THE MENSTRUAL CYCLE, CAFFEINE, AND VERBAL WORKING MEMORY

by

Emma Carter

Submitted in partial fulfillment of the requirements

for the degree of Masters of Arts in School Psychology

at

Mount Saint Vincent University

Halifax, Nova Scotia

August 2018

TABLE OF CONTENTS

List of Tables

Abstract

Verbal working memory is a domain of cognition critical to educational success. It is influenced by caffeine, with moderate doses generally resulting in improvements as measured by behavioural performance, as well as electroencephalographic (EEG) and functional MRI (i.e. neural) measures. Verbal working memory performance is also known to vary with fluctuations in sex hormones (i.e. estrogen and progesterone) such as those seen across the menstrual cycle. Generally, when estrogen levels are higher, so is performance on working memory tasks. To the best of our knowledge, the interaction between the menstrual cycle, caffeine, and verbal working memory has not been examined, despite there being individual relations between these variables. Employing a double-blind, placebo-controlled design, the present study examined EEG-derived event-related potentials (ERPs) to assess the effects of 200mg of caffeine (vs. placebo) across the luteal and menstrual phases of the menstrual cycle. Specifically, 16 participants completed the verbal n-back task and their corresponding P300 waveforms were analyzed. Results showed an overall effect of menstrual phase, due to quicker reaction times in the luteal (vs. menstrual) phase; this was predominantly observed during the 3-back task. Caffeine only improved reaction time during the 3-back task for participants in the menstrual phase. Participants in the menstrual phase had a larger P300 amplitude in response to the 1-back, while participants in the luteal phase displayed a greater P300 latency in response to the 1-back. Collectively, results showed a task dependent relation between caffeine, verbal working memory, and phases of the menstrual cycle.

Acknowledgements

Dr. Derek Fisher has been an exceptional supervisor throughout not one, but two theses. His expertise, guidance, and patience throughout the learning process are greatly appreciated.

Thanks to committee member, Dr. Sara King, for her knowledge, insightful comments, and support.

Thanks to EEG lab, particularly Kaitlyn Napier and Bronwen Schryver, for their work collecting exceptional EEG data.

CHAPTER ONE

Literature Review

Naturally occurring sex hormones alter the effects of caffeine (Lane, Steege, Rupp, & Kuhn, 1992), and the absorption of caffeine and its mechanisms within the body can alter the release and effects of sex hormones (Fenster et al., 1999). Of particular interest is how this interaction between caffeine and hormones affects cognitive function; specifically over the course of the menstrual cycle, as it is characterized by hormone fluctuation. Additionally, the menstrual cycle and caffeine have been found to individually enhance or impair cognitive performance (e.g. Hampson, 1990; Fan et al., 2005; Einöther & Giesbrecht, 2013; Barros, Tufik, & Andersen, 2015).

Menstrual Cycle

The human menstrual cycle involves variations in sex hormones (i.e. luteinizing hormone, follicle-stimulating hormone, progesterone, and estrogen) over an interval typically lasting 28-29 days (Sherman & Korenman, 1975). The menstrual (typically days 1-5) and early follicular phase are characterized by low levels of estrogen and progesterone, while the follicular phase continues until ovulation (approximately days 7-14) and is characterized by continually rising estrogen levels peaking roughly 1 day prior to ovulation (Farage, Osborn, & MacLean, 2008). At that time, the luteal phase begins (typically on days 15 to 28) and sees a rise in progesterone levels followed by a second peak in estrogen levels (Farage, Osborn, & MacLean, 2008). Both estrogen and progesterone hormones decrease during the late luteal phase until levels reach baseline, followed by the onset of menstruation and the beginning of a new cycle (Farage, Osborn, & MacLean, 2008). Physiologically, this cyclic fluctuation of hormones has a multitude of influences on the female body, including many physical implications (Farage, Osborn, & MacLean, 2008). In fact, nearly every organ in the body is affected by the variations in hormones throughout the menstrual cycle (Farage, Neill, & MacLean, 2009). Females may be cognizant of some of these physical changes and even some of the emotional states that correspond with the menstrual cycle, particularly during the pre-menstrual or menstrual phase. Anecdotal reports of emotional changes related to the menstrual cycle have been backed by scientific research concluding a broad range of evidence suggesting women's emotional state varies throughout their cycle (Farage, Neill, & MacLean, 2009). However, what many females may not be considering is how these same hormonal fluctuations also influence aspects of cognition.

A 1988 Science News article described these changes across the menstrual cycle as "common beliefs" (Weiss, 1988). This article stated that science had finally verified what was generally accepted as public knowledge: that women excel at some things and tend to do worse on others depending on the phase of their menstrual cycle (Weiss, 1988). Weiss (1988) declared "that a woman's monthly ebb and flow of gender-related biochemicals has predictable cognitive and behavioural effects" (Weiss, 1988, p.341). This article was based on the work of Canadian researchers Doreen Kimura and Elizabeth Hampson. Kimura and Hampson found that when a woman experiences low estrogen levels, typically during and immediately after menstruation, she does better on spatial tasks but scores poorly on complex motor tasks (Hampson, Kimura, & Thompson, 1988). Conversely, during periods of high estrogen levels, typically associated with phases in the middle of the cycle, participants showed improvements on tasks involving motor and verbal skills but showed difficulty with spatial relations (Hampson, Kimura, & Thompson, 1988). This research was among the first of its kind to describe the differences in cognitive function across the menstrual cycle.

Since that time, sex differences on cognitive tasks have been observed in various domains. This is hypothesized to be largely due to the effects of sex-steroids (Hausmann et al., 2000). For example, spatial skills, particularly mental rotation has been shown to be a task at which males out-perform females (Hyde, 2016). Conversely, females have shown an advantage in aspects of verbal skills such as vocabulary and essay writing (Hyde, 2016). This research has been influenced by studies which have observed that high levels of estradiol have enhancing effects on cognitive tasks in which females tend to excel and detrimental effects on tasks in which males tend to excel (Hampson & Kimura, 1988).

It is unknown whether these cognitive differences between males and females serves an evolutionary purpose or if it is merely a by-product of biology (Kimura & Hampson, 1994). It is hypothesized that in hunter-gatherer societies it was more beneficial to have enhanced spatial abilities in men when it was time to relocate or hunt (Kimura & Hampson, 1994). However, it is still unclear why changes in cognitive abilities across the menstrual cycle would be beneficial, indicating it may be a by-product of female biology.

Nonetheless, as sex-steroids fluctuate across the menstrual cycle, research has documented commensurate variations in cognitive abilities (i.e. Hampson, 1990; Barros, Tufik, & Andersen, 2015; Kromrey, Czoty, & Nader, 2015). Beginning with animal research, Kromrey, Czoty, and Nader (2015) examined 14 naturally menstruating monkeys. Cynomolgus monkeys share many characteristics with humans including a 28-day menstrual cycle with similar estrogen and progesterone fluctuations (Kromrey, Czoty, & Nader, 2015). Hormonal assays were conducted using blood samples from the monkeys (Kromrey, Czoty, & Nader, 2015). Using these samples menstrual phase was confirmed using estrogen and progesterone levels (Kromrey, Czoty, & Nader, 2015). The results found an inverse relation between progesterone and initial discrimination performance but no effect of menstrual phase on working memory (Kromrey, Czoty, & Nader, 2015). They found that monkeys with higher concentrations of progesterone were slower to learn a simple discrimination task, such that they required more trials and made more errors (Kromrey, Czoty, & Nader, 2015). The authors conclude that the effects of the menstrual cycle on cognition varies across tasks (Kromrey, Czoty, & Nader, 2015). This is demonstrated by Hampson (1990), who found that human females scored better on spatial ability tasks during menses (vs. follicular phase) when estradiol and progesterone hormone levels are lower than in the preovulatory phase. Conversely on motor tasks, participants scored better during the follicular phase as compared to menses (Hampson, 1990).

Hampson (1990) hypothesized that hormonal variations across the menstrual cycle would facilitate performance on certain cognitive tests, and not on others. This hypothesis was verified by testing naturally cycling women with a battery of six cognitive measures (Hampson, 1990). Women were tested once during the menstrual phase and once in the follicular phase when estrogen levels were higher (Hampson, 1990). Results found that women scored better on spatial tasks during the menstrual phase when estrogen and progesterone levels were lower (Hampson, 1990). Conversely, when hormone levels were higher women scored better on motor tasks (Hampson, 1990). This study confirms the theory of a task-dependent relationship between estrogen levels across the menstrual cycle and cognition (Hampson, 1990).

Hausmann et al. (2000) contributed to these findings by identifying that some studies found the menstrual cycle has an impact on spatial abilities, while other studies have found none. The authors sought to clarify these conflicting conclusions by eliminating potential confounding variables such as lack of menstrual cycle phase verification (Hausmann et al., 2000). Hausmann et al. (2000) demonstrated that different measures of spatial cognition are modified by the menstrual cycle and examined which hormones may be contributing to this phenomenon. Their participants were 12 young naturally cycling women whose hormones were analyzed using blood samples extracted multiple times in 3-day intervals over the course of the menstrual cycle (Hausmann et al., 2000). Participants were tested on their 2nd and 22nd days of their cycles in order to test during both menstrual and midluteal phases using three spatial tasks: the mental rotation test, the mirror pictures test, and the hidden figures test (Hausmann et al., 2000). Their most influential finding was a statistically significant difference between performance scores during the menstrual phase and midluteal phase on the Mental Rotation Test (MRT) with an increased ability during the menstrual phase when estrogen levels were lower (Hausmann et al., 2000). This finding was particularly relevant as previous studies had found conflicting results. However, Hausmann et al. (2000) controlled for cycle variations by validating cycle phase in order to confirm the findings. This conclusion is in agreement with the majority of previous studies and may explain the small body of literature that found contradictory results.

While Hausmann et al. (2000) found an increased ability to rotate objects mentally during the menstrual phase (vs. mid-luteal), Postma, Winkel, Tuiten, and van Honk (1999) found that during a spatial task, positional reconstruction scores (which require both spatial and working memory abilities) were poorer during the menstrual phase. These studies further confirm the relation between sex hormones and spatial abilities. Research in this area has been well documented with testosterone generally having a positive effect and estradiol (an estrogen steroid hormone) a negative effect on spatial ability (Symonds, Gallagher, Thompson, & Young, 2004). In fact, numerous studies have examined and described the role of estrogen in brain function (i.e. Wooley & McEwen, 1994; McEwen & Alves, 1999; Morrison, Briton, Schmidt, & Gore, 2006).

These findings have been corroborated by animal research, Kromrey, Czoty, and Nader (2015) examined 14 naturally menstruating monkeys. Cynomolgus monkeys share many characteristics with humans including a 28-day menstrual cycle with similar estrogen and progesterone fluctuations (Kromrey, Czoty, & Nader, 2015). Hormonal assays were conducted using blood samples from the monkeys (Kromrey, Czoty, & Nader, 2015). Using these samples menstrual phase was confirmed using estrogen and progesterone levels (Kromrey, Czoty, & Nader, 2015). The results found an inverse relation between progesterone and initial discrimination performance but no effect of menstrual phase on working memory (Kromrey, Czoty, & Nader, 2015). They found that monkeys with higher concentrations of progesterone were slower to learn a simple discrimination task, such that they required more trials and made more errors (Kromrey, Czoty, & Nader, 2015). The authors conclude that, just as in humans, the effects of the menstrual cycle on cognition varies across tasks (Kromrey, Czoty, & Nader, 2015).

Estrogen is the primary female sex hormone and presents as many different types, including estriol, estetrol, estrone, and estradiol. Estradiol is a sex steroid estrogen that when administered to menopausal women, has been shown to increase working memory (Epperson, Amin, Ruparel, Gur, & Loughead, 2012). Estrogen receptors are found in various parts of the brain and their widespread presence affects many systems (Sherwin, 2003). For instance, estrogen acts on the central nervous system by influencing multiple neurotransmitter systems, such as the dopaminergic, catecholaminergic, serotonergic, and cholinergic systems (McEwen, 2002). Deficits of estrogen in the brain have been linked with memory deficits, deficits in fine motor coordination, as well as depression and anxiety (McEwen & Alves, 1999). Estrogen receptors are commonly found in the hypothalamus and regions associated with reproduction (Gibbs & Gabor, 2003). Moreover, in a review of the literature McEwen (2002) highlights significant findings that multiple estrogen receptors have been found in areas of the brain that are not related to reproductivity. For instance, estrogen receptors have been found in areas such as the frontal and prefrontal cortex, and the hippocampus, which are areas associated with cognitive functions related to learning and memory (Islam, Sparkes, Roodenrys, & Astheimer, 2008).

Overall, the effect of estrogen on cognition, particularly for memory and learning that involves the hippocampus (Korol & Pisani, 2015), is becoming increasingly distinct. For example, Simon, Gallagher, Thompson, and Young (2004) proposed that neurocognitive functioning may be impaired during the luteal phase, when estrogen levels are reducing. This has been demonstrated in the literature including research examining functional connectivity in the brain throughout the menstrual cycle (Thimm, Weiss, Hausmann, & Sturm, 2014). In their study Thimm et al. (2014) point out that functional cerebral asymmetries have been found to be more pronounced in men than in women. However, previous studies (i.e. Hampson, 1990) have found that functional cerebral asymmetries in women vary across the menstrual cycle. These findings do not apply to postmenopausal women where functional cerebral asymmetries remain relatively stable; which serves to suggest that the fluctuations in hormones across the menstrual cycle (i.e. estrogen and progesterone) mediate the effects of changing functional cerebral asymmetries (Thimm et al., 2014). Although the underlying mechanism remains unclear, various studies have found conflicting cognitive differences during menstrual cycle, suggesting that the effects are task dependent (Thimm et al., 2014). One theory is that progesterone inhibits transmission of information through the corpus callosum through its effects on glutamate and GABA (Hausmann & Gunturkun, 2000).

