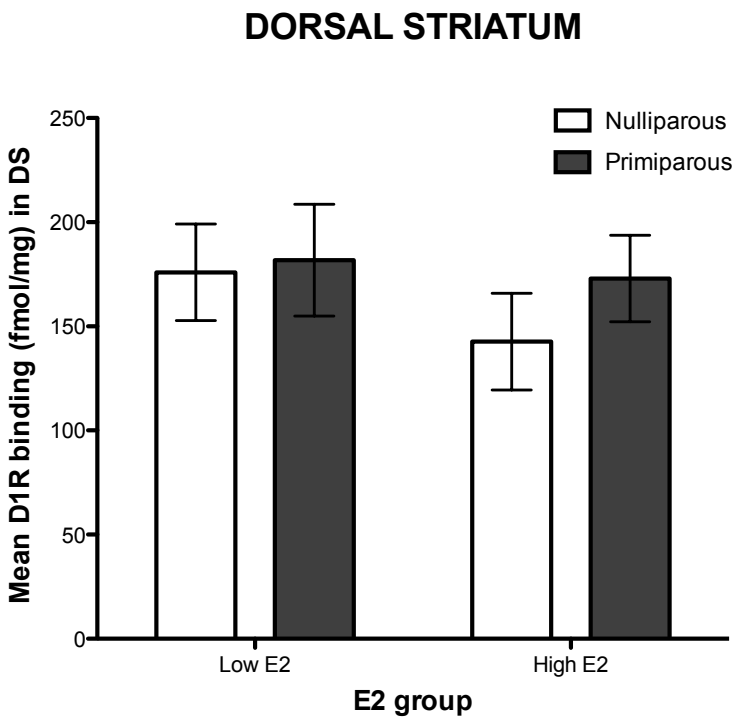


Figure 3.2. Mean DA D1R binding in the DS of nulliparous and primiparous rats receiving either low or high E2 replacement.

parameter. Similarly, there were no effects of parity or E2 group in D1R binding in the NAcc core or shell (see Figure 3.3). These results suggest that there is no difference in D1R binding in the DS and NAcc with reproductive experience or with exposure to different E2 levels.

3.3.2 D2R binding

Parity affected D2R binding in the DS, $F_{(1,8)} = 9.40$, $p = .015$, $\eta_p^2 = .540$; DS D2R binding was higher in nulliparous rats ($M = 40.93$, $SD = 12.82$) than in primiparous rats ($M = 19.93$, $SD = 5.59$), see Figure 3.4. The level of E2 given and the interaction between E2 and parity did not impact D2R binding in the DS.

Though the main effect of parity was not statistically significant in the NAcc core ($F_{(1,8)} = 4.24$, $p = .074$, $\eta_p^2 = .346$), there was a trend for higher D2R binding in nulliparous ($M = 23.28$, $SD = 10.48$) than in primiparous ($M = 11.02$, $SD = 6.92$) rats (see Figure 3.5). There was no statistically significant interaction or main effect of E2 group in D2R binding in the NAcc core. Finally, no statistically significant interaction or main effects were found in D2R binding in the NAcc shell. These results suggest that there is no difference in binding of D2Rs in the NAcc with reproductive experience or with exposure to different E2 levels.

3.4 Discussion

It was hypothesized that OVX rats with high E2 replacement would have greater D1R and D2R binding in the DS, as has been previously observed (Di Paolo et al., 1981; Le Saux et al., 2006; Lévesque & Di Paolo, 1989). It was also hypothesized that primiparous rats would have higher striatal DA receptor binding than nulliparous rats. The first hypothesis was not supported: E2 levels did not alter D1R nor D2R in the DS (see Figures 3.2 and 3.4). Contrary to the second hypothesis, parity did not affect D1R binding in the DS. Interestingly, nulliparous rats had twice as much D2R binding in the DS when compared to primiparous rats (see Figure 3.4). The present results suggest that D2R binding decreases with parity, and this seems to be a strong effect, given the large effect size ($\eta_p^2 = .540$). In addition, although no statistically significant results were found in D1R or D2R binding in the NAcc core and shell, there was a trend for higher D2R binding in the NAcc core of nulliparous rats (see Figure 3.5), which was associated with a moderate effect size ($\eta_p^2 = .346$). Taken together, these findings suggest that the E2 levels administered here

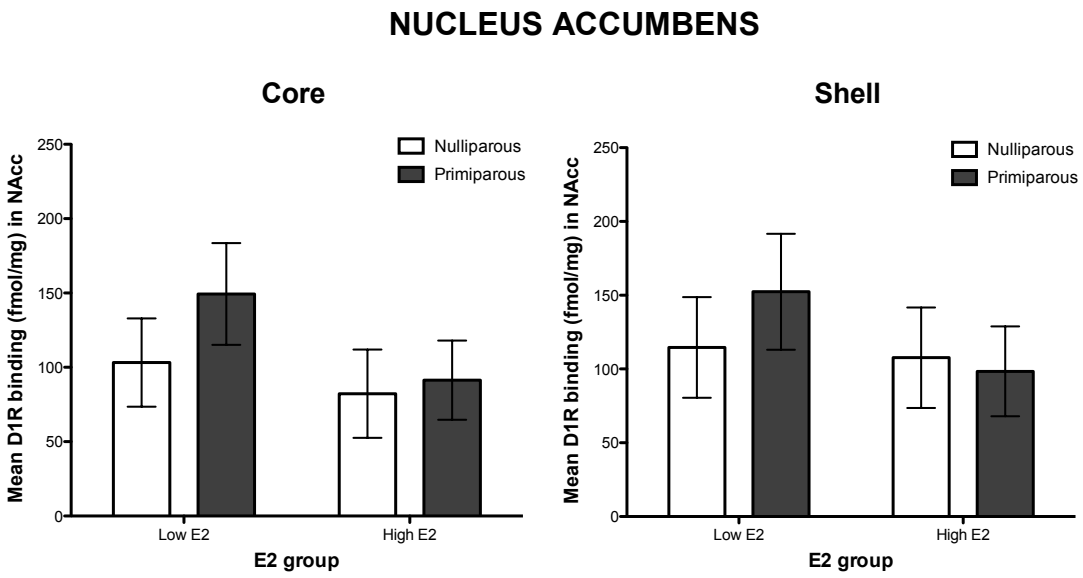


Figure 3.3. Mean DA D1R binding in the NAcc core (left panel) and shell (right panel) of nulliparous and primiparous rats receiving either low or high E2 replacement.

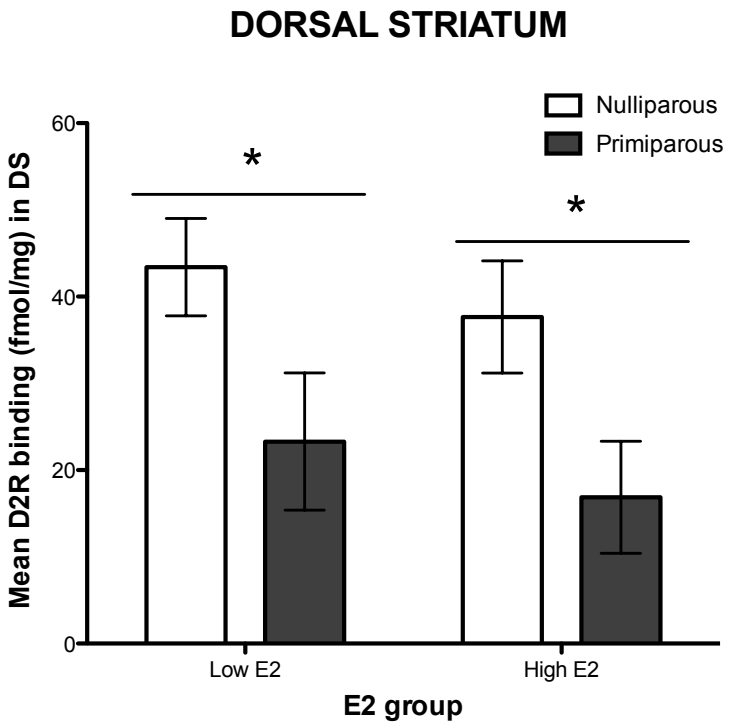


Figure 3.4. Mean DA D2R binding in the DS of nulliparous and primiparous rats receiving either low or high E2 replacement. D2R binding was significantly higher in nulliparous rats than in primiparous rats, $p = .015$. * indicates group difference.

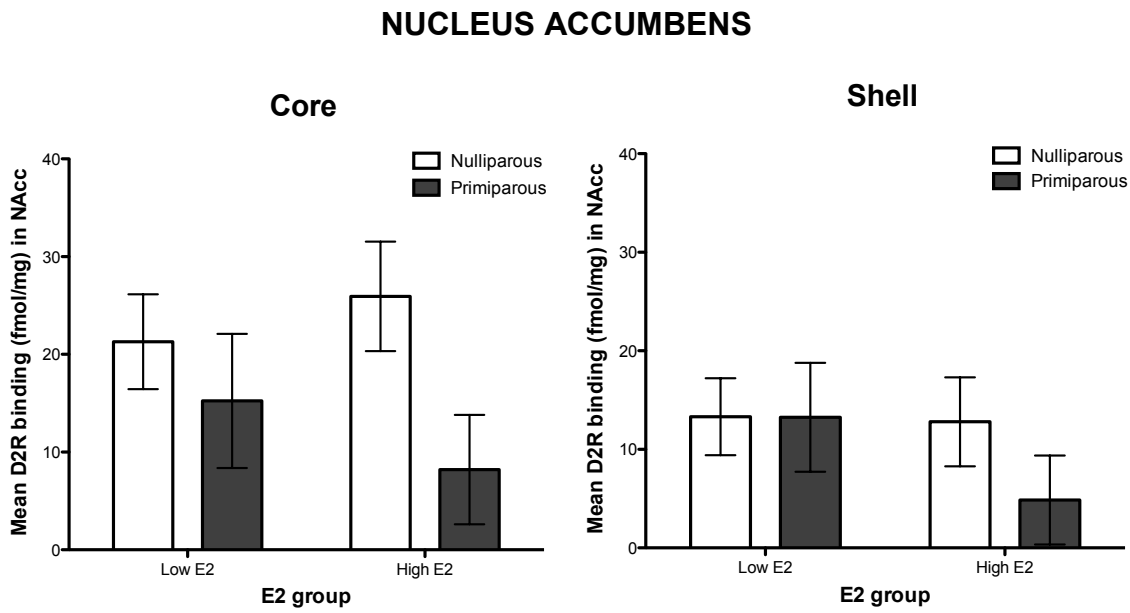


Figure 3.5. Mean DA D2 receptor binding in the NAcc core (left panel) and shell (right panel) of nulliparous and primiparous rats receiving either low or high E2 replacement.

did not affect DA receptor binding; however, parity did affect D2R binding was higher in the DS and NAcc core.

Previous research has shown that systemic as well as intra-striatal administration of both D1R and D2R antagonists interact with E2 to affect response memory (Daniel et al., 2006; Quinlan et al., 2013, 2008). However, the present results indicate that this interaction between E2 and striatal DAergic function is not mediated by estrogen-induced changes in D1R or D2R binding in the DS. There are multiple other mechanisms through which this interaction could occur; for example, E2 could be changing DA receptor kinetics rather than overall receptor levels to exert the observed behavioral effects. It is also possible that striatal DA effects that have been associated with response learning are occurring indirectly; for instance, E2 could be affecting DA function in another brain area with projections to the DS. This could potentially explain why DA receptor binding was equivalent across E2 groups. Indeed, it has been previously shown that infusing E2 directly in the medial prefrontal cortex, a brain area with projections to the DS, affects multiple memory system bias (Almey, Cannell, Bertram, & Filardo, 2014).

Others have reported that chronic E2 replacement in OVX rats leads to an increase in both D1R (Lévesque & Di Paolo, 1989) and D2R binding (Di Paolo et al., 1981; Le Saux et al., 2006). One probable reason for the discrepancy between the current finding and these earlier reports is the doses of E2 used. Di Paolo and colleagues used OVX rats given E2 doses ranging from 10 to 80 μg/mg; 10 μg/mg is equivalent to the high E2 dose given in the present study. That E2 regimen differs from the one used here, since no low E2 group was included here and the E2 doses were sometimes significantly higher in previous studies. Thus it is likely that the difference in E2 regimen and dose accounts for this discrepancy in striatal DA receptor binding between this and other studies.

Here, primiparous rats had lower striatal D2R, but not D1R, binding than nulliparous rats. This finding is incongruent with the hypothesis that reproductive experience would be related to higher DA receptor binding in the DS. It has previously been shown that parity is associated with an increase in striatal DA sensitivity in both intact and OVX rats (Byrnes et al., 2001). Specifically, parous females showed a greater behavioral response than nulliparous rats to the DA agonist apomorphine; in addition, higher DA and DOPAC (DA metabolite) levels were found in the DS of parous rats. As in

the present study, reproductively experienced rats in the Byrnes et al. (2001) study were tested several weeks following parturition, indicating that these parity effects are not just limited to the period immediately following birth. It is possible that the decrease observed in the current study is of presynaptic D2Rs, which would lead to a decrease in negative feedback of striatal DA synthesis and release and, hence, higher striatal DA levels. This would fall in line with the potentiated DA activity found in the Byrnes et al. (2001) study.

Furthermore, increased striatal DA levels have been shown in pregnant rats (Felicio et al., 1996); this increase in striatal DA during pregnancy and in the post-partum period could overstimulate DA receptors in the DS and consequently lead to increased DA sensitivity and, possibly, DA receptor downregulation. This is perhaps why D2R binding in the DS decreased by half in primiparous rats in the current study: high exposure to striatal DA during pregnancy could overstimulate D2Rs in the DS. Finally, one study has shown an increase in DA levels three months following parturition, indicating a more long-term effect of parity on DA function, though this was observed in the hippocampus (Macbeth et al., 2008). This further emphasizes the long-term effects of parity on DAergic function in the female brain.

Mirroring the DS effect, a trend was observed for higher D2R binding in the NAcc core of nulliparous rats, when compared to the primiparous group. Though not statistically significant, D2R binding in nulliparous rats was double that of primiparous rats in this brain region, and the effect size was moderate. No behavioral differences were observed when D2R antagonists were infused directly into the NAcc core of rats tested in the ambiguous t-maze task (Quinlan et al., 2013), which suggests that this brain region is not implicated in multiple memory system bias. However, the NAcc core is important in the motivation involved in reward seeking and completing tasks and is dependent on basolateral amygdala function (Grace et al., 2007; Ito & Hayen, 2011), whereas the NAcc shell is implicated in converging hippocampally-mediated spatial cues into reward-seeking behavior (Ito et al., 2008), and DA function is important for both (Ito & Hayen, 2011). It has also been found that NAcc core lesions lead to disrupted formation of stimulus-reward associations (Setlow, 1997). It is possible that there is a parity-induced decrease in D2R binding in the NAcc core, but not shell, and, consequently, disrupted motivation to complete a response task. This, along with the finding that D2R binding is also lower in the DS of primiparous rats, would

potentially explain why reproductively experienced rats show impaired response learning and a lower propensity to use response memory on an ambiguous t-maze task (Hussain et al., 2013).

In summary, the present study has revealed that, contrary to what was hypothesized, no difference was observed in D1R and D2R binding across chronic low versus chronic low plus intermittent high E2 female rats. In addition, D2R binding was lower in primiparous rats compared to nulliparous rats, and there was a similar trend in the NAcc core but not the NAcc shell. These results suggest that D2R binding decreases with reproductive experience, and that this could be a potential mechanism underlying previously observed changes in multiple memory system bias in reproductively experienced rats. These findings also emphasize the long-term impact of reproductive experience on the female brain that persists beyond pregnancy and the immediate post-partum period.

CHAPTER 4

Modulation of spatial and response strategies by phase of the menstrual cycle in women tested in a virtual navigation task

Dema Hussain, Sarah Hanafi, Kyoko Konishi, Wayne G. Brake, Véronique D. Bohbot

Preface

Previous findings show that E2 modulates multiple memory bias in both naturally cycling and OVX rats given E2 replacement. Low E2 levels are associated with enhanced DS-mediated response memory whereas high E2 promotes HPC-dependent spatial memory. In humans, E2 has been associated with improved performance on a variety of cognitive abilities in both cycling and postmenopausal women. However, other studies find no such E2-induced cognitive improvements while others show a deleterious effect of E2 on cognition. The effect of E2 on multiple memory system bias has not been investigated in women, though it has been shown that humans show bias in the type of memory system used. Therefore, the goal of the present study was to investigate multiple memory system bias across the menstrual cycle in order to see whether memory bias changes along with fluctuating ovarian hormones.

Abstract

Different memory systems are employed to navigate an environment. It has been consistently shown in rodents that estrogen impacts multiple memory system bias such that low estradiol (E2) is associated with increased use of a striatal-mediated response strategy whereas high E2 increases use of a hippocampal-dependent spatial memory. In addition, low E2 enhances performance on a response-based task whereas high E2 levels improve learning on a spatial task. The purpose of the present study was to investigate navigational strategies in young, healthy, naturally cycling women. Participants were split into either a follicular or luteal phase group, depending on the date of their menstrual cycle that they were in on the test day, and they were administered a virtual navigation task that can be solved by using either a response or spatial strategy. It was found that women tested in the luteal phase predominantly used a spatial strategy, whereas the opposite pattern was observed in the follicular group. Since E2 levels are generally lower in the follicular phase and higher in the luteal phase, these results show that, as in rodents, E2 may modulate multiple memory system bias such that low E2 levels may lead to a higher proportion of response strategy and high E2 maybe associated with a spatial strategy. Our data suggest that the type of memory system engaged in order to effectively navigate an environment shifts depending the phase of the menstrual cycle.

