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Effect of Menstrual Cycle Related Estrogen Fluctuations on Working Memory

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EFFECT OF MENSTRUAL CYCLE RELATED ESTROGEN FLUCTUATIONS ON WORKING MEMORY

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Abstract

Working memory (WM) is a dynamic brain system which allows for the on-line moment-to-moment maintenance, processing and monitoring of information involved in human cognition. Behavioural and neuroimaging studies have shown that the prefrontal cortex (PFC) plays an essential role in WM. Data suggest the PFC may be susceptible to modulation by estrogen. Behavioural studies examining whether PFC-dependent WM tasks exhibit estrogen sensitivity in postmenopausal women have shown a benefit of estrogen. The present study used hormone changes associated with the menstrual cycle to examine whether estrogen has a beneficial effect on WM function in reproductively aged women. Thirty-six women completed a battery of cognitive tasks including 3 WM tests in a repeated-measures design. The data showed that performance on the WM tasks was significantly better when estrogen levels were high compared to when they were low, suggesting that estrogen has the ability to modulate PFC-dependent WM function in young women.

Keywords: estrogen; menstrual cycle; prefrontal cortex; working memory.

Co-Authorship Statement

All research described in this thesis including the experimental design, data collect, data analysis and editing was carried out by Mia Segal under the supervisor of Dr. Elizabeth Hampson with the exception of the radioimmunoassays which were conducted by Bavani Rajakumar under the supervision of Dr. Elizabeth Hampson. All experiments are original research carried out for this Master's thesis.

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List of Abbreviations

^{125}I	Iodine-125
5-HT	Serotonin
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BA	Brodmann`s area
ChAT	Choline acetyltransferase
dlPFC	Dorsolateral prefrontal cortex
EIA	Enzyme immunoassay
ERα	Estrogen receptor alpha
ERβ	Estrogen receptor beta
fMRI	Functional magnetic resonance imaging
FSIQ	Full scale IQ
GnRH	Gonadotropin releasing hormone
NAART	North American Adult Reading Test
OVX	Ovariectomized
PET	Positron emission tomography
PFC	Prefrontal cortex
POMS	Profile of Mood States
RIA	Radioimmunoassay
SD	Standard deviation
SEM	Standard error of the mean
SOPT	Self-Ordered Pointing Task

SWPM	Spatial working memory task
Tukey HSD	Tukey's Honestly Significant Difference
WM	Working memory
WME	Working memory error

CHAPTER 1

INTRODUCTION

Working memory refers to the dynamic brain system which allows for the on-line moment-to-moment maintenance, processing and monitoring of information involved in human cognition (Baddeley, 1986; Goldman-Rakic, 1987). As such, the contents of working memory are continuously manipulated and updated in order to accurately guide subsequent actions. Working memory has been shown to play a fundamental role in an array of complex cognitive activities such as reasoning, fluid intelligence, language comprehension and mental calculation (Baddeley, 1986, 1994; Barett, Tugade, and Engle, 2004; Goldman-Rakic, 1987). An integral feature of these and indeed all working memory tasks, is their requirement for the temporary maintenance of task relevant information in a form which is readily accessible while simultaneously allowing for the performance of other cognitive decisions and operations on the stored material. For example, the decoding and integration of text necessary for accurate reading comprehension requires the reader to have access to semantic, pragmatic and syntactic information from previously encountered text (Daneman, and Carpenter, 1980)

The temporary relevance and usefulness of the information in working memory is the criterion which distinguishes the system from other forms of memory such as semantic (Tulving, 1972) or procedural (Squire, and Cohen, 1984) that involve longer-term representations. Additionally, unlike working memory, these other forms of memory are associative in that their contents are acquired by the repeated contiguity between stimuli and responses and/or consequences (Goldman-Rakic, 1995). In addition to their definitional differences, considerable evidence from studies in a number of diverse

scientific fields including neurobiology, neuropsychology, and cognitive neuroscience suggests a distinction between working and other forms of memory is also evident in the neuroanatomical structures which subserve them. Initial evidence for the neural basis of working memory was obtained through studies of nonhuman primates.

The Neural Basis of Working Memory

Early nonhuman primate studies which attempted to elucidate the neural underpinnings of working memory used conscious monkeys trained to perform a delayed response task adapted for use with nonhuman primates by Jacobsen (1936) from that originally devised by Hunter (1913) for human testing. On each trial of the task, the monkey watches the experimenter hide a food reward in one of two spatial locations (Figure 1.1). After a delay of several seconds, the monkey is allowed to retrieve the reward. During the delay, the monkey is prevented from physically orienting itself to the location of the target and from seeing the locations by an opaque screen. As such, the monkey must remember in which of the two spatial locations the food was hidden in order to be rewarded. The locations are identical in appearance eliminating the monkey's ability to use external cues to inform their decision. Therefore, the animal must rely solely on information stored in memory to guide their choice. Importantly, the spatial location of the hidden reward on each trial is varied and random. Thus in order to accurately guide behavior, the animal must not only temporarily store information regarding the spatial location of the target, but also update this information on a continual basis and then use the updated, internally stored representation to guide their subsequent actions.

It has been argued by Goldman-Rakic (1987; 1992) that the processes required for

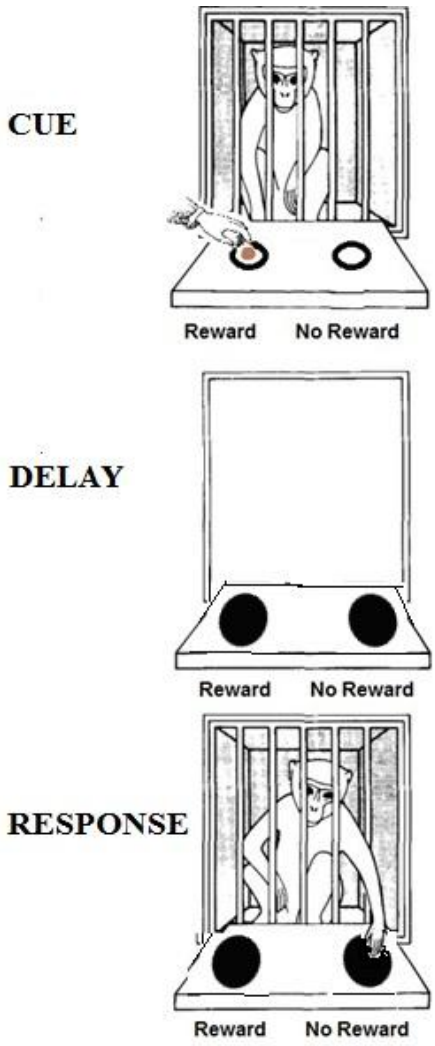

Figure 1.1. Schematic of the adapted version of the delayed-response task used with nonhuman primates. After watching the experimenter bait one of the two locations with a food reward during the cue phase and a delay phase during which the monkey can no longer see the locations, the monkey is allowed to make a response to retrieve the reward. The two possible bait locations are identical in appearance, thus the monkey must rely solely on information stored in memory to guide its response. Additionally, the location of the hidden reward is random and varied on each trial, requiring the monkey to update the information stored in memory on a trial-by-trial basis. (Adapted from Goldman-Rakic, 1987).

performance on the delayed response task in monkeys are analogous to those regarded as working memory in humans. Electrophysiological studies utilizing the delayed response task have consistently found neurons within the lateral prefrontal cortex (PFC) that become activated during specifically the delay period of the task (Funahashi, Bruce, and Goldman-Rakic, 1989; Fuster, and Alexander, 1971; Kubota, and Niki, 1971).

Pairing working memory tasks with a variety of neuroimaging techniques has allowed researchers to distinguish the perceptual and mnemonic roles played by the cortical regions involved in working memory. To date, a large number of such studies have been conducted (see D'Esposito et al., 1998 for a review). Importantly, such studies have consistently demonstrated that as in nonhuman primates, the PFC is engaged during working memory tasks in humans. In humans, the engagement of the lateral PFC while performing adapted versions of the delayed response task has been demonstrated using both positron emission tomography (PET; Jonides et al., 1993) and functional magnetic resonance imaging (fMRI; Courtney et al., 1997; Zarahn, Aguirre, and D'Esposito, 1999). These studies have used various methods to isolate those areas which play predominant roles in the mnemonic portion of working memory rather than the sensorimotor components of the tasks.

Researchers have also used fMRI and other neuroimaging techniques such as PET paired with more complex measures of working memory such as the 'n-back' task to further elucidate the neural underpinnings of the system. The spatial 'n-back' task involves participants indicating whether each item in a continuous series of visual stimuli is presented in the same or a different spatial location as a stimulus that was presented a

certain number (‘n’) steps earlier in the sequence of stimuli (Figure 1.2). The task requires participants to not only store information related to the spatial location of the stimuli, and to continually update this information as new items are incorporated, but also to monitor the stored information in order to make accurate decisions to guide their responses thus placing significant demands on working memory. McCarthy et al. (1994) had participants complete both the spatial ‘n-back’ as well as a sensorimotor control task which required participants to make simple perceptual judgements about a variety of irregular shapes while in an fMRI magnet. The same irregular shapes also served as the stimuli for the ‘n-back’ condition. The researchers found that compared to the control condition, there was a significant signal increase in Brodmann's area (BA) 46, located in the dorsolateral PFC (dlPFC), during the ‘n-back’ task. Similarly, using PET Smith et al. (1996) found the ‘n-back’ task to elicit significant increases in blood flow bilaterally in BA 46 when compared to a control task.

Both the delayed-response and the spatial ‘n-back’ tasks used in the previously described studies are considered to be *spatial* working memory tasks as the information which is required to be held within working memory pertains to the spatial locations of the target stimuli. There are other kinds of working memory tasks however such as verbal and visual which differ in the type of information that must be stored, manipulated and updated in order to accurately guide performance.

Using PET, Petrides et al. (1993b) measured cerebral blood flow while human participants performed both a control task and a digit randomization task which placed significant demands on verbal working memory. The same verbal response of reciting numbers was required for both tasks. For the digit randomization task, participants were

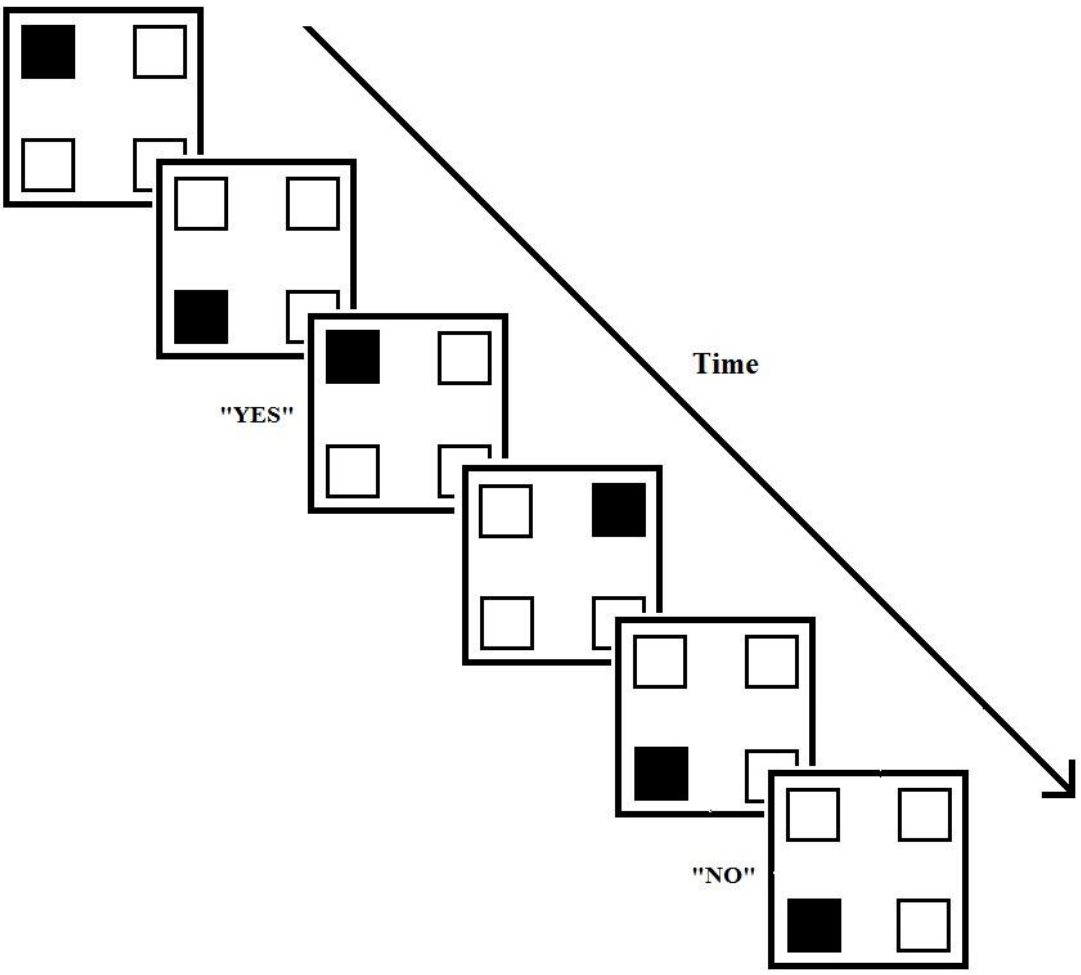

Figure 1.2. Schematic example of the spatial version of the n-back task. Depicted is a run of N-2 trials in which the participant is to respond “yes” or “no” indicating whether the filled square is in the same location as the stimulus that occurred two steps ago in the sequence of stimuli.

asked to say out loud the numbers from 1 to 10 in a random, self-generated order without repeating any numbers or leaving any numbers out. This task required participants to maintain within working memory the digits which they had already generated and update this information as they progressed through the task in order to accurately guide performance and avoid making errors. For the control task, participants counted out loud from 1 to 10 in ascending order. Thus the control task elicited the same processes of generating and vocalizing the numbers, but without a significant working memory component. When the researchers compared the patterns of activation elicited by the performance of the two tasks, the digit randomization task was found to evoke significant bilateral activation within the mid-dlPFC (BA 46/9) compared to the control task that was perceptually and motorically similar, but lacked a working memory component. These findings suggest that like spatial working memory tasks, the PFC is also essential to performance of non-spatial working memory tasks (Petrides, 1995b; Petrides, and Milner 1982). These findings are consistent with findings from similar work done with nonhuman primates (Petrides, 1995a; 2000a).