Thimm, Weiss, Hausmann, and Sturm (2014) studied the effects of menstrual cycle on attention, as it is a relatively understudied cognitive domain. Specifically, the authors examined selective attention through an fMRI experiment (Thimm et al., 2014). They assessed naturally cycling women with a go/no-go task over multiple testing sessions taking place during the menstrual phase, follicular phase, and luteal phase (Thimm et al., 2014). Thimm, Weiss, Hausmann, and Sturm (2014) found reduced functional connectivity in the brain throughout a go/no-go task during the luteal phase, suggesting hormone levels mediate the differences in functional connectivity.

In a review of the literature, Asthana (2003) determined that estrogen can aid in memory and cognition, particularly when used as replacement therapy for post-menopausal women. Though, prolonged use has been linked to increased risk of stroke, heart disease, and breast cancer (Asthana & Middleton, 2004). Research on estrogen replacement therapies have clearly documented the positive effects of estrogen on memory function (Yaffe, Grady, Pressman, & Cummings, 1998); specifically on tests of verbal memory, vigilance, reasoning, and motor speed (LeBlanc, Janowsky, Chan, & Nelson, 2001). Overall, estrogen has been shown to have many effects on the cognitive abilities of women. However, a commonly consumed substance can also affect aspects of cognition.

Caffeine

Caffeine is the most commonly consumed psychoactive substance in the world (Koppelstaetter et al., 2010). It is primarily consumed through food and beverage products such as coffee, tea, energy drinks, soft drinks, and chocolate bars (Lorist & Tops, 2003). After consumption caffeine is quickly absorbed into the gastrointestinal tract and into the body, reaching peak concentrations between 30-60 minutes (Ullrich, Vries, Kuhn, Repantis, Dresler, & Ohla, 2015). Caffeine is nearly 100% bioavailable (Koppelstaetter et al., 2010). It is a small molecule, which means it has the ability to easily pass through the blood brain barrier and into cerebrospinal fluid and the brain (Wong, Ye, Levy, Rothstein, Bergles, & Searson, 2013). Once absorbed, caffeine acts as an adenosine antagonist; which means that it slows down the inhibitory effects of adenosine, thereby promoting wakefulness and increased mood (Einöther & Giesbrecht, 2013).

Caffeine's ability to antagonize adenosine indirectly interferes with the dopaminergic system, which in turn plays a role in increased arousal and higher-order complex attention processes (Fan et al., 2005). By increasing neuronal activity in various regions of the brain, caffeine contributes to generally improved attention (Einöther & Giesbrecht, 2013). This is consistent with an earlier review which suggests that information processing is altered by caffeine by improving the ability to process relevant stimuli characteristics (Lorist & Tops, 2003). Despite this, relatively little is known about the relation between caffeine and cognition during the menstrual cycle.

What is known is that consumption of a variety of food and beverage products can alter cognition at various points during the menstrual cycle. This was demonstrated by Islam, Sparkes, Roodenrys, and Astheimer (2008), who found a relation between cognition and food-based soy isoflavens, as they interact with estrogen in the female body. Although the interaction between caffeine, cognition, and the menstrual cycle is unknown, it is recognized that caffeine is another food and beverage product with the ability to influence cognition. Beginning with animal research, Onaolapo and Onaolapo (2015) found caffeine injection improves non-spatial memory in female mice, but not males. They also found that spatial memory is improved in males but not females (Onaolapo & Onaolapo, 2015). They suggest that future studies examining caffeine and its effects on the central nervous system should take sex differences into consideration (Onaolapo & Onaolapo, 2015). This suggestion is demonstrated by human research that indicates sex differences on the subjective effects of caffeine. For example, Adan, Prat, Fabbri, and Sanchez-Turet (2008) found that the subjective-effects of caffeine (i.e., decreased drowsiness and greater activation) were more acute in men than women. This was despite the fact that all participants were given the same dose of caffeine; which resulted in a higher dosage in their female participants due to lower body mass (Adan, Prat, Fabbri, & Sanchez,Turet, 2008).

Caffeine has been shown to have a variety of different effects on the sexes. For example, research has shown that caffeine beverage increased anxiety in a dose-dependent manner in male mice, but not female (Botella & Parra, 2003). The observed sex differences may be due to the observation of caffeine's direct relation with the menstrual cycle. For example, researchers have determined that caffeine intake through any beverage source may be associated with improved menstrual cycle function as it reduces the risk of anovulation in premenopausal women (Schliep et al., 2016). In a study of 403 healthy premenopausal women, Fenster et al. (1999) found that women who consumed more than 300 mg of caffeine a day were less likely to have a long menses compared to their non-caffeine consuming participants. Additionally, the speed at which caffeine is eliminated from the body varies for women; for example, the rate of elimination is reduced when using oral contraceptives and in the luteal phase prior to menstruation (Lane, Steege, Rupp, & Kuhn, 1992). Although generally the effects of caffeine are felt when high levels are ingested, they can also occur when the rate of elimination is decreased and caffeine is repeatedly consumed throughout the day (Lane, Steege, Rupp, & Kuhn, 1992). These findings demonstrate a relation between caffeine and its effects on the female population at various points in the menstrual cycle.

Taken together, these studies confirm there is a relation between the hormonal fluctuations observed across the menstrual cycle and caffeine. What is missing is a connection between the effects of caffeine on cognition at various points in the menstrual cycle. Furthermore, many findings have been found in older, menopausal and post-menopausal women and generalized to the younger population while failing to consider the specific effects of caffeine during younger periods of life, particularly the adolescent stage. During adolescence, students are often facing the pressures of studying, exams, and the anxiety and stress of preparing for the future. As such, adolescents often turn to caffeinated beverages to enhance performance abilities (Ullrich, Vries, Kuhn, Repantis, Dresler, & Ohla, 2015). Therefore, it is important to examine the effects caffeine may be having on the adolescent brain. One aspect of cognition that may be particularly important during this time is verbal working memory, as it relates to many aspects of academic success.

Verbal Working Memory

Working memory is temporary memory that is resource limited and involves attending to, manipulating, and temporarily storing material in the brain (Moore, Li, Tyner, Hu, & Crosson, 2013). It is considered "one of the building blocks for higher cognitive functioning" (Den Bosch et al., 2014, p. 699) as it is essential for the completion of many higher order tasks such as language comprehension, learning, and reasoning (Baddeley, 1996). Working memory is described as the frontier between perception, attention, memory, and action (Baddeley, 1996). Furthermore, fMRI research has shown that children and adolescents rely heavily on the hippocampus to complete working memory tasks, whereas with brain maturation, adults rely more heavily on the prefrontal cortex, an area of the brain which largely develops in adulthood (Finn, Sheridan, Kam, Hinshaw, & D'Esposito, 2010). As there has been an increasing body of research establishing the presence of estrogen receptors in the hippocampus (Islam, Sparkes, Roodenrys, & Astheimer, 2008), this is a particularly important aspect of cognition to study across the adolescent menstrual cycle. Additionally, working memory has been shown to be a necessary cognitive process in nearly all aspects of learning and academic achievement (Bayliss, Jerrald, Baddeley, & Gunn, 2005).

Baddeley and Hitch's (1994) model of working memory focuses on this ability to temporarily store, process, and manipulate information for use in a variety of tasks. This model includes two specialized short-term buffers: the phonological loop and the visuospatial sketchpad (Baddeley, Hitch, Butters, Nelson, & Becker, 1994). The phonological loop is used to store verbal content such as that required for verbal working memory (Baddeley, Hitch, Butters, Nelson, & Becker, 1994). The phonological loop is used to passively store 1-2 seconds of phonological content such as sounds, words, or phrases which is then followed by an active rehearsal phase which is used to temporarily retain this information beyond the initial 1-2 seconds (Marvel & Desmond, 2012). The phonological loop allows us to do everyday things like follow a recipe, listen to directions, or remember a short list of things to pick up at the grocery store. In children and adolescents these tasks may be things like following multi-step directions given by the teacher, or remembering what is said long enough to write it down.

Verbal working memory tasks typically involve the maintenance and output of verbal information (Acheseon & MacDonald, 2009). One such task is that is often used to measure verbal working memory is the n-back task (i.e. McEvoy, Smith, & Gevins, 1998; Koppelstaetter et al., 2008; Lejbak, Crossley, & Vrbancic, 2011). The n-back task can measure different kinds of working memory, depending upon the version being used. For example, there are spatial, object, and verbal versions of the n-back (Lejbak, Crossley, & Vrbancic, 2011). This task is a continuous performance working memory measure that makes varying levels of demands (e.g. 1-back, 2-back, 3-back) on working memory capabilities by asking participants to recall stimuli previously presented (Lejbak, Crossley, & Vrbancic, 2011). In the verbal version of the n-back, a series of letters is presented and participants are required to respond as quickly as possible only when the letter on the screen matches the letter *n* stimuli back (i.e., for the 1-back and 3-back conditions, a target is any letter that is identical to the letter presented one or three trials back, respectively; Lejbak, Crossley, & Vrbancic, 2011). Overall the n-back task has been shown to have good psychometric properties with reasonable factorial structure, good criterion validity, and acceptable internal consistency (Forns et al., 2014).

Previous research has demonstrated that estrogen suppression is linked with decreased performance on both verbal and non-verbal n-back tests (Grigorova, Sherwin, & Tulandi, 2006). For example, in the middle of the menstrual cycle (i.e. follicular and luteal phases), when estrogen levels are high, scores on a verbal working memory task have been found to be higher when compared to scores during other phases of the menstrual cycle (Rosenberg & Park, 2002). These results were limited to naturally cycling women and not found in those using oral contraceptives (Rosenberg & Park, 2002); that said, only naturally cycling women showed an increase in estradiol across phases of the menstrual cycle (Mordecai, Rubin, & Maki, 2008). Contrary to the results reported by Rosenberg and Park (2002), Mordecai, Rubin, and Maki (2008) found that naturally cycling women had no change in verbal working memory across phases. Instead, they found that women who use oral contraceptives show better verbal working memory performance during active pill phases than non-active pill phases (Mordecai, Rubin, & Maki, 2008). Differing their predictions, naturally cycling women did not vary in working memory across the cycle (Mordecai, Rubin, & Maki, 2008). Overall, estrogen has been shown to improve short-term memory, thereby increasing the acuity of working memory (Sherwin, 2003).

The inconsistent results in the literature highlight the need for clarity in verbal working memory across the menstrual cycle in naturally cycling and hormone mediated cycles.

Nutritional neuroscience research has made large contributions in researching various products that affect women's cognition. For instance, research has found a connection between soy isoflavens, a type of phytoestrogens found mainly in soy beans and legumes and verbal working memory in women (Islam, Sparkes, Roodenrys, & Astheimer, 2008). Naturally-cycling women scored significantly worse on a verbal working memory task during menses (when estrogen was low) than during the luteal phase (Islam, Sparkes, Roodenrys, & Astheimer, 2008). The same group of participants did better on a verbal working memory task during menses when given soy supplementation which allowed their performance to be equivalent to when they were in their luteal phase (Islam, Sparkes, Roodenrys, & Astheimer, 2008). These findings suggest that nutritional intake involving estrogen can impact cognitive abilities such as verbal working memory.

There is lack of agreement among researchers that a deficiency of endogenous sex hormones (both estradiol and progesterone) in women is connected to a decline in verbal memory (Kramer, Delis, & Daniel, 1988). Given that women have demonstrated superior abilities in verbal memory compared to men (Hyde, 2016), it has been proposed that estrogen is a key component in this cognitive domain (Kramer, Delis, & Daniel, 1988). This is demonstrated in studies such as one conducted by Saeed, Hellström, and Sandberg (2009), which found that women scored better on working memory tasks during the ovulatory phase (late follicular and early luteal), when estrogen levels are high, than during the menstrual phase, when estrogen levels are low.

Caffeine also has been shown to be beneficial to working memory as demonstrated in a study comparing electroencephalographic (EEG) data to examine the effects of caffeine on working memory. EEG's measure event-related potentials (ERPs) which are electrical impulses created by the brain (Luck et al., 2011). They have been useful in caffeine studies as they are extremely temporally sensitive (Pan, Takeshita, & Morimoto, 2000). Bruce, Werner, Preston, and Baker (2014) showed an increased P450 amplitude (a positive ERP voltage deflection indicative of an increase in sustained attention) in participants who received a citicoline-caffeine (a major ingredient found in energy drinks) beverage as compared to the non-beverage control group (Bruce, Werner, Preston, & Baker, 2014). These findings also suggest improved working memory in those who consumed the citocoline-caffeine beverage (Bruce, Werner, Preston, & Baker, 2014). Though, discrimination between the ingredients was not possible and therefore the authors were unable to conclude which ingredient, or combination of ingredients, was responsible for the effects (Bruce, Werner, Preston, & Baker, 2014).

However, there are functional magnetic resonance imaging studies that have exclusively examined caffeine and working memory. For example, while assessing verbal working memory and caffeine, Koppelstaetter et al. (2008) examined fMRI signals during a 2-back verbal working memory task. The researchers found that compared to placebo, caffeine conditions showed an increased response in the frontopolar cortex ranging to the right anterior cingulate cortex (Koppelstaetter et al., 2008). This suggests caffeine produces changes in areas of the brain associated with executive and attentional functions during a verbal working memory task. Though the results of their study did not show a significant effect on the behavioural measures of accuracy or reaction time (Koppelstaetter et al., 2008).

Event-Related Potentials

Similar to Koppelstaetter et al. (2008), previous studies have examined verbal working memory using the N-back task with an electroencephalograph (EEG) to measure event-related potentials (ERPs) instead of an fMRI (i.e. Bruce, Werner, Preston, and Baker, 2014). Event-related potentials (ERPs) are electrical impulses created by the brain in response to external or internal stimuli (Luck et al., 2011). An ERP component is a compilation of positive and negative voltage deflection that is produced when a specific neural process occurs in regions of the brain (Luck et al., 2011). The many components produced by a stimulus are summed together to create ERP wave forms (Luck et al., 2011). ERPs can be useful in determining whether changes in behavioural performance are due to changes in neural vs. motor activity (Luck et al., 2011). ERPs provide information about higher mental processes such as those that involve memory, attention, changes in mental state, or expectation (Luck, 2011). ERP measures are often used because they can reflect ongoing instantaneous brain activity at the millisecond level and can be used by measuring specific brain waves prior to, during, and after a specific task (Luck, 2011).