4.1 Introduction

Multiple memory systems can be engaged to solve a task and aid in navigating a complex environment. Different learning systems were first documented by Tolman and colleagues (1946) who showed that rats utilize different strategies to find their way in a maze and complete a task (Tolman, Ritchie, & Kalish, 1946). Namely, two learning strategies can be used: the response strategy, which is a strategy that relies on body turns at specific points in the environment forming stimulus-response associations, and the spatial strategy, which is allocentric, i.e. independent of the position of the observer and relies on forming stimulus-stimulus associations between landmarks in order to create a cognitive map of the environment. These systems are dissociable, they can be competitive, and rely on different brain regions to function optimally. The hippocampus (HPC) is implicated in spatial memory (O'Keefe & Nadel, 1978) whereas the dorsal striatum (DS; which includes the caudate nucleus) is crucial for response memory (Packard, Hirsh, & White, 1989). These strategies were dissociated in rodents, in a series of seminal studies by McDonald & White in which it was demonstrated that spatial memory was significantly impaired when the hippocampal formation (fornix) was damaged and response memory was impaired when the DS was damaged (McDonald & White, 1993, 1994). Furthermore, it has been shown that rats initially use HPC-dependent spatial memory early on in a dual-solution maze task but this changes to DS-dependent response memory with additional training, suggesting that the HPC and DS have different temporal dynamics (Packard & McGaugh, 1996). Thus, these memory systems are dissociable, such that as one is inactivated, other systems are engaged to navigate an environment (Packard & McGaugh, 1992; Packard & White, 1991; Packard et al., 1989).

Evidence for the existence of multiple memory systems has also been observed in humans; as in rodents, spatial memory was associated with fMRI activity and grey matter in the HPC whereas response memory was associated with the caudate nucleus (Bohbot, Iaria, & Petrides, 2004; Bohbot, Lerch, Thorndycraft, Iaria, & Zijdenbos, 2007; Iaria, Petrides, Dagher, Pike, & Bohbot, 2003). Indeed, increased hippocampal volume is associated with enhanced navigational spatial skills (Maguire et al., 2000; Woollett & Maguire, 2011). Furthermore, individuals with a damaged HPC were shown to have impaired spatial memory (Bohbot et al., 1998; Holdstock et al., 2000) but did not show

impairment when using a response strategy to solve the task, which further demonstrates that this strategy does not rely on hippocampal function (Bohbot et al., 2004). Though multiple memory systems rely on specific brain structures, there exist individual differences in the type of strategies that are employed to navigate an environment. However, with time and practice, spatial learners tend to switch to the less cognitively demanding and faster response strategy. This has been observed in both humans (Iaria et al., 2003) and rats (Chang & Gold, 2003; Packard & McGaugh, 1996).

Interestingly, this switch from using a spatial strategy to use of a response strategy with time and practice is not observed in female rodents; multiple memory system bias seems to be modulated by estradiol (E2) levels such that high E2 levels are associated with spatial memory and low E2 levels with response memory. This has been shown in naturally cycling rats Korol, Malin, Borden, Busby, & Couper-Leo, 2004; McElroy & Korol, 2005) as well as ovariectomized rats receiving E2 replacement (Davis, Jacobson, Aliakbari, & Mizumori, 2005; Korol & Kolo, 2002; Quinlan et al., 2008). The effect of E2 on multiple memory systems has also been shown to occur directly in associated brain areas. It has been shown that infusing E2 directly into the HPC improves spatial memory (Zurkovsky, Brown, & Korol, 2006; Zurkovsky, Brown, Boyd, Fell, & Korol, 2007) whereas infusing E2 into the DS impairs response memory (Zurkovsky, Serio, & Korol, 2011), which suggests that E2 specifically enhances hippocampal function while it has the opposite effect in the DS. Hippocampal volume has also been shown to change across the estrous cycle in mice such that larger volumes correlate with proestrus, the phase of the cycle when E2 peaks, and spatial strategy use (Qiu et al., 2013). Furthermore, it has been shown that direct infusion of E2 into the medial prefrontal cortex of low E2 rats leads to a switch to spatial memory, suggesting that the prefrontal cortex also plays a role in multiple memory system bias (Almey et al., 2014). Thus, fluctuations of E2 in the female rat modulate whether a HPC-mediated spatial strategy or DS-mediated response strategy is employed to navigate an environment, and recent evidence suggests the medial prefrontal cortex could play a role in this E2-mediated shift between memory systems.

Based on the rat literature, it is clear that E2 levels modulate the use of memory systems; however, little is known about if and how this occurs in humans. It has been observed that E2 is associated with cognition in women; for example, E2 has been linked

with improved verbal memory (Maki, Rich, & Rosenbaum, 2002; Mordecai, Rubin, & Maki, 2008; Rosenberg & Park, 2002) whereas it is associated with impaired performance on mental rotation tasks (Hampson, 1990; Hausmann, Slabbekoorn, Goozen, Cohen-Kettenis, & Gunturkun, 2000). Structurally, one study revealed that hippocampal volume changes across the menstrual cycle in women: when endogenous E2 levels are high, there was an increase in hippocampal grey matter, compared to women tested when E2 levels are low (Protopescu et al., 2008). This, along with findings from animal research, lead to the hypothesis that E2 would promote spatial memory strategies in women. Therefore, in the current study, young, naturally cycling women were tested on a 4 on 8 virtual maze (4/8 VM) navigation task in which they could utilize either a spatial or response strategy to complete the experiment. Estradiol peaks towards the end of the follicular phase and then rises and plateaus across the luteal phase (for review see Hussain, Shams, & Brake, 2014; Mihm, Gangooly, & Muttukrishna, 2011); therefore, E2 levels are generally higher in the luteal phase compared to the follicular phase, which is marked by low E2 levels throughout menstruation. It was hypothesized that women tested during the luteal phase would be more likely to use a spatial strategy and utilize more landmarks to navigate whereas those tested in the follicular phase would use a response strategy more often. Furthermore, it was expected that the luteal phase would be associated with a higher number of errors and trials required to reach criterion, since spatial strategies are more cognitively demanding and are associated with increased errors on the 4/8 VM (Iaria et al., 2003).

4.2 Methods

4.2.1 Participant characteristics

A total of 54 healthy, right-handed, regularly cycling (i.e., a menstrual cycle lasting between 25 and 34 days) women were tested (age: $M = 30.35$; $SD = 3.32$; range = 23-36). All participants underwent a screening questionnaire over the phone to determine whether they were eligible to participate in this study. Participants who reported a history of psychological or neurological illness, drug or alcohol abuse, had been pregnant within the past two years, currently breastfeeding, or had taken contraceptive medication within three months of testing were excluded. Participants had, on average, 16.93 years of education ($SD = 2.71$) and a mean sleep score of 0.91 ($SD = 0.14$; sleep score = average number of hours of sleep over the past week / ideal number of hours of sleep for the individual). See

Table 4.1 for all participant demographics split by cycle phase group. Comparison groups did not differ in age, education, and sleep scores ($p > .05$). Informed consent was obtained from participants in accordance with local ethics guidelines. This study was approved by the Research Ethics Board at the Douglas Mental Health University Institute and was carried out in collaboration with the Center for the Study of Behavioral Neurobiology (CSBN) at Concordia University in Montreal, Canada.

4.2.2 Menstrual Cycle Phase Measurement and Hormonal Profile Questionnaire

As part of the screening questionnaire, participants were required to have kept track of the start dates and durations of their menses over at least the last six months and to provide the date of their last period. Based on the information given, it was determined whether the participant had a regular menstrual cycle, which is characterized as a cycle lasting between 25 and 34 days from the start of one period to just before the next one begins (Mihm, et al., 2011).

On the day of testing, a hormonal profile questionnaire was administered to participants in order to gather more detailed information regarding regularity of menstrual cycles, past pregnancies, contraceptive and synthetic hormone history, and general life habits (e.g., exercise, smoking, drinking). The dates of the last six periods were used to determine any given woman's average cycle length, current day in cycle (testing date – date of last period) and, consequently, estimate whether the participant is in a low or high E2 state when tested. If the participant was tested when in days 1-13 of her cycle, she was in the follicular phase of her menstrual cycle, which corresponds with low E2 levels (menses to ovulation). If the participant was tested from day 14 until the end of her cycle, she was considered to be in the luteal phase, when E2 levels are generally high. For the final analysis, there were 23 women included in the follicular phase group and 31 included in the luteal phase group.

4.2.3 Neuropsychological Tests, PSS, and Sleep Questionnaires

Participants were administered a battery of neuropsychological tests in order to control for possible confounds related to cognitive function, IQ, and memory. Questionnaires measuring stress and quality of sleep were also given to ensure that these variables would not differ across cycle phase group and have an effect on performance on the task.

The Rey Auditory Verbal Learning Test (RAVLT) is a standard neuropsychological

Table 4.1.

Participant Demographics, LSEQ, PSS, and Neuropsychological Measures

	Follicular		Luteal	
	n = 23		*n* = 31	
	M	*SD*	*M*	*SD*
Age (in years)	29.78	3.45	30.77	3.20
Number of years of education	17.26	2.51	16.68	2.87
Sleep ratio[a]	0.90	0.14	0.91	0.15
Hours of exercise/week	3.73	2.90	3.53	3.24
Number of alcoholic drinks/week	1.78	1.96	1.37	2.29
Number of cigarettes/week	5.00	15.23	6.21	18.24
Menstrual cycle data				
Age first period	12.28	1.90	12.74	1.34
Mean cycle length	28.83	2.41	29.51	2.81
Mean period length	5.01	1.12	5.32	0.99
Leeds Sleep Evaluation Questionnaire				
Getting to Sleep	57.07	14.35	51.54	15.62
Quality of Sleep	46.89	19.32	46.31	20.74
Awakening from Sleep	46.11	18.80	49.97	21.69
Behavior Following Wakefulness	52.18	19.69	55.00	19.25
Rey Auditory Verbal Learning Test[b]				
Pre-Interference (total score over 5 trials)	59.57	6.72	60.42	5.60
Post-Interference	12.57	1.95	12.84	1.75
Post-Delay	12.91	1.88	13.42	1.41
Rey-Osterrieth Complex Figure Task[c]				
Copy	35.26	1.10	34.76	1.66
Immediate Recall	24.33	7.45	25.07	4.84
Delay	23.87	5.90	24.97	4.90
IQ score	107.22	12.14	108.90	16.09
Perceived Stress Score[d]	23.89	7.40	22.95	5.91

Note. $N = 54$.

[a]Sleep Ratio = number of hours of sleep over last week/ideal number of hours of sleep.

[b]RAVLT: maximum score per trial = 15.

[c]RO: maximum score = 36.

[d]PSS: maximum score = 56 (higher score indicates higher perceived stress levels).

verbal memory test (Rey, 1941; Schmidt, 1996). A list of 15 words (list A) is read for five trials and after each trial the participant is asked to verbally recall as many words as they can remember. Next, an interference trial is provided whereby a different list of 15 words (list B) is read to the participant and again the participant is asked to recall as many words as they can remember. Following this interference trial, the participant is asked to recall as many words as they can from list A. Finally, after a 30 min delay, the participant is again asked to recall as many words as they can from list A. Performance was assessed with the number of words recalled after interference and after the 30 min delay. During the 30 min delay, the Rey-Osterrieth Complex Figure task (RO; Osterrieth, 1944) was administered. Participants were asked to copy a complex figure as accurately as possible. The figure was then taken away and the participant was asked to immediately draw it from memory (immediate recall). After a 30 min delay, participants were asked again to redraw the complex figure from memory. The Test for Non-Verbal Intelligence-3 (TONI; form A), a language-free test of intelligence that avoids problems associated with administering verbal tests of intelligence to non-native English or French speakers (Brown, Johnsen, & Sherbenou, 1997), was also administered in order to measure IQ scores. The perceived stress scale (PSS) was administered in order to measure participants' self-reported stress levels (Cohen, Kamarck, & Mermelstein, 1984). Finally, the Leed's Sleep Evaluation Questionnaire (LSEQ) was administered to evaluate sleep quality by focusing on four domains: ease with which an individual falls asleep, quality of sleep, ease with which an individual awakens following sleep, and behavior following wakening (Parrott & Hindmarch, 1978).

4.2.4 Behavioral task (4/8 VM)

A virtual navigational task was adapted from a commercially available computer game (Unreal; Epic Games, Raleigh, NC). The virtual environment was composed of an eight-arm radial maze with a central starting location (Bohbot et al., 2007; Iaria et al., 2003). The maze was surrounded by a landscape (mountains, sky, clouds), a tree, and a boulder. At the end of each arm, there was a staircase leading to the location where, in some of the arms, an object could be picked up. Objects were not visible from the central platform and only became visible when participants reached the end of the pathway. The experiment was carried out using a standard 17-inch computer screen while participants

were seated at a desk in a testing room. Participants used a keypad with forward, backward, left turn, and right turn buttons to move within the environment. Before testing, participants were given a habituation trial in which they spent a few minutes moving in a virtual room that was different from the experimental environment to practice the motor aspects of the task. Once the participants were comfortable using the keypad and navigating the virtual environment, the experimenter gave the instructions, and the experiment started.

The experiment consisted of successive trials that were composed of two parts. In Part 1, four of the eight arms were accessible with objects at the end of each arm; in Part 2, all arms were accessible and objects were present in the four arms that had been blocked in Part 1 (see Figure 4.1). The participants were told to retrieve all four objects from the accessible arms in Part 1 and remember which arms they visited because they will need to avoid these arms in order to find the objects in Part 2. An error consisted of an entry into an arm that did not contain an object. As such, part 2 errors consisted of the dependent variable because it involved memory for the four previously visited pathways out of eight choices. Two different sequences were used. In Part 1 of trial type A, arms 1, 3, 5, and 8 were accessible and contained an object; in Part 2, the four objects were located at the end of the four previously blocked arms (i.e., arms 2, 4, 6, and 7). In Part 1 of trial type B, arms 3, 4, 6, and 8 were accessible; in Part 2, the objects were located at the end of arms 1, 2, 5, and 7. A minimum of three trials were administered, with sequence order ABA. If the participant reached criterion (i.e., made no errors during Part 2 of any of the first three trials), a probe trial was administered. If criterion was not reached during the first three trials, up to five additional trials were given (sequence A) until the participant made no errors during Part 2. For every trial, participants completed the trial once the four objects had been picked up.

Part 1 of the probe trial was identical to trial type A (sequence A). In Part 2, however, the walls around the radial maze were raised to conceal the landscape, so that no landmarks were visible. Furthermore, unbeknownst to the participant, eight objects were present (one at the end of each arm). If a spatial strategy was employed, the landmarks present in the environment were necessary to perform the task; therefore, removing the landmarks from the environment in Part 2 of the probe trial should result in an increase in errors. Conversely, if participants were using a response strategy, no increase in errors

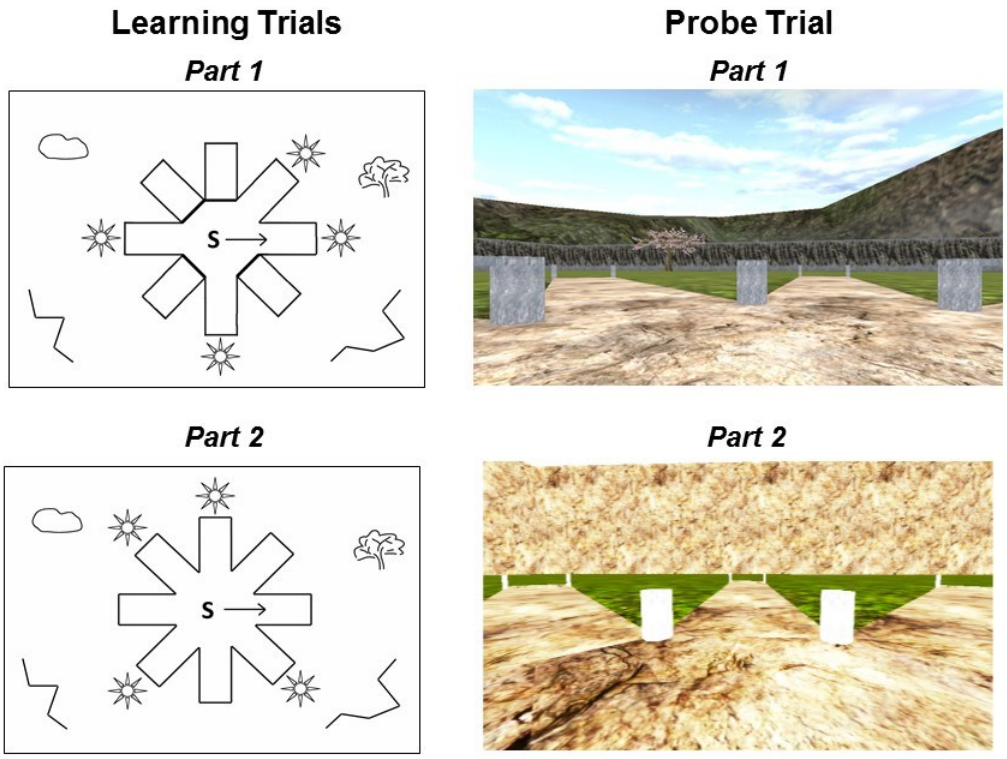


Figure 4.1. Illustration of 4/8 VM learning and probe trials. Sample trial of the 4/8 VM task showing location of landmarks around 8-arm radial maze (left). In Part 1, 4 of the 8 pathways are blocked and participant must collect objects in open pathways. In Part 2, all pathways are now accessible and participant must remember where the objects were located in part 1 and visit the previously blocked pathways to find objects. The arrow represents the starting position the participant begins each trial in. The stars represent the pathways that contain an object; participants must go to the end of the pathway in order to see the object. Sample screen shots of the 4-on-8 virtual maze during a probe trial (right). In Part 1, all landmarks are visible as in other trials. In Part 2, a wall obsurs the landscape surrounding the maze so participants can no longer use landmarks to orient themselves.

should occur with this change (Iaria et al., 2003). The pattern of visited pathways was used to assess the number of probe errors. Once the probe trial was completed, an additional type A trial was given in order to measure whether participants shifted their strategy following the probe trial.