Other studies of working memory within the field of neurobiology have relied heavily on the interpretation of deficits exhibited by humans and nonhuman primates with either naturally-occurring or surgically-induced PFC lesions. PFC injury has been shown to result in a variety of behavioural abnormalities and cognitive deficits including non-generalized impairments in mnemonic functioning (Fuster, 1997; Goldman-Rakic, 1987; Miller, and Cummings, 1999). Of particular importance to the current investigation are those studies in which marked deficits in working memory task performance were

observed following PFC lesions (Bauer, and Fuster, 1976; Milner, 1982; Petrides, 1989; Yener, and Zaffos, 1999). A sampling of such studies is reviewed below.

A large number of monkey studies have linked the integrity of the PFC to accurate performance of the delayed response task, beginning with the classic work by Jacobsen (1936) in which he was the first to describe the significant impairment of monkeys' ability to perform the delayed response task following bilateral excisions of the PFC. Since Jacobsen`s seminal study, a number of other studies have demonstrated that in addition to bilateral damage, unilateral damage to the PFC is sufficient to impair the monkey`s capacity to perform the classic delayed response task (Fuster, 1997; Goldman-Rakic, 1987). Such studies have also allowed for the source of the delayed response deficit to be localized to the cortex lining the sulcus principalis, especially its middle and caudal portions corresponding to BA 46 in the dlPFC (Butters, and Pandya, 1969; Goldman, and Rosvold, 1970; Gross, and Weiskrantz, 1962; Mishkin, 1957). Furthermore, research using rhesus monkeys has found that the deficits observed following the focal lesioning of the cortex within the sulcus principalis were as severe as those produced as a result of lesions to much larger portions of the PFC (Goldman, and Rosvold, 1970; Goldman et al., 1971; Mishkin, 1957). The specificity of the delayed response deficit to the dlPFC has been further supported by findings which show that cortical lesions which spare the sulcus principalis either did not result in observable deficits in delayed response task performance or only resulted in impairments when delay periods were extremely long (Goldman et al., 1971; Bauer, and Fuster, 1976; Jacobsen, 1936). In addition to permanent surgical lesions, researchers using either direct electrical stimulation (Stamm, 1961) or cryogenic cooling (Bauer, and Fuster, 1976) of the dlPFC

in non-human primates to induce temporary disruptions of the activity of neurons in the region have found these methods also induce errors in performance on delayed response tasks.

In order to determine the role of the dlPFC in non-spatial working memory in humans, Petrides and colleagues have studied the impact of unilateral frontal lobectomies performed in order to relieve pharmacologically intractable epilepsy on human patient's performance on the Self-Ordered Pointing Task (SOPT; Petrides, and Milner, 1982). Patients were presented with a set of stimuli consisting of either verbal (words) or visual (representational drawings or abstract designs) stimuli. An example of the stimuli used for this task are shown in Figure 1.3. The same set of stimuli were presented on a number of different pages in different spatial arrangements and patients were required to point to a different stimulus on each page until each item had been selected once but only once. A working memory error was scored when a patient reselected a stimulus they had already selected. The spatial locations of the stimuli were varied and random from page to page so that patients were unable to use a spatial strategy to keep track of those items which they had already selected. The patients were required to use memory for the figures or words themselves to guide their performance. According to Petrides, and Milner (1982), this task placed heavy demands on non-spatial working memory as it required patients to constantly monitor and update the identity of multiple items held in memory but did not require the maintenance or retrieval of spatial information. The comparison of the performance of patients with PFC excisions to those with temporal lobe excisions suggested that the SOPT was especially sensitive to PFC damage.

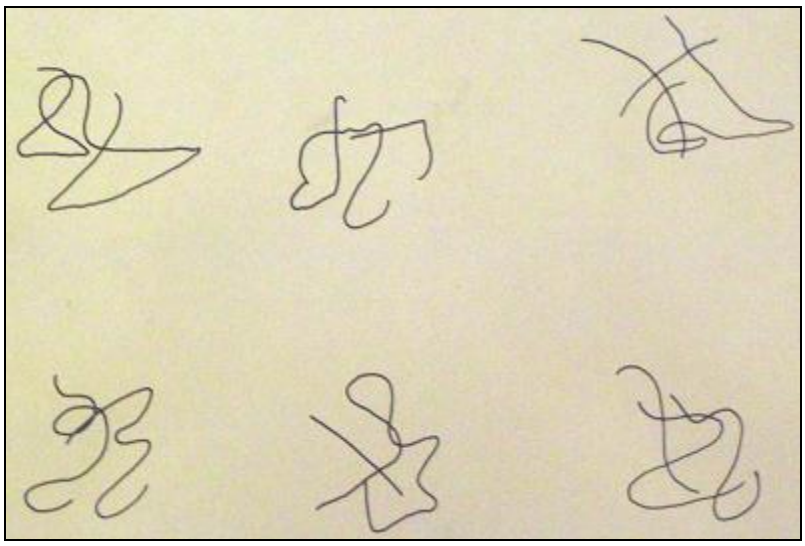

Figure 1.3. An example of the stimuli used by Petrides, and Milner (1982) in the 6 item abstract-design set of Self-Ordered Pointing Task stimuli.

Specifically, the researchers observed that patients with PFC excisions that included the dlPFC were markedly impaired on both the verbal and visual versions of the SOPT, while patients with unilateral excisions of the temporal lobe that did not include extensive damage to the hippocampal system were unimpaired. Patients with left frontal lobe excisions were impaired on both the verbal and nonverbal tasks while those with lesions of the right frontal lobe only showed deficits on the nonverbal tasks (Petrides, and Milner, 1982). These findings strongly support the role of the PFC in non-spatial working memory. The excisions experienced by these patients were made with the primary purpose of relieving them of epilepsy symptoms thus in most cases the resulting neural damage was not confined to a distinct neuroanatomical region within the PFC. For this reason the researchers were unable to specify the critical regions within the PFC responsible for the observed deficits on the self-ordering task.

In an attempt to more accurately isolate the critical prefrontal region Petrides (1995a) studied the performance of monkeys with more focal lesions on a modified version of the SOPT. It was found that those monkeys with lesions of the mid-dorsolateral cortex at BA 46 were impaired on the task when compared to those animals with lesions of the periarcuate cortex in the posterior frontal cortex. These findings led Petrides (1995a) to conclude that as in humans, the dlPFC played a critical role in working memory for non-spatial object information in the nonhuman primate brain.

Taken together, the results of the previously described animal, neuroimaging and lesion studies strongly suggest that the PFC plays an important and essential role in working memory function. Although the evidence is fairly new, recent neurophysiological and behavioural studies have begun to suggest that the functioning of

the adult primate PFC may be modulated by a number of metabolic factors including circulating hormones. Notably, accumulating evidence suggests that estrogens may exert regulatory effects on the adult primate PFC. This important metabolic influence has the potential to impact functions subserved by this region such as working memory.

Modulation of PFC Function by Circulating Hormones

A number of studies have shown that estrogen receptors are transiently expressed in the PFC of female rhesus monkeys during early brain development (Handa, Connolly, and Resko, 1988; MacLusky, Naftolin, and Goldman-Rakic, 1986; Pomerantz et al., 1985; Sholl, and Pomerantz, 1986). Specifically, MacLusky et al. (1986) observed levels of estrogen binding in the dlPFC of both late prenatal and early infant (less than 6 days postnatal) female rhesus monkeys to be comparable to the levels found in other cortical areas such as the visual, motor and somatosensory cortices. Additionally, evidence suggests that estrogen may also be present in the adult PFC (Hao et al., 2006). Expression of both estrogen receptors alpha (ERα) and beta (ERβ) messenger RNA fragments have been identified within the PFC of female rhesus monkey brain specimens (Pau, Pau, and Spies, 1998). Furthermore, the analysis of both young and aged rhesus monkey brain sections by electron microscopy indicates the presence of ERα in the dendritic spine and axon terminals in layer III of BA 46 (Wang et al., 2010). The presence of estrogen receptors within the PFC suggests that this region is a target of estrogens in adult non-human primate brains.

Examination of human brain specimens has also revealed evidence of estrogen binding within the PFC, although evidence is limited. Bixo et al. (1995) examined estradiol concentrations in different portions of the cortex in post-mortem brain samples

of adult human females and found the PFC to represent one of the densest binding sites for estradiol. Estrogen concentrations in the PFC were found to be approximately twice as high as those found in the temporal neocortex and more than seven times as high as those in the hippocampus. These findings suggest that the PFC is a principal location of estrogen in the adult human brain (Bixo et al., 1995).

The analysis of estrone sulfatase levels in the human female brain provides further evidence of estrogen activity in the PFC. Estrone sulfatase is the enzyme responsible for converting estrone sulfate, the major conjugated estrogen in female plasma, to the more biologically active estrogens, estrone and estradiol. Platia et al. (1984) found the levels of estrone sulfatase activity in the PFC to be equivalent to or greater than those levels in the endocrine hypothalamus. Taken together, these findings provide support for the hypothesis that the PFC is a primary target for estrogens within the primate brain even well into adulthood.

The presence of estrogen within the adult brain is clear evidence for the notion that the hormone plays an important role in the maintenance and expression of sex-typical or sexually differentiated behaviours into adulthood. Such effects of hormones (not limited to estrogen) which have the ability to act in an acute manner and occur later in life after the development of both the nervous system and sex organs are known as activational affects (Arnold, 2009; Breedlove, Cooke, and Jordan, 1999; Cooke et al., 1998). Such activational affects can be distinguished from the organizational effects of sex steroids which influence physiology and behaviour through developmental mechanisms in which hormones act during prenatal or early postnatal critical periods to mediate the permanent

sexually dimorphic development of brain morphology (LaCroix-Fralish, Tawfik, and DeLeo, 2005).

A great number of animal and human studies have shown that experimentally manipulating estrogen levels has significant effects on a wide variety of neurotransmitter systems that project to the PFC including serotonin, norepinephrine, and dopamine (Summer, and Fink, 1995; Kritzer, and Kohama, 1998). Many such studies have investigated the effects of estrogen replacement in ovariectomized (OVX) rats. For example, Summer, and Fink (1995) found the number of serotonin (5-HT) binding sites in the anterior frontal cortex of OVX rats to increase 41% within 24 hours of the administration of a single dose of estradiol. O'Malley et al. (1987) observed a significant reduction in acetylcholine synthesis in the frontal cortex of female rats following ovariectomy, an effect that was shown to be reversed within 5 days of estradiol replacement. Similarly, 28 weeks post-ovariectomy a 56% reduction in the activity of choline acetyltransferase (ChAT), one of the major enzymes responsible for acetylcholine synthesis, was found in the frontal cortex of OVX female rats (Singh et al., 1994). Importantly this reduction was prevented or reversed in OVX rats given exogenous estradiol replacement. An effect of estrogen replacement on ChAT levels was also observed in the basal forebrain of female rats, a major cholinergic afferent to the frontal cortex such that enzyme levels were significantly increased within 6-24 hours of estradiol treatment (McEwen, Luine, and Fischette, 1987). In both rodents and primates, activity of the nigrostriatal and mesolimbic dopaminergic system both of which have projections that terminate in the PFC has also been shown to be affected by estradiol at relatively short latencies (Di Paolo, 1994). Within 15 minutes of treatment Thompson and Moss

(1994) found an increase in both dopamine release and uptake within the mesolimbic dopamine pathway projecting to the PFC in female rats. Estradiol administration has also been found to decrease the activity of tyrosine hydroxylase, the enzyme responsible for the conversion of tyrosine to dopamine's precursor, L-DOPA, in the limbic forebrain of OVX rats 24 hours after hormone injection (Hernandez et al., 1991).

Findings of the effects of estrogen on neurotransmitter systems are not limited to rodent models. Evidence also suggests that estrogens have the ability to regulate neurotransmitter activity in the PFC of nonhuman primates. Ovariectomized adult monkeys have been found to display a dramatic decrease in the density of dlPFC fibers which are immunoreactive for tyrosine hydroxylase and choline acetyltransferase, enzyme markers for dopaminergic and noradrenergic fibers, and cholinergic fibers respectively (Kritzer, and Kohama, 1998; 1999). An opposite effect of ovariectomy was found for axons immunoreactive for dopamine β-hydroxylase and 5-HT such that there was an increase in their density within the dlPFC. These findings suggests that ovarian hormones might regulate various neurotransmitter afferents in the PFC. This hypothesis was tested by examining the density of immunolabelling in the dlPFC of the same OVX monkeys given either estrogen or estrogen plus progesterone hormone replacement. It was found that monkeys receiving hormone replacements had labelling densities similar to those observed in hormonally intact control animals. Specifically, estrogen was found to be as effective as the combination of estrogen and progesterone in stimulating normal prefrontal immunoreactivity for choline acetyltransferase and dopamine β–hydroxylase (Kritzer, and Kohama, 1998; 1999). These findings suggest that estradiol was the critical hormone regulating and maintaining cholinergic, noradrenergic, and dopaminergic

activity within the PFC of female primates. Taken together, these studies strongly suggest that the modulatory effects of estrogen on various neurotransmitter systems active within the PFC may be a mechanism by which circulating estrogens modulate the PFC and thus the functions subserved by the region.

It is noteworthy that both dopamine and serotonin, and to some extent norepinephrine and acetylcholine, play important roles in working memory processes (Seamans, and Yang, 2004; Robbins, 2005). Experimental manipulation of dopamine transmission in the dlPFC of nonhuman primates has been shown to affect the performance of spatial working memory tasks (Luciana, Collins, and Depue, 1998). Dopamine depletion in the PFC of both rats and monkeys, specifically from the sulcus principalis, results in impaired performance on the spatial delayed response task (Brozoski et al., 1979). Additionally, the administration of a selective 5-HT receptor agonist has been shown to impair working memory on a radial arm maze task in rats (Luciana, Collin, and Depue, 1998; Winter, and Petti, 1987).

A number of recent neuroimaging studies support the hypothesis that human PFC function might be influenced by circulating estrogen levels. Such studies have found PFC activation to change systematically with the hormonal, specifically estrogen status although most existing studies have focused only on postmenopausal women (Berman et al., 1997; Shaywitz et al., 1999; Smith et al., 2006). One of the few investigations to study young women was conducted by Berman et al. (1997) and used PET to investigate the changes in regional cerebral blood flow patterns elicited by the Wisconsin Card Sorting Test associated with three pharmacologically controlled hormonal conditions. The researchers found the characteristic increase in regional cerebral blood flow in the

PFC elicited during the performance of the Wisconsin Cart Sorting Test to be attenuated in young women following the administration of a gonadotropin releasing hormone (GnRH) agonist which supressed ovarian function resulting in estrogen and progesterone levels which were comparable to those of postmenopausal women. Furthermore, a normalization of the regional cerebral blood flow pattern was observed when estrogen was administered in concert with the GnRH agonist.