Previous studies have used an electroencephalogram during a working memory task. For example, Marchand, Lefebvre, and Connolly (2006) used the Digit Span task from the Wechsler Adult Intelligence Scale – Third Edition (WAIS-III) to examine the ERP responses of 20 participants. Their main focus was to assess the validity of a statistical method which allows neuropsychological test ERP responses to serve in lieu of behavioural responses in participants with communication impairments (Marchand, Lefebvre, & Connolly, 2006). Specifically, Marchand, Lefebvre, and Connolly (2006) measured working memory with the P300 component.

With respect to the N-back task, previous studies have consistently examined the P300 event-related potential (Kim & Kim, 2016). Many previous ERP studies have found that ERPs elicited by correctly identified repeated stimuli are more positive than those elicited by correctly identified new items (O'Reilly, Cunningham, Lawlor, Walsh, & Rowan, 2004). This is called the ERP repetition effect and includes the P300 component in visual recognition memory tasks (O'Reilly, Cunningham, Lawlor, Walsh, & Rowan, 2004). The P300 is often used in measurements of verbal working memory and is a positive peak that occurs over central-parietal sites approximately 300 ms after the presentation of stimuli, hence the term P300 (Kim & Kim, 2016). This ERP is thought to be elicited by tasks that involve a decision, such as those in a verbal working memory task (McEvoy, Smith, & Gevins, 1998). It has been suggested that the P300 reflects the categorization of stimuli, updating of memory, and attentional allocation (Kim & Kim, 2016). Previous studies have concluded that increasing working memory demands result in an attenuated P300 amplitude (McEvoy, Smith, & Gevins, 1998). It has been suggested that this inverse relation between the P300 and the working memory task demands reflect the reallocation of resources from stimulus classification and decision-making towards maintaining and rehearsing the information presented (McEvoy, Smith, & Gevins, 1998). Many previous ERP studies have found that ERPs elicited by correctly identified repeated stimuli are more positive than those elicited by correctly identified new items (O'Reilly, Cunningham, Lawlor, Walsh, & Rowan, 2004). This repetition effect includes the P300 component in visual recognition memory tasks (O'Reilly, Cunningham, Lawlor, Walsh, & Rowan, 2004).

Not only has the P300 ERP been used in working memory tasks, it has also been documented in research investigating the effect of the menstrual cycle on cognitive function (i.e. Pan, Takeshita, & Morimoto, 2000; O'Reilly, Cunningham, Lawlor, Walsh, & Rowan, 2004). Although estrogen is generally known to increase cortical excitability, the P300 amplitude has been shown to be larger during the menstrual phase despite estrogen being at its lowest (O'Reilly, Cunningham, Lawlor, Walsh, & Rowan, 2004). However, whether this is reflective of improved function during the menstrual phase or more efficient processing (i.e. requiring less cognitive resources to perform the same task) during the luteal phase is unclear.

Finally, the P300 has also been used to measure the effects of caffeine. Pan, Takeshita, and Morimoto (2000) studied how caffeine affects P300 measurements in response to repeatedly presented stimuli. Their participants were nine healthy males who were administered either caffeine or a placebo in one of two double-blind counterbalanced sessions (Pan, Takeshita, & Morimoto, 2000). Participants then responded to the auditory oddball paradigm in order to elicit the P300 (Pan, Takeshita, & Morimoto, 2000). Their most relevant finding was that caffeine ingestion produced an attenuated P300 amplitude and a shorter latency overall compared to placebo (Pan, Takeshita, & Morimoto, 2000), suggesting more efficient cognitive performance.

Collectively, research has examined the effects of caffeine on cognition, the effects of caffeine on the menstrual cycle, and the effects of the menstrual cycle on cognition in various domains. Despite the breadth of literature on these variables, there is comparatively very little research examining all of these variables as they interrelate. Particularly, little to no research has examined the effects caffeine and the menstrual cycle may have on the adolescent population; despite this age group regularly consuming this readily available psychoactive drug.

CHAPTER TWO

Electrophysiological Investigations of the Menstrual Cycle, Caffeine, and Verbal Working Memory

Caffeine is one of the most popular pharmacologically active substances (Fenster et al., 1999) and among the most consumed natural alkaloids (Lane, Steege, Rupp, & Kuhn,1992). Its consumption among adolescents in particular is common and on the rise (O'Neill et al., 2016) as adolescents increasingly consume readily available energy drinks, sodas, chocolate, coffee, and other caffeinated products. One study found that over half of their adolescent participants were classified as having high or moderate caffeine intake (Orbeta, Overpeck, Ramcharran, Kogan, & Ledsky, 2006). Caffeine is eagerly consumed by adolescents for its seemingly beneficial effects on physiological and cognitive performance (Temple, 2009). However, what adolescents may not be considering is how naturally occurring hormones alter the effects of caffeine (Lane, Steege, Rupp, & Kuhn, 1992) and how the absorption of caffeine and its mechanisms within the body make it likely to alter hormones (Fenster et al., 1999). Of particular interest is how caffeine's interaction with hormones affect cognitive function, which can affect performance in the classroom. Both the menstrual cycle and caffeine have been found to both enhance and impair cognitive performance (e.g. Hampson, 1990; Fan et al., 2005; Einöther & Giesbrecht, 2013; Barros, Tufik, & Andersen, 2015). However, relatively little research has examined how psychoactive drugs, caffeine in particular, may affect cognition across the menstrual cycle.

Verbal Working Memory

One aspect of cognition that may be affected by caffeine and the menstrual cycle is working memory. Working memory is temporary memory that is resource limited and involves attending to, manipulating, and temporarily storing material in the brain (Moore, Li, Tyner, Hu,

& Crosson, 2013). Baddeley and Hitch's (1994) model of working memory includes two specialized short-term buffers: the phonological loop and the visuospatial sketchpad (Baddeley, Hitch, Butters, Nelson, & Becker, 1994). The phonological loop is used to store verbal content such as that required for verbal working memory (Baddeley, Hitch, Butters, Nelson, & Becker, 1994).

In general, working memory has a significant effect on educational success as it has been tied with many aspects of learning as well as basic skills such as remembering instructions (Alloway & Alloway, 2010). It is involved in reading, acquiring new skills, and even skills such as solving basic math equations (Van de Weijer-Bergsma, Kroesbergen, & Van Luit, 2015). For example, when solving an equation, students must hold the relevant information in mind while simultaneously manipulating it to solve the problem, requiring working memory (Van de Weijer-Bergsma, Kroesbergen, & Van Luit, 2015). Overall, working memory is so strongly linked to learning that it predicts academic achievement independently and at times more strongly than IQ (Alloway & Alloway. 2010).

In particular, verbal working memory has been associated with classroom learning such as the acquisition of vocabulary and grammar in both first language and second language learning (Adams & Gathercole, 1996; Verhagen & Leseman, 2016).Additionally, verbal working memory has been shown to have a strong predictive value in many math domains (Van de Weijer-Bergsma, Kroesbergen, & Van Luit, 2015). This strong predictive value increases as students progress through grades so that by the time they are in grade five/six, verbal working memory is a stronger predictor of math success than visual-spatial working memory (Van de Weijer-Bergsma, Kroesbergen, & Van Luit, 2015). This is likely due to working memory, specifically verbal working memory, increasing with adolescent development as demonstrated by increased functional connectivity in various regions of the brain (Den Bosch, Marroun, Schmidt, Tibboel, Manoach, Calhoun, & White, 2014).

Previous research has demonstrated that estrogen suppression is linked to decreased performance on both verbal and non-verbal n-back tests (Grigorova, Sherwin, & Tulandi, 2006). For example, in the middle of the menstrual cycle (i.e. follicular and luteal phases), when estrogen levels are high, scores on a verbal working memory task have been found to be higher when compared to scores during other phases of the menstrual cycle (Rosenberg & Park, 2002). These results were limited to naturally cycling women and not found in those using oral contraceptives (Rosenberg & Park, 2002); that said, only naturally cycling women showed an increase in estradiol across phases of the menstrual cycle (Mordecai, Rubin, & Maki, 2008). However, contrary to the results reported by Rosenberg and Park (2002), Mordecai, Rubin, and Maki (2008) found that naturally cycling women had no change in verbal working memory across phases. Instead, they found that women who use oral contraceptives show better verbal working memory performance during active pill phases than non-active pill phases (Mordecai, Rubin, & Maki, 2008). Contrary to predictions, naturally cycling women did not vary in working memory across the cycle (Mordecai, Rubin, & Maki, 2008). These inconsistent results highlight the need for clarity in verbal working memory across the menstrual cycle.

It has been proposed that estrogen is a key component in verbal memory (Kramer, Delis, & Daniel, 1988). This is demonstrated in studies such as Saeed, Hellström, and Sandberg (2009), which found that women scored better on working memory tasks during the ovulatory phase (late follicular and early luteal), when estrogen levels are high, than during the menstrual phase, when estrogen levels are low.

In addition to estrogen, caffeine also has been shown to be beneficial to working memory. A study comparing electroencephalographic (EEG) data showed an increased response in participants who received a citicoline-caffeine (a major ingredient found in energy drinks) beverage as compared to the non-beverage control group (Bruce, Werner, Preston, & Baker, 2014). While assessing verbal working memory and caffeine, Koppelstaetter et al. (2008) examined fMRI signals during a 2-back verbal working memory task. The researchers found that compared to placebo, caffeine conditions showed an increased response in the frontopolar cortex ranging to the right anterior cingulate cortex (Koppelstaetter et al., 2008). This suggests caffeine produces changes in areas of the brain associated with executive and attentional functions during a verbal working memory task, although the results of their study did not show a significant effect on the behavioural measures of accuracy or reaction time (Koppelstaetter et al., 2008).

With respect to the N-back task, previous studies have consistently examined the P300 event-related potential (Kim & Kim, 2016). The P300 is often used in measurements of verbal working memory and is a positive peak that occurs over central-parietal sites approximately 300 ms after the presentation of stimuli, hence the term P300 (Kim & Kim, 2016). This ERP is thought to be elicited by tasks that involve a decision, such as those in a verbal working memory task (McEvoy, Smith, & Gevins, 1998). It has been suggested that the P300 reflects the categorization of stimuli, updating of memory, and attentional allocation (Kim & Kim, 2016). Previous studies have concluded that increasing working memory demands result in an attenuated P300 amplitude (McEvoy, Smith, & Gevins, 1998). It is though that this inverse relation between the P300 and the working memory task demands reflect the reallocation of resources from stimulus classification and decision-making towards maintaining and rehearsing the information presented (McEvoy, Smith, & Gevins, 1998).

Previous research has found that caffeine ingestion results in a decreased P300 amplitude and shorter P300 latencies compared to placebo (Pan, Takeshita, & Morimoto, 2000). These findings suggest that caffeine increases the speed of mental processing and decreases the demand of attentional resources required for cognitive task performance (Pan, Takeshita, & Morimoto, 2000).

Caffeine

As an adenosine antagonist, caffeine slows down the inhibitory effects of adenosine thereby promoting wakefulness and increased mood (Einöther & Giesbrecht, 2013). Although commonly consumed for these arousing properties, caffeine has been known to affect cognition (which may be a downstream effect of enhanced arousal) in generally an inverted U shape, with moderate doses of caffeine having a positive effect while high and low doses of caffeine have a negative effect or no effect at all (Anderson & Revelle, 1983).

Caffeine's ability to antagonize adenosine also indirectly interferes with the dopaminergic system, which in turn plays a role in increased arousal and higher-order complex attention processes (Fan et al., 2005). By increasing neuronal activity in various regions of the brain, caffeine contributes to generally improved attention (Einöther & Giesbrecht, 2013). This is consistent with an earlier review which suggests that information processing is altered by caffeine by improving the ability to process relevant stimuli characteristics (Lorist & Tops, 2003).

Consumption of caffeine amongst adolescent females is common, with an average intake of 317.9 mg/day (Rudolph, Faerbinger, & Koenig, 2014). This consumption is drastically more than in previous studies where similar age groups (both sexes) were found to have an average intake of 41.7, 65.9, and 70 mg/day in earlier years (Rudolph, Faerbinger, & Koenig, 2014). For example, a survey of 5 to18-year olds indicated that 98% were caffeine consumers (Morgan, Stults, & Zabik, 1982). Another study found that in 6 to18-year-old participants daily cola consumption resulted in an average caffeine intake of 192.88 mg of caffeine a day from this source alone (Herin-Hanit, & Gadoth, 2003), not accounting for coffee, chocolate, tea, energy drinks or the copious other caffeine sources available. To put that in perspective, in the early 1990's the average daily per capita caffeine consumption in Canada was 238 mg amongst adults (Herin-Hanit, & Gadoth, 2003). Although being consumed in copious amounts relatively little is known about the relation between caffeine and cognition during the menstrual cycle.

What is known is that sex can be a determinant in the way caffeine influences the body. Research indicates sex differences on the subjective effects of caffeine. For example, caffeine beverage increases anxiety in a dose-dependent manner in male mice but not female (Botella & Parra, 2003). Moreover, researchers have determined that caffeine intake through any beverage source may be associated with improved menstrual cycle function as it reduces the risk of anovulation in premenopausal women (Schliep et al., 2016). Women who consumed more than 300 mg of caffeine a day showed less likely to have a long menses compared to their non-caffeine consuming participants. Additionally, the speed at which caffeine is eliminated from the body varies for women; the rate of elimination is reduced when using oral contraceptives and in the luteal phase prior to menstruation (Lane, Steege, Rupp, & Kuhn, 1992). Although generally the effects of caffeine are felt when high levels are ingested, they can also occur when the rate of elimination is decreased and caffeine is repeatedly consumed throughout the day (Lane, Steege, Rupp, & Kuhn, 1992). These findings demonstrate a relation between caffeine and its effects on the female population at various points in the menstrual cycle.

Collectively, these studies confirm there is a relation between the hormonal fluctuations observed across the menstrual cycle and caffeine and that adolescents are consuming caffeine in copious and increasing amounts. However, there is very little research to connect the effects of caffeine on cognition across the menstrual cycle. As adolescents in particular are consuming vast amounts of caffeine, there is one aspect of cognition that may be significant to female adolescents in the classroom as it is a domain of cognition important to educational success: verbal working memory.