At the end of the experiment, participants were debriefed and asked a series of questions about how they solved the task from beginning to end. Participants were categorized as using a response strategy if they used a sequence or counting strategy (i.e., numbered the pathways) from a single starting point. If they used at least two landmarks and relied on the spatial relationship among these landmarks and the objects, without using a pattern, they were categorized as using a spatial strategy. The initial strategy that was used by the participant was assessed. Two raters independently evaluated the verbal reports and assigned participants to a particular strategy group corresponding to the method used when navigating the environment. The independent judgments of the raters were correlated to evaluate their consistency. Aside from probe errors, errors made during all Part 2 trials (which assess memory for the location of objects in the maze), number of landmarks mentioned in the verbal report, and trials to criterion were also measured.

4.2.5 Statistical Analysis

A series of independent samples *t*-tests were carried out in order to compare the follicular and luteal groups on the neuropsychological measures: RAVLT (total recall for first 5 trials, recall following interference, recall following delay) and RO (copy, immediate recall, delay). Similarly, participants were compared across groups on IQ score (TONI), PSS, and all LSEQ measures.

For the 4/8 VM task, independent samples *t*-tests were carried out to compare follicular and luteal groups on the average number of errors committed during all part 2 trials, number of trials to criterion, number of probe errors, and number of landmarks mentioned in the verbal report. These 4/8 VM task measures were also compared between response and spatial learners in order to observe whether spatial learners make more errors, require more trials to reach criterion, and mention more landmarks than response learners. Levene's test for equality of variances was carried out for every *t*-test. Finally, a Chi square test was carried out to compare proportions of participants in the follicular and luteal groups using an initial spatial or response strategy in the task.

4.3 Results

4.3.1 Neuropsychological Tests, PSS, and Sleep Questionnaires

The follicular and luteal groups did not differ on any of the neuropsychological test measures. Also, there were no differences in IQ and PSS scores ($p > 0.05$). None of the four LSEQ domains differed across the two groups; however, when individual questions were examined, women tested during the follicular phase differed significantly from those tested in the luteal phase for two questions in the LSEQ. Participants in the follicular phase reported greater ease ($t_{(52)} = 2.15$, $p = .036$, $d = 0.59$) and speed ($t_{(52)} = 2.12$, $p = .039$, $d = 0.58$) for falling asleep compared to participants tested in the luteal phase. This suggests that a lower E2 state could be associated with an easier time falling asleep.

4.3.2 Behavioral task (4/8 VM)

Chi-square results revealed that participants tested in the luteal phase, high in E2, predominantly used a spatial strategy (Spatial = 64.5%) compared to a response strategy in the 4/8 VM task, whereas the opposite was observed in the follicular group (Spatial = 30.4%); $X^2 = 6.14$, $p = .013$ (see Figure 4.2). This suggests that when women are tested in a high E2 state, they were more likely to use a spatial strategy than a response strategy to complete the task. Conversely, when women are tested when E2 levels are low, they are more likely to use a response strategy than a spatial strategy.

Consistent with these findings, participants tested during the luteal phase were more likely to use landmarks than participants tested in the follicular phase. This was evidenced by a significantly higher number of probe errors made by women in the luteal phase ($M = 0.32$, $SD = 0.54$) than the follicular phase group ($M = 0.09$, $SD = 0.29$), $t_{(47.80)} = -2.06$, $p = .045$, $d = -0.57$. The assumption of equal variances was violated for this *t*-test (Levene's test: $F = 17.43$, $p < .001$); corrected values were therefore used. When probe errors were compared between response and spatial learners, as categorized by verbal reports, no difference was found ($t_{(52)} = 0.00$, $p = 1.00$, $d = 0.00$). These results indicate that women in the luteal group made more probe errors compared to women tested during the follicular phase, suggesting that women in the luteal phase high in E2 relied more on landmarks. While the number of landmarks reported was not different in women in the luteal and follicular groups, ($t_{(52)} = -0.07$, $p = .948$, $d = -0.02$), spatial learners mentioned significantly more landmarks in the verbal report ($M = 2.74$, $SD = 0.81$) compared to response learners

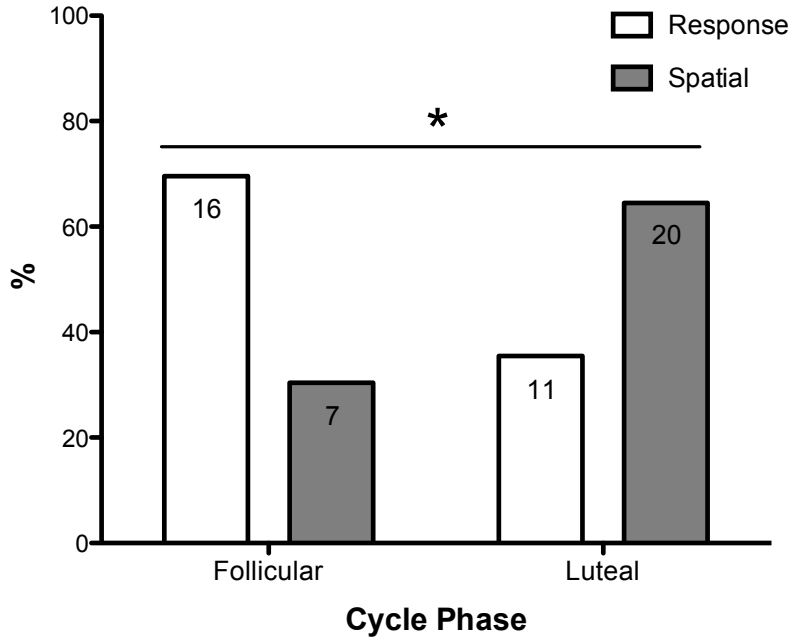


Figure 4.2. Bar graph representing proportion of participants using an initial spatial or response strategy in the follicular and luteal phases of the cycle. Women tested in the follicular phase predominantly used a response strategy whereas the opposite was observed in women tested during the luteal phase; $X^2 = 6.14$, $p = .013$.

(M = 2.15, SD = 0.95; $t_{(52)}$ = 2.46, p = .017, d = 0.67), as has been previously observed (Schwabe, Bohbot, & Wolf, 2012).

There were no group differences in terms of the general learning variables, such as errors and trials to criterion. Follicular and luteal groups did not differ in terms of the average number of errors ($t_{(52)}$ = -1.34, p = .187, d = -0.37), number of trials to criterion ($t_{(52)}$ = -0.87, p = .390, d = -0.24). Spatial and response learners did not differ in terms of errors ($t_{(52)}$ = -2.09, p = .835, d = -0.57) or in number of trials to criterion ($t_{(52)}$ = 1.12, p = .269, d = 0.30).

In summary, when women were tested during their luteal phase, high in E2, the chi-square revealed a significantly higher number of spatial learners than response learners. The opposite was found when women were tested during their follicular phase. Consistent with these findings, women tested during their luteal phase were more likely to rely on landmarks, as evidenced by a significantly higher number of errors in the probe trial, when landmarks were removed.

4.4. Discussion

In the current study, it was found that women tested in the luteal phase use a spatial strategy significantly more than a response strategy. In contrast, a higher proportion of response strategy use was observed in women during the follicular phase (see Figure 4.2). Women tested in the luteal phase also made significantly more probe errors compared to women tested during the follicular phase. This is consistent with the fact that the spatial strategy, the strategy that women in their luteal phase predominantly use, is generally associated with the use of landmarks and increased probe errors when these are removed (Iaria et al., 2003).

Interestingly, contrary to the hypothesis that the luteal and follicular groups would differ in terms of 4/8 VM measures, no group differences were observed apart from probe errors. Despite the difference observed in strategy across the cycle, the luteal and follicular groups did not differ in the number of trials needed to reach criterion or the number of errors made in the task. This may be because the sample tested in this study was comprised of young, healthy participants; perhaps the task was not difficult enough to observe a high number of errors or differences in trials required to reach criterion. Indeed, out of the 54 participants tested, only four of them required any extra trials (above the minimum 3 trials

given before the probe) to reach criterion. It is therefore possible that group differences were obscured by the low variability in this measure.

The present findings indicate that E2 modulates memory systems such that the phase of the cycle favoring a higher E2 state, promotes the use of HPC-dependent spatial strategies and decreases the use of caudate nucleus-dependent response strategies. To the best of our knowledge, this is the first study to demonstrate an E2 phase-mediated effect on multiple memory system in humans. This suggests that E2 may act in the HPC and caudate nucleus to impact the type of strategy used by women when navigating a virtual environment. These results are in line with a previous study showing a correlation between high E2 and increased hippocampal grey matter in cycling women (Protopescu et al., 2008). Furthermore, it has previously been found that estrogen receptors are present in the human HPC (Osterlund, Gustafsson, Keller, & Hurd, 2000; Osterlund, Keller, & Hurd, 2000). Thus, E2 could be structurally altering the HPC and binding to estrogen receptors within this brain area to promote spatial memory.

Current results corroborate previous findings observed in rodents showing that E2 directly modulates multiple memory system bias in naturally cycling and ovariectomized females given E2 replacement, such that low E2 levels bias a female to use response memory and high E2 levels are associated with the use of spatial memory (Hussain et al., 2013; Korol & Kolo, 2002; Korol et al., 2004; Quinlan et al., 2008; Zurkovsky et al., 2006, 2007, 2011). The current results demonstrate that this effect potentially translates to humans such that memory system bias is affected by menstrual cycle phase. Furthermore, it has previously been demonstrated that a response strategy used in the 4/8 VM task corresponds with enhanced caudate nucleus activity and spatial strategy use correlates with enhanced hippocampal activity (Iaria, et al., 2003). Similarly, response learners have increased grey matter in the caudate nucleus and decreased hippocampal grey matter; this pattern is reversed in spatial learners (Bohbot, et al., 2007). Thus, in concordance with findings from the rat studies, it appears that a low E2 state biases women towards using a caudate-dependent response strategy to solve a task or navigate an environment whereas a high E2 state promotes HPC-dependent spatial memory.

Recent findings hint at the possibility that this E2 phase-mediated shift in strategy could be occurring due to an interaction with neurotransmitter systems. A recent study

revealed that cycle-dependent changes in cognitive function could be due to E2 interacting with prefrontal dopamine levels, such that the direction of E2's effect on performance on a working memory task depends on baseline dopamine levels. Specifically, individuals with low basal dopamine levels showed impaired performance on the task when endogenous E2 levels were low whereas a low E2 state was associated with improved working memory in individuals with higher basal dopamine levels (Jacobs & D'Esposito, 2011). This finding is consistent with data from rodent studies that show an interaction between E2 and dopamine transmission in the DS and how this impacts multiple memory system bias. Specifically, it has been demonstrated that directly blocking dopamine binding with a locally administered dopamine receptor antagonist in the DS leads to a switch in the strategy employed by rats in a dual solution maze task, such that low E2 rats switched from response to spatial memory and high E2 rats switched from spatial to response (Quinlan et al., 2013). In addition, E2 exacerbates the impairments caused by striatal dopamine blockade on response learning (Daniel, Sulzer, & Hulst, 2006). It is therefore possible that an interaction between a low E2 state and optimal dopamine transmission in the caudate nucleus is promoting response memory in women.

The current findings provide a novel contribution to the literature on cognition across the menstrual cycle in young women. Generally, studies show that when women are tested when levels of E2 are low (e.g., during menstruation), they tend to perform better on tasks sensitive to a male advantage, such as mental rotation (Courvoisier et al., 2013; Hausmann et al., 2000; Maki et al., 2002). Conversely, when E2 is higher (e.g., luteal phase), performance on tasks sensitive to a female advantage, such as verbal fluency and memory, improves (Maki et al., 2002). However, many studies fail to find such effects (for review, see: Sundström Poromaa & Gingnell, 2014) and no cycle difference in verbal memory (as measured by the RAVLT) was found in the present study. These inconsistencies could be due to the different types of tests were used across studies. The present results suggest that the phase of the cycle where E2 is highest, is associated with cognitive functions that go beyond the sexually dimorphic tasks discussed above and can engage different brain systems depending on cycle phase.

Participants in this study provided detailed and thorough information related to their menstrual cycle and thus, the estimated cycle phase on testing day should be robust and

reliable. However, it would be advantageous to directly measure hormone levels in order to deal with possible limitations of self-report data. It would also be of interest to investigate the role of other hormones that fluctuate across the menstrual cycle, particularly progesterone. There has been growing interest in the role of progesterone on cognitive function in both animals and humans (Barros, Tufik, & Andersen, 2015); since progesterone is high in the luteal phase and low in the follicular phase, it is possible that this hormone also plays a role in the cycle phase differences in strategy use observed in the present study. Moreover, with an increasing number of women in the Western world spending multiple years on contraceptive medication and living a larger proportion of their lives after menopause, it would also be of interest to broaden this research question to investigate multiple memory system bias in women taking contraceptives as well as peri- and postmenopausal women.

The present study has revealed that, as in rodents, gonadal hormones influence the type of memory system that is likely to be engaged by women when solving a task or effectively navigating a virtual environment. The luteal phase is associated with a significant increase of women using a spatial strategy, and a significant increase in women using landmarks in a maze when remembering the position of objects in the environment. Conversely, women tested during the follicular phase used a response strategy to solve the 4/8VM task. These findings support the growing body of research showing that cognitive function is modulated by and change with fluctuating hormones across the menstrual cycle.

CHAPTER 5

Learning and memory changes across the menstrual cycle; differences between mothers and non-mothers

Dema Hussain, Sarah Hanafi, Kyoko Konishi, Wayne G. Brake, Véronique D. Bohbot

Preface

In the previous chapter, it was revealed that multiple memory system bias changes across the menstrual cycle, which shows that ovarian hormones modulate which memory system is more likely to be used to navigate an environment. In Chapter 2, it was shown that, in rats, the association between E2 levels and memory bias is altered by parity. However, it is unknown whether a similar effect would be found in humans. Very little research has been done on the long-term effects of reproductive experience on cognition in women; however, improvements in verbal and working memory have been observed in mothers up to two years after giving birth. Moreover, studies carried out with rodents have also provided evidence for long-term changes related to parity. It is therefore possible that multiple memory system bias across the menstrual cycle could be altered by parity. Thus, the goal of the present study was to investigate the effect of parity on memory bias in cycling women.

Abstract

A hippocampus-mediated spatial or a striatum-mediated response memory system can be employed to navigate and environment or solve a task. Moreover, these memory systems are modulated by 17β-Estradiol (E2) such that high levels of E2 promote spatial memory whereas low E2 levels promote response memory. Yet this effect changes with reproductive experience in rats. In humans, memory bias changes across the menstrual cycle such that women predominantly use a spatial strategy during the luteal phase, when E2 is high. In the present study, the effect of reproductive experience on cycle-dependent memory system bias was investigated in women. Fifty-four young women with (n = 28) and without (n = 26) maternal experience were tested either during the follicular (n = 23) or luteal (n = 31) phase of the menstrual cycle on the 4-on-8 virtual maze, which can be solved by either using a spatial or response strategy. Mothers tested in the luteal phase predominantly used a spatial strategy (70.6%), whereas the opposite pattern was observed in the follicular group (spatial = 27.3%). The same pattern was observed in women without reproductive experience; however, this was not statistically significant. In addition, non-mothers needed more trials to reach criterion in the luteal than in the follicular phase, whereas the opposite pattern was observed in mothers. These findings suggest that women tend to rely on spatial memory when tested during the luteal phase whereas they predominantly use response memory during the follicular phase, and this is effect is pronounced in mothers. This indicates that multiple memory system bias changes with fluctuating hormone levels across the menstrual cycle; however, unlike in rodents, this does not change with reproductive experience.

5.1 Introduction

It has been shown in both rodents and humans that multiple memory systems can be engaged in order to solve a task for a reward or navigate an environment. Namely, a hippocampus- (HPC) dependent place, or spatial, strategy relies on allocentric cues to form a cognitive map whereas a dorsal striatum- (DS; or caudate nucleus in humans) dependent response strategy relies on egocentric, stimulus-response cues to navigate an environment. These different strategies are independent and competitive, such that as one memory system is compromised (e.g., by lesion or inhibitory drug infusions), the other becomes dominant (McDonald & White, 1994; Packard, Hirsh, & White, 1989; Packard & McGaugh, 1992, 1996; Packard & White, 1991; White & McDonald, 2002). Similarly, in humans, it has been shown that individuals can navigate an environment by using either a spatial strategy, which involves memorizing the locations of several landmarks and the spatial relationship between them, or a response strategy, which involves memorizing body turns (e.g., left or right) from a start position (Iaria, Petrides, Dagher, Pike, & Bohbot, 2003). Just as in rodents, these strategies have been shown to rely on specific brain regions; spatial strategy use is correlated to increased hippocampal grey matter and activity whereas response strategy use is correlated with higher activity and grey matter in the caudate nucleus (Bohbot, Lerch, Thorndycraft, Iaria, & Zijdenbos, 2007; Iaria et al., 2003).