More recently, Smith et al. (2006) used a randomized, double-blind, placebo-controlled crossover study to find task-induced prefrontal activity as measured by fMRI to change systematically with the hormonal status of female participants. The participants consisted of a group of postmenopausal women whose ovaries had ceased functioning leading to a significant decrease in endogenous estrogen levels. Each woman was tested and scanned twice; once while receiving hormone replacement therapy and once while receiving a non-hormone containing placebo. The researchers used a subtraction method to isolate those areas which were more highly activated during the spatial working memory task while participants were receiving hormone therapy than when they were receiving the placebo. It was discovered that the hormone therapy was associated with a more pronounced activation in the ventrolateral PFC bilaterally. Using a similar experimental design, Shaywitz et al. (1999) found the differences in the patterns of activation observed when the women were receiving either the hormone replacement or the placebo treatment to be the same regardless of whether the working memory task was verbal or spatial in nature. Specifically, the researchers observed the degree of PFC activation in the superior frontal gyrus during the performance of the verbal working memory task to be significantly greater when the women were tested after having

received 21-days of hormone replacement therapy than the same length of a placebo therapy. Taken together, these findings directly demonstrate that the estrogen status of human females modulates cognition-related neural activity.

Estrogen Status Correlates with PFC-Dependent Cognitive Tasks on a Behavioural Level in Both Nonhuman and Human Primates

The possibility that the estrogen status of a female may also correlate with her level of performance on PFC-dependent cognitive tasks receives preliminary support from behavioral studies with nonhuman primates. Roberts et al. (1997) compared the performance of age-matched groups of pre-menopausal, menopausal and post-menopausal female monkeys on the classic delayed response task. Both the menopausal and post-menopausal groups were found to display significant impairments in performance at a delay as short as 1-second. Furthermore, test performance was found to be significantly correlated with the hormonal status of the animal such that those with lowest estrogen metabolite levels also had the lowest performance scores (Roberts et al., 1997).

In order to test whether surgical menopause and subsequent estrogen replacement had an influence on cognitive outcomes of normal aging including working memory decline, Rapp, Morrison, and Roberts (2003) compared the performance of two groups of aged rhesus monkeys on a delayed response test of spatial working memory. The researchers found the performance of OVX monkeys who had received a regimen of low-dose, cyclic estradiol replacement to be substantially better than that of vehicle-injected monkeys on the delayed response task. Additionally, the reversal of age-related impairments in cognitive functioning by hormone replacement appeared to be specific to

the PFC dependent delayed response task, as there was only a very modest recovery of recognition memory which has been shown to be dependent on an intact medial temporal lobe (Rapp, 1993). These findings clearly suggest that a nonspecific estrogen influence on general performance factors such as motivation or perceptual ability is unlikely to account for the benefits of estrogen treatment observed on the working memory task.

Behavioral investigations with human participants provide further support for the existence of an association between estrogen levels and performance on tasks with prominent working memory components. If in fact such an association exists, then it is logical to hypothesize that performance on tasks which place significant demands on working memory would differ between groups of individuals who are exposed to different levels of estrogen. To date however, very few studies have been conducted in order to test this hypothesis. One of the few studies which has investigated the potential activational effects of estrogen on working memory performance was conducted by Duff, and Hampson (2000). The researchers compared the performance of three groups of post-menopausal women on the Spatial Working Memory Task (SPWM), a novel multi-trial spatial working memory task that was designed to be sufficiently sensitive to detect small to moderate individual differences within the normal range of performance, and Digit Randomization, tasks with strong spatial and verbal working memory components respectively. All three groups were post-menopausal. One group of women was not receiving any hormone replacement therapy. The other two groups were taking hormone replacement therapies consisting of either estrogen only, or a combination of estrogen and a progestin. The researchers found that both groups of women receiving hormone replacement therapy performed better than women not receiving estrogen on the tasks

that had prominent working memory components, but not on control tasks. Furthermore, no significant difference in performance between the two groups of women who received hormone replacement was observed. These findings support the hypothesis that it was specifically the estrogen component of the hormone replacement therapy that was responsible for the improved performance of those women receiving exogenous hormones.

Similarly, the results of a study conducted by Keenan et al. (2001) yielded evidence for disruptions of a variety of pre-frontally mediated cognitive processes including working memory in post-menopausal women not receiving hormone replacement therapy as compared to aged matched controls receiving replacement therapy. Specifically, untreated menopausal women were relatively impaired in inhibiting inappropriate responses in the form of perseverative errors, and in the performance of an auditory-verbal version of the N-back test of working memory. Further support for the hypothesis that estrogens have the ability to modulate working memory function is provided by a double-blind within-subject study by Krug, Born, and Rasch (2006). The researchers found the performance of post-menopausal women on tasks involving PFC dependent memory function to be significantly better after they had received transdermal estrogen replacement for as little as 3 days as compared to a placebo treatment. Importantly, estrogen was found not to affect hippocampus-dependent functions of memory retention. Thus the findings of both Duff, and Hampson (2000) and Krug, Born, and Rasch (2006) indicate that in postmenopausal women, a transient increase in plasma estrogen concentration acutely improves PFC-dependent cognitive functions. These data are consistent with both the notion that estrogen is active within the PFC and that it is

capable of influencing the function of the cognitive systems subserved by that region in postmenopausal women.

Research by Grigorova, Sherwin, and Tulandi (2006) suggests that the same association between estrogen levels and PFC mediated cognitive function could potentially exist even in premenopausal women who have not undergone long-term estrogen deprivation. The researchers investigated the influence of sex steroid hormone suppression given to a group of premenopausal women to chemically suppress ovarian function as a treatment for various benign gynecological disorders. The hormone suppression treatment suppressed estrogen levels to the postmenopausal range whereas progesterone levels fell to levels typical of the lower limit of the menstrual cycle range. Each woman completed a battery of tasks including both a verbal and non-verbal version of the N-back working memory task before and after 4 weeks of treatment. The comparison of each woman's pre-treatment baseline to their post-treatment performance, revealed a decline in performance on both working memory measures. Importantly, further analysis using post-treatment declines in progesterone, mood, or other health-related symptoms as covariates revealed that the relative declines in working memory performance were associated with post-treatment declines in estrogen concentrations, but not these other factors. Thus these findings provide preliminary evidence that as was observed in postmenopausal women, estrogen is also influential in the maintenance of working memory function in premenopausal women. Such conclusions must be made cautiously however as these data are preliminary and to date the research by Grigorova and colleagues (2006) is the only basis on which conclusions can be made regarding the

effect of estrogen levels on PFC dependent cognitive functioning on a behavioural level in young women.

Summary and Hypothesis

Numerous lines of research have demonstrated that the PFC, specifically its dorsolateral region, is critical for working memory function. Furthermore, convergent evidence from neuroendocrine and neuroimaging studies in both nonhuman and human species suggests that the adult female PFC, including its dorsolateral region, may be susceptible to the activational effects of estrogens. Thus, it appears that estrogens may have the ability to modulate the function of the PFC. More recently, behavioural investigations have begun to provide support for the notion that the activational effects of circulating estrogens on the PFC may influence PFC-dependent working memory processes at a functional level.

Two standard paradigms are commonly used by researchers to investigate the potential activational effects of estrogens on cognitive functions in women. The first is the study of surgically menopausal or naturally postmenopausal women. The second paradigm takes advantage of the natural fluctuations in ovarian hormones across the menstrual cycle. Such methods allow for the investigation of the effects of estrogen on cognition in young women in a non-invasive and naturalistic manner.

The menstrual cycle refers to a recurrent sequence of ovarian changes and hormone output that occurs cyclically in women of reproductive age under the guidance of stimulation from the hypothalamus and pituitary, when conception has not occurred. The exact timing of the endocrine events varies from woman to woman and cycle to cycle, but the menstrual cycle is often divided into two broad phases, the follicular and

the luteal phase (Wilson et al., 1998). In women who have a 28-29 day cycle, the follicular or proliferative phase is marked by the first day of menstruation (cycle Day 1) and extends until cycle Day 14-15 when ovulation occurs (Figure 1.4). The beginning of the follicular phase is characterized by low serum concentrations of both progesterone and 17β-estradiol, the most abundant and potent form of estrogen. While progesterone levels remain low, a preovulatory peak in estradiol occurs just prior to ovulation. The end of the follicular phase is marked by ovulation. The luteal or secretory phase is characterized by high endocrine concentrations of both estrogen and progesterone and extends from cycle Days 15 to 28. The length of the luteal phase is relatively fixed at 13-15 days, regardless of the length of the overall menstrual cycle. An estimation of the hormonal state of a female can be obtained by counting backward from cycle Day 1, the first day of menstruation. This reverse counting method has been used in a number of previous studies, however investigations of the validity of the approach have determined it to be associated with error rates of 15% or higher (Stern, and McClintock, 1995). As such, the confirmation of expected hormone concentrations for each menstrual cycle phase by radioimmunoassay (RIA) or enzyme immunoassay (EIA) has become a critical part of the methodology for studies using menstrual cycle related hormone fluctuations as a variable. Such retrospective endocrine validation of hormone levels allows for greater accuracy in determining menstrual phase at the time of testing.

The objective of the current study was to further extend the limited body of research that has investigated estrogen's actions within the PFC and its ability to influence the functions subserved by the region. This aim was achieved using the natural

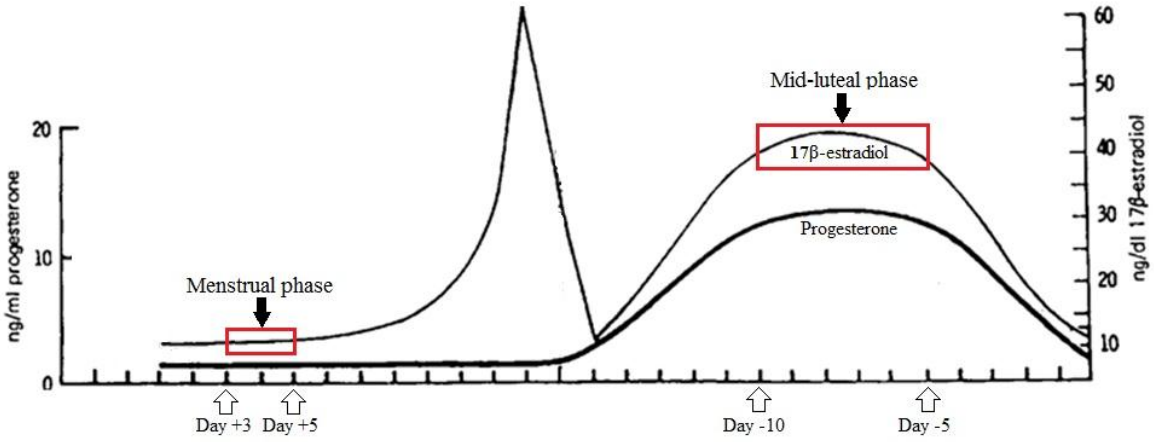

Figure 1.4. An illustration of the typical changes in progesterone and 17β-estradiol concentrations that occur across a 28 day menstrual cycle. Days +3 and +5 correspond to the menstrual phase when estrogen concentrations are low. Days -5 to -10 correspond to the mid-luteal phase when estrogen levels are high.

estrogen fluctuations associated with the female menstrual cycle as a noninvasive and ecologically valid method of manipulating the hormone levels to which the participants were exposed. Because previous evidence suggests that the PFC is susceptible to estrogen and that estrogen has a facilitatory effect on working memory function, it was hypothesized that women would perform selectively better on measures of working memory (make fewer working memory errors) when tested under the influence of high estrogen during the mid-luteal phase of the menstrual cycle than when tested under the influence of low estrogen during the menstrual phase of the cycle. Such findings would support previous theoretical findings of a beneficial effect of high estrogens on the PFC and the functions it subserves.

CHAPTER 2

METHOD

Participants

The participants were 36 healthy female university staff, graduate and undergraduate students ranging in age from 21 to 35 years (*M* = 24.75 years, *SD* = *3.93*) with a mean estimated IQ of 108.78 (*SD* = 7.12). All participants had regular menstrual cycles between 21 (minimum) and 35 (maximum) days in length (*M* = 29.22, *SD* = 1.93). Participants had not used oral or other types of hormonal contraceptives for at least 4 months. Participants who indicated histories of health conditions including attention deficit disorder, seizures or epilepsy, major depression, schizophrenia, bipolar disorder, over or underactive thyroid glands, diabetes, head injuries, or other serious neurological or psychiatric conditions on a confidential health questionnaire were not eligible to participate.

Procedure

Prospective participants were recruited via posters displayed throughout the University of Western Ontario campus. After indicating their interest in participating, volunteers completed a confidential online demographic and reproductive health questionnaire in order to determine eligibility. Women meeting the above criteria were contacted for testing at a later date. Eligible participants were tested on two separate occasions the timing of which were individually targeted to coincide with either the menstrual phase of the cycle when estrogen levels are lowest (cycle days +3 to +5 relative to the day of menstrual onset), or the mid-luteal phase of the cycle when estrogen levels peak (cycle days -5 to -10). Cycle phase for the first session was randomly assigned and counterbalanced across participants. Due to the fact that the menstrual cycle is not perfectly reliable, each woman's cycle stage at each test session was confirmed retrospectively. Testing was considered successful if RIA of saliva collected during the test sessions provided independent confirmation of the levels of progesterone and 17β-estradiol, the major estrogen in women of reproductive age (see *Radioimmunoassays*, below). In the group of women reported here, the mean level of 17β-estradiol was 3.56pmol/L (*SD* = 1.76) during the menstrual phase and 6.20pmol/L (*SD* = 1.81) during the mid-luteal phase. Associated levels of progesterone were 37.91pg/mL (*SD* = 12.22) and 145.83pg/mL (*SD* = 63.31), respectively. Mean salivary hormone concentrations at each phase of the cycle were in agreement with previous reports based on healthy women with ovulatory menstrual cycles (Bao et al., 2003; Ellison, 1993; Gandara, Leresche, and Mancl, 2007; Oinonen, and Mazmanian, 2007; Shirtcliff et al., 2000; Worthman, Stallings, and Hofman, 1990).

Each woman was tested individually. Both sessions began and ended with the collection of a saliva specimen for hormonal analysis. In order to ensure the purity of the saliva samples, women were asked not to smoke, chew gum, brush their teeth, eat or drink anything except plain water for 1 hour prior to their appointment time. For the sample provided at the start of the session, approximately 7 mL of saliva were collected into a polystyrene culture tube pre-treated with sodium azide. A stick of inert sugarless gum was used as a saliva stimulant. For the sample provided at the end of the session, approximately 2 mL of saliva were collected into a borosilicate tube by passive drool. No saliva stimulant was used in order to permit analysis of the saliva using enzyme-linked immunosorbent assay (not reported here). All specimens were frozen at −20°C until the end of the study then analyzed in a single assay.