Menstrual Cycle

The human menstrual cycle involves variations in sex hormones (mainly estrogen and progesterone) over an interval typically lasting 28-29 days (Sherman & Korenman, 1975). For instance, the menstrual phase is characterized by low levels of estrogen and progesterone, while the luteal phase (typically days 15 to 28) sees a rise in progesterone levels followed by a second peak in estrogen levels (Farage, Osborn, & MacLean, 2008). Both estrogen and progesterone hormones decrease during the late luteal phase until levels reach baseline, followed by the onset of menstruation and the beginning of a new cycle (Farage, Osborn, & MacLean, 2008). Physiologically, this cyclic fluctuation of hormones has a multitude of influences on the female body including physical and emotional (Farage, Neill, & MacLean, 2009). Yet, what many females, adolescents in particular, may not be cognizant of is how these same hormonal fluctuations also influence aspects of cognition.

Canadian researchers Doreen Kimura and Elizabeth Hampson found that when a woman experiences low estrogen levels, typically during and immediately after menstruation, she does better on spatial tasks but scores poorly on complex motor tasks (Hampson, Kimura, & Thompson, 1988). Conversely, during periods of high estrogen levels, typically associated with phases in the middle of the cycle, participants showed improvements on tasks involving motor and verbal skills but showed difficulty with spatial relations (Hampson, Kimura, & Thompson, 1988).

Sex differences on cognitive tasks have been observed in various domains likely due in part to the effects of sex-steroids (Hausmann et al., 2000). For example, females have shown an advantage over males in aspects of verbal skills such as essay writing and vocabulary (Hyde, 2016). In fact, some studies have observed that high levels of estradiol (a common type of estrogen) have enhancing effects on cognitive tasks in which females tend to excel and detrimental effects on tasks in which males tend to excel (Hampson & Kimura, 1988). Given sex-steroids like estrogen fluctuate across the menstrual cycle, research has documented commensurate variations in cognitive abilities (i.e. Hampson, 1990; Barros, Tufik, & Andersen, 2015; Kromrey, Czoty, & Nader, 2015). For instance, in animal research cynomolgus monkeys demonstrated an inverse relation between progesterone and initial discrimination performance, but no effect of menstrual phase on working memory (Kromrey, Czoty, & Nader, 2015). The authors conclude that the effects of the menstrual cycle on cognition varies across tasks (Kromrey, Czoty, & Nader, 2015). This is demonstrated by Hampson (1990), who found that human females scored better on spatial ability tasks during menses (vs. follicular phase) when estradiol and progesterone hormone levels are lower than in the preovulatory phase. Conversely on motor tasks, participants scored better during the follicular phase as compared to menses (Hampson, 1990). On the contrary, during the menstrual phase women scored better on spatial tasks (Hampson, 1990), confirming the theory of a task-dependent relationship between estrogen levels across the menstrual cycle and cognition (Hampson, 1990).

Whereas Hausmann et al. (2000) found an increased ability to rotate objects mentally during the menstrual phase (vs. mid-luteal), Postma, Winkel, Tuiten, and van Honk (1999) found that during a spatial task, positional reconstruction scores, which require both spatial and working memory abilities, was poorer during the menstrual phase. Overall, well documented research holds that testosterone generally has a positive effect and estradiol a negative effect on spatial ability (Symonds, Gallagher, Thompson, & Young, 2004). In fact, numerous studies have investigated and described the role of estrogen in brain function (i.e. Wooley & McEwen, 1994; McEwen & Alves, 1999; Morrison, Briton, Schmidt, & Gore, 2006).

Estrogen receptors are found in various parts of the brain and their widespread presence affects many systems (Sherwin, 2003). Deficits of estrogen in the brain have been linked with memory deficits as well as deficits in fine motor coordination (McEwen & Alves, 1999). In addition to being found in regions associated with reproduction, estrogen receptors have been found in areas of the brain that are not related to reproductivity. For instance, estrogen receptors have been found in areas such as the frontal and prefrontal cortex, and the hippocampus: areas associated with cognitive functions related to learning and memory (Islam, Sparkes, Roodenrys, & Astheimer, 2008). Overall, the effect of estrogen on memory and learning that involves the hippocampus (Korol & Pisani, 2015) is becoming increasingly distinct. For example, Simon, Gallagher, Thompson, and Young (2004) proposed that neurocognitive functioning may be impaired during the luteal phase, when estrogen levels are reducing. Thimm et al. (2014) indicate that functional cerebral asymmetries have been found to be more pronounced in men than in women. Other studies (i.e. Hampson, 1990) have found that functional cerebral asymmetries in women vary across the menstrual cycle in premenopausal women only, suggesting that the fluctuations in hormones across the menstrual cycle (i.e. estrogen and progesterone) facilitate the differences (Thimm et al., 2014). Although the underlying mechanism remains unclear, various studies have found conflicting results of cognitive skills depending on menstrual cycle phase, suggesting that the effects are task dependent (Thimm et al., 2014).

In a review of the literature Asthana (2003) determined that estrogen can aid in memory and cognition. Research on estrogen replacement therapies have clearly documented the positive effects of estrogen on memory function (Yaffe, Grady, Pressman, & Cummings, 1998) specifically on tests of verbal memory, vigilance, reasoning, and motor speed (LeBlanc, Janowsky, Chan, & Nelson, 2001). These findings had previously only been found in older post-menopausal women and generalized to younger women and not taking into consideration differences in hormones or the role of commonly consumed drugs like caffeine.

Current Study

Similar to Koppelstaetter et al. (2008), we examined verbal working memory using an N-back task. However, the present study used an electroencephalograph (EEG) to measure event-related potentials (ERPs), as did the study by Bruce, Werner, Preston, and Baker (2014). Using ERP measures is appropriate for this study, as previous research has established the complex relation between caffeine and its behavioural effects which can be convoluted by individual differences and the nature of the task at hand (Lorist & Tops, 2003). By using an EEG to directly examine cortical activity many of these complications associated with examining behavioural data alone can be circumvented (Lorist & Tops, 2003). An EEG provides superb temporal resolution which allows the researcher to track cortical change at the millisecond level. The effects of caffeine have been measured using EEG's for an extensive period, including a study published nearly 75 years ago by Gibbs and Maltby (1943). Using these measures, we examined caffeine's effects during a verbal working memory task.

Therefore, the present research examined the relations between the menstrual cycle, caffeine, and cognition. The aim is to provide an understanding of the effect(s) of caffeine on a largely understudied group in neuroscience research. Female participants in general are underrepresented in neuroscience research, largely owing to neurochemistry, hormones, and changes during the menstrual cycle (Kromrey, Czoty, & Nader, 2015). By examining how natural and hormone-mediated menstrual cycles influence the brain's response to caffeine, the goal of this research is to provide evidence-based information for girls and young women in their educational and occupational environments. This could be particularly relevant during the adolescent phase when both menstrual cycles and increased caffeine consumption may be relatively novel. This study will emphasize caffeine's potential effect(s) on female adolescents in the classroom by focusing on a domain of cognition important to educational success: verbal working memory.

The hypotheses in the present study are consistent with behavioural studies of caffeine's effects on n-back indexed working memory. We expect caffeine will reduce reaction times and will reduce P300 latency, with the strongest effects being observed during the menstrual phase when baseline reaction times (RT's) and amplitudes are expected to be delayed and smaller, respectively.

Method

Participants

Participants were 16 (Table 4) females between the ages of 18 and 41 with a mean of 23.5 years old and a median of 21 years old. There was not a statistically significant difference in age between groups. Participants had normal or corrected to normal vision and were recruited using an information sheet (Appendix D). Each participant attended the EEG lab at MSVU (3^{rd}

floor of Evaristus Hall) for two morning testing sessions. Participants were women with regular menstrual cycles who were randomly assigned to attend during either the menstrual (day 2-4 of cycle) or mid-luteal (3-9 days before onset of new cycle) phase of their cycle, which is consistent with previous EEG research by Walpurger, Pietrowsky, Kirschbaum and Wolf (2004). Participants were equally distributed among cycle phase so that there were eight in the luteal phase and eight in the menstrual phase of their cycle. Of the 16 participants, 13 had hormone-mediated menstrual cycles while three were naturally cycling. Due to lack of variability in the data, cycle type (i.e. natural vs. hormone mediated) was unable to be included as a variable.

Measures

Verbal Working Memory. Replicating previous research in this area (i.e. Koppelstaetter et al., 2008; Jacobs et al., 2017) this paradigm used two randomized conditions of the verbal N-Back task (Figure 1). Each condition presented identical stimuli and response demands but consisted of increasing levels of working-memory load. In the verbal version of the n-back a series of letters is presented and participants are required to respond as quickly as possible only when the letter on the screen matches the letter *n* stimuli back (i.e., for the 1-back and 3-back conditions, a target is any letter that is identical to the letter presented one or three trials back, respectively; Lejbak, Crossley, & Vrbancic, 2011).

EEG Acquisition and Analysis. An electroencephalogram was used to collect ERP data. The P300 ERP was extracted from EEG activity at electrode site P_Z occurring following the presentation of a target (i.e. a letter previously presented *n* back).

Procedure

This study used a randomized, placebo-controlled, double-blind, repeated measures design. Specifically, this study examined P300 waveforms elicited by the verbal version of the n-back task. The primary outcome measures were ERP amplitudes and latencies, as well as behavioural measures such as accuracy (correct hits) and reaction time. All testing procedures were carried out in accordance with the Declaration of Helsinki and following the approval of the Research Ethics Board of Mount Saint Vincent University.

Participants attended two testing sessions with approximately 28 days between each session so that each participant attended during the same menstrual phase but under different drug conditions (i.e. placebo or caffeine). The second testing session follower the same procedures as the first (less the demographic survey, questionnaires, and informed consent). During both sessions the interaction between caffeine and menstrual phase was assessed. Neural (ERPs) and behavioural (hits, reaction time) correlates of verbal working memory were examined.

Upon arrival at the laboratory, following self-report of adherence to pre-testing abstinence, informed consent (Appendix A), completion of Caffeine Consumption Questionnaire (CCQ; Landnum, 1992), and the Morningness-Eveningness Questionnaire (MEQ; Horne & Ostberg, 1976), participants were provided with a small pill (either placebo or 200 mg of caffeine). Administration was counterbalanced with half the participants receiving caffeine at the first session and placebo during the second, and the other half of participants receiving the reverse. Following this, EEG electrodes were applied and participants were asked to fill two small tubes with saliva for later analysis of estradiol and progesterone levels as part of the larger research project. This process took between approximately 30-60 minutes; which is the average time it takes for caffeine to reach peak plasma levels after consumption (Lorist & Tops, 2003). Volunteers were then completed a computerized neurophysiological test battery which included the n-back as well as tasks from the larger project: visual sustained attention, an auditory oddball paradigm, and a visual search task.

As verbal working memory tasks typically involve the maintenance and output of verbal information (Acheseon & MacDonald, 2009), the task that is often used to measure verbal working memory is the n-back task (i.e. McEvoy, Smith, & Gevins, 1998; Koppelstaetter et al., 2008; Lejbak, Crossley, & Vrbancic, 2011). The n-back task can measure different kinds of working memory, depending upon the version being used. This task is a continuous performance working memory measure that makes varying levels of demands (e.g. 1-back, 2-back, 3-back) on working memory capabilities as it requires participants to recall stimuli previously presented (i.e. 1-back, 2-back, 3-back) (Lejbak, Crossley, & Vrbancic, 2011). As verbal working memory tasks have been shown to rely heavily on many interworking areas of the brain, including the hippocampus (Faraco et al., 2011), and given the many estrogen receptors found in the hippocampus (Bean, Ianov, & Foster, 2014), this task is an appropriate one for measuring working memory as it relates to the menstrual cycle. Therefore, the n-back was administered to participants by having a series of letters was presented via a computer screen and participants were required to respond as quickly as possible by pressing a button only when the letter on the screen matched the letter *n* stimuli back (i.e., for the 1-back and 3-back conditions, a target is any letter that is identical to the letter presented one or three trials back, respectively). Behavioural endpoint measures included accuracy (correct target detections) and reaction time (ms) to targets.

Participants were required to abstain from caffeine-containing food and drink, illicit drugs, medications (except for oral contraceptives) and alcohol, beginning at 8:00 p.m. of the previous day. To facilitate source localization, all participants were right handed with a regular menstrual cycle. Participants were excluded if they reported DSM-5 illness, current history of drug use or dependence, head injury resulting in loss of consciousness within the last year, a neurological disorder, regular use of any medication (excluding oral contraceptives) within two weeks of participation, cardiac illness, pregnancy within the last year, or currently breastfeeding. Participants were included or excluded based on a screening questionnaire (Appendix B)

EEG activity was recorded using Brain Vision Recorder software and a Brain Vision BrainAmp MR amplifier. EEG activity was recorded from an electrode cap with Ag+/Ag+Cl- ring electrodes at 32 scalp sites (see Appendix C) with bandpass settings of 0.1 and 100 Hz, digitized at 500 Hz, and stored for later analysis. Electrical activity was elicited using electrogel as a conductive medium. Electrodes were placed on the supra-sub orbital and external canthi of the eyes to record biopolar electrooculogram (EOG) activity, on the mastoid processes (reference), on the nose, and another electrode placed mid-forehead (ground).

ERPs were extracted from the EEG recording using Brain Vision Analyzer. Prior to ERP analysis electrical activity was averaged separately for each stimulus type and filtered digitally offline with a bandpass of 0.1 Hz to 30 Hz. Electrical epochs were corrected for residual eye movement and eye blink activity using an algorithm operating in the time and frequency domain (Gratton et al., 1983) and then baseline corrected using a window of pre-stimulus activity.

Results

Analysis

Neurophysiological (amplitude and latencies) and behavioural (accuracy and reaction times) endpoint measures were analyzed separately. Analyses were carried out using the Statistical Package for the Social Sciences software- 24.0 (SPSS). Neuropsychological data were analyzed using a repeated measures ANOVA procedure with three within-group factors: task (2 levels: 1-back and 3-back), drug (2 levels: caffeine and placebo), and scalp sites (3 levels: Pz, P3, and P4), and one between-group factor of phase (2 levels: luteal and menstrual phase). Behavioural data analyses were nearly identical, with the exception of the removal of scalp site from the ANOVA. A Huynh-Feldt epsilon correction was applied. Partial eta squared (η_p^2) was used to report effect size of main effects, while Cohen's *d* was used for pairwise comparisons. We will report significant findings with a p-value less than .05 and non-significant findings with a Cohen's *d* of .6 and greater.