Since a spatial strategy is more cognitively demanding and less efficient than a response strategy in a familiar environment, both rodents and humans tend to eventually switch from spatial to response memory with practice (Chang & Gold, 2003; Iaria et al., 2003). However, this does not occur in females, as multiple memory system bias has been consistently shown to be affected by 17β-Estradiol (E2) levels. Several studies have shown that, in both naturally cycling and ovariectomized female rats given replacement E2, no or low E2 levels bias a female to use a response strategy while high levels of E2 are associated with use of spatial strategy in appetitively motivated tasks (Korol, Malin, Borden, Busby, & Couper-Leo, 2004; Marriott & Korol, 2003; Quinlan, Hussain, & Brake, 2008; Quinlan et al., 2013). Furthermore, low E2 levels lead to enhanced performance on tasks that rely on response learning and high E2 enhance performance on spatial based tasks (Hussain, Hoehne, Woodside, & Brake, 2013; Korol & Kolo, 2002). This E2-mediated effect appears to impact multiple memory system bias by directly affecting the HPC and DS (Marriott,

2003; McElroy & Korol, 2005; Zurkovsky, Serio, & Korol, 2011; Zurkovsky, Brown, Boyd, Fell, & Korol, 2006). In addition, hippocampal volume in mice increases in proestrus, the phase of the mouse estrous cycle in which E2 peaks and decreases in estrus, when E2 is low; these hippocampal volume changes correlate with use of spatial and response strategies, respectively (Qiu et al., 2013). It should also be noted that E2 has been shown to act directly in the prefrontal cortex to affect multiple memory system bias (Almey et al., 2014), which suggests that brain regions other than the HPC and DS are play a role in memory bias.

This body of research emphasizes the key role of E2 on multiple memory system bias; however, this effect had until recently not been investigated in humans. In fact, there are a limited number of studies that have investigated how cognition changes in reproductively aged women across the menstrual cycle and with varying levels of circulating E2. Indeed, women experience changes in E2 levels across the menstrual cycle, beginning with low levels during menstruation, a peak in E2 prior to ovulation, followed by elevated levels of E2 and progesterone (P) in the second half of the cycle (Mihm, Gangooly, & Muttukrishna, 2011). The menstrual cycle and cognition literature has shown that there is either no difference in cognitive functioning across the cycle, or an enhancement of male-typical tasks, such as mental rotation, when E2 levels are low and increased proficiency in female-typical tasks, such as verbal memory, when E2 is high (Duff & Hampson, 2000; Hampson, 1990; Rosenberg & Park, 2002; Sundström Poromaa & Gingnell, 2014). Recent findings from our laboratory have shown that women tested during the second half of their menstrual cycle (luteal phase) predominantly use a spatial strategy to navigate a virtual maze task whereas those tested in the first half of the cycle (follicular phase) were more likely to use a response strategy (Hussain, Hanafi, Konishi, Brake, & Bohbot, in prep). This suggests that, as in rodents, there is a cycle-dependent bias in the type of memory system engaged by women to solve a task.

Cognition can also potentially be affected by reproductive experience; the process of pregnancy, birth, and the post-partum period is marked by a dramatic change in hormonal levels, brain plasticity, and behavior in both rodents and humans (Macbeth & Luine, 2010). Indeed, in rodents, parity is associated with enhanced spatial memory in a radial arm maze compared to nulliparous (without reproductive experience) rats (Kinsley et

al., 1999; Pawluski, Walker, & Galea, 2006); interestingly, this effect was not observed in rats exposed to pups, which indicates that the observed changes are associated with the brain changes that occur through pregnancy and birth (Pawluski, Vanderbyl, Ragan, & Galea, 2006). This parity-associated improvement of spatial memory has also been shown to be long-term (Gatewood et al., 2005; Love et al., 2005), lasting up to 16 months after weaning (Lemaire et al., 2006), and counteracting age-related memory decline (Macbeth et al., 2008). Related to this behavioral effect, reproductive experience is also associated with an increase in dendritic spine density (Kinsley et al., 2006) and long-term potentiation of cells, which is associated with learning and memory function (Tomizawa et al., 2003), in the HPC. Furthermore, reproductively experienced rats show increased sensitivity to E2 compared to nulliparous rats (Bridges & Byrnes, 2006) and reduced dopamine D2 receptor binding in the dorsal striatum (Hussain, Graham, Woodside, & Brake, in prep). Taken together, these findings demonstrate that reproductive experience is associated with potentiated hippocampal function, E2 sensitivity and, consequently, spatial memory. This would suggest that parity would bias females to use a hippocampal spatial strategy over a response strategy. Indeed, it has been shown that reproductively experienced rats do not show differences in strategy type used or in acquisition of place or response learning, regardless of E2 levels (Hussain, Hoehne, Woodside, & Brake, 2013).

In humans, the experience of pregnancy has long been associated with a subjectively rated and experimentally tested cognitive decline (for review, see: Macbeth & Luine, 2010), though little is known about the long-term effect of motherhood on the brain and cognitive function in women. Several studies have shown that these cognitive impairments are temporary and are enhanced again within 12 months of giving birth (Mickes, Wixted, Shapiro, & Scarff, 2009; Silber, Almkvist, Larsson, & Uvnäs-Moberg, 1990) and comparative to controls without children (Crawley, Dennison, & Carter, 2003). In addition, one study revealed that cognitive changes associated with reproductive experience are long-term and persist up to two years postpartum; women's attention as well as verbal and working memory improved compared to pregnancy levels, though this was correlated with improvements in mood and anxiety levels (Buckwalter, Buckwalter, Bluestein, & Stanczyk, 2001).

Though the research findings are sparse, it appears that reproductive experience modifies the brain and behavior long-term; also, the E2-mediated effect on multiple memory system bias in rats changes with parity such that strategy differences are no longer observed. However, the impact of reproductive experience on multiple memory systems in women has not yet been investigated. In the present study, young, naturally cycling women who had given birth to at least one child and were not on contraceptive medication were tested on a 4 on 8 virtual maze (4/8 VM) task that can be solved by using either a spatial or response strategy. Participants were tested either during the follicular or luteal phase of the menstrual cycle. Age- and education-matched women who had never had children were also tested as a comparison group. It was also ensured that at least two years had elapsed since birth and that mothers were not currently breastfeeding in order to investigate long-term effects of reproductive experience and so the menstrual cycle and associated hormonal levels would be comparable to the control group.

Since it's been previously observed that women tend to use a spatial strategy during the luteal phase and the opposite is observed in the follicular group, it was hypothesized that the same pattern would be observed in the control group in the current study. It was hypothesized that, since the luteal phase is associated with use of a spatial strategy, this group would make errors during the probe trial when all landmarks are removed (Iaria et al., 2003). In addition, this group would make more errors throughout the 4/8 VM task, need more trials to learn the task, and mention more landmarks. Finally, it was also hypothesized that this pattern would no longer be observed in the reproductive experience group, since it's been shown in rodents that the E2-mediated effect on strategy use disappears with parity.

5.2 Methods

5.2.1 Participant characteristics

A total of 54 healthy, right-handed, regularly cycling (i.e., a menstrual cycle lasting between 25 and 34 days) women with (n = 28) and without (n = 26) reproductive experience were tested (age: M = 30.35; SD = 3.32; range = 23-36). All participants completed a screening questionnaire in order to determine whether they were eligible to participate in this study. Individuals were excluded if they reported a history of psychological or neurological illness, drug or alcohol abuse, had been pregnant within the

past two years, were currently breastfeeding, or had taken contraceptive medication within three months of testing. Age, education, and sleep score were controlled for across comparison groups to ensure that participants with or without reproductive experience were as similar as possible (see Table 5.1 for complete participant demographics). Participants' phase of the cycle on testing day was determined using a hormonal profile questionnaire and detailed information regarding individual menstrual cycle duration and regularity, as well as other health-related information. Detailed description of menstrual cycle phase determination can be found elsewhere (Hussain et al., in prep). In the total sample, there were 23 women included in the follicular phase group and 31 included in the luteal phase group. Informed consent was obtained from participants in accordance with local ethics guidelines. This study was approved by the Research Ethics Board at the Douglas Mental Health University Institute.

5.2.2 Neuropsychological Tests, PSS, and Sleep Questionnaires

Participants were administered a battery of neuropsychological tests in order to control for possible confounds or group differences related to cognitive function, IQ, and participants were using a response strategy, no increase in errors should occur with this memory. Questionnaires measuring stress and quality of sleep were also given in order to investigate if mothers rate their stress levels and quality of sleep differently than women with no reproductive experience. The neuropsychological test battery given included the Rey Auditory Verbal Learning Test (RAVLT; Rey, 1941; Schmidt, 1996), the Rey-Osterrieth Complex Figure task (RO; Osterrieth, 1944), the Test for Non-Verbal Intelligence-3 (TONI; form A; Brown, Johnsen, & Sherbenou, 1997), the perceived stress scale (PSS; Cohen, Kamarck, & Mermelstein, 1984), and the Leed's Sleep Evaluation Questionnaire (LSEQ), which is split into four domains: ease with which an individual falls asleep, quality of sleep, ease with which an individual awakens following sleep, and behavior following wakening (Parrott & Hindmarch, 1978). These tests have been described in more detail elsewhere (Hussain et al., in prep). Means and standard deviations for neuropsychological tests, IQ, PSS, and LSEQ in mothers and non-mothers can be found in Table 5.1.

Table 5.1.

Participant demographics for mothers and non-mothers

	NON-MOTHER		MOTHER	
	M	*SD*	*M*	*SD*
Age (in years)	29.58	3.06	31.07	3.43
Number of years of education	17.12	2.86	16.75	2.61
Sleep ratio[a]	0.92	0.14	0.89	0.15
Hours of exercise/week	3.85	3.32	3.40	2.87
Number of alcoholic drinks/week	2.50	2.54	0.66	1.17
Number of cigarettes/week	6.73	19.28	4.73	14.60
Menstrual cycle data				
Age first period	12.69	1.38	12.41	1.80
Number of months without period[b]	2.13	6.31	3.14	5.84
Mean cycle length (days)	29.24	2.72	29.20	2.63
Mean period length (days)	4.97	0.99	5.39	1.08
Contraception history				
Number of months on	69.24	41.96	61.05	45.30
Number of months since off	66.29	47.88	67.86	48.34
Reproductive experience				
Number of children	-	-	1.50	0.69
Months elapsed since last pregnant	-	-	43.75	23.89
Leeds Sleep Evaluation Questionnaire[c]				
Getting to Sleep	51.04	18.22	56.54	11.47
Quality of Sleep	47.37	22.20	45.80	18.02
Awakening from Sleep	46.50	21.08	50.02	20.02
Behavior Following Wakefulness	51.35	19.47	56.07	19.22
Rey Auditory Verbal Learning Test[d]				
Pre-Interference (total score over 5 trials)	59.12	6.29	60.93	5.81
Post-Interference (out of 15)	12.85	2.09	12.61	1.57
Post-Delay (out of 15)	13.27	1.85	13.14	1.43

	NON-MOTHER		MOTHER	
	M	*SD*	*M*	*SD*
Rey-Osterrieth Complex Figure Task				
Copy Score (out of 36)	35.12	1.45	34.84	1.47
Immediate Recall Score (out of 36)	25.19	6.33	24.34	5.84
Delay Score (out of 36)	24.42	5.43	24.57	5.31
IQ Score	105.85	12.74	110.36	15.77
Perceived Stress Score (out of 56)[e]	24.54	6.34	22.25	6.64

[a]Sleep ratio is calculated by dividing average hours of sleep per night over last week by ideal number of hours of sleep per night to feel rested.

[b]The number of months that period was interrupted (not including pregnancy).

[c]Scores are out of a total 100, with higher numbers indicating better sleep.

[d]Values represent number of words recalled.

[e]Higher values indicate higher perceived stress.

5.2.3 Behavioral task (4/8 VM)

A virtual navigational task was adapted from a commercially available computer game (Unreal; Epic Games, Raleigh, NC). The virtual environment was composed of an eight-arm radial maze with a central starting location (Bohbot et al., 2007; Iaria et al., 2003), which was consisted of successive trials that were composed of two parts. In Part 1, four of the eight arms were accessible with objects at the end of each arm; in Part 2, all arms were accessible and objects were present in the four arms that had previously been blocked in Part 1. Participants were told to retrieve all four objects from the accessible arms in Part 1 and remember which arms they visited to avoid them in Part 2. An error consisted of an entry into an arm that did not contain an object. At least three trials were administered; if the participant reached criterion (i.e., made no errors during Part 2 of any of the first three trials), a probe trial was administered in which the walls around the radial maze were raised to conceal the landscape, so that landmarks were no longer visible. The purpose of this probe trial was to determine whether participants were using a spatial or a response strategy throughout the task. If a spatial strategy was employed, the landmarks present in surrounded by various landmarks (e.g., mountains, a tree, a boulder). The experiment the environment were relevant to perform the task; therefore, the change in the environment observed in the probe trial should result in an increase in errors. Conversely, if change (Iaria et al., 2003). Once the probe trial was completed, an additional trial was given in order to observe whether participants changed their strategy following the probe trial.

At the end of the experiment, participants were debriefed and asked a series of questions about how they solved the task from beginning to end. Participants were categorized as using a response strategy when they associated the arms with numbers or letters, or if they counted the arms (clockwise or counterclockwise) from a single starting point. If they used at least two landmarks and did not mention a response strategy, they were categorized as using spatial memory. Participants who mentioned using several landmarks at the beginning and later shifted to counting were placed into the “shift group”. The initial strategy that was used by the participant was measured. Two experimenters independently evaluated the verbal reports and assigned participants to a particular strategy group depending on the method used to navigate the environment. The independent judgments of the experimenters were correlated to evaluate their consistency. Aside from

probe errors, which are errors made in relation to the sequence pattern being rotated in the probe trial, errors made during all Part 2 trials, number of landmarks, and trials to criterion were also measured. For a more detailed description of the 4/8 VM task, see: Hussain et al., submitted.

5.2.4 Statistical Analysis

A series of 2 (cycle phase) X 2 (reproductive experience) independent factorial analysis of variance (ANOVA) tests were carried out in order to compare the neuropsychological measures across reproductive experience and phase groups: RAVLT (total recall for first 5 trials, recall following interference, recall following delay) and RO (copy, immediate recall, delay). Similarly, participants were compared across groups on IQ score (TONI), PSS, and all LSEQ measures.

For the 4/8 VM task, four 2 (cycle phase) X 2 (reproductive experience) independent factorial ANOVAs were carried out to compare the average number of errors committed during all part 2 trials, number of trials to criterion, number of probe errors, and number of landmarks mentioned in the verbal report across cycle phase and reproductive experience group. Levene's test for equality of variances was carried out for every ANOVA. Finally, two Chi square tests were carried out to compare proportions of participants in the follicular and luteal groups using an initial spatial or response strategy in the task in mothers and in non-mothers.

5.3 Results

5.3.1 Neuropsychological Tests, PSS, and Sleep Questionnaires

Cycle phase and reproductive experience groups did not differ on any of the neuropsychological test measures or IQ. However, there was a significant reproductive experience group by cycle phase interaction for PSS ($F_{(1,50)} = 5.41$, $p = .024$, $\eta_p^2 = 0.10$). In the follicular phase group, mothers reported lower perceived stress ($M = 20.32$, $SD = 7.89$) than non-mothers ($M = 27.17$, $SD = 5.34$) whereas in the luteal phase group, mothers' reported stress levels were higher ($M = 23.50$, $SD = 5.58$) than in non-mothers ($M = 22.29$, $SD = 6.43$). This suggests that reproductive experience is associated with lower perceived stress, but only in the follicular phase of the menstrual cycle (see Figure 5.1).

None of the four LSEQ domains differed across groups; however, cycle phase has an effect for two questions in the LSEQ questionnaire. Namely, participants tested in the

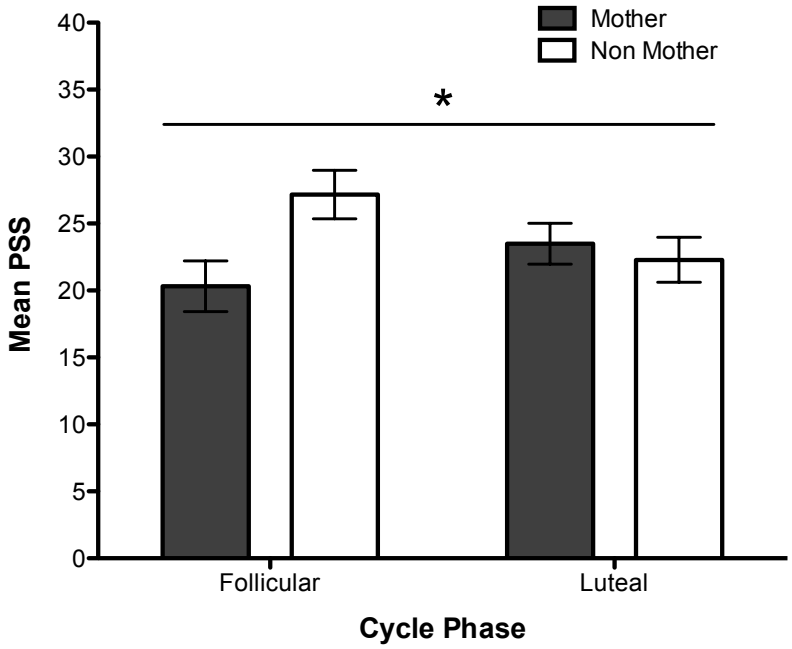


Figure 5.1. Average levels of perceived stress (as measured by PSS) in mothers and non-mothers across the cycle. * indicates significant interaction: stress levels vary by cycle phase and by maternal experience group.

follicular phase reported greater ease ($F_{(1,50)} = 5.15$, $p = .028$, $\eta_p^2 = 0.09$) and speed ($F_{(1,50)} = 4.72$, $p = .035$, $\eta_p^2 = 0.09$) for falling asleep compared to participants tested in the luteal phase. This suggests that a lower E2 state could be associated with an easier time falling asleep, but general quality of sleep does not differ between mothers and non-mothers.