Each test session took approximately 1 hour during which the battery of tasks described below was administered. Each task was administered once at each of the two test sessions with the exception of the North American Adult Reading Test (NAART) which was only administered at the first test session.

Cognitive and Memory Tasks

Working Memory Tasks

At each of the two test sessions, participants completed three tasks which had significant spatial, verbal or figural working memory components:

Spatial Working Memory Task (SPWM; Duff, and Hampson, 2000). This task was developed as a more cognitively demanding version of those spatial working memory tasks which have been shown previously to depend on PFC in nonhuman (Passingham, 1985) and human primates (Owen et al., 1990; 1995). The SPWM was adapted for

humans from a search task used by Passingham (1985), who found that rhesus monkeys who had surgical lesions in the vicinity of the sulcus principalis (BA 46) were severely impaired, relative to control animals (i.e., they produced high numbers of working memory errors). The performance of younger adults on the SPWM has previously been shown to correlate significantly with performance on Digit Ordering, a verbal WM task (Duff, and Hampson, 2001) and with performance on SOPT (Hampson et al., 2010).

Participants were seated at a desk on which a board containing a 4 x 5 array of 10 different coloured dots (red, purple, green, blue, orange, pink, black, white, fuchsia, yellow). Each dot was 3 cm in diameter and were arranged in a random order on a neutral backing (45 cm long, 41 cm high). Figure 2.1 shows an illustration of the SPWM board. The board containing the coloured dots was placed in front of participants at eye level. Each item in the array was completely concealed beneath a hinged flap (8.0 cm long, 4.5 cm wide) and only became visible when the corresponding flap was temporarily lifted by the participant. Two dots of each colour were hidden beneath the hinged flaps, and participants were instructed to find all 10 matching pairs of dots, in as few choices as possible, by lifting the flaps two at a time. When a flap was not being lifted by the participant, it was closed, concealing the item beneath. As such, the participants were required to maintain within WM the locations of the pairs of dots they had already found and update this information iteratively as they found further pairs of dots.

Participants were familiarized with the complete set of colours before they began the task by showing them a token of each colour on a piece of black felt placed to the side

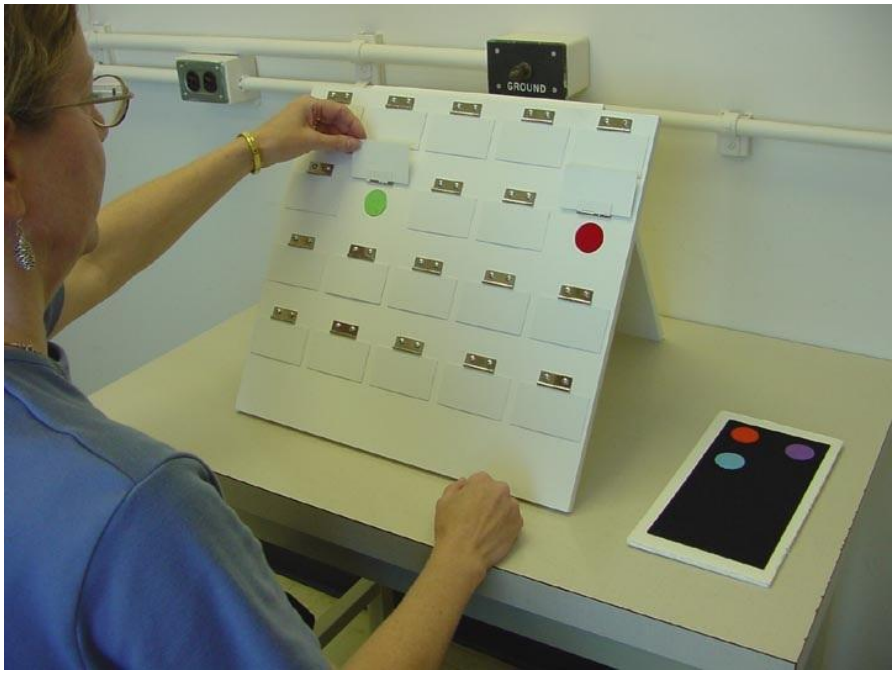

Figure 2.1. A photograph of a participant selecting a non-matching pair on the spatial working memory (SPWM) board. The board consists of 20 hinged doors under which 10 pairs of coloured dots are hidden. Participants are instructed that their goal is to find all 10 pairs of matching dots in as few tries as possible, lifting the doors two at a time. Participants are required to update the locations stored in memory as they lift the doors and find matching pairs. A working memory error is counted anytime the participant selects a pair of doors that they have already selected, or a pair of doors which they already know do not contain a pair.

of the array. Before the participants began their search, the tokens were removed but during the task, as each pair was discovered, the experimenter replaced the corresponding token on the felt. By the end of each trial, a token of each of the 10 colours appeared on the felt. The tokens provided visual feedback to the participants eliminating the need for them to remember which colours they had already found. As such, the participants were only required to remember the *locations* of the matched or not matched dots as the task progressed.

Participants were told that they would be timed on the task, but that their primary objective was to locate the matching pairs in the fewest tries possible. Each participant completed three consecutive trials of the same array. Alternate forms of the task were presented on each of the two testing days and which arrangement of the dots the women received at their first test session was counterbalanced within each phase of cycle. A trial was considered complete when all 10 matching pairs of dots were found. The dependent variables were: the number of working memory errors and the time to completion of each trial in seconds. A working memory error was committed anytime a participant chose a pair of locations that had already been searched but did not match, or re-searched an already matched pair.

Digit Ordering (Petrides et al., 1993b). Previous PET studies have used this task with the aim of mapping the patterns of neural activation elicited by verbal working memory tasks. The task has been shown to elicit significant activation in the mid-dlPFC bilaterally (Petrides et al., 1993a, b).

In the present study, participants were asked to say out loud the numbers from 1 to 10 in a random order without repeating or missing any digits. Participants completed

10 different randomization trials during each of the two test sessions. The dependent variables were: the total time to complete each trial as well as the total number of working memory errors committed. For the purpose of statistical analysis, the total number of working memory errors summed across the 10 trails was used. A working memory error was defined as the omission or repetition of any digit within a trial.

Self-Ordered Pointing Task (SOPT; adapted from Petrides, and Milner, 1982). Performance on the SOPT has been shown in studies of neurological patients to be extremely sensitive to frontal lobe damage (de Zubicaray, 1997; Petrides, and Milner, 1982; Wiegersma, van der Scheer, and Hijman, 1990). Imaging work in humans or nonhuman primates has confirmed activation in the right mid-dorsolateral frontal cortex (BA 46) as well as a weaker response within this same region in the left hemisphere, consistent with its recruitment of the working memory system (Petrides, 2000a; Petrides, 2000b; Petrides, 1989; Petrides et al., 1993).

The task required participants to complete a series of trials in which the goal was to point to all items in a set of stimuli once and only once. Participants completed a 4 item practice set, followed by 8, 10, 12 and two 14 item sets. Different visual stimuli were used for each set size. All stimuli, other than the 4 item practice stimuli which depicted food items, were abstract, and were chosen to be easily visually distinguishable from one another but difficult to verbally encode. All items measured 5 cm in height and 4.1 cm in width. Each set of stimuli was printed on large-sized photopaper that ranged in size from 21.59 x 27.94 cm to 29.72 x 41.91 cm. An example of one of the pages from the 10 item set is shown in Figure 2.2.

Each set size was bound separately and was presented directly in front of the

Figure 2.2. The stimuli used for the 10 item set of the Self-Ordered Pointing Task. Each of the 10 pages in the set contained the same 10 images, but the spatial location of each image was random and varied on each page. The participant's goal was to point to one item on each page of the stimulus set but to point to each item once and only once. This required the participants to continually update the object identity information but not spatial information stored in working memory in order to accurately guide their performance. (Imaged cropped and adapted from http://infolific.com)

participant by the experimenter after the completion of the previous set. Each set had a number of pages corresponding to the number of items in the set (e.g., 12 pages for the 12 item set). On each page the items were arranged in a fixed two-column layout. Each page in a given set contained the complete set of stimuli at that set size, and thus had the same number and identity of items, but the spatial location of each item within the two-column layout was randomized and varied unpredictably on each page.

The participant was required to initiate and execute a sequence of pointing responses. In order to avoid making errors, the participant had to maintain an internal representation of the responses made and continually update this record as they monitored their own performance. Due to the fact that the spatial location of items on each page of the set varied randomly, it was not possible to perform the SOPT using a spatial strategy. Instead, participants had to maintain the figural identity of the items which they had already selected in working memory. Thus the task required the temporary maintenance and updating of object identity but not spatial information throughout the task.

Subjects were instructed that they could point to the items in any order they wished, but that they were not to point to any items they had selected on previous pages. Before beginning each set size, the participants were given between 5 and 30 seconds depending on the set size to familiarize themselves with the appearance of the visual stimuli. The demand on working memory was increased by having participants complete three trials at each set size consecutively during each testing session. The same set of stimuli was used for each of the three trials.

The dependent variables were: the time to completion (in seconds) at each set size and the number of working memory errors at each set size. A working memory error was counted anytime a participant reselected an item they had already selected within a trial.

Span Control Tasks

Because it is the frontally mediated executive functions of working memory that were hypothesized to be influenced by estrogen, control tasks were used that required the passive short-term retention and retrieval of verbal, spatial and visual information similar to that used in the working memory tasks but without monitoring, active maintenance, manipulation or reworking of the stored information. These control tasks enabled the alternative possibility that estrogen-related effects on the passive storage processes of working memory are the basis for any facilitative effect of estrogen observed on the working memory tasks described above to be tested.

Digit Span (Wechsler Adult Intelligence Scale – Revised, Wechsler, 1981). This task required only the passive retention of the digits in memory without any active manipulation of the stored information. Previous neuroimaging research has shown the type of short-term storage of verbal information required for performance of this task to involve the recruitment of the posterior perisylvian cortex, but not the PFC (Postle, Berger, and D'Esposito, 1999; Shallice, and Vallar, 1990). Furthermore, patient studies have shown performance on the forward digit span task to be unaffected by excisions or lesions of the PFC (Canavan et al., 1989a; D'Esposito, and Postle, 1999; Petrides, 1995b).

The Forward Digit Span portion of the WAIS-R Digit Span subtest was administered in the standard manner. Participants were asked to repeat a sequence of digits of progressively increasing length as it was presented to them verbally by the

examiner. A maximum of two tries were allowed at each sequence length. The digit span score was the maximum number of digits repeated correctly. This measure allowed for the quantification of any changes in the participant's ability to temporarily store and retrieve verbal information throughout the menstrual cycle which could contribute to or account for any differences on the Digit Ordering task at the two cycle phases investigated.

Corsi Block-Tapping (Milner, 1971). This task is a visuospatial analogue of the Forward Digit Span task. Patient research indicates that performance on the Corsi block-tapping task is unaffected by lesions to the PFC and implicated other more posterior cortical areas such as the inferior parietal cortex as being essential for accurate task performance (Baldo, and Dronkers, 2006; D'Esposito, and Postle; 1999). The task involved participants observing the examiner tap a progressively longer spatial sequence on a set of 10 identical 3 cm cubes painted black and randomly arranged on a 27.7 x 22.8 cm wooden platform. Immediately after each sequence was shown to the participant, they were required to reproduce the exact sequence demonstrated by the examiner, in order. The score was the maximum sequence length that a participant could reproduce in the correct order. A maximum of two tries were allowed at each sequence length. The task required participants to temporarily store a sequence of spatial locations but did not require the active manipulation or on-line maintenance of the stored information. This measure allowed for the quantification of any changes in the participant's ability to temporarily store and retrieve spatial information throughout the menstrual cycle which could contribute to or account for any differences on the SPWM at the two cycle phases investigated.

Pattern Span. This task was developed as a control task for the SOPT. One page of figural stimuli from the 12 item set of the SOPT was placed on the desk in front of the participant. The participant watched while the examiner pointed to a sequence of the figural items of progressively longer length. The participant was then required to reproduce the exact sequence of items demonstrated by the examiner. The score was the maximum sequence length the participant could reproduce in the correct order. Comparison of the participant's score on this task at the two phases allowed for the quantification of any changes in the passive storage of a set of figural items of the same sort participants were required to store during the SOPT

Additional Control Tasks

Mooney-Harshman Closure Task (Adapted from Mooney, and Ferguson, 1951). This closure task has been validated in clinical assessments of patients with brain lesions. Studies have shown that performance on this task is extremely sensitive to posterior temporal lobe lesions, but is unaffected by frontal lobe lesions (Kolls, Milner, and Taylor, 1983; Lansdell, 1968; Milner, 1980; Newcombe, and Russel, 1969; Wasserstein et al., 1984). Additionally, a neuroimaging study by Grutzner et al. (2010) has implicated a variety of brain areas in the performance of the Mooney-Harshman Closure Task in which faces served as the stimuli (Mooney, 1957). Importantly, the PFC was not one of the areas which were found to play an important role in task performance. These findings support the fact that the task does not significantly recruit working memory functions subserved by the PFC, but rather visual object recognition processes which rely on the integrity for more posterior cortical regions.

Two parallel forms of the task were created. Each consisted of 13 black and white images printed individually on 21.59 x 27.94 cm cards, which were presented on the desktop in front of the participant. Each image depicted a common object with parts of the picture missing or incomplete. The participant's task was to identify the object. In order to minimize practice effects, alternate forms of the task were used on the two testing sessions. Which of the two forms each participant was shown during their first test session was counterbalanced across participants and within phase of cycle. Participants were allowed up to 20 seconds to identify each item. Responses were recorded verbatim by the examiner. The scores taken were the number of correctly identified items (maximum = 13) and the time in seconds taken by the participant to make a correct identification of the image shown in the picture (maximum = 20 sec).

Previous studies have found women tested during their menstrual phase to identify significantly more figures correctly than those tested during the mid-luteal phase (Hampson et al., 2005). It was anticipated that the same effect would be found in the present study. This is a reverse menstrual cycle effect compared to that which was hypothesized for the working memory tasks. As such, this task served as an important contrast, allowing the potential to demonstrate selectivity in any menstrual cycle effects observed. A reversal in the direction of the menstrual cycle effect on task, with differing cognitive requirements would help to rule out the possibility that any effect found on the working memory measures is due to an across-the-board facilitative effect of estrogen on all brain regions.