P300 Amplitude

Overall, there was not a statistically significant main effect of drug (caffeine) on P300 amplitude. However, results showed that there was a statistically significant main effect of scalp site, $F(1,13) = 14.62$, $p<.001$, $\eta_p^2 = 0.69$, due to larger amplitudes being present at the P3 (left region) scalp site in comparison to the Pz (midline region) and P4 (right region) scalp sites. The mean amplitude was significantly larger in the left region (P3; M=10.29 µV, SE= 1.03) compared to the midline region (Pz; M=8.81 µV, SE= .73) and the right region (P4: M= 8.92 µV, SE= .97).

There was a significant main effect of task on P300 amplitude $F(1,14)= 10.96$, $p= .005$, $\eta_p^2 =.439$.This was due to overall larger amplitudes in response to the 1-back versus the 3-back. There was also a significant drug by task interaction, $F(1,14) = 5.18$, $p=.039$, $\eta_p^2 =.270$. However, these findings are consistent with the significant effect of task reported above, due to larger amplitudes in response to the 1-back compared to the 3-back in both the caffeine (p=.002) and placebo (p=.032) conditions. Followed up further, our analyses showed that the mean amplitude of the P300 at the midline region (Table 1) during the menstrual phase differed significantly between tasks (1 back vs. 3 back) $F(1,14) = 12.39$, $p=.003$, $\eta_p^2 = .468$. This finding was true regardless of drug, as larger amplitudes for the 1-back (vs. 3-back) were observed under both caffeine (1-back: *M*=13.37, *SE*= 2.84 vs. 3-back: *M*= 7.01, *SE*= 1.12) and placebo (1-back: *M*=13.17, *SE*= 2.67 vs. 3-back: *M*= 7.57, *SE*= 1.76) conditions.

An overall difference in P300 amplitudes between tasks was not observed in the luteal phase of the menstrual cycle *p*=.260. Participants in the luteal phase did, however, show a large effect size between tasks (1-back task > 3-back task) during the caffeine condition *d*=1.11. These results were only seen during the caffeine condition and not during the placebo condition where the difference between tasks did not approach statistical significance (*p*= .780), nor have a large effect size (*d*= 0.32).

Furthermore, in the luteal phase there was a large effect size observed at the left parietal site (P3) during the 3-back between drug conditions (*d*=0.66) such that the mean amplitude of the 3-back task during the placebo condition (*M*=9.97 µV, *SE*=3.28), was greater than the mean amplitude during the caffeine condition (*M*=6.90 µV, *SE*=1.16). This was not seen in the menstrual phase, during the 1-back task, or at any other scalp site.

In response to the 3-back task, there was also a large effect size (*d*= 1.00) between phases. In pairwise comparisons at the left region (P3 scalp site) during the 3-back task under the placebo condition, there was a large effect size between phases where the larger amplitudes were present in the luteal phase (*M*=9.97 µV, *SE*= 1.26) as compared to the menstrual phase (*M*= 7.57 µV, *SE*= 1.76). These differences approaching statistical significance were not seen during the caffeine condition.

P300 Latencies

Overall, results revealed that there was no main effect of drug on P300 latency $F(1, 14) = 3.06$, p=.950, $\eta_p^2 = .000$. However, results showed that during the placebo condition, there was a significant difference of latency between tasks, $F(1,14)= 6.76$, p=.021, $\eta_p^2 = .326$, where the 1-

back had a greater latency (*M*= 341.50 ms, *SE*= 10.29) than the 3-back (*M*=312.50 ms, *SE*=11.20). These differences were not present in the caffeine condition (p= .660), due to both a (non-significant) shortening of latency during the 1-back task and increased latency during the 3-back task. A Cohen's *d* calculation revealed that these differences were not only significant but had a moderate effect size (*d*= 0.67). Additionally, these findings were only significant in the luteal phase (p= .024), and not in the menstrual phase (p= .270). Comparatively, under the caffeine condition, mean latencies were not statistically significantly different between the 3-back task (*M*=323.75 ms, *SE*= 13.26) and the 1-back task (*M*=329.38 ms, *SE*=9.42).

Behavioural Data

All participants completed two conditions of the n-back task (1-back and 3-back). Accuracy (Table 2) and reaction time (Table 3) endpoint measures were analyzed with respect to phase (menstrual vs. luteal), drug (placebo vs. caffeine), and task (1-back vs. 3-back).

In terms of accuracy (correct target identification), there was no main effect of phase (p=.686) or drug (p=.541). However, there was an effect of task, $F(1,14)$ = 0.41, $p < .001$ η_p^2 = .030. Predictably, participants were more accurate in detecting targets in the 1-back (*M*= 0.95, *SE*= 0.02) than the 3-back (*M*=0.56, *SE*= 0.03). This was true regardless of phase or drug.

With regards to reaction time, again, there was a main effect of task, $F(1,14)$=38.63, $p<.001$, η_p^2 =.847, with participants being quicker to respond to the 1-back (*M*=428.42 ms, *SE*=20.78) than the 3-back (*M*=600.63 ms, *SE*=31.59). This was true regardless of phase or drug.

Overall, there was a significant main effect of phase, $F(1,13)$=7.58, p=.016, η_p^2 =.368, with participants responding quicker during the luteal phase (*M*=436.61ms, *SE*=35.39) than the menstrual phase (*M*=570.05 ms, *SE*=33.11). This was observed overall under both drug conditions and for both tasks, though follow up comparisons of the phase x drug x task interaction revealed a significant difference between groups (luteal > menstrual) for the 3-back only under both caffeine ($p = .036$) and placebo ($p = .024$) conditions.

Discussion

The aim of the present study was to examine the effects of caffeine on verbal working memory in women during different phases of the menstrual cycle. The goal was to predominantly provide information that can be generalized to the adolescent population as caffeine consumption continues to rise steeply amongst this age group. Data was analyzed using the P300 waveform in response to the commonly used n-back task in women during the luteal or menstrual phases of the menstrual cycle and separately under placebo and caffeine conditions. The hypotheses in the present study were that we expected caffeine would reduce reaction times and will reduce P300 latency, with the strongest effects being observed during the menstrual phase when baseline reaction times (RT's) and amplitudes are expected to be delayed and smaller, respectively. The hypotheses were generally verified as demonstrated by our results.

Amplitudes

Overall, there was not a significant main effect of drug on P300 amplitude, contrary to the original hypothesis. It was predicted that caffeine would reduce amplitudes, particularly during the menstrual phase. The lack of findings is contrary to previous findings (i.e. Pan, Takeshita, & Morimoto, 2000) which document caffeine-treatment conditions resulting in smaller P300 amplitudes.

There was a main effect of scalp site, due to larger amplitudes at the P3 (left region) site in comparison to the Pz (midline) and P4 (right) scalp sites. The mean amplitude was significantly larger in the left region suggesting an overall larger P300 amplitude in response to the verbal working memory task in that region of the brain compared to the midline and right regions. These findings suggest increased processing in the left region in response to the verbal working memory task, which is not surprising considering the copious amounts of research which indicate that language is highly lateralized in the left hemisphere of the brain (i.e. Sperry 1982; Waldie & Mosley, 2000; Vlachos, Andreou, & Delliou, 2013). Furthermore, previous research examining sLORETA EEG data during the n-back found increased activity in response to the 3-back in left cortices of the brain but not right (Imperatori et al., 2013).

The present results demonstrate a significant difference between tasks with larger amplitudes in response to the 1-back compared to the 3-back during the menstrual phase. During this phase, mean P300 amplitudes differed significantly between tasks regardless of drug. The decreased amplitude in response to the 3-back is consistent with previous findings that suggest increasing working memory demands result in an attenuated P300 amplitude (McEvoy, Smith, & Gevins, 1998). Moreover, larger P300 amplitudes have been observed in the menstrual phase (as compared to the ovulatory phase; O'Reilly, Cunningham, Lawlor, Walsh, & Rowan, 2004), which may contribute to the findings not being present in the luteal phase.

Although these results were only found in the menstrual phase, during the luteal phase, results showed a large effect size between tasks during the caffeine condition. These non-significant results are mentioned as they support significant latency and behavioural findings. Although only approaching significance (albeit with a large effect size), results indicated that the amplitude of the P300 was larger in response to the 1-back than the 3-back during the luteal phase and only under the caffeine condition. This suggests that caffeine may contribute to a decrease of P300 amplitude in the 3-back condition. This is consistent with previous findings that caffeine ingestion results in a decreased P300 amplitude (Pan, Takeshita, & Morimoto, 2000), but is different than the findings during the menstrual phase which found an increased

P300 amplitude in the 1-back in both drug conditions. This suggests that an increased P300 amplitude during the 1-back is observed in the menstrual phase regardless of drug but may only be present in the luteal phase under the influence of caffeine. These findings will be further analyzed with more statistical power (i.e. larger sample size) when the larger research project is complete.

In response to the 3-back, there was also a large effect size between phases with larger amplitudes present in the luteal phase as compared to the menstrual phase. These differences were not seen during the caffeine phase. This may propose a difference in P300 amplitude such that in the luteal phase, amplitude is increased under the placebo condition in response to the 3-back. This would mean that there is a greater P300 amplitude in response to the more complex task (the 3-back) during the luteal phase of the cycle, but only during the placebo condition and not during the caffeine condition. Taken together with the above results, this may be evidence to suggest that during the luteal phase, caffeine decreases P300 amplitude in response to the 3-back but may increase P300 amplitude in response to the 1-back. This would mean that during the luteal phase, caffeine invokes greater attention on a verbal working memory task only when the task is simple and greater arousal would be beneficial, as a larger P3 amplitude is thought to reflect greater attention (Sur & Sinha, 2009). However, these preliminary results should be interpreted with caution as they only approached significance and therefore definitive conclusions should not be drawn at this time. Due to large effect sizes, these findings should be duplicated with a larger sample size in order to establish significance.

P300 Latencies

Overall, there was no main effect of drug on P300 latency indicating caffeine did not improve latency in response to either the 1-back or the 3-back, contrary to the original hypothesis which held that caffeine would reduce P300 latency. This is inconsistent with findings which suggest that caffeine results in a shorter latency overall compared to placebo groups (Pan, Takeshita, & Morimoto, 2000). The inconsistencies may be due to a difference in dosage as the present study gave all participants 200 mg of caffeine, Pan et al., (2000) administered participants 5mg per 1 kg of bodyweight which would likely result in higher dosages (i.e. ~295 mg for a participant weighing 130 lbs, or 59 kg). Furthermore, reduced P300 latencies in this previous work were found in male participants only, and did not consider the hormonal fluctuations across the menstrual cycle.

The present results showed that during the placebo condition the 1-back had a significantly greater latency than the 3-back meaning participants had a quicker P300 response to the 3-back, which is counter to previous reports that P300 latency does not change across different n-back conditions (Watter, Geffen & Geffen, 2001). These differences had a moderate effect size and were not present in the caffeine condition. Furthermore, these findings were only significant in the luteal phase and not in the menstrual phase. As shorter latencies are thought to reflect superior mental performance (Sur & Sinha, 2009) and increased cognitive ability (Martin, Delpont, Suisse, Richelme, & Dolisi, 1993) these results suggest that participants are demonstrating better mental performance on the 3-back task than the 1-back task during the luteal phase, but only under the placebo condition, and not during the caffeine condition. This suggests that caffeine may serve to impair performance on the more complex 3-back during the luteal phase which is consistent with the above trends in amplitude findings. Conversely, caffeine may increase motivation to perform the relatively simple 1-back, as reflected by quicker processing.

Behavioural Data

In terms of accuracy (correct target identification), there was no main effect of phase or drug. This is contrary to our hypothesis that caffeine would reduce reaction times, but again is consistent with findings that caffeine may not change behavioural performance on accuracy despite changing P300 amplitude and latency (Pan, Takeshita, & Morimoto, 2000).

The results did show a significant main effect of task on accuracy. Predictably, participants were more accurate in detecting targets in the 1-back than the 3-back. This is not surprising considering the increased work load in the 3-back. Moreover, participants were significantly quicker to respond to the 1-back than the 3-back. This was true regardless of phase or drug. There is a notable difference between P300 latency and behavioural reaction time, as participants responded quicker to the 1-back (vs. the 3-back) despite having a slower P300, at least under placebo conditions. This suggests a disconnection between the recognition of a target and the initiation of motor responses to indicate such recognition.

Overall, there was a significant main effect of phase on reaction times, with participants responding quicker during the luteal phase than the menstrual phase. These findings are consistent with Symonds, Gallagher, Thompson, and Young (2004) who found that participants responded quicker to a continuous performance task involving letter detection during the luteal phase than the follicular phase, despite no significant differences in target detection. The findings here are a similar comparison showing faster response times in the luteal phase than the menstrual phase in a verbal working memory task despite lack of differences on accuracy. The larger research project will assess if these findings are also found in comparison to the follicular phase as found by Symonds, Gallagher, Thompson, and Young (2004). During the 3-back this interaction was significant with participants responding quicker during the luteal phase than the menstrual phase. This is consistent with the above latency findings that showed during the luteal phase under the placebo condition the 1-back had a significantly greater latency than the 3-back meaning participants were quicker to respond to the 3-back. As shorter latencies are thought to reflect superior mental performance (Sur & Sinha, 2009) these results suggest that participants are demonstrating better mental performance on the 3-back task than the 1-back task during the luteal phase, as demonstrated by the decreased reaction times.

There was also a significant interaction of phase, drug, and task so that in the menstrual phase during the 3-back task participants responded quicker during the caffeine condition than the placebo condition. This interaction was not present in the 1-back task or the luteal phase. This is consistent with our hypotheses which expected that caffeine would reduce reaction times and with the strongest effects being observed during the menstrual phase. However, we did not account for these effects to only be observed during the 3-back task.