5.3.2 Behavioral task (4/8 VM)

Though not statistically significant, there was a trend for a probe error effect of cycle phase, such that participants tested during the follicular phase made fewer probe errors ($M = 0.09$, $SD = 0.46$) than the luteal phase group ($M = 0.33$, $SD = 0.46$), $F_{(1,50)} = 3.54$, $p = .066$, $\eta_p^2 = 0.07$. These results suggest that women in the luteal group made more probe errors, regardless of whether they have children or not.

A statistically significant interaction was found for number of trials required to reach criterion $F_{(1,50)} = 6.38$, $p = .015$, $\eta_p^2 = 0.11$; in the luteal phase, non-mothers ($M = 2.36$, $SD = 1.45$) needed more trials to reach criterion than mothers ($M = 1.47$, $SD = 0.87$). The opposite was observed in the follicular phase: mothers ($M = 1.91$, $SD = 1.04$) needed more trials to reach criterion when compared to non-mothers ($M = 1.33$, $SD = 0.65$). The same pattern was observed in average number of errors committed during part 2 trials; however, this interaction was not statistically significant, $F_{(1,50)} = 2.65$, $p = .110$, $\eta_p^2 = 0.05$. Furthermore, results revealed that mothers ($M = 2.67$, $SD = 0.89$) mentioned significantly more landmarks in the verbal report than non-mothers ($M = 2.17$, $SD = 0.88$); $F_{(1,50)} = 4.21$, $p = .045$, $\eta_p^2 = 0.08$. In addition, mothers and non-mothers were almost equivalent in the follicular phase whereas the mothers mentioned, on average, one more landmark in their verbal report than non-mothers in the luteal phase (interaction trend: $F_{(1,50)} = 3.59$, $p = .064$, $\eta_p^2 = 0.07$). See Figure 5.2 for all 4/8 VM behavioral results.

Finally, in women without reproductive experience, participants tested in the follicular phase predominantly used an initial response strategy (66.7%) compared to a spatial strategy (33.3%) in the 4/8 VM task whereas the opposite was observed in the luteal group (Spatial = 57.1%); however, this was not statistically significant, $X^2 = 1.47$, $p = .225$. The same pattern was observed in the reproductive experience group: participants tested in the follicular phase predominantly used an initial response strategy (72.7%) compared to a spatial strategy (27.3%) in the 4/8 VM task whereas the opposite was observed in the luteal group (Spatial = 70.6%), $X^2 = 5.04$, $p = .025$. This suggests that women tested in the second

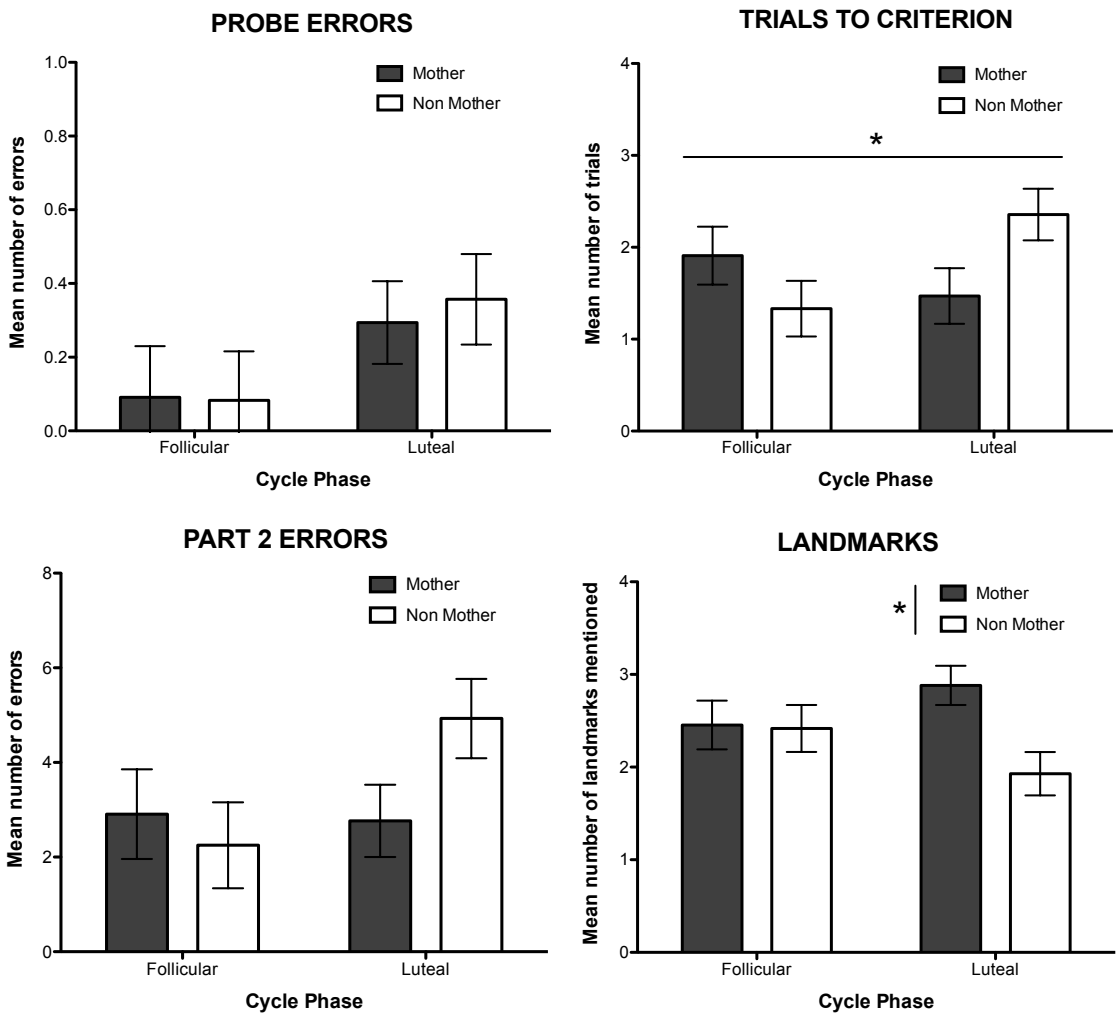


Figure 5.2. Performance on 4/8 VM learning measures in mothers and non-mothers across cycle phase. Top left panel. Average number of errors made by participants in the probe trial. Top right panel. Average number of errors made in all Part 2 trials of 4/8 VM task across cycle phase and maternal experience group. Bottom left panel. Average number of trials taken to reach criterion. * indicates significant interaction. Bottom right panel. Average number of landmarks mentioned by participants in the verbal report. * indicates significant reproductive experience effect: mothers mentioned more landmarks than non-mothers.

half of the cycle tend to use a spatial strategy to complete the task, whereas women tested in the follicular phase of their cycle tend to use a response strategy. This happens whether they are reproductively experienced or not, though the effect is more pronounced in mothers. See Figure 5.3 for strategy results.

5.4 Discussion

The main goal of this study was to investigate multiple memory system bias in cycling women with and without reproductive experience. Findings revealed that the luteal phase was associated with spatial strategy and the follicular phase with response in both mothers and non-mothers, though this was statistically significant only in mothers (see Figure 5.3). This contradicts what has previously been found in rodents: not only did the E2-mediated effect on multiple memory system bias not disappear in mothers, it was stronger with reproductive experience. There are multiple reasons why this could have occurred. First, the only study that had looked at the relationship between E2 and parity on multiple memory systems was done with rodents (Hussain et al., 2013). The human mother experiences a myriad of psychological and social experiences that could be having additional effects on cognitive function. Second, in the rat study, subjects were ovariectomized and given replacement E2. Perhaps being exposed to the natural flux of ovarian hormones across the cycle has different effects on which memory system will be engaged to navigate an environment. In addition, it is possible that circulating P, along with E2, plays a role in multiple memory system bias. It is important to note that behavioral results did not differ between naturally cycling and ovariectomized rats given E2 (Korol & Kolo, 2002; Korol et al., 2004); there could, however, be a difference in how reproductive experience modifies this effect across species. Finally, the results show that the highest proportion of spatial strategy use observed was in mothers tested in the luteal phase (see Figure 5.3). As stated above, both high E2 levels and reproductive experience has been associated with potentiated hippocampal function and improved spatial learning; it is therefore possible that being a mother and being exposed to higher E2 levels has an additive effect on hippocampal function and, consequently, a higher likelihood of using a HPC-dependent spatial strategy.

In general, it appears that the luteal phase is marked by a higher spatial strategy use, which was expected to correlate with a higher number of probe and part 2 errors, trials to

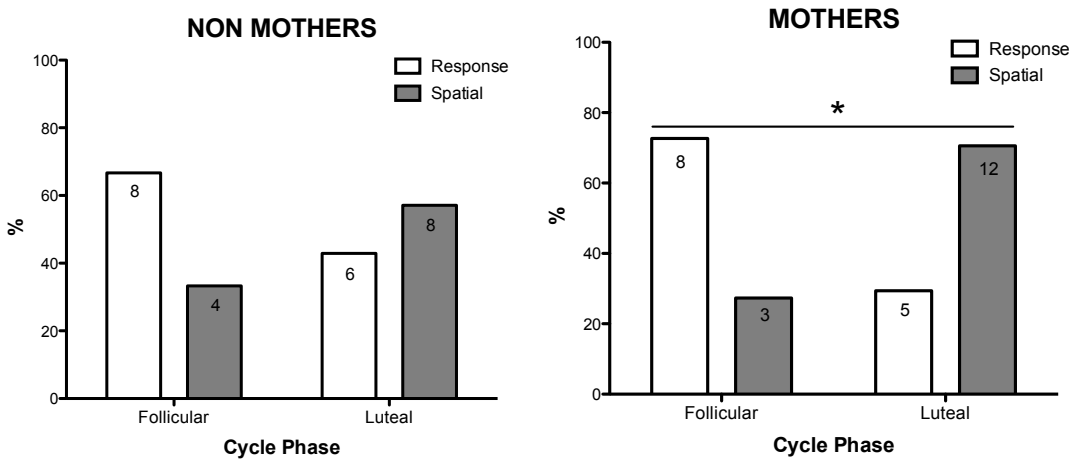


Figure 5.3. Percentage of women with (right panel) and without (left panel) reproductive experience tested in the follicular or luteal phase using an initial response or spatial strategy in the 4/8 VM task. * indicates significant difference in proportion of participants using response or spatial between cycle phase groups. Numbers represent *n* for each bar.

criterion, and landmarks mentioned in participants' verbal reports. This was partially observed; though not significant, there was a trend indicating a higher number of probe errors made by women tested during the luteal phase than the follicular phase (see Figure 5.2). In general, participants did not make many errors in the task, as can be seen in the group means, which are all under one error and indicative of a floor effect. This, combined with high within group variability, could explain why no effects were observed here. In addition, results show that non-mothers tested in the luteal phase needed more trials to reach criterion than those in the follicular phase; this pattern was reversed in mothers. These findings indicate that the difficulty of the task, as measured by how many trials were needed to learn it, differed significantly across reproductive experience group and cycle phase. Indeed, the effect of menstrual cycle phase on learning is reversed in mothers. Interestingly, the number of errors made during part 2 trials, when all pathways are open and participants must remember where to locate the objects, matched this pattern (see Figure 5.2), with non-mothers tested in the luteal phase making the most errors and needing the most trials to reach criterion.

Finally, the number of landmarks mentioned by participants in the verbal report did not differ across cycle phase. However, there was a significant effect of reproductive experience, such that mothers mentioned more landmarks than non-mothers. This is interesting, since the total proportion of women using a spatial strategy (across phase) was equivalent between mothers and non-mothers (around 50%), yet mothers reported noticing more landmarks while completing the task. This could be due to enhanced hippocampal function and spatial memory in mothers, which has previously been shown to occur in reproductively experienced rats (Kinsley et al., 1999; Kinsley et al., 2006; Pawluski et al., 2006a; 2006b) and could play an important evolutionary role (e.g., mothers having enhanced spatial memory in order to be able to locate food for their young). In the context of the current results, mothers noticed more landmarks but did not necessarily use spatial memory more than non-mothers. Indeed, as seen in Figure 5.3, strategy use in mothers changed across cycle phase, which indicates that ovarian hormone levels and menstrual cycle phase impacted strategy use despite higher overall memory for landmarks.

Participants scored similarly on most neuropsychological tests, indicating that they did not differ in any significant way other than in reproductive experience and phase of the

cycle they were tested in. Interestingly, a statistically significant interaction was found in PSS (see Figure 5.1); perceived stress levels were higher in the follicular phase than in the luteal phase for non-mothers, whereas stress levels were more equal across the cycle in mothers. The largest difference can be seen between mothers and non-mothers in the follicular phase: perceived stress was about 7 points higher in non-mothers than in mothers. These results fall in line with previous research showing that E2 and P, which are higher in the luteal phase of the menstrual cycle, are associated with decreased anxiety (Frye, Walf, Rhodes, & Harney, 2004; Koss, Gehlert, & Shekhar, 2004). In addition, reproductive experience has also been shown to play an anxiolytic role in both rodents and humans (Heron, O'Connor, Evans, Golding, & Glover, 2004; Love et al., 2005; Wartella et al., 2003). Thus, it is possible that, in women who have not been exposed to reproductive experience, ovarian hormones play a role in modulating anxiety and stress levels across the cycle. However, motherhood could be altering hormonal stress modulation such that this cyclic fluctuation in perceived stress is no longer observed in mothers.

Though the regularity of participants' menstrual cycles was carefully measured in order to get an accurate estimate of the exact day in the cycle that each woman was tested on, the information obtained is still subject to issues related to self-report data. It would therefore be beneficial to also measure endogenous circulating levels of ovarian hormones on testing day in order to directly compare strategy use across different E2 levels. In addition, the luteal phase is not only marked by elevated E2 levels, but is also related to high P levels (Mihm et al., 2011). Progesterone has been implicated in mediating E2's effects on cell proliferation in the HPC (Pawluski et al., 2009; Tanapat, Hastings, & Gould, 2005) and could therefore play a role in spatial memory and learning.

The present study revealed that reproductive experience and fluctuating hormones across the menstrual cycle have an impact on women's performance on a virtual navigation task as well as the type of cognitive strategy that is engaged to solve it. It would be warranted to further explore these effects. For example, some studies have shown that not only is there a difference between nulliparous and reproductively experienced females, but the number of times a female becomes pregnant and gives birth can also affect brain function in both rodents and humans (Paris & Frye, 2008; Tu, Lupien, & Walker, 2006). Number of children was not controlled for in the current study; however, the data suggests

that the association between cycle phase and strategy observed in mothers is strongest in women with two or more children. It is therefore possible that the association between the menstrual cycle and multiple memory system bias could change with additional pregnancies. It would be of interest to further investigate this effect in future studies.

Furthermore, in the current study, young adults were tested; since women are becoming pregnant and choosing to have children later in life, it would be interesting to study how the effects found here are affected by age. In addition, it is currently not known how long the observed parity-related effects on cognitive function last. It is possible that previously observed enhanced hippocampal function and performance on spatial tasks associated with parity (Kinsley et al., 1999, 2006; Lemaire et al., 2006; Love et al., 2005; Pawluski et al., 2006a; 2006b) persist into old age and help prevent age-related hippocampal and cognitive decline. With an increasingly aging population and menopause-related cognitive decline (see: Sherwin, 2012), it would be of great interest to investigate a possible protective role of motherhood on the aging female brain.

In conclusion, the present results reveal cycle-dependent changes in memory system bias and performance in a navigational task in women with and without reproductive experience. These findings emphasize the key role of fluctuating ovarian hormones on the type of memory system engaged to navigate an environment and solve a task. Additionally, they provide novel evidence for the long-term effects of reproductive experience on brain and cognitive function in women and can potentially provide clues for future directions in understanding the role of hormones and parity on post-menopausal cognitive decline in women.

CHAPTER 6

Multiple memory system bias across the menstrual cycle is modulated by progesterone, testosterone, and reproductive experience

Dema Hussain, Sarah Hanafi, Kyoko Konishi, Wayne G. Brake, Véronique D. Bohbot

Preface

It was observed in Chapters 4 and 5 that multiple memory system bias shifts across the menstrual cycle such that response memory is promoted during the follicular phase and spatial memory is used more often in the luteal phase. Moreover, this effect is enhanced in women with reproductive experience. It is clear that ovarian hormones play a key role in this cycle-dependent memory system bias; however, it was unknown whether this was due to changes in E2, P, or an interaction between these two hormones.

In the present study, serum E2, P, and T levels were analyzed and compared across spatial and response learners with or without reproductive experience in order to investigate which hormones are contributing to response and spatial strategy use and whether this differs with parity.

Abstract

Multiple memory systems can be used to navigate an environment. Namely, hippocampus-mediated spatial memory, which relies on the spatial relationship among landmarks, or caudate nucleus-mediated response memory, which relies on specific stimulus-response associations. Previous work from our laboratory has revealed that, in women, memory system bias shifts across the menstrual cycle such that response memory is promoted during the follicular phase (first half of the cycle) and spatial memory is promoted during the luteal phase (second half of the cycle); this effect is also observed in reproductively-experienced women. The goal of the current study was to investigate the role of ovarian hormones in cycle-dependent changes in memory bias in participants completing a virtual navigation task. Serum levels of 17β-estradiol (E2), progesterone (P), and testosterone (T) were measured across cycle phase in young, cycling women with or without reproductive experience. The results revealed that there was no significant difference in E2 levels between spatial and response learners. Interestingly, there were differences in P and T levels, and this changed with reproductive experience. In non-mothers, response memory was associated with high P and low T, whereas spatial memory use was marked by low P and higher T. In mothers, response memory was marked by low P and high T levels; spatial memory was associated with high P and low T. These findings suggest that multiple memory system bias in women is modified by changes in P and T, rather than E2. Moreover, this is underscored by a different hormonal profile in mothers and non-mothers, which hints at a long-term effect of reproductive experience on the link between ovarian hormones and cognition.