Profile of Mood States (POMS; McNair et al., 1971). The POMS is a self-report mood inventory suitable for use in healthy as well as clinical populations. Participants

were asked to rate how accurately each of 65 mood descriptors (e.g., “Nervous”, “Carefree”, “Sad”) described their mood on the day of testing. Items were rated on a 5-point Likert scale ranging from “Not at all” to “Extremely”. A total score for each of the six POMS subscales was computed: Tension, Depression, Anger, Vigor, Fatigue, and Confusion. As certain mood states such as clinical depression have been associated with lower scores on objective measures of declarative memory recall (for review, see Cassens et al., 1990), the POMS was administered in order to address the possibility that any changes in working memory performance were simply a reflection of menstrual cycle-related changes in general mood state. However, research has shown that differences in mood between the menstrual and mid-luteal phases are infrequent in healthy populations and not normally of clinical proportions (Abplanalp et al., 1979a; Abplanalp et al., 1979b; Ainscough, 1990; McFarlane, Martin, and Williams, 1988; Zimmerman, and Parlee, 1973).

North American Adult Reading Test (NAART; Blair, and Spreen, 1989). This task required participants to read aloud a list of 61 irregularly spelled English words, or foreign words imported into English that have kept their foreign pronunciation and spelling. Each word was scored for accuracy of pronunciation according to American and Canadian pronunciation rules (maximum = 61). Full Scale IQ (FSIQ) scores were estimated using the actuarial prediction equations developed by the creators of the NAART. The NAART has been validated as a predictor of the IQ scores of the Wechsler Adult Intelligence Scale (Blair, and Spreen, 1989). Participants completed the NAART only during their first test session. Estimated FSIQ scores allowed for the comparison of the two counterbalanced groups (women tested at the menstrual phase first and women

tested at the mid-luteal phase first) to confirm that they were evenly matched in general intellectual ability. Additionally, previous research using other vocabulary scores which show strong positive correlations with general IQ (Vernon, 1971; Wechsler, 1981) have found crystallized intelligence to be unaffected by the levels of circulating hormones (Broverman et al., 1981; Hampson, 1990; Sommer, 1972; Souza et al., 2012). Based on these findings, no effect of menstrual cycle phase was predicted on the participants NAART scores.

Radioimmunoassays

The saliva was thawed and centrifuged at 3000rmp (4^{o}C) for 15 minutes prior to analysis. To quantify progesterone, 1 mL of centrifuged saliva was extracted twice with diethyl ether then evaporated at 35-40^{o}C under a nitrogen stream and reconstituted in phosphate buffered saline. The samples were analyzed in a single run using a ^{125}I Coat-A-Count progesterone radioimmunoassay kit (Siemens Healthcare Diagnostics, Deerfield IL) modified for saliva according to an established laboratory protocol (Mead, and Hampson, 1997; Oinonen, and Mazmanian, 2007). The sensitivity of the assay was 8.2 pg/mL and the intra-assay coefficient of variation, averaged across low, medium, and high pools was 7.7%. For estradiol, the samples were analyzed without extraction using the DSL4800 Ultra-Sensitive Estradiol RIA (Immunotech, Prague, Czech Republic) adapted for saliva. Briefly, a 300µL aliquot of saliva was incubated at 4^{o}C for 18-24hr using ^{125}I tracer (1:2) and antiserum (1:3) diluted with buffer (pH 8.0), and referenced to a standard curve that ranged from 0.625 to 20pg. The sensitivity across two runs averaged <0.25pg/mL and the intra-assay coefficient of variation averaged 3.8%.

Women were considered to have been tested successfully at the targeted phases of the menstrual cycle if the following criteria were met: (a) both test sessions fell on days of the woman's cycle consistent with the menstrual (between days +3 and +5) and mid-luteal (between days -3 and -12) phases. The day of onset of the subsequent menstrual period was used retrospectively to confirm the timing of the luteal session. (b) the assays revealed elevated estradiol at the mid-luteal phase and a mid-luteal progesterone concentration at least twice as high as the menstrual phase concentration, consistent with the presence of an ovulatory cycle; (c) concentrations of both ovarian hormones were within acceptable physiological boundaries at each phase of the menstrual cycle. Five participants met the first two criteria but failed to meet the third criterion. One woman had a mid-luteal estradiol concentration >6 SD above the group mean. Four others were excluded whose salivary progesterone level was unacceptably high at the menstrual phase of the cycle (*cf.*, Schultheiss et al., 2003; for review see Ellison, 1993). Based on these criteria, the final sample consisted of 15 women tested at the menstrual phase first and 21 tested at the luteal phase first.

CHAPTER 3

RESULTS

All data were analyzed with SPSS 18.0 for Windows unless otherwise specified.

NAART

A *t*-test for independent samples confirmed that the two groups of women (those tested at the menstrual phase first or at the mid-luteal phase first) were evenly matched on estimated FSIQ based on the NAART. The mean FSIQ for the menstrual first group was

108.20 (*SD* = 7.04) and for the luteal first group was 109.19 (*SD* = 7.32), $t(34) = 0.41$, $p = 0.686$.

Cognitive and Memory Tasks

Working Memory Tasks

Scores on the working memory measures were analyzed in separate 2 x 2 mixed effects ANOVAs with phase at first session (menstrual or mid-luteal) as the between-subjects factor and menstrual cycle phase (menstrual or mid-luteal) as the within-subjects factor. For the SPWM and SOPT, trial (or set size) was included as a second within-subjects factor. Post-hoc analysis was performed where indicated using the Tukey HSD test.

SPWM. Scores on the SPWM task were analyzed in a 2 x 2 x 3 ANOVA that included trial (1, 2, or 3) as a within-subjects factor. Analysis of the number of working memory errors on each of the three trials of the SPWM revealed a significant main effect of trial, $F(2, 68) = 3.42$, $p = 0.038$, whereby fewer errors were made by the third trial, and a main effect of cycle phase, $F(1, 34) = 30.28$, $p < 0.001$ (Figure 3.1). As predicted, participants made significantly fewer working memory errors when tested during the mid-luteal phase than during the menstrual phase. Post-hoc analysis with Bonferroni correction indicated that the effect of phase was significant on all three trials of the SPWM, all $p < 0.016$, two-tailed. The interaction between phase and the phase at first test session was also significant, $F(1, 34) = 10.79$, $p = 0.002$, indicating a practice effect occurred on the task on session two (Figure 3.2). As a result of the additive effect of practice, the effect of phase of cycle appeared larger among those women whose mid-luteal testing occurred on session two.

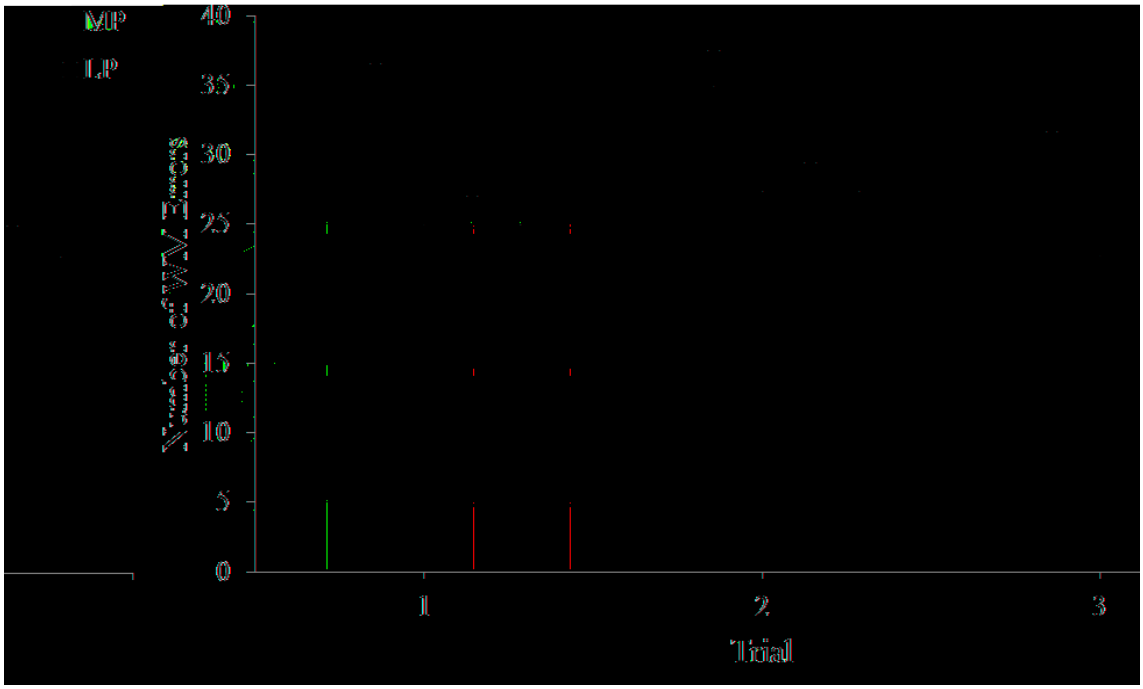

Figure 3.1. The mean number of working memory (WM) errors committed on the Spatial Working Memory Task (SPWM) by women tested at the menstrual phase (MP) compared to at the mid-luteal phase (LP) of their menstrual cycle. Error bars represent SEM. Women's performance was less accurate, committing significantly more WM errors, at the MP than the LP test session.

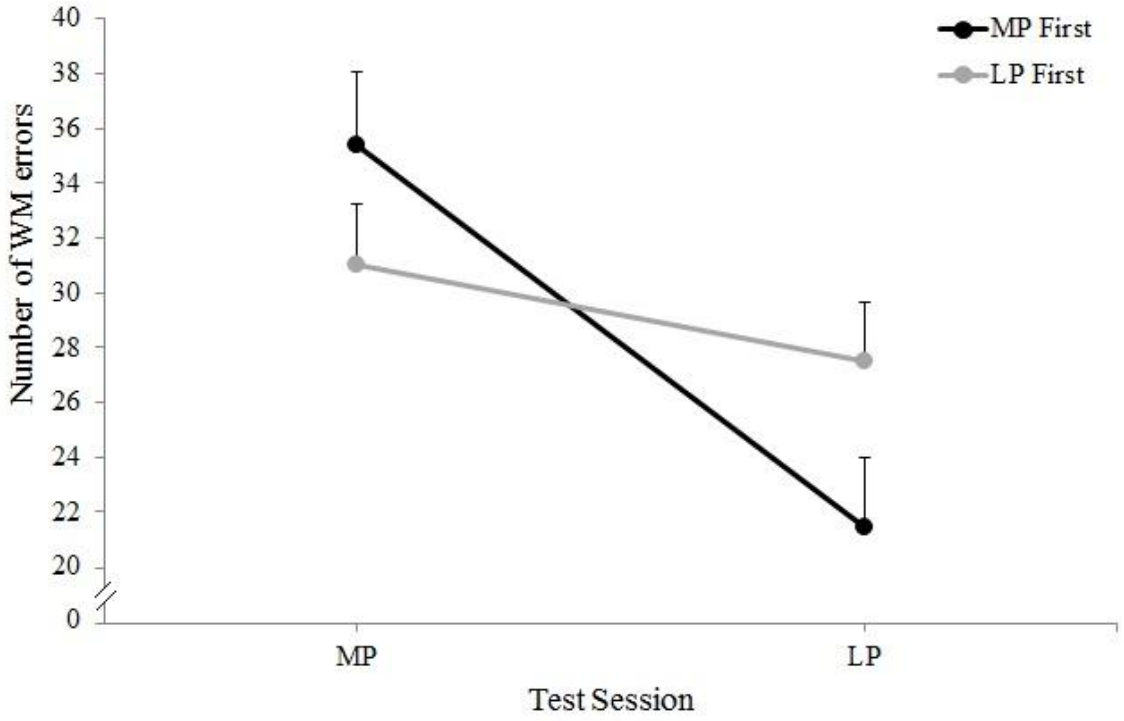

Figure 3.2. Mean number of working memory (WM) errors commited at each phase of the cycle by women who were tested at the menstrual phase (MP) first compared to those tested at the mid-luteal phase (LP) first. The apparent size of the menstrual cycle effect on the SPWM was larger in women in the MP First group, who were tested at the mid-luteal phase on the second session. Error bars represent SEM.

ANOVA of the time scores on the SPWM revealed that there was not a significant effect of cycle phase on time to completion $F(1, 34) = 2.39$, $p = 0.132$ (Figure 3.3) although, as for the number of errors, there was a significant main effect of trial, $F(2, 68) = 16.64$, $p < 0.001$, with faster performance on the SPWM as the task progressed from trial 1 to trial 3. Thus the amount of time required for the participants to complete the trials of the SPWM did not depend on their cycle phase at the time of testing.

Digit Ordering. Consistent with the results of the SPWM, there was a significant main effect of cycle phase on the Digit Ordering task, $F(1, 34) = 31.33$, $p < 0.001$ (Figure 3.4). As predicted, women committed significantly more working memory errors in their menstrual phase test session than their mid-luteal phase session. The interaction between phase and testing order was also significant, $F(1, 34) = 5.54$, $p = 0.025$, consistent with the presence of a practice effect on session two. As a result, the apparent size of the phase effect was greater for women whose second test session occurred during the mid-luteal phase of their cycle than for women tested in the reverse order.

As with the SPWM, an ANOVA of time scores from the Digit Ordering task revealed no significant effect of cycle phase on time to completion, $F(1, 34) = 0.035$, $p = 0.853$ (Figure 3.5). In the present sample, the amount of time required for the women to complete the 10 trials of the Digit Ordering task failed to show a significant difference regardless of their cycle phase at the time of testing (menstrual phase: $M = 115.85$, SEM = 6.68, mid-luteal phase: $M = 116.75$, SEM = 6.26).