Together, the results highlight the complex relationship between caffeine, the menstrual cycle, and cognition. Caffeine had an overall affect on reaction times, but not on ERP amplitude or latencies. Menstrual cycle phase had some impact on one version of the 1-back, but different results for the 3-back. Results showed differences between the 1-back and the 3-back such that caffeine and menstrual cycle phase may not only alter cognition in a domain-dependent manner, but may also alter different measurements of cognition (i.e. behaviours vs. neural), and depend on the complexity of the task (i.e. 1-back vs. 3-back). Therefore, results presented only begin to uncover the relations between these variables and necessitate future research in this area.

Implications

Phase. Collectively, the findings suggest that performance during the 1-back consistently elicited an increased P300 amplitude during the menstrual phase, which is consistent with the literature and indicative of increased attention. This is also indicative of a task dependent relationship between the simple 1-back and the more complex 3-back. These task-dependent findings would also be consistent with the literature which found that mensural phase affects performance depending on the task (Thimm et al., 2014). Behavioural data also showed that participants were quicker to respond during the luteal phase, regardless of drug and task, despite no differences in accuracy. This suggests that females during the menstrual phase may display increased attention to simple verbal working memory tasks. However, participants during the luteal phase are quicker to respond to verbal working memory tasks. As there has been an increasing body of research establishing the presence of estrogen receptors in the hippocampus (Islam, Sparkes, Roodenrys, & Astheimer, 2008), and estrogen levels are increasing in the luteal phase compared to the menstrual phase, which is when estrogen is at its lowest baseline level (Farage, Osborn, & MacLean, 2008), the present results suggest this increase in estrogen during the luteal phase is interacting with the estrogen receptors in the hippocampus, which in turn increases reaction times in response to a verbal working memory task.

Caffeine. In the menstrual phase during the 3-back task participants responded with quicker reaction times during the caffeine condition than the placebo condition. This suggests that caffeine increases response times in the menstrual phase during complex verbal working memory tasks. However, during the luteal phase caffeine may only increase attention during the 1-back. Although the amplitude findings only showed large effect sizes, during the luteal phase, the latencies during the luteal phase definitively showed that under placebo conditions participants had significantly shorter latencies reflecting better performance on the 3-back. This was consistent with behavioural findings that found participants responded quicker to the 3-back during the luteal phase. This would indicate that caffeine may increase attention (as indicated by increased P300 amplitude) in response to the 1-back but impairs (increases P300 latency) during the 3-back. Despite this, participants responded quicker during the caffeine condition, regardless of task, particularly in the luteal phase.

The present research points to the potential necessity for caffeine-consuming adolescents to consider the effects this seemingly innocuous drug may have on their academic achievement, particularly as it relates to the hormonal fluctuations across the menstrual cycle. Although presently only preliminary findings and needing clarity, conclusions in this study would suggest that young females consider abstaining from or reducing caffeine intake during the luteal phase of their menstrual cycle when they need to perform complex verbal working memory tasks as it may impair performance. This finding was shown with significantly delayed P300 latencies. Although caffeine increased reaction time during this phase, it did not increase accuracy. Furthermore, the results showed that not only does caffeine and the menstrual cycle influence performance on various tasks, but may also differently alter behavioural (i.e. reaction times) and cognitive mechanisms (i.e. amplitude and latencies) within the same task. This lack of clarity points to the complexity of this phenomenon, the task dependent nature, and overall, a need for more research.

Although eminent psychologist Doreen Kimura warns that even though the female menstrual cycle has such effects, it is no reason to believe that males are excluded from differences across various cycles, as she references research that indicates differences across male biological cycles (Kimura, as cited in Weiss, 1988). As females are largely understudied in neuroscience (Kromrey, Czoty, & Nader, 2015) it is important to continue research with this population. Continuing research in this area will elucidate the effects of caffeine on cognition across the menstrual cycle and will allow females to make informed decisions when consuming this lifelong drug.

Limitations

Some caveats to this research include the breadth of data available to date which would allow the inclusion of the follicular phase, natural vs. hormone-mediated menstrual cycles, and an overall larger sample size to be analyzed. At present, many of the findings in the present study are limited due to lack of statistical power, and therefore final conclusions are reserved until the final phase of the larger research project. Furthermore, time constraints did not allow for salivary analysis to take place in order to verify menstrual cycle phase, which is important when doing menstrual cycle research as demonstrated by Hausmann et al. (2000). These caveats may contribute to the lack of clear findings. Limitations also include a one-time dosage of caffeine (200 mg) preventing dose-dependent differences from being assessed and a single raw dosage for all participants (rather than dosing using mg/kg).

Future Research

As part of a larger research project, the data from this project in combination with additional data, will be analyzed. In addition to the analyses presented in this project, the larger research project will analyze salivary samples to verify menstrual phase. Furthermore, the larger research project will analyze the follicular phase in addition to the menstrual and luteal phase. The sample will also be large enough to analyze natural versus hormone-mediated menstrual phases. Other aspects of cognition will also be analyzed, including sustained attention and selective attention. The larger research project is also collecting data on regular caffeine consumption by requiring each participant to complete a Caffeine Consumption Questionnaire (CCQ) to assess caffeine consumption, including the source of caffeine consumption (tea, soda, coffee, chocolate, etc.) and time period of caffeine consumption. This will provide more in-depth information regarding the effects caffeine has on cognition throughout the menstrual cycle.

Although the focus of this research was to generalize the information to adolescents, the study population was pre-menopausal women between the ages of 16 and 41. Future research should seek to take this one step further, specifically studying the effects caffeine has on adolescent cognition throughout the menstrual cycle and examining a sample comprised entirely of adolescent participants (i.e. 15-19-year-olds). This will allow for the effects of caffeine on this population to be more fully investigated understood.

Conclusion

Caffeine is a drug which can be consumed over the entirety of a lifetime. Specifically, adolescents are increasingly consuming caffeine with the suggestion that adolescents represent the highest population of energy drink consumers (Rudolph, Faerbinger, & Koenig, 2014). Baring this in mind, this drug can influence cognition and the menstrual cycle, however the way in which these variables interact is still under investigation.

By continuing this research, perhaps the perception of caffeine as a seemingly harmless drug can shift to a perception that considers the implications of consuming such a readily available product. It is hoped that research in this area may contribute to health education for young females so that they can make informed decisions about their caffeine consumptions and its effects on their cognitive abilities.

Clinical Implications. Although only preliminary findings, this research suggests that women should be considering the effects caffeine has on cognition, particularly on various tasks across the menstrual cycle. Therefore, further research is needed to inform educational material so pre-menopausal women can make informed decisions regarding caffeine consumption throughout their menstrual cycle.

Specifically, it is hoped that further research in this area will consider the need for the distribution of information amongst adolescents as they are increasingly consuming vast amounts of caffeine. As school psychologists are typically the most qualified mental health professionals working in schools (Sheridan & Gutkin, 2000) it is important that they be included in disseminating these findings. One recommendation would be for this information to be distributed by school psychologists in the educational system. As researchers, school psychologists may be better able to access and understand the information through their own conference or professional development days. School psychologists can then disperse the information to teachers throughout their job or through their own professional development presentations. Secondary (junior high and high school) teachers may then incorporate this material into their health curriculum.

Additionally, depending upon the findings of future research, school psychologists may need to take into consideration a female adolescents caffeine intake during phases of the menstrual cycle when conducting or interpreting certain psychoeducational assessment tasks. For instance, some tasks within the psychoeducation assessment may be impaired by increased caffeine intake during a particular phase of the menstrual cycle. Future research may show such an impact on various tasks that it may be necessary for school psychologists to consider asking adolescents their caffeine consumption before beginning assessment procedures. N-back task performance may be specifically crucial to psychoeducational assessments as training on the n-back has been associated with improved matrix reasoning (Jaeggi et al., 2010), a task frequently used by school psychologists as part of the WISC-V to interpret fluid reasoning and overall cognitive abilities (Roberts, 2017). This means that there may be overlapping cognitive abilities required for the n-back and matrix reasoning (Jaeggi et al., 2010), making findings surrounding this task applicable to school psychologists in their everyday practice.

Figure 1. N-Back task.

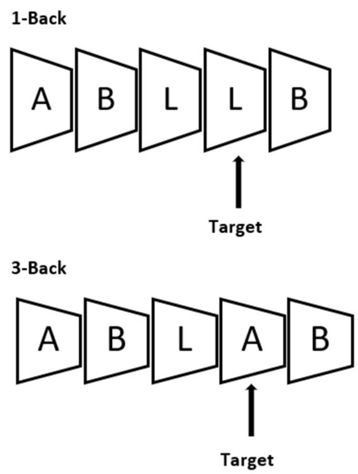


Table 1

Means and Standard Error of P300 Amplitude (in microvolts μV) at the Pz Scalp Site, N=16.

Drug	Phase	Task	M	*SE*
Caffeine	Menstrual	1-Back	13.37	2.84
		3-Back	7.01	1.12
	Luteal	1-Back	11.86	1.01
		3-Back	8.23	1.28
Placebo	Menstrual	1-Back	13.17	2.67
		3-Back	7.57	1.76
	Luteal	1-Back	11.11	1.22
		3-Back	9.97	1.26

Table 2

Means and Standard Error of N-Back Accuracy, N=16.

Drug	Phase	Task	M	*SE*
Caffeine	Menstrual	1-Back	0.92	0.05
		3-Back	0.55	0.08
	Luteal	1-Back	0.98	0.01
		3-Back	0.60	0.09
Placebo	Menstrual	1-Back	0.93	0.03
		3-Back	0.56	0.03
	Luteal	1-Back	0.96	0.02
		3-Back	0.54	0.06

Table 3

Means and Standard Error of N-Back Reaction Time (ms), N=16.

Drug	Phase	Task	M	*SE*
Caffeine	Menstrual	1-Back	474.09	40.63
		3-Back	633.97	47.67
	Luteal	1-Back	340.36	54.31
		3-Back	487.37	36.75
Placebo	Menstrual	1-Back	471.72	28.57
		3-Back	700.40	47.00
	Luteal	1-Back	410.69	14.66
		3-Back	508.01	56.19

Table 4

Participant Demographic Data.

Phase	**Average Caffeine Consumption (CCQ)**	**Morningness-Eveningness (MEQ)**
Menstrual, N=8	*M*=1692.69mg/week,	Neither, N=5
	SE=433.18	Moderately Morning, N=2
		Moderately Evening, N=1
Luteal, N=8	*M*=1308.61 mg/week,	Neither, N=6
	SE=237.13	Moderately Morning, N=1
		Moderately Evening, N=1

References

Acheson, D.J., & MacDonald, M.C. (2009). Verbal working memory and language production: Common approaches to the serial ordering of verbal information. *Psychol Bull, 135* (1), 50-68.

Adams, A. M., & Gathercole, S. E. (1996). Phonological working memory and spoken language development in young children. *Quarterly Journal of Experimental Psychology, 49*, 216–233.

Adan, A., Prat, G., Fabbri, M., & Sanchez-Turet, M. (2008). Early effects of caffeinated and decaffeinated coffee on subjective state and gender differences. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 32*, 1698 –1703.

Alloway, T.P., & Alloway, R. G. (2010). Investigating the predictive roles of working memory and IQ in academic attainment. *Journal of Experimental Child Psychology, 106*(1), 20-29.

Anderson, K.J. & Revelle, W. (1983). The interactive effects of caffeine, impulsivity and task demands on a visual search task. *Personality and Individual Differences, 4,* 127-134

Asthana, S. (2003). Estrogen and cognition: The story so far. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 58*(4), M322-M323.

Asthana, S., & Middleton, W. (2004). Estrogen and cognition: A true relationship? *Journal of the American Geriatrics Society, 52*(2), 316-318.

Baddeley, A. (1996). The fractionation of working memory. *Proc Natl Acad Sci USA,93*, 13468–13472.

Baddeley, A., Hitch, G., Butters, Nelson, & Becker, James T. (1994). Developments in the Concept of Working Memory. *Neuropsychology, 8*(4), 485-493.

Barros, L.A., Tufik, S., and Andersen, M.L. (2015). The role of progesterone in memory: An overview of three decades. *Neuroscience and Biobehavioral Reviews, 49,* 193-204.

Bayliss, D.M., Jarrold, C., Baddeley, A.D., Gunn, D.M .(2005). The relationship between short-term memory and working memory: Complex span made simple? *Memory,13*414–421.

Bean, L., Ianov, L., & Foster, T. (2014). Estrogen receptors, the hippocampus, and memory. *The Neuroscientist, 20*(5), 534-545.

Botella, P., & Parra, A. (2003). Coffee increases state anxiety in males but not in females. *Human Psychopharmacology: Clinical and Experimental, 18*(2), 141-143.

Bruce, S., Werner, K., Preston, B., & Baker, L. (2014). Improvements in concentration, working memory and sustained attention following consumption of a natural citicoline-caffeine beverage. *International Journal of Food Sciences and Nutrition, 65*(8), 1003-7.

Corrigan, E. (1985). Gender differences in alcohol and other drug use. *Addictive Behaviors, 10*(3), 313-7.

Curran, C., & Marczinski, C. (2017). Taurine, caffeine, and energy drinks: Reviewing the risks to the adolescent brain. *Birth Defects Research, 109*(20), 1640-1648.

Den Bosch, G., Marroun, H., Schmidt, M., Tibboel, D., Manoach, D., Calhoun, V., & White, T. (2014). Brain connectivity during verbal working memory in children and adolescents. *Human Brain Mapping, 35*(2), 698-711.

Einöther, S., & Giesbrecht, J. (2013). Caffeine as an attention enhancer: Reviewing existing assumptions. *Psychopharmacology, 225*(2), 251-274.

Epperson, C., Amin, Z., Ruparel, K., Gur, R., & Loughead, J. (2012). Interactive effects of estrogen and serotonin on brain activation during working memory and affective processing in menopausal women. *Psychoneuroendocrinology, 37*(3), 372-82.

Fan, J., Mccandliss, B., Fossella, J., Flombaum, J., & Posner, M. (2005). The activation of attentional networks. *NeuroImage, 26*(2), 471-9.