6.1 Introduction

Individuals can use multiple memory systems to navigate an environment; namely, a hippocampus- (HPC) dependent spatial memory, which involves creating spatial associations among landmarks in an environment to form a cognitive map, or a dorsal striatum- (DS; or caudate nucleus in humans) dependent response memory, which involves relying on stimulus-response cues and specific body turns in order to learn how to navigate an environment (Bohbot, Iaria, & Petrides, 2004; Bohbot, Lerch, & Thorndycraft, 2007; Iaria, Petrides, Dagher, Pike, & Bohbot, 2003; Maguire et al., 1998; O'Keefe & Nadel, 1978). These different memory systems have been shown to be distinct and competitive in nature, such that as one system is enhanced, the other is impaired. For instance, damage to the HPC not only leads to impaired spatial memory, but also improved performance on a response-based task. Similarly, damage to the DS leads to impaired response memory but has also been shown to improve spatial memory (McDonald & White, 1994; Packard & McGaugh, 1996; Packard & White, 1991; White & McDonald, 2002).

Interestingly, multiple memory system bias is modulated by 17β-Estradiol (E2) in the female rat. In both naturally cycling and ovariectomized rats given replacement E2, it has been established that low levels of E2 bias a female to use a response strategy to solve a dual solution maze task, whereas high E2 is associated with use of a spatial strategy (Hussain, Hoehne, Woodside, & Brake, 2013; Korol, Malin, Borden, Busby, & Couper-Leo, 2004; Quinlan, Hussain, & Brake, 2008; Zurkovsky, Brown, Boyd, Fell, & Korol, 2006). Furthermore, low E2 levels are associated with enhanced performance on a response task and high E2 levels improve performance on a place task (Hussain et al., 2013; Korol & Kolo, 2002). These effects have been shown to be due to E2 acting directly in the HPC to enhance place memory and in the DS to impair response memory (Zurkovsky, Serio, & Korol, 2011; Zurkovsky et al., 2007; Zurkovsky, Brown, & Korol, 2006). In addition, it has also been shown that infusing E2 directly in the medial prefrontal cortex affects memory bias (Almey et al., 2014). Thus, E2 appears to modulate memory system bias directly in the HPC and DS, and through prefrontal activation of these brain areas.

Research has shown that E2 alters hippocampal and striatal function, which could underscore the association between E2 and memory bias. Indeed, the proestrus phase of the rat estrous cycle, which is marked by high E2 levels, is associated with a higher number of

excitatory synapses and dendritic spines in the HPC and, hence, enhanced hippocampal function (Gould, Woolley, Frankfurt, & McEwen, 1990; Woolley & McEwen, 1992; Woolley, Wenzel, & Schwartzkroin, 1996). It has also been revealed that hippocampal volume increases with the phase of the cycle associated with high E2 levels in both mice and humans (Protopescu et al., 2008; Qiu et al., 2013); this hippocampal volume change is also associated with use of a place strategy in mice (Qiu et al., 2013). Moreover, E2 may impact response memory in the DS by mediating dopamine (DA) function in this brain area. It is known that E2 is related to increased DA release and striatal DA-dependent behaviors in the rat (Becker, 1999; Becker & Beer, 1986; Becker & Cha, 1989; Castner, Xiao, & Becker, 1993) and impacts decision making and reward seeking in humans (Bayer, Bandurski, & Sommer, 2013; Dreher et al., 2007). In young, cycling women, it has been shown that high E2 is associated with enhanced performance on female typical tasks, such as verbal fluency and memory (Rosenberg & Park, 2002) whereas low E2 is linked to better performance on male typical tasks, such as mental rotation (Hampson, 1990; Hausmann, Slabbekoorn, Goozen, Cohen-Kettenis, & Gunturkun, 2000). However, these studies are scarce and little is known about how multiple memory system bias is affected in women by fluctuating E2 levels across the menstrual cycle.

Multiple memory bias is not only impacted by varying levels of E2, but also by reproductive experience (also known as parity). It was recently observed that the link between E2 and memory bias is no longer present in reproductively experienced rats, such that both strategy types are equally employed on a dual solution task and there is no difference in performance on response- or place-based tasks across low and high E2 levels (Hussain et al., 2013). The female brain undergoes a dramatic hormonal and organizational change during pregnancy (for review, see: Macbeth & Luine, 2010), but little is known about the long-term impact of reproductive experience on cognition and behavior. A few studies have shown that parity is associated with increased sensitivity to E2 (Bridges & Byrnes, 2006), enhanced spatial learning (Love et al., 2005), and increased striatal DA levels (Byrnes, Byrnes, & Bridges, 2001). In humans, one study has shown that mothers have higher verbal and working memory two years after giving birth compared to when they were pregnant (Buckwalter, Buckwalter, Bluestein, & Stanczyk, 2001). It is therefore

possible that the female brain becomes permanently altered following reproductive experience thus changing how E2 impacts multiple memory bias.

In a recent study carried out by the authors, it was found that memory bias changes in women across the menstrual cycle and with reproductive experience. When women were tested during the follicular phase of the menstrual cycle, they were more likely to use a response strategy to solve a virtual navigation task. Conversely, women were more likely to use a spatial strategy when tested in the luteal phase of the cycle (Hussain et al., submitted). Interestingly, this pattern was observed whether participants were reproductively experienced or not, which contradicts findings observed in rodents (Hussain et al., in prep; Hussain et al., 2013). These findings show that multiple memory bias changes depending on the phase of the cycle, which emphasizes the important role of fluctuating ovarian hormones and how they impact cognition in women. However, unlike what had previously been observed in rodents, this pattern did not change with reproductive experience.

Most research has focused on the role of E2 in female cognition and in multiple memory system bias. It is important to note that the menstrual cycle is also marked by changes in progesterone (P) levels such that P levels are low throughout the follicular phase while they peak and plateau in the luteal phase, before dropping at the onset of menstruation (Mihm, Gangooly, & Muttukrishna, 2011). Thus, P could be playing a role in the observed results, since this hormone also increases in the luteal phase. In addition, though less studied than E2, studies have shown that P is associated with both enhancing (Maki, Zonderman, & Resnick, 2001; Natale, Albertazzi, Zini, & Di Macco, 2001) and disruptive (Freeman, Weinstock, Rickels, Sondheimer, & Coutifaris, 1992; Honjo et al., 2005) effects on memory in women; however, the majority of studies that are focused on hormones and cognition in women are carried out with a postmenopausal sample taking hormone replacement. Furthermore, P has been shown to rapidly increase hippocampal spine density, but this decreases within a few hours and P blocks the aforementioned E2-induced increase in hippocampal synapses (Woolley & McEwen, 1993). Indeed, P's actions are dependent on induction of E2 receptors (Lydon et al., 1995) and seem to oppose some of E2's actions (Choi et al., 2003; Honjo et al., 2005). This suggests that E2 and P act in concert to affect brain function and, possibly, multiple memory system bias.

In the present study, the aim was to investigate how serum hormone levels across the menstrual cycle relate to memory system bias in young, naturally cycling women with and without reproductive experience. Since it was previously revealed that the strategy used to solve a virtual navigation task changed depending on whether participants were tested during the follicular or luteal phase of the cycle, and that pattern was more pronounced in mothers, we wanted to directly observe whether serum E2 levels could explain these differences. In addition, though participants' menstrual cycle information was thoroughly measured and, therefore, approximated phase of the cycle on testing day was considered robust, serum hormone levels were collected in order to verify this data. Thus, following testing on a 4-on-8 virtual maze (4/8 VM) task that can be solved by using either a spatial or response strategy, blood samples were collected from participants and analyzed for levels of E2 and P; this was done in order to determine if both hormones are contributing to the previously observed spatial strategy bias in the luteal phase (when E2 and P are high). In addition, testosterone (T) was also measured, since low levels of T are produced by the ovaries, have been found in some areas of the female brain, and can be converted into E2 (Davis & Tran, 2001; Vierhapper, Nowotny, & Waldhäusl, 1997). Additionally, T has been shown to influence cognitive abilities in cycling women (Hausmann et al., 2000).

First, it was hypothesized that serum hormone levels would match menstrual cycle data such that the follicular phase would be marked by low E2 and P levels whereas the luteal phase would correlate with high E2 and P. Since mothers were tested at least two years after giving birth, hormone levels were expected to be back at baseline and, therefore, this hormonal profile should be similar in mothers and non-mothers. Second, as has been repeatedly shown in rodents, it was expected that high E2 would be associated with use of a spatial strategy and low E2 would be related to response. Thus, it was hypothesized that participants who used a response strategy on the 4/8 VM task would have low serum E2 levels. Conversely, spatial learners were expected to have higher serum E2 levels. Finally, since previous results have shown that multiple memory bias across cycle phase did not differ with reproductive experience, it was expected that hormonal patterns across strategy groups would not differ between mothers and non-mothers.

6.2 Methods

6.2.1 Participant characteristics

A sample consisting of 49 right-handed women with (n = 23) and without (n = 26) reproductive experience were tested. All participants were regularly cycling (i.e., a menstrual cycle lasting between 25 - 34 days) and had not been using contraceptive medication for at least three months before testing. Participants were, on average, 30 years of age (M = 30.16; SD = 3.27; range = 23-36) and had 17 years of education (M = 17.10; SD = 2.66). Age, education, and sleep quality were controlled for across comparison groups to ensure that participants with or without reproductive experience were as similar as possible. All participants completed a screening questionnaire in order to determine whether they were eligible to participate in this study. Phase of the cycle on testing day was determined using a hormonal profile questionnaire and detailed information regarding individual menstrual cycle duration and regularity, as well as other health-related information. Detailed description of exclusion criteria and menstrual cycle phase determination can be found elsewhere (Hussain et al., submitted). In this sample, 21 women were included in the follicular phase group and 28 included in the luteal phase group. Informed consent was obtained from participants in accordance with local ethics guidelines. This study was approved by the Research Ethics Board at the Douglas Mental Health University Institute.

6.2.2 Neuropsychological Tests, PSS, and Sleep Questionnaires

Participants were administered a battery of neuropsychological tests in order to control for possible confounds or group differences related to verbal and visual memory and non-verbal IQ: this test battery included the Rey Auditory Verbal Learning Test (RAVLT; Rey, 1941; Schmidt, 1996), the Rey-Osterrieth Complex Figure task (RO; Osterrieth, 1944), and the Test for Non-Verbal Intelligence-3 (TONI; form A; Brown, Johnsen, & Sherbenou, 1997). Questionnaires measuring perceived stress and quality of sleep were also given in order to investigate whether there was a reproductive experience effect on stress and sleep quality levels, since this could impact performance on the 4/8 VM task. Stress was measured using the Perceived Stress Scale (PSS; Cohen, Kamarck, & Mermelstein, 1984). Sleep quality was assessed using the Leed's Sleep Evaluation Questionnaire (LSEQ), which is split into four sections: ease with which an individual falls

asleep, quality of sleep, ease with which an individual awakens following sleep, and behavior following wakening (Parrott & Hindmarch, 1978). These tests have been described in more detail elsewhere (Hussain et al., submitted).

6.2.3 Behavioral task (4/8 VM)

The 4/8VM task consists of an 8-arm radial maze situated in a virtual environment composed of various landmarks (e.g., a tree). The task consisted of a series of trials that consisted of two parts. In Part 1, in four arms are open and four are blocked, and participants have to retrieve objects located at the end of the open arms. Then, in Part 2, all eight arms are open and participants have to retrieve objects now located in the previously blocked arms. If the participant reached criterion (i.e., made no errors during Part 2 of any of the first three trials), a probe trial was administered in which the walls around the radial maze were raised to conceal the landscape, so that landmarks were no longer visible. If a spatial strategy was employed by the participant, the landmarks present in the environment were relevant to perform the task; therefore, the change in the environment observed in the probe trial should result in an increase in errors (Iaria et al., 2003). Once the probe trial was completed, an additional trial was given in order to observe whether participants changed their strategy following the probe trial. Strategy use was assessed with a verbal report once the task is completed. Participants were categorized as using a response strategy when they associated the arms with numbers or letters, or if they counted the arms (clockwise or counterclockwise) from a single starting point. If they used at least two landmarks and relied on the relationship between them to navigate the virtual environment, they were categorized as using spatial memory. Aside from probe errors, which are errors made in relation to the sequence pattern being rotated in the probe trial, errors made during all Part 2 trials, number of landmarks, and trials to criterion were also measured. For a more detailed description of the 4/8 VM task, see: Hussain et al., in prep.

6.2.4 Blood Hormone Analysis

Once testing was completed, a blood sample (18mL; 3 tubes of 6mL) was collected from participants; plasma was later analyzed and serum levels of E2, P, and T analyzed and quantified. A final sample of 49 blood samples were collected; sample attrition was due to participants changing their mind about providing blood, complications during the blood

extraction process (e.g., the nurse having difficulty finding the participant's vein), and unusable samples from which it was impossible to extract a large enough plasma sample.

6.2.5 Statistical Analysis

A series of Pearson's correlation coefficients were computed in order to test whether serum hormone levels (E2, P, T) correlated with any of the neuropsychological test measures, IQ, sleep measures, or PSS. Additionally, a Pearson's correlation coefficients were computed in order to observe the relationships between hormone levels and 4/8 VM task measures: average number of errors committed during all part 2 trials, number of trials to criterion, number of probe errors made during part 2 of the probe trial, and number of landmarks mentioned in the verbal report.

A series of 2 (cycle phase) X 2 (reproductive experience) independent factorial analysis of variance (ANOVA) tests were carried out to compare serum E2, P, and T levels across cycle phase in order to assess accuracy of self-reported cycle phase information. Furthermore, a series of 2 (reproductive experience) X 2 (strategy) independent factorial ANOVAs were carried out to compare serum E2, P, and T levels between spatial and response learners with and without reproductive experience. Partial eta squared (η_p^2) values were computed as a measure of effect size.

Data from three participants was removed due to presence of outliers in serum hormone levels (one each for E2, P, and T levels). In all three cases, serum hormone levels were more than three standard deviations higher than the mean. Thus, the final sample used for analysis consisted of 46 participants (20 mothers and 26 non-mothers).

6.3 Results

6.3.1 Correlations between Serum Hormone Levels and Neuropsychological Tests

Serum E2, P, and T levels did not correlate with any of the neuropsychological test measures (RAVLT, RO). There was also no association between endogenous hormone levels and IQ, PSS, or any of the LSEQ measures.

6.3.2 Correlations between Serum Hormone Levels and 4/8 VM Measures

None of the 4/8 VM task measures correlated with serum hormone levels. However, as can be seen in Table 6.1, there was a strong positive correlation between the number of trials taken to reach criterion and the number of errors made during part 2 trials (r = .67, p

Table 6.1

Correlations for serum hormone levels and 4/8 VM measures

	1	2	3	4	5	6	7
1. Estradiol	-						
2. Progesterone	.015	-					
3. Testosterone	.202	-.172	-				
4. Probe errors	-.098	.145	-.035	-			
5. Errors in Part 2 trials	.253	.252	-.065	.062	-		
6. Trials to Criterion	-.055	.161	-.087	.013	.672**	-	
7. Landmarks	.185	-.165	.004	-.014	-.010	-.022	-

Note. ** $p < .01$

< .01); the more difficult the task (as measured by how many trials are needed to learn it), the more errors a participant will make (see Table 6.1).

6.3.3 Hormone Levels across the Menstrual Cycle

Serum hormone results showed that, contrary to what was hypothesized, there was no difference in E2 levels between the follicular (M = 174.63, SD = 131.30) and the luteal (M = 158.12, SD = 87.49) groups. There was a significant difference in P levels between the follicular (M = 0.24, SD = 0.25) and luteal (M = 5.79, SD = 5.67) groups, $F_{(1,42)} = 20.47$, $p < .001$, $\eta_p^2 = 0.328$. No differences were observed for T levels and mothers across cycle phase. In addition, serum hormone levels did not differ between mothers and non-mothers. Overall, these results suggest that E2 and T levels did not differ between the follicular and luteal phase, with T levels being very low throughout the cycle. As expected, P levels are high in the luteal phase but almost negligible in the follicular phase (see Table 6.2).

6.3.4 Hormone Levels by Strategy Use and Reproductive Experience

When comparing serum hormone levels in spatial and response learners, a different relationship emerges. Though not statistically significant, a trend was present for higher serum E2 levels in women who initially used a response strategy (M = 200.40, SD = 139.20) compared to women who initially used a spatial strategy (M = 133.81, SD = 56.65), $F_{(1,42)} = 3.83$, $p = .057$, $\eta_p^2 = 0.083$ (see Figure 6.1). Thus, contrary to the hypothesis, higher E2 levels are not associated with use of a spatial strategy and are higher in response learners.