SOPT. Scores on the SOPT were analyzed in a 2 x 2 x 6 repeated measures ANOVA which included set size (4, 8, 10, 12, 14 trial 1, 14 trial 2) as a within-subjects factor. A significant main effect of cycle phase was found for the number of working

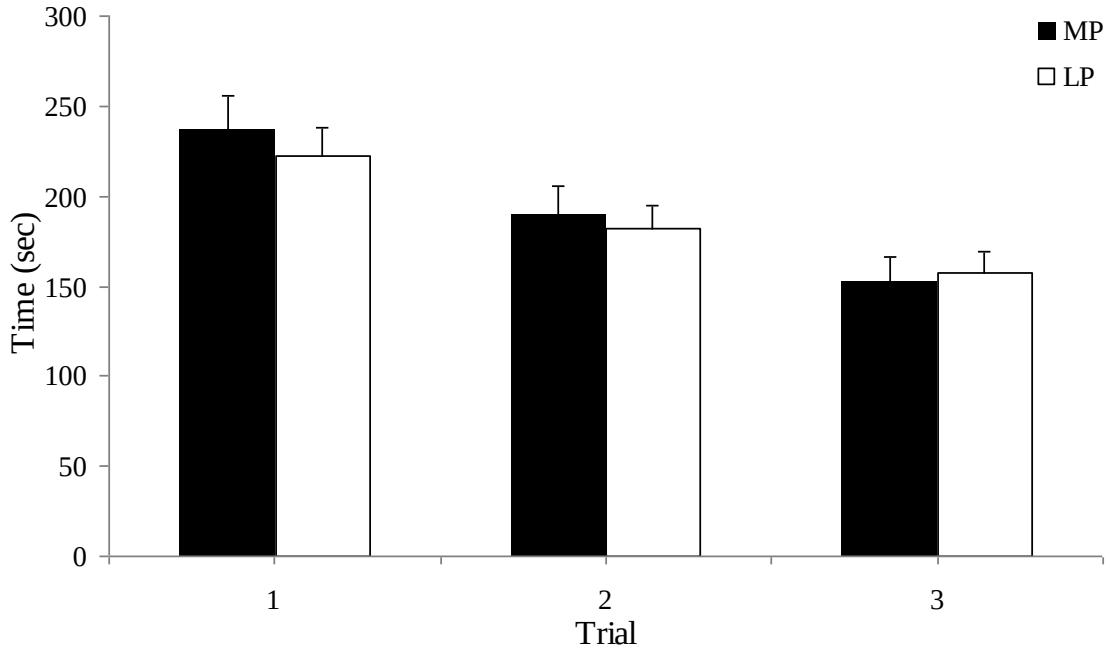

Figure 3.3. The mean amount of time required for women to complete Trials 1, 2 and 3 of the SPWM at the menstrual phase (MP) compared to the mid-luteal phase (LP) of their cycle (n = 36). Error bars represent SEM. There was no significant effect of phase on the time required to complete the SPWM.

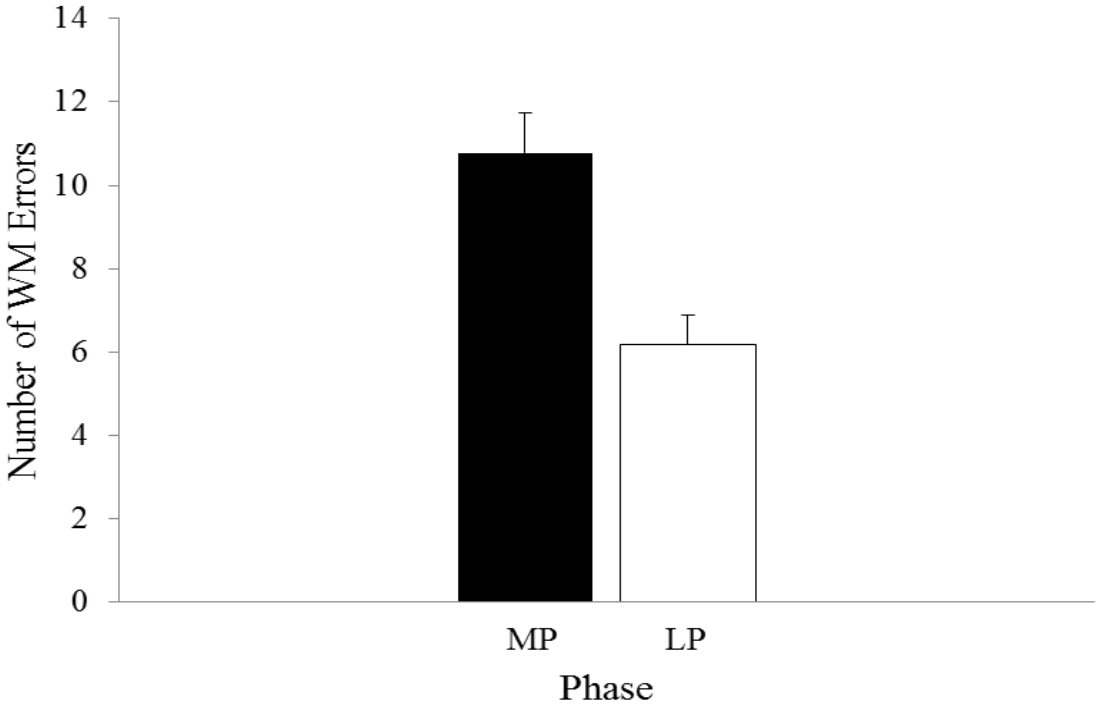

Figure 3.4. The mean number of working memory (WM) errors committed by women tested at the menstrual phase (MP) of their cycle compared to their performance at the mid-luteal phase (LP) of their cycle on the Digit Ordering task (n = 36). Error bars represent SEM. Women committed significantly more WM errors when tested at the MP than the LP.

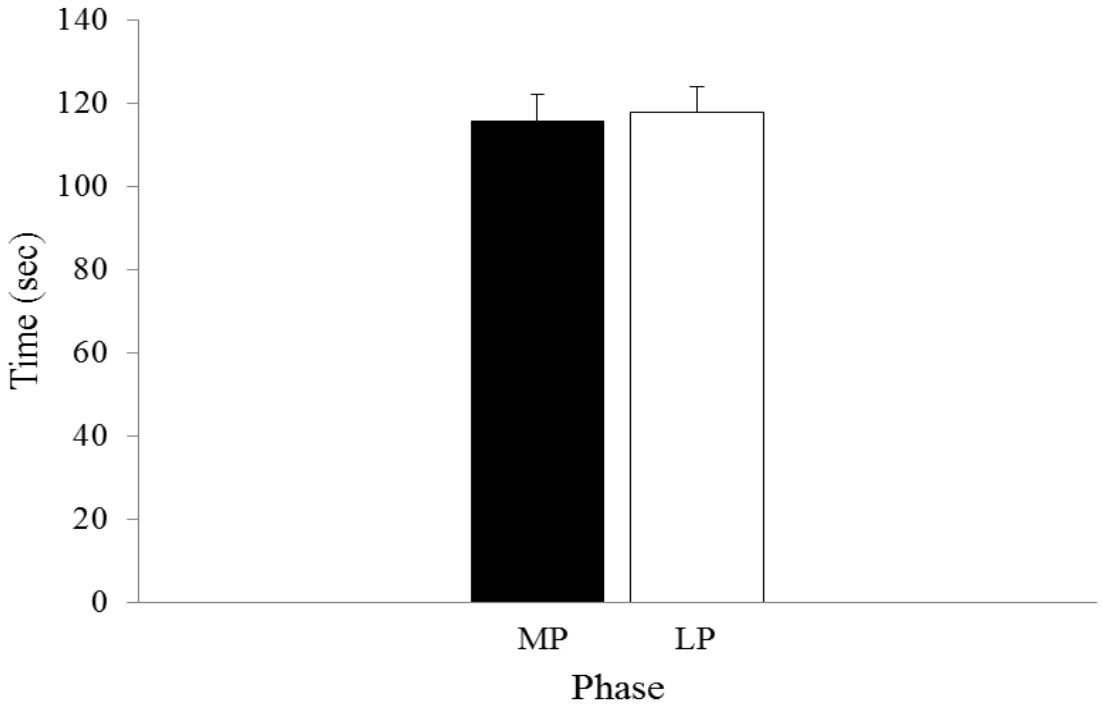

Figure 3.5. The mean amount of time required for women to perform the 10 trials of the Digit Ordering task at the menstrual phase (MP) compared to the mid-luteal phase (LP) of their cycle (n = 36). Error bars represent SEM. The time required to complete the Digit Ordering task did not differ significantly based on the phase of the menstrual cycle.

memory errors made on the SOPT, $F(1, 34) = 50.16$, $p < 0.001$ (Figure 3.6). Women made significantly more errors during their menstrual than during their mid-luteal test session. There was no evidence of a practice effect on the SOPT, but there was a main effect of set size, $F(5, 170) = 95.36$, $p < 0.001$, and a significant interaction between phase and set size, $F(5, 170) = 5.14$, $p < 0.001$. Post-hoc tests showed that the phase of cycle effect was significant for all but the 4 item and 8 item set size.

No significant main effect of cycle phase was found for the time data from the SOPT, $F(1, 34) = 3.56$, $p = 0.068$ (Figure 3.7).

Correlations between hormone concentrations and working memory tasks. The results of a number of studies have supported the hypothesis that cognitive processes may be influenced by circulating hormone levels. We hypothesized that the higher levels of estradiol found during the mid-luteal phase would elicit a detectable improvement in women's performance on tasks that taxed the executive components of working memory relative to the menstrual phase when estradiol is very low. However, absolute estradiol concentrations show substantial differences across women, and from one cycle to another within a given individual. Progesterone also rises during the mid-luteal phase and in the present study was correlated with the rise is estrogen levels ($r = 0.302$). Therefore, the relation between absolute hormone concentrations and working memory performance was investigated by conducting Pearson product-moment correlations between participants' salivary estrogen and progesterone concentrations and their individual scores on the SPWM, Digit Ordering, and SOPT tasks. The results are shown in Table 3.1.

Both estradiol and progesterone correlated significantly with the number of working memory errors committed on all three working memory tasks, all $p < 0.05$. The

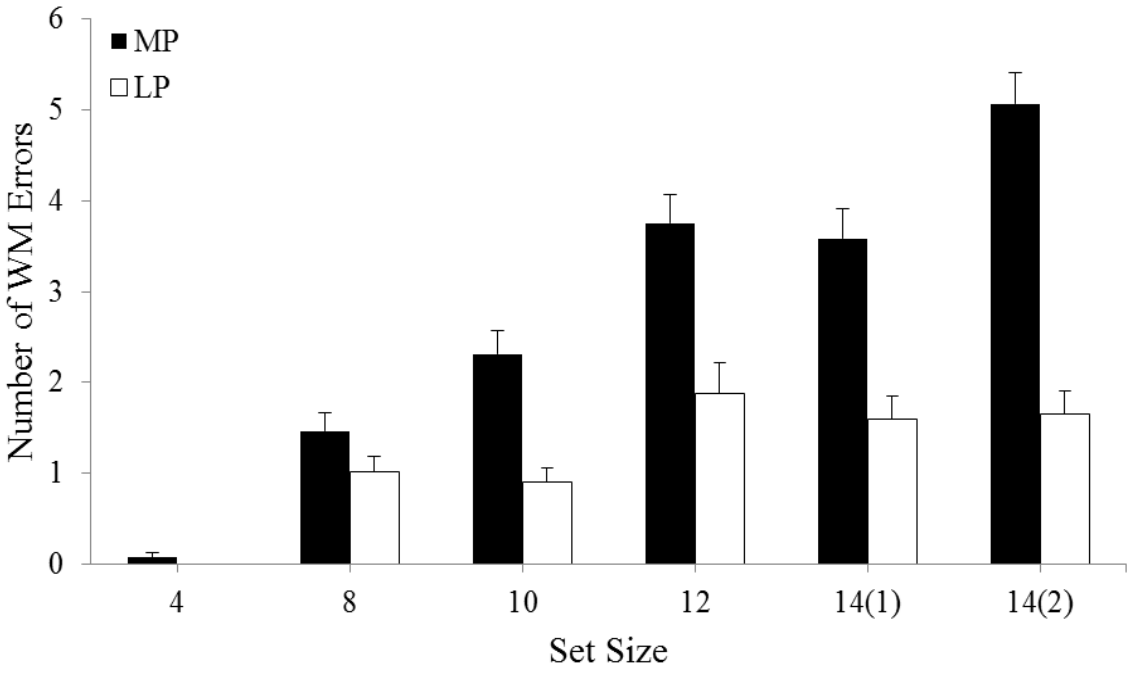

Figure 3.6. The mean number of working memory (WM) errors committed on the 4, 8, 10, 12, and two 14 item (14(1) and 14(2)) sets of the Self-Ordered Point Task (SOPT) when women were tested at the menstrual phase (MP) of their cycle compared to their performance at the mid-luteal phase (LP) (n = 36). Error bars represent SEM. The number of WM errors was significantly greater when women were tested at the MP of their cycle.

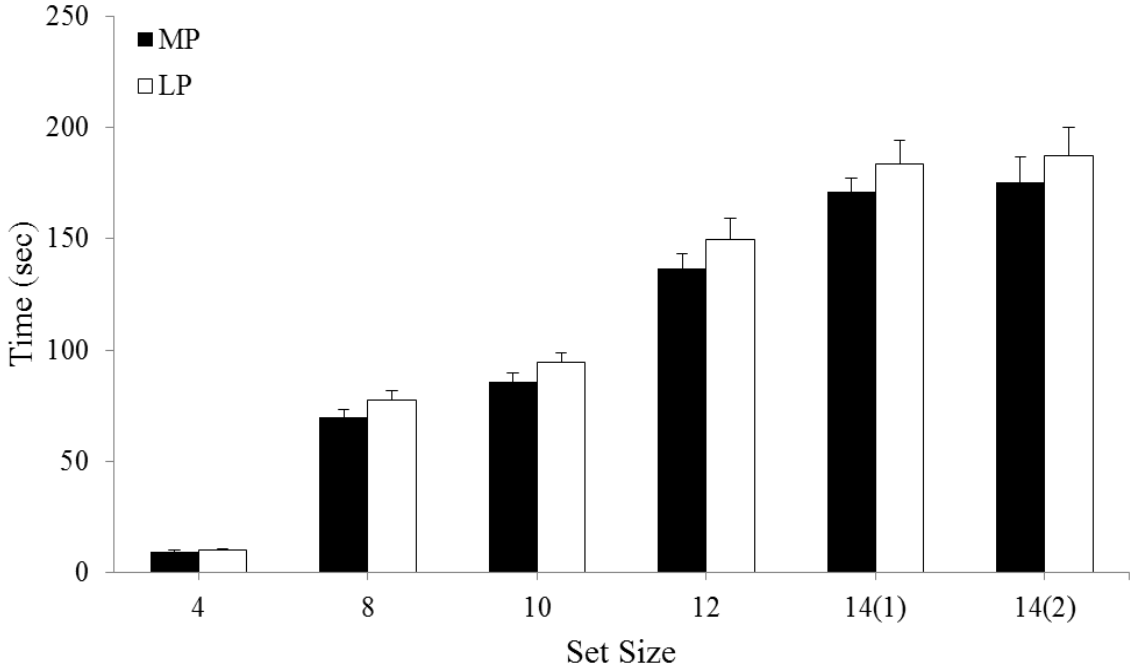

Figure 3.7. The mean amount of time required for women to complete the 4, 8, 10, 12, two 14 item (14(1) and 14(2)) set sizes of the Self-Ordered Point Task (SOPT) at the menstrual phase (MP) compared to the mid-luteal phase (LP) of their cycle (n = 36). Error bars represent SEM. There was no significant difference in the mean time required during the MP and LP of the cycle.