Faraco,C.C., Unsworth, N., Langley, J., Terry, D., Li, K., Zhang, D., Liu, T., & Miller, L.S. (2011). Complex span tasks and hippocampal recruitment during working memory. *Neuroimage* . *55*,773–787.

Farage, M.A., Neill, S., & MacLean, A.B. (2009). Physiological changes associated with the menstrual cycle: A review. *Obstetrical and Gynecological Survey, 64 (1),* 58-72.

Farage, M., Osborn, A., & MacLean, T. (2008). Cognitive, sensory, and emotional changes associated with the menstrual cycle: A review. *Archives of Gynecology and Obstetrics, 278*(4), 299-307.

Fenster, L., Quale, C., Waller, K., Windham, G., Elkin, E., Benowitz, N., & Swan, S. (1999). Caffeine consumption and menstrual function. *American Journal of Epidemiology, 149*(6), 550-7.

Ferré, S. (2008). An update on the mechanisms of the psychostimulant effects of caffeine. *Journal of Neurochemistry, 105*(4), 1067-1079.

Finn, A.S., Sheridan, M.A., Kam ,C.L., Hinshaw ,S., & D'Esposito, M. (2010). Longitudinal evidence for functional specialization of the neural circuit supporting working memory in the human brain. *J Neurosci, 30,* 11062–11067.

Forns, Esnaola, López-Vicente, Suades-González, Alvararez-Pedrerol, Julvez, . . . Sunyer. (2014). The n-back Test and the Attentional Network Task as Measures of Child Neuropsychological Development in Epidemiological Studies. *Neuropsychology, 28*(4), 519-29.

Gibbs. R.B., & Gabor, R. (2003)/ Estrogen and cognition: Applying preclinical findings to clinical perspectives. *J Neurosci Res. 74*, 637–643.

Gibbs, F.A., & Maltby, G.L. (1943). Effect on the electrical activity of the cortex of certain depressant and stimulant drugs—barbiturates, morphine, caffeine, benzedrine and

adrenalin. *Journal of Pharmacology and Experimental Therapeutics, 78* ,1-10

Grigorova, M., Sherwin, B.B., & Tulandi, T. (2006). Effects of treatment with leuprolide acetate depot on working memory and executive functions in young premenopausal women. *Psychoendocrinology, 31,* 935-947.

Hampson, E. (1990). Estrogen-related variations in human spatial and articulatory-motor skills. *Psychoneuroendocrinology, 15*(2), 97-111. doi:10.1016/0306-4530(90)90018-5

Hampson, E., & Kimura, D. (1988). Reciprocal effects of hormonal fluctuations on human motor and perceptual-spatial skills. *Behavioral Neuroscience, 102*, 456-459.

Hampson, E., Levy-Cooperman, N., & Korman, J. (2014). Estradiol and mental rotation: Relation to dimensionality, difficulty, or angular disparity? *Hormones and Behavior, 65*(3), 238-248.

Harnack, L, Stang, J, & Story, M. (1999). Soft drink consumption among US children and adolescents: Nutritional consequences. *Journal of the American Dietetic Association, 4* (99), 436-441.

Hausmann M. & Gunturkun, O. (2000) Steroid fluctuations modify functional cerebral asymmetries: The hypothesis of progesterone-mediated interhemispheric decoupling. *Neuropsychologia 38*,1362–1374.

Hausmann, M., Slabbekoorn, D., Van Goozen, S., Cohen-Kettenis, P., Güntürkün, O., & Gallagher, Michela. (2000). Sex hormones affect spatial abilities during the menstrual cycle. *Behavioral Neuroscience, 114*(6), 1245-1250.

Hering-Hanit, R., & Gadoth, N. (2003). Caffeine-induced headache in children and adolescents. *Cephalalgia, 23*(5), 332-335.

Horne, J.A., & Ostberg, O. (1976). A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. *International Journal of Chronobiology,4,* 97-110.

Hyde, J. (2016). Sex and cognition: Gender and cognitive functions. *Current Opinion in Neurobiology, 38*, 53-6.

Imperatori, C., Farina, B., Brunetti, R., Gnoni, V., Testani, E., Quintiliani, M., . . . Della Marca, G. (2013). Modifications of EEG power spectra in mesial temporal lobe during n-back tasks of increasing difficult. *Frontiers in Human Neuroscience, 7* (2013), 109.

Islam, F., Sparkes, C., Roodenrys, S., & Astheimer, L. (2008). Short-term changes in endogenous estrogen levels and consumption of soy isoflavones affect working and verbal memory in young adult females. *Nutritional Neuroscience, 11*(6), 251-262.

Jacobs, E., Weiss, B., Makris, N., Whitfield-Gabrieli, S., Buka, S., Klibanski, A., & Goldstein, J. (2017). Reorganization of functional networks in verbal working memory circuitry in early midlife: The impact of sex and menopausal status. *Cerebral Cortex, 27(5)*, 2857-2870.

Jacola, L., Willard, V., Ashford, J., Ogg, R., Scoggins, M., Jones, M., . . . Conklin, H. (2014). Clinical utility of the N-back task in functional neuroimaging studies of working memory. *Journal of Clinical and Experimental Neuropsychology, 36*(8), 1-12.

Jaeggi, Susanne M., Studer-Luethi, Barbara, Buschkuehl, Martin, Su, Yi-Fen, Jonides, John, & Perrig, Walter J. (2010). The Relationship between N-Back Performance and Matrix Reasoning--Implications for Training and Transfer. *Intelligence, 38*(6), 625-635.

Kim, S., & Kim, M.S. (2016). Deficits in verbal working memory among college students with attention-deficit/hyperactivity disorder traits: An event-related potential study. *Clinical Psychopharmacology and Neuroscience : The Official Scientific Journal of the Korean College of Neuropsychopharmacology, 14*(1), 64-73.

Kimura, D., & Hampson, E. (1994). Cognitive pattern in men and women is influenced by fluctuations in sex hormones. *Current Directions in Psychological Science, 3*(2), 57-61.

Koppelstaetter, Poeppel, Siedentopf, Ischebeck, Verius, Haala, . . . Krause. (2008). Does caffeine modulate verbal working memory processes? An fMRI study. *NeuroImage, 39*(1), 492-9.

Korol, D., & Pisani, S. (2015). Estrogens and cognition: Friends or foes?: An evaluation of the opposing effects of estrogens on learning and memory. *Hormones and Behavior,74*, 105-15.

Kramer J.H., Delis D.C., & Daniel M. (1998). Sex differences in verbal learning. J. *Clin. Psychol., 44,* 907-915.

Kromrey, S., Czoty, P., & Nader, M. (2015). Relationship between estradiol and progesterone concentrations and cognitive performance in normally cycling female cynomolgus monkeys. *Hormones and Behavior, 72*, 12-19.

Landnum, R.E. (1992). College students' use of caffeine and its relationship to personality. *College Student Journal, 26,* 151-155.

Lane, J., Steege, D., Rupp, F., & Kuhn, S. (1992). Menstrual cycle effects on caffeine elimination in the human female. *European Journal of Clinical Pharmacology, 43*(5), 543-546.

LeBlanc, E. S., Janowsky, J., Chan, B. K. & Nelson, H. D. (2001). Hormone replacement therapy and cognition: systematic review and meta-analysis. *Journal of the American Medical Association 285,* 1489–1499.

Lokken, K., & Ferraro, F. (2006). The Relationship Between Menopausal Status, Phase of Menstrual Cycle, and Replacement Estrogen on Cognition in Healthy Women Without Dementia. *The Journal of Psychology, 140*(6), 533-47.

Lorist, M.M. & Tops, M. (2003). Caffeine, fatigue, and cognition. *Brain and Cognition, 53(1),* 82-94.

Luck, S. J., Mathalon, D. H., O'Donnell, B. F., Hämäläinen, M. S., Spencer, K. M., Javitt, D. C., & Uhlhaas, P. J. (2011). A roadmap for the development and validation of event-related potential biomarkers in schizophrenia research. *Biological Psychiatry, 70,* 1, 28-34.

Marchand, Y., Lefebvre, C., & Connolly, J. (2006). Correlating digit span performance and event-related potentials to assess working memory. *International Journal of Psychophysiology : Official Journal of the International Organization of Psychophysiology, 62*(2), 280-9.

Marvel, Cherie L., & Desmond, John E. (2012). From storage to manipulation: How the neural correlates of verbal working memory reflect varying demands on inner speech. *Brain and Language, 120*(1), 42-51.

McEvoy, L., Smith, M., & Gevins, A. (1998). Dynamic cortical networks of verbal and spatial working memory: Effects of memory load and task practice. *Cerebral Cortex, 8*(7), 563-574.

McEwen, B.S. (2002). Estrogen actions throughout the brain. *Recent Prog Horm Res, 57*, 357–384

McEwen, B.S. & Alves, S.E.(1999). Estrogen actions in the central nervous system. *Endocr. Rev., 20*, 279-307

Moore, A., Li, Z., Tyner, C., Hu, X., & Crosson, B. (2013). Bilateral basal ganglia activity in verbal working memory. *Brain and Language, 125*(3), 316-23.

Mordecai, K., Rubin, L., & Maki, P. (2008). Effects of menstrual cycle phase and oral contraceptive use on verbal memory. *Hormones and Behavior, 54*(2), 286-93.

Morgan, K.J., Stults, V.J., & Zabik, M.E. (1982). Amount and dietary sources of caffeine and saccharin intake by individuals ages 5 to 18 years. *Regulatory Toxicology and Pharmacology, 2* (4), 296-307.

Morrison, J.H., Briton, R.D., Schmidt, P.J., Gore, A.C. (2006). Estrogen, menopause, and the aging brain: how basic neuroscience can inform hormone therapy in women. *J Neurosci . 26,*10332–10348.

Mucignat-Caretta, C. (1998). Changes in female cognitive performance after energetic drink consumption: A preliminary study. *Progress in Neuro-psychopharmacology & Biological Psychiatry, 22*(6), 1035-42.

Onaolapo, A.Y., & Onaolapo, O.J. (2015). Caffeine's influence on object recognition and working-memory in prepubertal mice and its modulation by gender. *Pathophysiology : The Official Journal of the International Society for Pathophysiology, 22*(4), 223-30.

O'Neill, C., Newsom, R., Stafford, J., Scott, T., Archuleta, S., Levis, S., . . . Bachtell, R. (2016). Adolescent caffeine consumption increases adulthood anxiety-related behavior and modifies neuroendocrine signaling. *Psychoneuroendocrinology, 67*, 40-50.

O'Reilly, M., Cunningham, C., Lawlor, B., Walsh, C., & Rowan, M. (2004). The effect of the menstrual cycle on electrophysiological and behavioral measures of memory and mood. *Psychophysiology, 41*(4), 592-603.

Orbeta, R.L., Overpeck, M.D., Ramcharran, D., Kogan, M.D., & Ledsky. R*p*. (2006). High caffeine intake in adolescents: Associations with difficulty sleeping and feeling tired in the morning. *Journal Of Adolescent Health, 38*(4), 451-453.

Pan, J., Takeshita, T., & Morimoto, K. (2000). Acute caffeine effect on repeatedly measured P300. *Environmental Health and Preventive Medicine, 5*(1), 13-17.

Peralta, L., Rowling, L., Samdal, O., Hipkins, R., & Dudley, D. (2017). Conceptualising a new approach to adolescent health literacy. *Health Education Journal, 76*(7), 787-801.

Postma, A., Winkel, J., Tuiten, J.,& van Honk, V.J. (1999). Sex differences and menstrual cycle effects in human spatial memory. *Psychoneuroendocrinology, 24*, 175-192.

Rapp, P.R., Morrison, J.H., & Roberts, J.A. (2003). Cyclic estrogen replacement improves cognitive function in aged ovariectomized rhesus monkeys. *J Neurosci . 23*, 5708–5714.

Roberts, C. (2017). Wechsler intelligence ccales for children – Fifth edition (WISC-V). *Educational Psychology in Practice, 33*(1), 108-109.

Rosenberg, L., & Park, S. (2002). Verbal and spatial functions across the menstrual cycle in healthy young women. *Psychoneuroendocrinology, 27*(7), 835-41.

Rudolph, E., Faerbinger, A., & Koenig,J. (2014). Caffeine intake from all sources in adolescents and young adults in Austria. European Journal of Clinical Nutrition, 68(7), 793-798.

Saeed, M., Hellström, B., & Sandberg, E. (2009). Effects of the menstrual cycle on verbal working memory in young women. (Unpublished dissertation). Stockholms Universitet, Stockholm, Sweden.

Schliep, K., Schisterman, E., Wactawski-Wende, J., Perkins, N., Radin, R., Zarek, S., . . . Mumford, S. (2016). Serum caffeine and paraxanthine concentrations and menstrual cycle function: Correlations with beverage intakes and associations with race, reproductive hormones, and anovulation in the BioCycle Study. *The American Journal of Clinical Nutrition, 104*(1), 155-63.

Sheridan, S. M., & Gutkin, T. B. (2000). The ecology of school psychology: Examining and changing our paradigm for the 21st century. *School Psychology Review, 29*, 485-502.

Sherman, B.M., & Korenman, S.G. (1975). Hormonal characteristics of the human menstrual cycle throughout reproductive life. *The Journal of Clinical Investigation, 55 (4),* 699-706.

Sherwin, B.B. (2003). Estrogen and cognitive functioning in women. *Endocrine Reviews, 24 (2)*, 133-151.

Sperry, R.W. (1982). Some effects of disconnecting the cerebral hemispheres. *Science, 217,* 1223-1227.

Sur, S., & Sinha, V. K. (2009). Event-related potential: An overview. *Industrial Psychiatry Journal*, *18*(1), 70–73. http://doi.org/10.4103/0972-6748.57865

Symonds, C., Gallagher, P., Thompson, J., & Young, A. (2004). Effects of the menstrual cycle on mood, neurocognitive and neuroendocrine function in healthy premenopausal women. *Psychological Medicine, 34*(1), 93-102.

Temple, J. (2009). Caffeine use in children: What we know, what we have left to learn, and why we should worry. *Neuroscience and Biobehavioral Reviews, 33*(6), 793-806.