There was a significant strategy by reproductive experience interaction for P levels, $F_{(1,42)} = 5.52$, $p = .024$, $\eta_p^2 = 0.116$ (see Figure 6.2). Results show that, in mothers, spatial learners had higher serum P levels (M = 6.06, SD = 5.82) than response learners (M = 0.80, SD = 0.98). The opposite pattern was observed in non-mothers: spatial learners had lower serum P levels (M = 1.90, SD = 3.98) than those using a response strategy (M = 3.42, SD = 5.59). Finally, a statistically significant strategy by reproductive experience interaction ($F_{(1,42)} = 6.20$, $p = .017$, $\eta_p^2 = 0.129$) as well as a trend for a main effect of strategy ($F_{(1,42)} = 3.94$, $p = .054$, $\eta_p^2 = 0.086$) was found for serum T levels (see Figure 6.3). Testosterone levels were generally higher in response learners (M = 0.19, SD = 0.12) than in spatial learners (M = 0.13, SD = 0.13). This difference can be observed in

Table 6.2

Blood hormone levels by total sample and by cycle phase

	Total Sample ($N = 46$)		Follicular Phase ($n = 21$)		Luteal Phase ($n = 25$)	
	M	*SD*	*M*	*SD*	*M*	*SD*
Estradiol	165.66	108.69	174.63	131.30	158.12	87.49
Progesterone	3.25	5.00	0.26*	0.25	5.79*	5.67
Testosterone	0.16	0.13	0.17	0.13	0.15	0.13

Note. E2 levels shown in pg/mL; P and T levels shown in ng/mL. * denotes statistically significant P level difference between follicular and luteal groups, $p < .001$. Serum hormone levels did not differ between non-mothers and mothers.

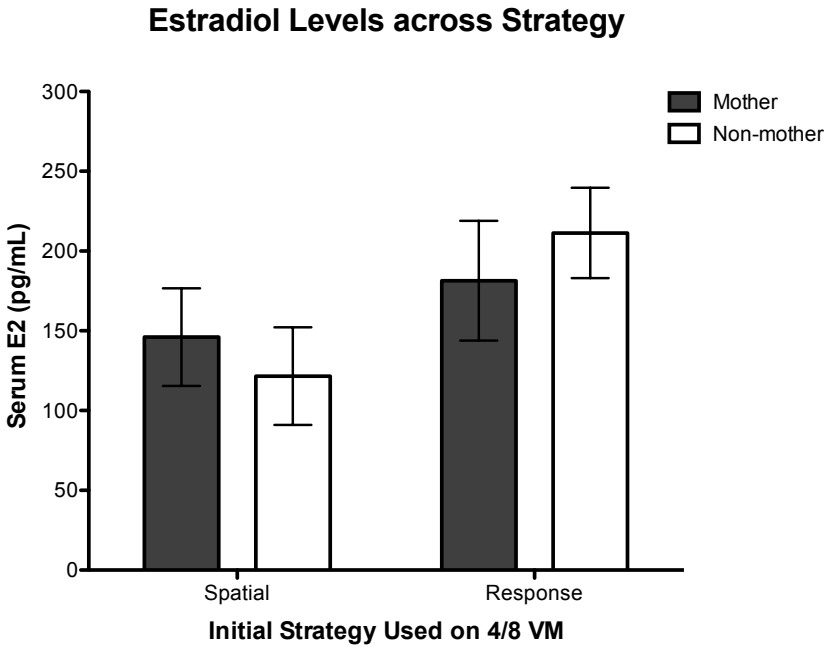


Figure 6.1. Serum E2 levels in mothers and non-mothers across strategy type.

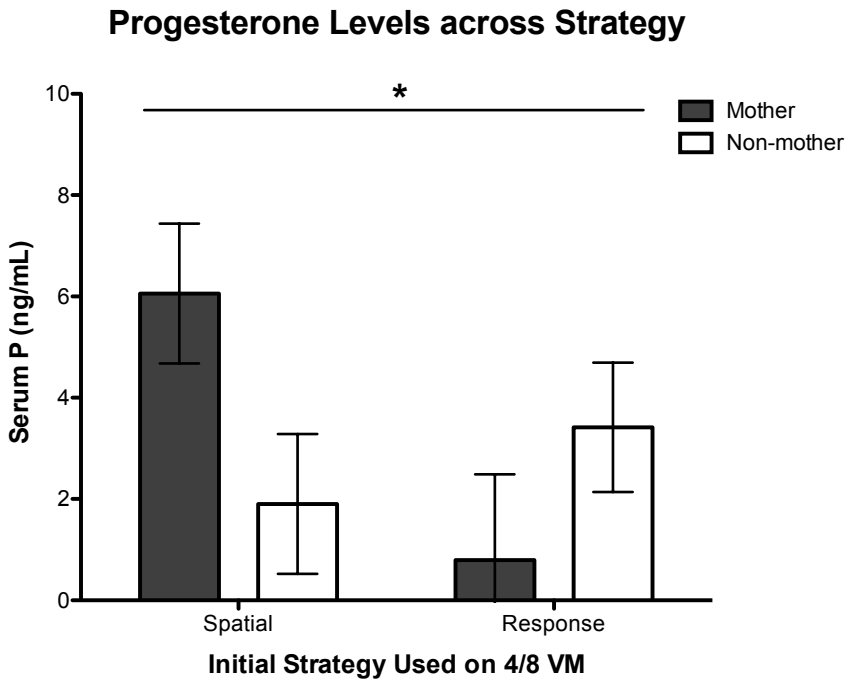


Figure 6.2. Serum P levels in mothers and non-mothers across strategy type. * indicates a statistically significant interaction between strategy and reproductive experience, p = .024.

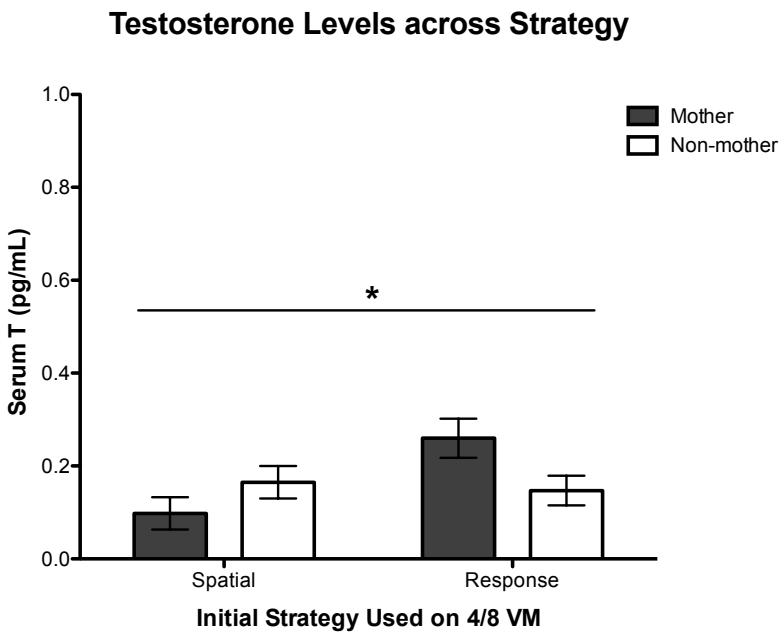


Figure 6.3. Serum T levels in mothers and non-mothers across strategy type. * indicates a statistically significant interaction between strategy and reproductive experience, p = .017.

mothers (spatial: $M = 0.10$, $SD = 0.08$; response: $M = 0.26$, $SD = 0.12$) whereas the pattern switches in non-mothers (spatial: $M = 0.17$, $SD = 0.17$; response: $M = 0.15$, $SD = 0.10$). Overall, these results suggest that endogenous levels of E2, P, and T all play a role in the type of strategy used by women across the cycle, and this changes with reproductive experience.

6.4 Discussion

The present findings reveal that multiple memory system bias across the menstrual cycle is underscored by an interaction between endogenous fluctuations of E2, P, and T. Contrary to what was expected, it appears that spatial memory is associated with low E2 and response memory with high E2 levels. Moreover, this interaction between hormones and memory bias is modified by reproductive experience such that P and T, but not E2, have contrasting effects on memory bias in mothers and non-mothers.

Serum hormone analysis showed that the follicular phase is marked by low P levels whereas the luteal phase is associated with high P, as was expected. However, contrary to what was hypothesized, E2 levels did not significantly differ across cycle phase (see Table 6.2). Moreover, T levels were low and also did not change across cycle phase. This suggests that the luteal phase is indicative of a high P state, rather than high E2, and the follicular phase is marked by low P. Furthermore, mothers and non-mothers did not differ in terms of hormone levels across cycle phase; therefore, all women had similar hormonal profiles across the menstrual cycle. This shows that the effect of reproductive experience on memory bias is not due to differences in endogenous hormone levels. Previous findings from this laboratory showed that women tested during the luteal phase of the cycle predominantly used a spatial strategy on the 4/8 VM while the follicular phase was marked by a higher proportion of response strategy use (Hussain et al., in prep). The present findings would suggest that this cycle-dependent memory system bias is associated with different P and T levels, rather than differences in E2.

Though studies have previously shown a link between verbal skills and high E2 in cycling women (Maki et al., 2002; Rosenberg & Park, 2002), this was not observed in the present study. It is possible that E2's effects on verbal skills in women are subtle and not detectable across different types of tests used. Indeed, many studies do not find a link between E2 and verbal memory (Hatta & Nagaya, 2009; Jacobs & D'Esposito, 2011;

Maki et al., 2002; Phillips & Sherwin, 1992). Hormone levels also did not correlate with any of the other neuropsychological tests (RO, IQ), perceived stress, or LSEQ measures. Finally, hormone levels did not correlate with the 4/8 VM measures (see Table 6.1), which indicates that task and probe errors, trials to criterion, and number of landmarks mentioned by participants are not associated with hormone levels despite the observed strategy effect.

When spatial and response learners' E2, P, and T levels were compared, it was revealed that memory bias across the menstrual cycle goes beyond the effects of E2. Contrary to what has been observed in the rat literature (Hussain et al., 2013; Korol & Kolo, 2002; Korol et al., 2004; Quinlan et al., 2008), spatial memory was not associated with high E2 levels. Interestingly, the opposite pattern was observed: though not statistically significant, participants who used a response strategy had higher serum E2 compared to spatial learners (see Figure 6.1). Thus, it is not E2 changes across the cycle that seems to be modulating memory bias in women.

In addition, P and T levels both differed across strategy use and reproductive experience. The results showed that, in women with no reproductive experience, spatial learners had low P levels whereas response learners had high P levels; T levels were low across strategy (see Figures 6.2 and 6.3). Conversely, mothers who used a spatial strategy had high P levels and low T levels. In mothers who used a response strategy, P levels were low and T levels were high (see Figures 6.2 and 6.3). Thus, P and T both differ in spatial and response learners, but this link between hormone levels and memory bias changes depending on whether participants are reproductively experienced.

The present findings reveal that the effects of E2 and P on memory system bias are dissociated in humans, such that they differentially impact the type of strategy used to solve a task. This contradicts what has previously been observed in rodents; results consistently show that high E2 levels are associated with spatial memory and low E2 with response memory whether given alone to ovariectomized rats (Hussain et al., 2013; Korol & Kolo, 2002; Marriott & Korol, 2003; Quinlan et al., 2008) or when naturally fluctuating along with P in intact rats (Korol et al., 2004). This could be due to E2 and P peaking concurrently in the rat estrous cycle, whereas they do not overlap in the same

way in the menstrual cycle (Hussain, Shams, & Brake, 2014). Thus, it is possible that E2 and P act differently in humans such that they have contrasting effects on memory bias.

Interestingly, despite hormonal profiles being similar in women with or without reproductive experience, P and T levels were found to have different effects on strategy use in mothers and non-mothers. The literature on long-term postpartum changes in brain function and cognition in women is limited. One study showed no correlation between E2, P, or T and performance on a variety of cognitive tests (e.g., verbal memory, visuospatial skills, IQ) during pregnancy and two months following birth (Buckwalter et al., 1999). This study contradicts the current findings; however, there are two possible explanations for this discrepancy. First, as mentioned earlier, studies focusing on long-term effects of parity in women are uncommon and usually focus on comparing performance in participants from pregnancy to a period within a year of giving birth. It is possible that parity-induced changes in cognition persist and continue to change for years following birth. This is supported by work done in rodents (Gatewood et al., 2005; Love et al., 2005; Macbeth et al., 2008) and humans (Buckwalter et al., 2001), which show long-lasting parity-associated differences in cognitive function. Second, the cognitive function measured in the current study differs from what has previously been studied in reproductively experienced women. Thus, it is possible that the balance between different memory systems is altered differentially by hormones in mothers than in non-mothers, and this difference is long-term.

The present findings reveal that reproductive experience changes the association between hormones and multiple memory bias. It was recently observed that mothers and non-mothers show the same pattern of memory bias across the menstrual cycle: the follicular phase was associated with predominant use of a response strategy whereas the luteal phase was associated with a higher likelihood of using a spatial strategy, and this was more pronounced in mothers (Hussain et al., in prep). It is apparent from the current results that this association between the luteal phase and spatial memory is not underscored by high E2, but rather by high P combined with low T levels in the mother group. It is possible that these hormones are playing a role in the HPC to exert this effect that is different from non-mothers. Indeed, studies have shown that pregnancy and birth are associated with changes to hippocampal morphology (Kinsley & Lambert, 2008;

Kinsley et al., 2006; Pawluski et al., 2009) and P receptors are present in this brain region (Guerra-Araiza, Coyoy-Salgado, & Camacho-Arroy, 2002). Since the experience of pregnancy is marked by chronic exposure to high levels of P (Nicholas & Hartmann, 1981), it is possible that P receptors could upregulate and hence sensitivity to P could be enhanced in mothers.

Testosterone levels did not vary much across strategy in non-mothers, yet in the mother group, they were lower in spatial learners than in response learners. Like with E2 and P, mothers undergo changes in exposure to T throughout pregnancy and birth such that T peaks during the postpartum period (Macbeth & Luine, 2010); this could be increasing sensitivity to T in mothers. In addition, it has been found that reproductively experienced rats show an increase in hippocampal spine density compared to nulliparous rats, and this is correlated with the number of male pups in the litter (Pawluski & Galea, 2006), which suggests that exposure to T could be underscoring this effect. The sex of the children was not measured in this study, but it would be interesting to investigate whether there would be a T-driven difference in strategy use between mothers who have had male children compared to female children. Since T metabolizes into E2, such effects could be due to a combination of T and E2 actions in the brain. Indeed, current results show that the hormonal profile of a mother using a spatial strategy is marked by low E2 and T, whereas both hormones are higher in mothers using a response strategy. Moreover, there is some evidence that the ratio of E2 and T is implicated in cognitive function (Nyborg, 1983), which suggests that the interaction between these two hormones may be implicated in memory bias.

In conclusion, the present study reveals an interactive effect of E2, P, and T on multiple memory system bias in naturally-cycling women, and this balance between cycling hormones and memory bias is modified in women who have undergone reproductive experience. Moreover, this study reveals that multiple memory bias extends beyond the effects of E2 in women and is significantly influenced by levels of P and T. Thus, it is perhaps the relationship between hormones that is key in determining whether a HPC-dependent spatial or caudate-dependent response strategy is adaptive, and this is disrupted with reproductive experience. These findings strongly suggest that hormones impact cognition in young, cycling women such that different brain areas and memory

systems are activated at different points in the menstrual cycle. Furthermore, this study adds to the limited body of literature that shows that motherhood can change the female brain permanently. This could have an impact on studying the effects of reproductive experience on the aging female brain.

CHAPTER 7

General Discussion

7.1 Summary of findings

The present thesis has revealed that ovarian hormones and reproductive experience have a significant impact on the female brain and multiple memory system bias in both rodents and humans. In chapter 2, it was shown that nulliparous ovariectomized rats given low 17β-Estradiol (E2) replacement learned a response-based plus-maze task faster than a place-based task. No learning differences were observed in high E2 or primiparous rats, with primiparous rats given low E2 needing significantly more days to reach criterion on the response task compared to nulliparous low E2 rats. Thus, this study showed that low E2 levels enhance response learning, but this effect disappears in parous rats. In chapter 3, it was revealed that D2 receptor (D2R) binding in the dorsal striatum (DS) is significantly lower in primiparous rats compared to nulliparous rats, regardless of E2 dose. This suggests that parity is associated with decreased D2R binding in the DS, which could potentially underscore the difference in response learning observed in chapter 1.

In chapter 4, it was shown that multiple memory system bias also changes in women with varying hormone levels. Women tested during the follicular phase of the menstrual cycle, when E2 and progesterone (P) levels are low, were more likely to use response memory to solve a virtual navigation task. Participants tested during the luteal phase, when both hormones are high, predominantly used spatial memory. In chapter 5, it was revealed that this pattern between cycle phase and memory bias is not only similar, but more pronounced, in reproductively experienced women. Finally, in chapter 6, it was shown that this difference in memory bias across the cycle is due to variations in P and testosterone (T). The observed shift in memory bias from the follicular to the luteal phase is likely underscored by altered P levels, rather than E2. Furthermore, in non-mothers, response memory was associated with high P whereas spatial memory was linked to low P levels. In mothers, this relationship changes: response memory was associated with low P and high T levels whereas this is reversed in spatial learners. These results indicate that mothers and non-mothers show a similar memory bias pattern across the menstrual cycle, but this is underscored by different hormonal profiles.

7.2 Role of E2 in multiple memory system bias

It has been established that multiple memory systems rely on distinct and competitive brain areas. Indeed, spatial memory is dependent on the hippocampus (HPC) whereas response memory relies on the DS such that spatial memory is impaired when the HPC is damaged and response memory is impaired when the DS is damaged (White & McDonald, 2002). Moreover, when one region is impaired or damaged, the competing memory system is employed and enhanced in rats (Packard & McGaugh, 1996). It has been shown that these memory systems are also modulated by neurotransmitter activity in these brain regions. For example, high acetylcholine (ACh) levels in the HPC and DS are associated with use of spatial and response memory, respectively (Chang & Gold, 2003; McIntyre, Marriott, & Gold, 2003). Similarly, glutamate has been shown to improve spatial and response learning when infused into the HPC and DS, respectively (Packard, 1999). Thus, it appears that these memory systems are associated with increases in excitatory neurotransmitters within the HPC and DS. However, evidence has shown that multiple memory system bias is also mediated by the medial prefrontal cortex (mPFC), a region that has reciprocal projections to the HPC and DS (White & McDonald, 2002). Specifically, neuronal activity within the mPFC is associated with a switch from one memory system to another, but not with response or spatial learning (Ragozzino, Detrick, & Kesner, 1999; Rich & Shapiro, 2007, 2009). It therefore appears that spatial and response memory are not only modulated by increased activity within the HPC and DS, but also rely on the mPFC in switching between these two memory systems.