Table 3.1.

Correlations between estrogen or progesterone concentrations and working memory errors.

	Estrogen		Progesterone	
Task	Correlation	*p* value	Correlation	*p* value
SPWM	-0.322	0.006	-0.247	0.036
Digit Ordering	-0.454	< 0.001	-0.252	0.033
SOPT	-0.493	< 0.001	-0.272	0.021

Note. p values shown are for two-tailed tests of significance.

n = 36 for all tasks.

total number of errors on each task, summed across all trials or set sizes, was used as the variable for analysis. As hypothesized, all estrogen concentrations and working memory task performance correlations were negative such that higher hormone levels were associated with lower numbers of errors on the tasks and therefore better performance. In support of the hypothesis that estrogen and not progesterone is the more important hormone for supporting working memory function, a partial correlation between estrogen concentrations and working memory scores controlling for progesterone only very slightly changed the strength of the correlations, all $p < 0.05$ (Table 3.2). In contrast, the partial correlation between progesterone concentrations and working memory scores controlling for estrogen changed the strength of the correlations to the point where all three were no longer significant, all $p > 0.05$ (Table 3.3).

Span Control Tasks

Estradiol was hypothesized to influence the frontal executive components of the working memory system, and not the capacity for passive short-term store (corresponding to the 'buffer' systems within working memory described by Baddeley (1996; 1986)). Therefore a significant effect of the menstrual cycle on the three span tasks was not anticipated.

Digit Span. There was no effect of cycle phase on the length of Forward Digit Span, $F(1, 34) = 2.17$, $p = 0.150$ (Table 3.4). This suggests that the effect of cycle phase on the Digit Ordering task was not due to significant changes in women's capacity to passively hold verbal information in short-term store across the two phases.

Corsi Block-Tapping. The Corsi Block-Tapping task did not show a significant effect of cycle phase, $F(1, 34) = 1.99$, $p = 0.167$ (Table 3.4). This suggests that

Table 3.2.

Partial correlations between estrogen concentrations and working memory errors controlling for progesterone concentrations.

Task	Correlation	*p* value
SPWM	-0.268	0.024
Digit Ordering	-0.410	< 0.001
SOPT	-0.448	< 0.001

Note. p values shown are for two-tailed tests of significance.

n = 36 for all tasks.

Table 3.3.

Partial correlations between progesterone concentrations and working memory errors controlling for estrogen concentrations.

Task	Correlation	*p* value
SPWM	-0.166	0.165
Digit Ordering	-0.135	0.263
SOPT	-0.149	0.216

Note. p values shown are for two-tailed tests of significance.

n = 36 for all tasks.

Table 3.4.

Means (± SD) on the control measures of working memory span.

Test	MP (n =36)	LP (n =36)
Digit Span	6.67 ± 1.20	6.86 ± 1.22
Corsi Block-Tapping	6.39 ± 0.93	6.17 ± 1.06
Pattern Span	5.72 ± 0.91	5.78 ± 0.72

variations in women's ability to temporarily retain a set of spatial locations are unlikely to be a basis for the effect of cycle phase that was found on the SPWM.

Pattern Span. A repeated measures ANOVA of the pattern span data revealed no significant main effect of cycle phase, $F(1, 34) = 0.14$, $p = 0.714$ (Table 3.4).

Additional Control Tasks

Mooney-Harshman Closure Task. Performance on the Mooney-Harshman Closure Task was analyzed using a 2 x 2 mixed effects ANOVA with phase of cycle as a within-subjects factor and phase tested first as the between-subjects factor. The number of items correctly identified on the Mooney-Harshman Closure Task did not show a significant phase of cycle effect, $F(1, 34) = 1.54$, $p = 0.223$. The phase of their menstrual cycle the women were in at the time of testing did not have an impact on their degree of accuracy in identifying the incomplete images included in the closure task. On the other hand, the mean time required for participants to generate a correct identification did differ significantly based on phase of cycle at the time of testing, $F(1, 34) = 54.67$, $p < 0.001$ (Figure 3.8). As predicted, women took longer to make a correct identification of the incomplete images when tested during the mid-luteal than the menstrual phase of the cycle. The phase of cycle effect observed on the Mooney-Harshman Closure Task is the reverse of that observed on the working memory tasks, in that more proficient performance was seen at the menstrual phase. The existence of a menstrual cycle effect which is the opposite of that seen on the working memory tasks indicates that the working memory findings are selective. The same pattern was not replicated on a task that had a different set of computational demands.

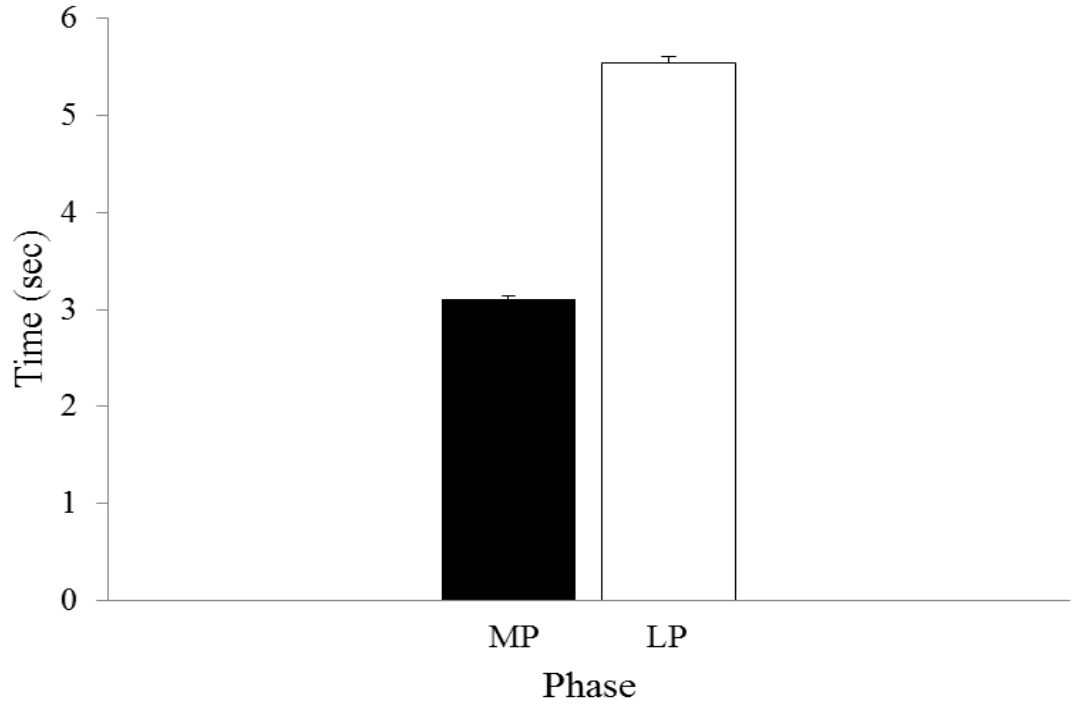

Figure 3.8. Mean time required for women to correctly identify the incomplete images on the Mooney-Harshman Closure Task. Error bars represent SEM. Women took significantly less time to generate a correct identification when tested during the menstrual phase (MP) than the mid-luteal (LP) of their menstrual cycle.

Profile of Mood States. A 2 x 2 multivariate ANOVA was performed on the POMS scores, with phase as the within-subjects factor and phase tested first as a between-subjects factor, and the six subscales of the POMS (tension, depression, anxiety, confusion, vigor, anger) serving as the dependent variables. The multivariate effect of phase failed to reach significance, $F(6, 29) = 2.33$, $p = 0.058$, therefore the univariate results were not interpreted. However, Table 3.5 gives the means and *SD*s for each subscale and shows the univariate results (uncorrected for the number of comparisons).

In order to examine whether the effect of menstrual phase was still significant when the mood variables were treated as covariates, a repeated measures ANCOVA was performed using R version 2.15.1 for Windows. The covariate used was a composite "negative affect" score which was comprised of the three negative affect subscales which had the lowest univariate *p* values (tension, depression and anger) and were therefore most likely to impact the working memory results. All three variables were summed and weighted equally for the composite score as there was no a priori reason for expecting one subscale to have a greater impact on the working memory scores than another. The results of the repeated measures ANCOVA with the composite negative affect score as the covariate indicated a significant covariate effect ($p = 0.022$, 0.021, 0.030 on the SPWM, Digit Ordering, and SOPT respectively). However, the main effect of menstrual cycle phase was still significant for all three working memory tasks when the mood subscales were treated as a covariate, all $p < 0.001$. It is therefore unlikely that differences in mood at the time of the two test sessions were the basis for the cognitive advantage observed on the working memory tasks during the mid-luteal phase.

Table 3.5.

Means (± SD) on the six subscales of the Profile of Mood States.

Scale	MP (n =36)	LP (n =36)	Univariate *p* value
Tension	9.86 ± 6.05	7.92 ± 5.83	0.041
Depression	8.67 ± 10.51	6.58 ± 9.27	0.050
Anger	6.03 ± 7.04	5.22 ± 6.51	0.306
Vigor	11.78 ± 4.50	13.64 ± 6.60	0.080
Fatigue	8.67 ± 6.29	7.03 ± 6.24	0.096
Confusion	7.61 ± 4.70	7.58 ± 4.23	0.983

Note. Higher scores indicate more extreme endorsements of items for a particular subscale.

The multivariate effect of phase on POMS scores was not significant ($p = 0.058$).

CHAPTER 4

DISCUSSION

The purpose of the current investigation was to examine the possibility that working memory function is sensitive to estrogen in reproductively aged females and thus may have the ability to modulate their performance on tasks with significant working memory components. This hypothesis was based on previous neurophysiological and neuroimaging data which suggests that the PFC plays an important role in working memory function and that this area is a target for estrogen binding. Behavioural evidence has suggested that estrogen status in postmenopausal women has an impact on PFC dependent cognitive functions such that hormone replacement therapies have a beneficial effect on working memory performance. To date however very little research has been conducted examining the behavioural effect of estrogen status on PFC dependent cognitive functioning in younger females of reproductive age. The investigation of changes in working memory performance throughout the menstrual cycle allows for the examination of the effects of estrogen on cognitive performance in this age group in a noninvasive, ecologically-valid manner.

The results of the present study indicate that, as was hypothesized, the estrogen status of naturally cycling reproductively aged females had a significant effect on their performance on a variety of tasks with significant working memory components. Specifically, higher estrogen was associated with a change in PFC dependent working memory performance such that the young women performed significantly better on measures of verbal (Digit Ordering), spatial (SPWM) and figural working memory (SOPT) when tested during the mid-luteal phase of the menstrual cycle when estrogen

levels were high, than during the menstrual phase of the menstrual cycle when estrogen levels are at their lowest. These findings are consistent with what has been observed previously in postmenopausal women (Duff, and Hampson, 2000; Keenan et al., 2001; Krug, Born, and Rasch, 2006; for an exception see Grigorova, Sherwin, and Tulandi, 2006), Specifically, women committed approximately 25%, 35% and 40% more errors on the SPWM, Digit Ordering and SOPT respectively when tested during the menstrual than the mid-luteal phase indicating that the phase of cycle effect is potentially quite large. An effect of the menstrual cycle on working memory such that performance on tasks with prominent working memory components is better during the mid-luteal phase when estrogen is high is consistent with the growing body of evidence which suggests that estrogen is capable of modulating the activity of the PFC (Berman et al., 1997; Hao et al., 2006; Kritzer, and Kohama, 1998; 1999; Shaywitz et al., 1999; Smith et al., 2006; Summer, and Fink, 1995).

Although these three working memory tasks differ in the type of information which is required to be held in the working memory system, they all require the information to be held in an on-line manner so that the active manipulation and updating of information required for the performance of the task can occur, therefore placing demands on the cognitive processes known to depend on the PFC. In neurosurgical patients with cortical excisions, performance on both the Digit Ordering and the SOPT has been linked to the integrity of the dlPFC (Petrides et al., 1993; Petrides, and Milner, 1982), the area of the cortex which physiological evidence suggests is a site in which estrogen is active (Bixo et al., 1995; MacLusky et al., 1986; Platia et al., 1984). Thus the results of the current investigation are consistent with both the findings of previous work,

which support a beneficial effect of estrogen replacement therapy on working memory functioning in post-menopausal women (Duff, and Hampson, 2000; Keenan et al., 2001; Krug, Born, and Rasch, 2006) and with the novel hypothesis that the estrogen fluctuations associated with the menstrual cycle have the ability to modulate PFC-mediated working memory function in reproductively-aged women, which was the focus of the current investigation.

The specificity of the effect of the menstrual cycle on the frontally-mediated active manipulation component of working memory is strengthened by the results of the control measures. No significant effect of menstrual cycle phase was observed for the verbal (digit span), spatial (Corsi Block-Tapping) or pattern span tasks which require little or no active manipulation or transformation of the stored information. Previous research has indicated that these span tasks are impaired by lesions of posterior perisylvian cortex (Baldo, and Dronkers, 2006; D'Esposito, and Postle; 1999; Postle, Berger, and D'Esposito, 1999; Shallice, and Vallar, 1990) but are unimpaired by lesions that affect the frontal lobes (Canavan et al., 1989b; D'Esposito, and Postle, 1999; Petrides, 1995b). Taken together, the results of the working memory tasks and those of the control tasks support the interpretation that estrogen modulates specifically the active processes in working memory that have been shown to depend substantially on the PFC.