Thimm, M.,Weis,S.,Hausmann, M., & Sturm, W. (2014). Menstrual cycle effects on selective attention and its underlying cortical networks. *Neuroscience, 258*, 307-17.

Ullrich, S., Vries, Y.C. de, Kuhn, S., Repantis, D., Dresler, M., & Ohla, K. (2015). Feeling smart: Effects of caffeine and glucose on cognition, mood and self-judgment. *Physiology and Behavior, 151*, 629-637.

Van de Weijer-Bergsma, E., Kroesbergen, E., & Van Luit, J. (2015). Verbal and visual-spatial working memory and mathematical ability in different domains throughout primary school. *Memory and Cognition, 43*(3), 367-378.

Verhagen, Josje, & Leseman, Paul. (2016). How do verbal short-term memory and working memory relate to the acquisition of vocabulary and grammar?: A comparison between first and second language learners. *Journal of Experimental Child Psychology, 141*, 65-82.

Vlachos, Filippos, Andreou, Eleni, & Delliou, Afroditi. (2013). Brain hemisphericity and developmental dyslexia. *Research in Developmental Disabilities: A Multidisciplinary Journal,34*(5), 1536-1540.

Waldie, K., & Mosley, J. (2000). Hemispheric specialization for reading. *Brain and Language, 75,* 108-122.

Walpurger, V., Pietrowsky, R., Kirschbaum, C., & Wolf, O. (2004). Effects of the menstrual cycle on auditory event-related potentials. *Hormones and Behavior, 46*(5), 600-6.

Watter, S., Geffen, G.N., & Geffen, L.B. (2001). The n-back as a dual task: P300 morphology under divided attention. *Psychophysiology, 38* (6), 998-1003.

Weiss, R. (1988). Women's skills linked to estrogen levels. *Science News, 134*(22), 341.

Wolk, B. J., Ganetsky, M., & Babu, K. M. (2012). Toxicity of energy drinks. *Curr Opin Pediatr, 24,* 243–251.

Wong, A., Ye, M., Levy, A., Rothstein, J., Bergles, D., & Searson, P. (2013). The blood-brain barrier: An engineering perspective. *Frontiers in Neuroengineering, 6*, 7.

Woolley, C.S., McEwen, B.S. (1994). Estradiol regulates hippocampal dendritic spine density via an N-methyl-D-aspartate receptor-dependent mechanism. *J Neuroscience, 14*,7680–7687.

Yaffe, K., Grady, D., Pressman, A., & Cummings, S. (1998). Serum estrogen levels, cognitive performance, and risk of cognitive decline in older community women. *Journal of the American Geriatrics Society, 46*(7), 816-821.

Appendix A

CONSENT FORM

Title of Proposal: The Impact of Caffeine on Cognition Across the Menstrual Cycle

Principal Investigator: Derek Fisher, Ph.D.

Associate Investigators: Tara Perrot, Ph.D.

Statement: I, ________________, agree to participate in the above described research project, the nature and possible complications of which have been explained to me as outlined in the attached Information Letter of which I have received a copy.

I agree not to consume alcohol, recreational drugs and medications (except oral contraceptives) beginning at 8pm on the evening prior to the test session.

I understand the risks and benefits of the study that have been explained to me.

I understand that I may skip any questions I do not wish to answer and may withdraw from the study at any time without penalty.

I will not be identified in any scientific presentation or publication.

I may keep a copy of this consent form (with one copy being kept by the study investigators) and I may withdraw from participation at any time.

________________________	____________________	_________________
Name of Volunteer (printed)	Signature of Volunteer	Date
________________________	____________________	_________________
Name of Person Conducting	Signature	Date

Appendix B

CAFFEINE AND COGNITION ACROSS THE MENSTRUAL CYCLE

Screening Questionnaire

Date: __________________ ID#: ______________

Name: ______________________________ Age: _____ DOB (dd/mm/yy): ____________

Sex: _____ Education:____________________________

Classification: *HCM/ HCF/ HCL NCM/ NCF/ NCL*

Handedness: *Left* / *Right* Normal hearing: *Y* / *N* Vision: *Normal* / *Corrected*

Telephone: (h) ____________________________

EXCLUSION CRITERIA

- Do you have a regular menstrual cycle? Y/ N
- Have you been pregnant in the last year or intending to become pregnant in the next 3 months? Y / N
- Are you currently breastfeeding? Y / N
- Are you currently on medication on a regular basis for any physical condition? Y / N
- Are you currently using pain medication on any basis? ____________________
- Steroid use within the past 3 months (If so, delay testing)?
- Have you ever been diagnosed with a psychiatric or mental illness e.g. depression, anxiety? Y / N

If yes: Are you still being seen for treatment? _______

Do you currently receive medication for these problems? ________

- When was your last medical check up
- Have you ever been diagnosed with a learning disability?

If yes: What disorder was diagnosed?

- Have you had a head or brain injury in the past 6 months? _______

If yes, did you lose consciousness for one or more hours? _______

- Do you work shift work? Y / N
- Do you have a regular sleep cycle? Y / N Average hours per night? _______

To the best of your knowledge:

- Do you have any other neurological disorders such as epilepsy, dementia, Parkinson's disease? Y / N
    - If yes, which: ____________
- What is your daily (or weekly) alcohol consumption? _______
- Have you ever smoked? Y / N If yes:
        - Do you currently smoke?: _____________
        - How many cigarettes/day: ______
- How many cups of caffeinated beverage do you drink per day? ______(min. 1 cup/day)
- Do you use street drugs? _______

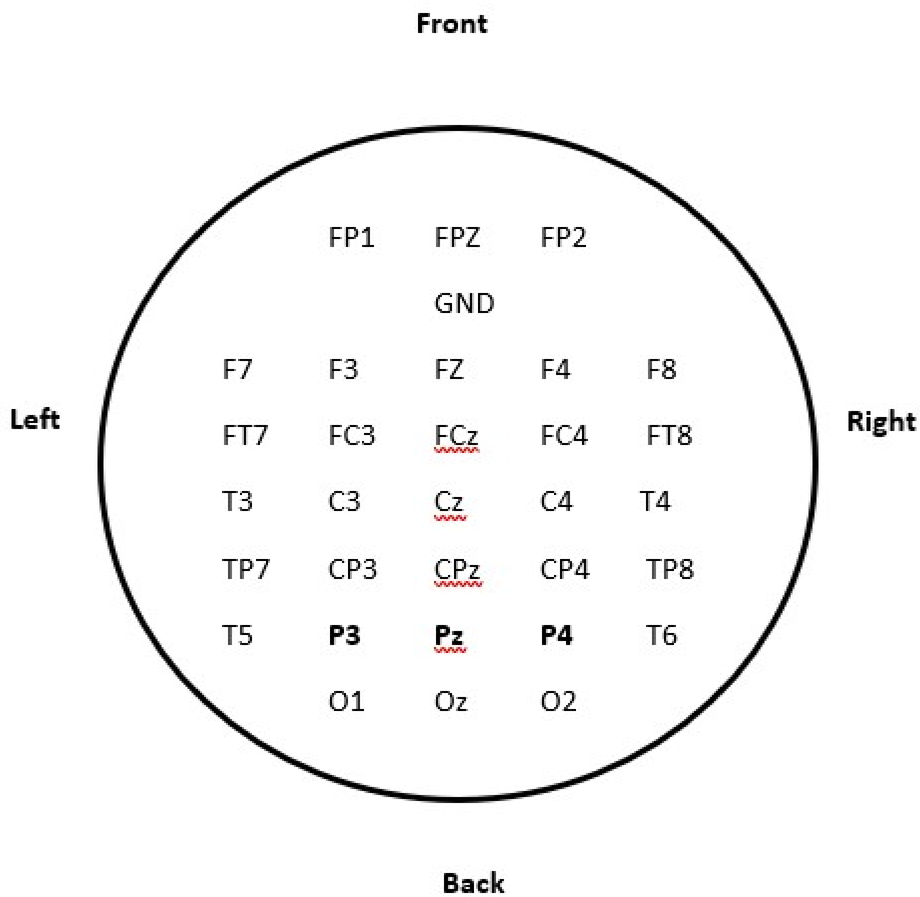
If yes: How often? _______

What drugs? _________________________

Verbal assent to abstain: _________________________

Appendix C

Scalp Site Map



Appendix D

INFORMATION SHEET

STUDY TITLE: The Impact of Caffeine on Cognition Across the Menstrual Cycle

PRINCIPAL INVESTIGATOR: Derek Fisher, Ph.D.

Department of Psychology, Mount Saint Vincent University

EVAR 430 – 166 Bedford Hwy, Halifax, NS

P: 457.5503; E: derek.fisher@msvu.ca

ASSOCIATE INVESTIGATORS: Tara Perrot, Ph.D.

Department of Psychology and Neuroscience, Dalhousie University

INTRODUCTION

Please read and carefully consider the following information before you give your consent to participate in this study. This information sheet describes the purpose and procedures and the possible risks and benefits of the proposed research. You are encouraged to discuss any questions with the study investigators and other members of the research laboratory. You will receive a copy of this information sheet to keep for your records if you consent to participate.

BACKGROUND

Caffeine is the most used psychoactive drug in Canada, with regular consumption by 88% of the adult population. Nearly all caffeine comes from dietary sources, including coffee, tea, cocoa beverages, chocolate bars, and soft drinks. After oral consumption, 99% of the ingested caffeine is rapidly absorbed from the gastrointestinal tract into the bloodstream, and is able to pass through all biological membranes including the blood-brain barrier and the placental barrier, allowing widespread distribution throughout the body.

The human menstrual cycle (HMC) is characterized by variations in sex hormones (estradiol, progesterone) over a period typically lasting 28-29 days, although there is significant inter-individual variation. The HMC is often conceptualized as occurring in distinct phases, each with its own defined sex hormone levels. While there is a relatively small, but important, body of research reporting differences in cognitive performance across varying menstrual phases, there is virtually no work examining how psychoactive drugs may impact cognition within this context.

PURPO*SE*

The primary purpose of this research is to examine how caffeine impacts cognitive processes across the menstrual cycle in both naturally-cycling (NC) and hormonal birth control medicatedcycling (HC) women. This study will employ a randomized, placebo-controlled, double-blind, repeated measures design.

PROCEDURES

You will be asked to abstain from caffeine-containing food & drink (coffee, tea, energy drinks, pop, chocolate), alcohol, nicotine, and recreational drugs beginning at 8pm on the evening prior to the test session.

You will be asked to participate in two sessions with approximately 28 days (depending on the length of your typical menstrual cycle) between each session. Upon arrival at the laboratory, you will fill a small tube with saliva for later analysis on estradiol and progesterone levels. On one of the days you will be asked to swallow a pill containing 200mg of pure caffeine, while in the other session you will be asked to swallow a pill containing a placebo substance. On both test days, neither you nor the researchers will know what drug you received. Following this, EEG electrodes will be applied to different areas of the scalp and face and, 30 minutes after drug administration (approx. time for absorption of caffeine) you will be assessed on three different cognitive measures: 1) Visual search; 2) Visual sustained attention; 3) Verbal working memory; and 4) Auditory change detection. All of the tasks will require responding (by the pressing of a computer key) when you detect specific visual stimuli. Each session will be identical, with the exception of the contents of the pill.

Questionnaires regarding caffeine consumption and demographic information will also be completed at the beginning of the first test session. You have the right to skip any questions you do not wish to answer. Women who do not consume any caffeine will be excluded from the study.

PARTICIPATION

Your participation is voluntary and you may withdraw from the study at any time without penalty. You will be compensated $50 ($25 at the end of each session) to cover meals/snacks and transportation/parking costs; withdrawing from the study once it has commenced will have no impact on this compensation.

RISKS

While the amount of caffeine administered in this study (200mg) is equivalent to the amount found in a large Tim Hortons coffee (according to published data on timhortons.com), there is a risk you may feel physically ill. If you feel unwell, or you feel that you are unusually sensitive to caffeine and will therefore experience more than usual discomfort, please inform the research assistant immediately so that the session may be temporarily halted or discontinued.

The electrode sensors which are applied to the scalp and face may result in temporary redness and irritation of the skin that will disappear in a few hours.

BENEFITS

There are no immediate benefits for participating in this study, however, you will be contributing to a better understanding of how the menstrual cycle may affect the use of psychoactive drugs, such as caffeine. You will not be paid to be in this study. You will be compensated $25 per session to cover meals and transportation costs on the study visit day.

HOW WILL THIS DATA BE U*SE*D?

The data will be analyzed by the study investigators and will potentially form the basis for future published manuscripts and conference presentations.

CONFIDENTIALITY

Information will be coded and your privacy will be protected. Any scientific publication or presentation resulting from this work will be presented so that you cannot be identified as a test participant. Information collected from the study will be stored in a password-protected secure server housed at Mount Saint Vincent University (computer tasks) and in a locked filing cabinet with the principal investigator's office (study questionnaires). Data will be kept for 7 years, this includes EEG files, questionnaires, and saliva samples, which will only contain your participant code. After the 7-year period it will be destroyed by physical or digital shredding. Saliva samples will be disposed of via incineration after data analysis is complete. At all times, only the principal investigator (Fisher), associate investigators (Perrot) and research assistants currently working in the lab will have access to this information.

INFORMATION

If you have any specific questions about this study, you should contact the principal investigator, Derek Fisher (902.457.5503; derek.fisher@msvu.ca)

If you have any questions regarding the conduct of this study and/or wish to speak to someone at arm's length from this study, you may contact the Research Office at Mount Saint Vincent University (research@msvu.ca; 902.457.6350).

If you feel distressed after participating in this study, you may contact the study investigators. Alternatively, MSVU offers free and confidential help through Counselling Services for students. Psychological counselling is located on the 2nd floor of Evaristus, Room 218. You can make an appointment by calling 902.457.6567 or emailing counselling@msvu.ca. If you cannot reach anyone at by telephone or email and you are experiencing a crisis that calls for immediate attention or are not a student at MSVU, you can call the Mental Health Mobile Crisis Team (902-429-8167 or 1-800-429-8167).

If you feel unwell after participating in this study, you should contact your personal physician or the MSVU Health Office, 2nd floor of Assisi Hall. For appointments, phone 902.457.6354.