As has been shown in the present thesis and by previous studies, multiple memory system bias is affected by E2 in the female rat; the findings from chapter 2 support this. In general, the proestrus phase of the rat estrous cycle, which is the point at which E2 peaks, is associated with enhanced spatial learning and a preponderance to rely on spatial memory to solve a dual solution maze task. Conversely, low levels of E2, which mark the estrus phase of the cycle, are linked to improved performance on a response task as well as a higher proportion of rats using response memory (Korol & Kolo, 2002; Korol, Malin, Borden, Busby, & Couper-Leo, 2004; Quinlan, Hussain, & Brake, 2008; Zurkovsky, Brown, Boyd, Fell, & Korol, 2007). This effect occurs due to E2 directly acting in the HPC to promote spatial memory, whereas E2 has been shown to rapidly impair response

memory through its action in the DS (Zurkovsky, Serio, & Korol, 2011; Zurkovsky, Brown, Boyd, Fell, & Korol, 2007).

Studies carried out in mice and humans have also shown that hippocampal volume changes across the cycle, such that a high E2 state correlates with increased volume and low E2 with decreased volume (Protopescu et al., 2008; Qiu et al., 2013), which demonstrates that E2 is associated with potentiated hippocampal function and, hence, enhanced spatial memory. However, less is known in terms of DS morphology and function across the cycle. It would appear, however, that E2's impact on DS dopamine (DA) activity is implicated in response memory. Indeed, previous findings have shown that administration of DA antagonists directly into the DS disrupt multiple memory bias in both low and high E2 groups (Quinlan et al., 2013). It has also been shown that DA receptor (DAR) density, DA release, and DA-related behaviors change across the estrous cycle (Becker & Cha, 1989; Becker, Robinson, & Lorenz, 1982; Lévesque, Gagnon, & Di Paolo, 1989). It is therefore possible that cycle-dependent alterations in DS DA activity impacts response memory. Moreover, it has been shown that E2 acts in the mPFC to modulate switching between memory systems: infusing a high dose of E2 directly into this brain region leads to a rapid switch from predominant use of response memory to spatial memory (Almey et al., 2014). Thus, it appears that the mPFC is a critical region involved in the switch between memory systems, and E2 plays a key role in this brain region and acts directly in the HPC and DS to impact memory bias. A general overview of the DS, HPC, and mPFC under low and high E2 conditions can be seen in Figure 7.1.

7.3 Parity alters E2's impact on multiple memory system bias

Until recently, little was known about the long-term effects of parity, especially concerning multiple memory system bias. A few studies conducted in rodents have shown that parity is associated with long-term enhanced sensitivity to E2 (Bridges & Byrnes, 2006), improved spatial learning (Gatewood et al., 2005; Love et al., 2005), as well as higher DA levels and sensitivity (Byrnes et al., 2001), when compared to nulliparous rats. Furthermore, in a dual solution t-maze task in which rats exposed to low E2 rely on response memory and high E2 rats use spatial memory, primiparous rats were

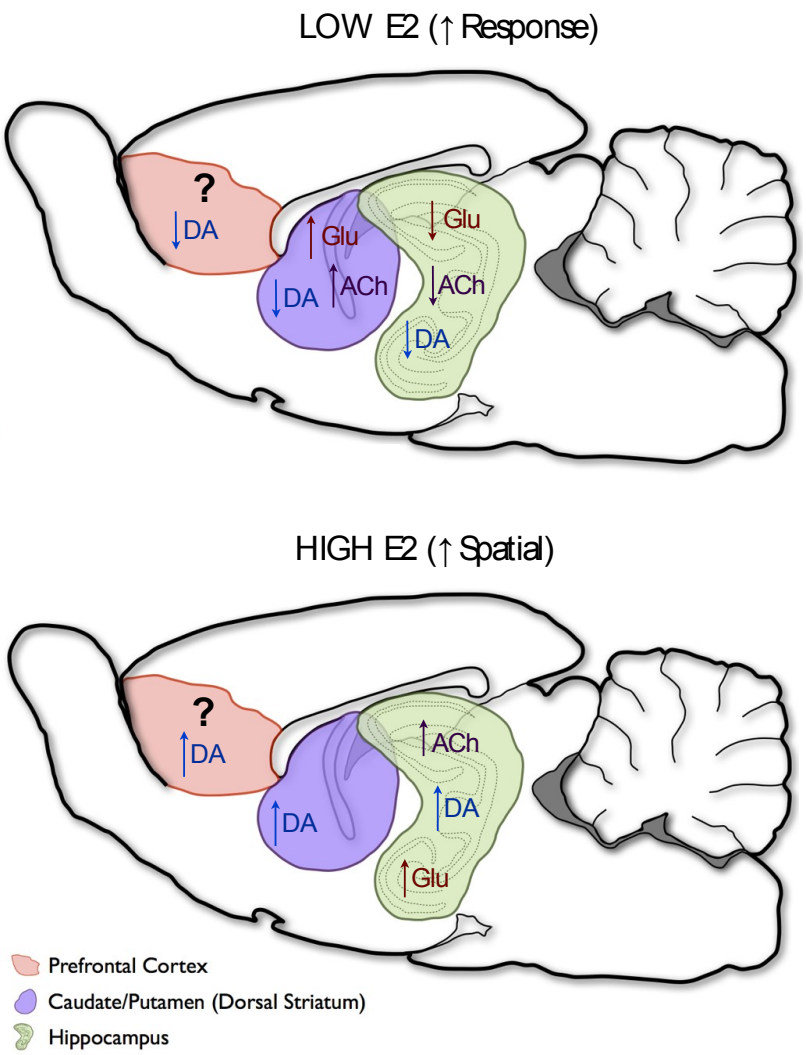


Figure 7.1 Schema representing neurotransmitter levels in the PFC, DS, and HPC of the female rat brain (sagittal view) under low and high E2 states, when response and spatial memory are optimized, respectively. The PFC, DS, and HPC during a low E2 state (top panel). Neurotransmitter levels decrease in the PFC and HPC, whereas they are generally increased within the DS. DA function is lower within the DS, which is associated with potentiated response memory. The PFC, DS, and HPC during a high E2 state (bottom panel). High E2 is associated with increases in neurotransmitter levels in all three brain regions. Increased DA levels within the DS are associated with impaired response memory; hence, high E2 levels are optimal for HPC-dependent spatial memory. It is currently unknown how E2 and its effect on DA (and, possibly, other neurotransmitters) within the PFC impacts multiple memory system bias.

shown to use both memory systems equally regardless of E2 dose (Hussain et al., 2013). The results of the present thesis reveal a similar pattern; in chapter 2, it was shown that there are no differences in learning on a response- or place-based task in primiparous rats, regardless of E2 level. Specifically, the low E2 advantage observed in nulliparous rats for the response task disappears with parity, indicating that the interaction between E2 and DA function in the DS is altered.

Findings from chapter 3 shed some light on this behavioral result: D2R, but not D1R, binding was shown to be lower in the DS of primiparous rats than in nulliparous rats. Thus, it is possible that changes in DS D2R function underlies the parity-induced difference in response learning observed in chapter 2. In nulliparous rats, low E2 levels could be optimally stimulating DS D2Rs to enhance response learning. However, this association between low E2 and improved response learning is not observed in primiparous rats; this could be because of decreased DS D2R binding either because D2Rs are downregulated following parity, or it is presynaptic D2R binding that is affected. Furthermore, it has been shown that that these is an increase in ERs within the DS in primiparous rats compared to nulliparous rats (Byrnes, Babb, & Bridges, 2009); hence, parity-induced differences in response memory and DS D2R binding could also be disrupted by changes in E2 binding to DS ERs. Interestingly, it has been shown that ERs are not colocalized with DA neurons in the DS, but with cholinergic neurons (Almey et al., 2014). Since ACh has effects on striatal DA (Threlfell & Cragg, 2011), this would suggest that low E2 levels could be indirectly stimulating response memory via cholinergic neurons. It would therefore be of interest to investigate DS ACh activity and how this changes with parity.

7.4 Multiple memory system bias across the menstrual cycle

It is established that multiple memory system bias in rodents is modulated by E2 and, as has been more recently shown, parity. The present thesis has also revealed that memory systems are modulated by fluctuating ovarian hormones across the menstrual cycle in women. Moreover, as in rats, this effect changes with reproductive experience. The existing literature has shown that humans also rely on multiple memory systems in order to navigate an environment. As in rodents, spatial memory is dependent on the HPC whereas stimulus-response memory relies on the caudate nucleus (Bohbot et al., 2007;

Bohbot et al., 2004; Holdstock et al., 2000; Iaria et al., 2003; O'Keefe & Nadel, 1978). Furthermore, though there are individual differences in the type of memory system an individual will rely on, it has been shown that, in general, participants who are spatial learners will often switch to the less cognitively demanding and more efficient response memory with practice (Iaria et al., 2003). However, as is demonstrated in chapters 4, 5, and 6, the memory system a woman uses is affected by the phase of the cycle she is tested in.

Findings from chapter 4 showed that women tested during the follicular phase predominantly used a response strategy on the 4/8 VM task. Women who were tested during the luteal phase tended to rely on spatial memory. Thus, the changes in hormonal profile across the menstrual cycle must affect multiple memory systems such that different strategies become optimal. In chapter 5, it is shown that this pattern does not change with reproductive experience; also, this split in strategy across cycle phase was more pronounced in mothers than in non-mothers. This shows that reproductive experience affects multiple memory system bias differently in women than in female rats. Interestingly, results from chapter 5 show that, despite the similarities in strategy use, there were differences in 4/8 VM learning measures between mothers and non-mothers. For instance, in the luteal phase group, mothers required on average one fewer trial to reach criterion but mentioned more landmarks in their verbal report than non-mothers. This indicates that there are parity-induced differences in learning to navigate the 4/8 VM task, but the overall memory bias pattern across the cycle is similar.

7.5 Role of P and parity in modulating multiple memory system bias

The differences associated with reproductive experience are further emphasized by the findings revealed in chapter 6. When hormone levels were compared between spatial and response learners, it was found that E2 levels were higher in the response strategy group, though this was not statistically significant. This contradicts findings from rodents; it would appear that in humans, E2 does not modulate multiple memory bias in the same way. Also, since P levels were found to be higher in the luteal group than in the follicular group, memory bias is possibly underscored by P such that a low P state is associated with response memory and high P with spatial memory. Finally, it was revealed that the interaction between hormones that contributes to predominant use of a

spatial or response strategy on the 4/8 VM task is different in mothers and non-mothers. In non-mothers, high P and low T levels promoted response memory whereas low P and high T promoted spatial memory. However, in mothers, the relationship between P, T, and memory bias was reversed. Interestingly, E2 levels did not significantly differ with parity, which suggests that changes in P and T following parity could be playing a larger role in multiple memory system bias. The findings from chapters 4, 5, and 6 suggest that hormones act in the female brain to modify multiple memory system bias across the menstrual cycle. Furthermore, the way in which these hormones exert their effects on memory bias changes dramatically with reproductive experience, which suggests that there may be a long-term parity-induced change in P and T receptor function.

Taken together, it would appear that multiple memory system bias is modified by E2 and parity in rodents, whereas the interaction between reproductive experience and P and T plays a larger role in modulating memory bias in cycling women. Though it is known that estrogen receptors (ER) are widely distributed in the mammalian brain (for review, see: Hussain, Shams, & Brake, 2014) and some evidence has surfaced indicating that ER distribution in the rodent brain changes with parity (Byrnes et al., 2009), it remains unknown whether ERs change following reproductive experience in women and whether this could be permanent. It is possible that, unlike in rodents, ERs are not affected by parity in humans past pregnancy and birth. This could potentially explain why E2 did not differentially influence memory bias in mothers tested here, since at least two years had elapsed since birth. It is therefore especially interesting that P and T levels did affect memory bias differently in mothers; this indicates that P and T are not only important in modulating multiple memory bias, but P and T receptors could permanently change with parity.

Indeed, progesterone receptors (PR) have been observed in many areas of the rat brain, including the cerebral cortex, hippocampal formation, and caudate putamen (McEwen & Alves, 1999; Schumacher et al., 2014), which suggests that P could be acting directly in the HPC and caudate nucleus to affect multiple memory bias. However, it is presently unknown whether PR distribution is the same in the human brain or whether it changes following parity. It is therefore possible that the effect of P within the HPC and DS is altered with reproductive experience, such that different levels of P

become optimal in promoting spatial or response memory in mothers. It is important to note that P is often related to E2 action since activation of the PR depends on prior induction by ERs; however, this is mostly observed within the hypothalamus and studies have shown that PR activation does not depend upon E2 action in brain regions involved in multiple memory system bias (Camacho-Arroyo & Pérez-Palacios, 1994; Maclusky & McEwen, 1978; Parsons, Rainbow, & Maclusky, 1982). Thus, P could be acting independently of E2 in the HPC and DS to affect spatial and response memory, respectively. Moreover, as has been shown with E2 (Almey et al., 2014), P could also be acting in the mPFC to affect switching between memory systems. Finally, these mechanisms could change following reproductive experience such that different levels of P activate these memory systems.

7.6 Future directions

It is evident from the results of chapter 2 that E2's effect on multiple memory system bias, particularly DS-mediated response memory, changes with parity. Though there is a growing body of literature showing that ERs are widely distributed throughout the rodent brain, little is understood about how ER distribution and function is impacted by parity. Studies have revealed that ER levels fluctuate across the estrous cycle (Mitterling et al., 2010) and change with age (Mehra, Sharma, Nyakas, & Vij, 2005; Waters et al., 2011). As previously mentioned, ERα levels also change in the DS with parity (Byrnes et al., 2009). Thus, it would be of interest to further elucidate the effect of parity on other ER subtypes (ERβ and GPER1) within the DS as well as in the HPC and mPFC. This could potentially explain the observed difference in how E2 modulates memory bias in nulliparous and primiparous rats.

Following on from this, it is clear that the role of P in multiple memory system bias warrants more attention, especially its interaction with reproductive experience. The findings from the present thesis show that, despite similar E2 levels and pattern of memory bias across the menstrual cycle, different levels of P seem to contributing to this effect in mothers and non-mothers. It would therefore be interesting to investigate PR distribution in the female brain and how this changes following parity. Moreover, colocalization of PRs onto DA neurons in the DS should be examined: could P directly affect DA transmission in the DS and, hence, response memory? Furthermore, the

findings from chapter 6 show that T levels also differ between spatial and response learners depending on reproductive experience, though T levels did not significantly change across the cycle. Thus, T could also be playing a role in modifying multiple memory system bias in women. Whether this is occurring due to T converting into E2 is unknown, though some evidence has shown that this does not impact cognition (Shah et al., 2006). However, the Shah et al., (2006) study was carried out in an E2-treated postmenopausal population; this could differ in a reproductively aged sample in which E2 levels fluctuate. Furthermore, it is currently unknown whether aromatase activity changes in the female brain following reproductive experience. Thus, examining the potential effect of aromatization of T into E2 on cognition in young women, and whether parity impacts this, could provide an interesting avenue for future research.

The results from chapter 3 revealed that D2R binding in the DS and NAcc change with parity. It would be interesting to measure D2R mRNA using in situ hybridization in order to assess whether this difference can potentially be in presynaptic D2Rs. Furthermore, as previously mentioned, the mPFC plays a key role in orchestrating the switch between memory systems (Almey et al., 2014; Ragozzino et al., 1999; Rich & Shapiro, 2007, 2009). It would therefore be of interest to follow-up the experiment carried out in chapter 3 by examining D1R and D2R binding within this brain area. This would shed more light on the mechanisms underlying memory system switching within the mPFC.

It is clear that reproductive experience alters brain function and, consequently, cognition, in both rodents and humans. However, in chapters 2 and 3, primiparous rats were compared to nulliparous rats. In chapters 4, 5, and 6, the number of children that women in the mother group had was not controlled for. Therefore, the findings from this thesis do not extend to whether the number of births has an effect on multiple memory system bias. It has been shown in both rodents (Paris & Frye, 2008) and humans (Parsons et al., 2004) that there are differences in cognitive function between primiparous and multiparous females, which indicates that the number of births can have additive effects on cognition in women. Thus, future studies could examine whether multiple memory system bias changes with the number of pregnancies that a woman has experienced.

Furthermore, does ER or PR distribution differ between primiparous and multiparous females, and would the relationship between E2, P, and T and memory bias be affected?

The present thesis has revealed that hormones play a significant role in modulating multiple memory system bias in reproductively aged rodents and women and that parity alters this effect. Importantly, these findings hint at a long-term effect of reproductive experience on cognition. A critical future research direction would be to investigate whether these long-term effects are permanent. A large portion of the research on E2 and cognition has focused on postmenopausal women and whether providing hormone replacement therapy (HRT) is beneficial or detrimental. It would be interesting to observe whether multiple memory system bias changes with menopause: would menopausal women be more likely to use one memory system over another? How would E2, P, or T replacement impact memory bias? Furthermore, would the parity-induced effects observed here also be found decades following birth, and would multiple births affect this? Finally, since spatial memory may be protective against age-related degeneration of the HPC (Bohbot et al., 2007), studying multiple memory system bias in older women could provide clues about which brain regions show age-related decline and whether HRT could prevent such impairments. In conclusion, gaining a better understanding of how hormones and reproductive experience affect cognition in young women could shed light on ways to alleviate aging and menopause related cognitive decline in the growing postmenopausal population.

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