The results of the analysis of the Mooney-Harshman Closure Task data strongly indicate that a generalized benefit of estrogen on cognitive functioning across the board can be ruled out as an alternative interpretation of the menstrual cycle effect on the working memory task error measures found in the current investigation. The phase of cycle effect on the closure task was found to be significant for the mean time required for

the women to correctly identify the incomplete images. Importantly however, the effect of menstrual cycle phase on this task was the reverse of the effect observed on the working memory tasks. Specifically, performance on the Mooney-Harshman Closure Task was found to be better when women were tested during their menstrual than the mid-luteal phase. The women were able to correctly identify what was depicted in the incomplete image more adeptly when they were tested during the menstrual phase, when estrogen levels are lowest, rather than the mid-luteal phase of their cycle. In a previous study using the Mooney-Harshman Closure Task, Hampson et al. (2005) found a menstrual cycle effect which followed the same pattern as that found here. This pattern of results is consistent with other studies which have found a menstrual phase advantage on other types of complex spatial and visual-perceptual tasks, including the accuracy of perceptual judgments of horizontality or verticality (Hampson, and Kimura, 1988; McCourt et al., 1997), speed and accuracy of mental rotation (Hausmann et al., 2000; Maki, Rich, and Rosenbaum, 2002), and on various measures of spatial visualization (Hampson, 1990; Phillips, and Silverman, 1997). Together, these findings indicate that the effect of menstrual cycle phase is dependent upon the type of task, the cognitive processes and thus the neuroanatomical areas it recruits.

The use of a repeated measures design in the current investigation allows for differences in demographic characteristics or general intelligence to be effectively ruled out as a plausible basis for any observed menstrual cycle effect. Although no menstrual-cycle related effects on mood were expected, a multivariate analysis of the participants' scores on a self-report mood inventory in the current investigation was nearly significant. Follow-up analysis examining the effect of phase on working memory scores with those

subscales which were closest to significance as a covariate remained significant indicating that the decrease in the participants' accuracy on the working memory measures at the menstrual phase of the cycle was not explained by an estrogen-related decrease in their mood. Only a small number of women had raised scores, which appeared to be random over the various subscales of the POMS, and for most, higher scores at one phase of the cycle were matched by higher scores at the other. These findings are consistent with a large body of previous research which has consistently found no significant differences in scores on various self-reported measures of mood across the menstrual cycle in healthy non-depressed populations (Abplanalp et al., 1979a; Abplanalp et al., 1979b; Ainscough, 1990; McFarlane, Martin, and Williams, 1988; Zimmerman, and Parlee, 1973). Further, research has shown mood to be driven more strongly by daily life events than by physical symptoms or the degree of physiological changes associated with the menstrual cycle (May, 1976). Thus although certain mood states such as major depression have been shown by other researchers to be associated with lowered scores on measures of short-term recognition and recall and long-term episodic and semantic memory (Burt, Zembar, and Niederehe, 1995; Cassens, Wolfe, and Zola, 1990), the POMS results from the current investigation suggest that negative affect is not a viable explanation for the menstrual cycle effect found here.

The attribution of the effect of the menstrual cycle phase on working memory function to changes in estrogen concentrations specifically is complicated by the nature of the relative changes in the two major ovarian hormones at the phases which were examined here. At the mid-luteal phase, both estrogen and progesterone concentrations are elevated relative to those at the menstrual phase and in the current investigation,

estrogen and progesterone concentrations were found to be significantly correlated with each other (r = 0.302). Furthermore, the increase in progesterone levels is typically much more marked than that of estrogen. Consistent with this, the average progesterone levels at the mid-luteal phase in the current investigation were nearly 4 fold higher than those at the menstrual phase while average estrogen levels were slightly less than 2 fold higher. In spite of this, progesterone has generally been found to be relatively inert in terms of its ability to affect cognition (Duff, and Hampson, 2000; Hampson, 1990; Hausmann et al., 2000; Maki, Rich, and Rosenbaum, 2002). Duff and Hampson (2000), for example, found no significant difference in the working memory performance of a group of postmenopausal women who took estrogen-only hormone replacement therapy and those who took a combination estrogen and progestin replacement. In combination, previous findings lead to the hypothesis that estrogen and not progesterone is the primary hormone responsible for any observed menstrual cycle effect on working memory function. In order to examine this, the correlation was examined between the concentration of each hormone and the number of working memory errors produced by each woman. As would be expected based on the current hypothesis, estrogen concentrations were significantly correlated with the number of working memory errors on each of the three working memory tasks. Additionally, all correlations were negative such that higher estrogen levels were associated with fewer working memory errors as was predicted a priori. Consistent with its significant covariation with the measured level of estradiol in our sample, progesterone was also significantly negatively correlated with the number of working memory errors on the SPWM, Digit Ordering and SOPT. However the strength of the correlations between progesterone and all three working memory tasks were

weaker than those for the correlations between estrogen and the same tasks. Furthermore, when the partial correlations between estrogen and the tasks were examined while controlling for progesterone levels, the correlation coefficients decreased only slightly and the correlations remained highly significant. However, when the partial correlations between progesterone concentrations and the working memory tasks were calculated controlling for estrogen levels, all three correlations no longer reached significance. The lack of a distinct effect of progesterone on working memory performance found in the current investigation is consistent with previous studies of working memory in postmenopausal women (Duff, and Hampson, 2000) and studies of other types of cognitive functions over the menstrual cycle (Hampson, 1990; Hausmann et al., 2000; Maki, Rich, and Rosenbaum, 2002). As a result of the significant correlation between estrogen and progesterone concentrations, it is difficult to further disentangle the relative roles of the two hormones in influencing working memory function, but it does appear that if progesterone does exert a small influence on working memory function, it is far less significant than the effect of estrogen concentration.

The possibility that progesterone in addition to estrogen may have an impact on working memory function in reproductively aged women is an issue that is important to examine more closely in the future. As previously described, the examination of effects of estrogen and progesterone in isolation using the menstrual cycle as a naturalistic method is complicated by the nature of the changes in the two hormones across the menstrual cycle. In general, the pattern of changes in progesterone concentrations is mirrored by that of estrogen. As there is no time during the menstrual cycle when increases in progesterone are not paralleled by increases in estrogen, it impossible to

study the effects of progesterone on cognition in isolation using the menstrual cycle method. Unlike progesterone, there is one time point during the menstrual cycle when the effects of estrogen on cognition can be studied independently of the influence of progesterone. This occurs just prior to ovulation when there is a marked peak is estradiol concentrations without a corresponding increase in progesterone concentrations. Having women complete the working memory measures during this preovulatory estradiol peak would allow for the clearer identification of the effect of estradiol on task performance apart from any effect of progesterone. The hurdle associated with employing such a methodology, and the reason it was not used in the current investigation is the difficulty in targeting this estrogen peak as it is extremely transient, lasting only about 24-36 hours, and its timing depends on the exact menstrual cycle length of the individual in a given cycle, which can vary within a 2 to 4 day window of variation in most women. Thus while specifically targeting the preovulatory peak would help to clarify the differential impact of estrogen on working memory task performance, it would require prospective participants to closely monitor their cycle for months prior to testing in order to increase the likelihood of accurately timing the test session to the estrogen peak resulting in a significant increase in the amount of time and effort required to complete the investigation over the methods that were used in the current investigation.

The current data raise a number of interesting questions regarding male performance on tasks with significant working memory components. If estrogen does have a beneficial influence on working memory in women, it is logical to hypothesize that this influence may give rise to a sex difference in performance because males, on average, are exposed to lower estrogen concentrations than women. In direct support of

this hypothesis, Duff and Hampson (2001) compared male and female performance on the same spatial and verbal working memory tasks that were used in the current investigation. The researchers found females to perform significantly better than males, making fewer working memory errors on both the SPWM and Digit Ordering task. Importantly, the effect of sex on the participants' performance on the tasks remained even after other mnemonic and non-mnemonic processes that could hypothetically explain the female advantage were examined and covaried out. The effect of sex on the SPWM was confirmed by Lejbak et al. (2009) using geometric forms, common objects, or abstract shapes as stimuli. Additionally, it is noteworthy that the sex difference was found in these studies even though the menstrual cycle phase of the female participants and thus estrogen concentration was not controlled. The results of the current investigation indicate that the averaging of the number of working memory errors committed by women across menstrual cycle phase could result in either an attenuation or inflation of the size of the sex difference. Additionally, the dynamic variability in working memory task performance related to changes in hormone levels may not be unique to females. Males experience diurnal and seasonal fluctuations in testosterone levels (Resko, and Eik-Nes, 1966). Like estrogen in women, testosterone may have a beneficial effect on working memory performance in men. In the only test of this hypothesis so far, Janowsky, Chavez, and Orwoll (2000) found older men to exhibit improvements in SOPT performance following testosterone supplementation compared with placebo. Thus the size of the observed sex difference is likely to fluctuate with the hormonal status of the individuals included in the sample, potentially in both sexes not just females.

The changes in hormone levels which occur in women across the menstrual cycle are predictable, known to the participant and may potentially be associated with cultural stereotypes which may provide a possible confound for the observed pattern of results. Research conducted in the 1970s and 1980s found cultural stereotypes to influence both behaviours and symptom reporting such that more negative symptoms and expectations of poorer performance were generally associated with the menstrual phase of the cycle (Englander-Golden, Parlee, 1974; Whitmore, and Dienstbier, 1978; Olasov, and Jackson, 1987; AuBuchon, and Calhoun, 1985; McFarland, Ross, and DeCourville, 1989). The negative stereotype was prevalent even though prospective studies of the same era that monitored daily mood variations in women over extended periods demonstrated no true association with the menstrual cycle (Abplanalp et al., 1979a; Abplanalp et al., 1979b; Ainscough, 1990; McFarlane, Martin, and Williams, 1988; Zimmerman, and Parlee, 1973; see Schwartz et al., 2012 (in press) for a recent validation of these findings). Interestingly, however, while many women in those early studies believed that their performance was universally negatively affected at the menstrual phase of their cycle, behavioural measures of cognitive and physical performance showed no difference across cycle phases on most measures (Moos, 1968; Sommer, 1973). Recent research supports the notion that some residual negative stereotypes regarding both the premenstrual and menstrual phase of the menstrual cycle persist today (Chrisler et al., 2006). Interestingly, this more contemporary research has found the negative stereotypes about the menstrual cycle to exist only in relation to mood rather than to cognition (Chrisler et al., 2006; Chrisler, and Levy, 1990). Thus, even if some women in the current investigation maintained negative stereotypes regarding the effects of the menstrual cycle, these more

recent findings suggest that these beliefs would only extend to their scores of mood related measures such as the POMS and not their performance on the working memory tasks. This may explain why the POMS scores showed an effect of menstrual cycle phase in a subset of the women tested when none was hypothesized. Additionally, Chrisler et al. (2006) found significant self-serving biases such as illusionary optimism which led participants to express support for negative menstrual cycle related stereotypes even though they believed that they were not themselves affected negatively by their own menstrual cycle. These findings support the notion that even if the women in the current investigation believed that the menstrual cycle negatively effects the performance of others, they were unlikely to believe that their own performance is impaired by their menstrual cycle. As the majority of the research on the topic of menstrual cycle related cultural stereotypes was conducted at least two to three decades ago, it is difficult to ascertain whether such beliefs still exist. In contrast to the possibility that the participants in the current investigation were exhibiting the good-subject tendency is the fact that their performance on the Mooney-Harshman Closure Task showed a reverse menstrual cycle effect, where improved performance was observed at the menstrual phase not the mid-luteal phase. This is consistent with contemporary neuroendocrine studies, which show that if the menstrual cycle does exert effects on cognitive function, the effects are seen only on specific narrowly defined tasks, namely those on which a sex difference in performance is normally found, and that depending on the particular cognitive function assessed, improved performance may occur at different phases of the menstrual cycle including the menstrual phase. If the participants in the current investigation did hold a stereotyped belief that performance is poorer during the menstrual phase of the cycle,

then the direction of the effect of menstrual phase would be negative and expected to be consistent on all tasks. As this was not the case, it seems unlikely that cultural stereotypes were the basis for the menstrual cycle effect on working memory task performance observed here.

In summary, the results of the present study suggest that fluctuations in estrogen concentrations associated with the menstrual cycle may have the ability to modulate PFC dependent working memory function in naturally-cycling reproductively aged females. As hypothesized, women`s performance on tasks with significant working memory components was better when tested during the mid-luteal phase of the cycle when estrogen levels are raised. A reverse effect of menstrual cycle phase on the closure task suggests a generalized improvement in cognitive functioning does not occur during the mid-luteal phase. These findings extend the body of evidence supporting the regulatory actions of estrogens in the PFC and their ability to modulate those behaviours known to be subserved by the region.

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Use of Human Subjects - Ethics Approval Notice |

Review Number	**11 06 08**	**Approval Date**	11 06 28
Principal Investigator	**Elizabeth Hampson/Mia Segal**	**End Date**	12 06 30
Protocol Title	Estrogen, mood and cognition		
Sponsor	n/a		

This is to notify you that The University of Western Ontario Department of Psychology Research Ethics Board (PREB) has granted expedited ethics approval to the above named research study on the date noted above.

The PREB is a sub-REB of The University of Western Ontario's Research Ethics Board for Non-Medical Research Involving Human Subjects (NMREB) which is organized and operates according to the Tri-Council Policy Statement and the applicable laws and regulations of Ontario. (See Office of Research Ethics web site: http://www.uwo.ca/research/ethics/)

This approval shall remain valid until end date noted above assuming timely and acceptable responses to the University's periodic requests for surveillance and monitoring information.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the PREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of research assistant, telephone number etc). Subjects must receive a copy of th information/consent documentation.

Investigators must promptly also report to the PREB:
a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) all adverse and unexpected experiences or events that are both serious and unexpected;
c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information,'consent documentation, and/or recruitment advertisement, the newly revised information consent documentation, and or advertisement, must be submitted to the PREB for approval.

Members of the PREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on. such studies when they are presented to the PREB.

Clive Seligman Ph.D.

Chair, Psychology Expedited Research Ethics Board (PREB)

The other members of the 2010-2011 PREB are: Mike Atkinson (Introductory Psychology Coordinator), David Dozois, Vicki Esses. Riley Hinson Albert Katz (Department Chair), and Tom O'Neill (Graduate Student Representative)

CC: UWO Office of Research Ethics

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Dekaban, G. A., Snir, J., Shrum, B., de Chickera, S., Willert, C., **Merrill, M**., Said, E. A., Sekaly, R., Foster, P. J., & O'Connell, P. J. (2009). Semiquantitation of mouse dendritic cell migration in vivo using cellular MRI. *Journal of Immunology, 32,* 240-251.

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Abstracts Accepted:

de Chickera, S., Snir, J., Willert, C. R., **Merrill, M.**, Rohani, R., Foster, P. J., Dekaban, G. A. (2009). Labelling dendritic cells with Feridex has implications for in vivo migration according to magnetic resonance imaging. Abstract submitted to the World Molecular Imaging Congress.

Willert, C., De Chickera, S., **Merrill, M**., Snir, J., Shrum, B., Foster, P., O'Connell,P., Dekaban, G. (2008) Impact of MR contract agent Feridex on murine dendritic cell phenotype, function and migration. Abstract submitted to the 7th Annual Imaging Symposium.