The Effect of Cycling Female Estradiol Levels on Retinal-Based Smooth Pursuit Eye Movements (SPEM) and Post-Target Offset Persistence

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Abstract

Estradiol has primarily been associated with the female reproductive system; however, estradiol receptors have been located in the retinas of male and female humans, as well as throughout the central nervous system (CNS) including lower brainstem areas associated with oculomotor functioning. The present study investigated the significance of estradiol in relation to smooth pursuit eye movements (SPEM) with the specific intention of localizing estradiol effects on realtime and on extrapolated SPEM tracking after target termination. This was accomplished in two ways. First, testing occurred at two opposing stages of the female menstrual cycle: the late follicular and the late luteal phases, where estradiol levels are known to be high and low respectively. Second, we selected participants symptomatically categorized into three groups: women who did not have Premenstrual Syndrome (PMS)-type symptoms and who were not using hormonal contraception within the last three months (control group), women who were hormonally sensitive due to the presence of PMS-type symptoms and were not using hormonal contraception (PMS-type group), and women who were using the hormonal contraceptive Alesse™ or the generic form Aviane™ (Alesse™ user group).

Horizontal SPEMs were measured using a 60 Hz infrared eye tracker. We found deviations from nominal sinusoidal eye movement patterns between our control, PMS-type and Alesse™ user groups in which the hormonally sensitive and regulated groups (PMS-type and Alesse™ users) showed shorter durations of persistence (defined as duration of movement that statistically followed an extrapolated track after target termination), greater amplitude excursions, and a left-to-right excursion bias. These findings suggest that further efforts should be made in order to better understand the

modulatory interactions of stable estradiol- or low progesterone-based affective symptoms and their underlying common neural circuitry with SPEM maintenance. The relevant SPEM neural circuitry includes the cerebellar vermis and flocculus, the medial vestibular nucleus, and other areas where estradiol has been reported to exert its effects.

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Table 8 *Percent Amplitude Accuracy decay with respect to the theoretical Extrapolated Target Position for the 1,0 Hz condition. Details as in Table 7.*

The Effect of Cycling Female Estradiol Levels on Retinal-Based Smooth Pursuit Eye Movement Persistence

Estradiol receptors are located throughout the mammalian central nervous system (Kaja, Yang, Wei et al., 2003; McEwen & Milner, 2007) and they have been located in the retinas of both male and female humans (Munaut, Lambert, Noel, Frankenne & Deprez, 2001; Ogueta, Schwartz, Yamashita et al., 1999; Wickham, Gao, Toda et al., 2000). Retinal signals can be processed through a number of pathway circuits including oculomotor systems involved with smooth pursuit eye movements (SPEM) and higher end cortical systems such as those devoted to image formation. Therefore, it is logical to assume that cycling estradiol concentration levels and/or the degree to which one is hormonally sensitive to changing hormone levels across the menstrual cycle may have an influential role on a number of vision-based systems. Further, the presence of estradiol receptors in the cerebellum (Andreescu et al., 2007) and in the lower brainstem (Medina & Lisberger, 2007; Raffi, Squatrito, & Maioli, 2007; Schlack, Hoffmann, & Bremmer, 2003; Sparks, 2002) may demonstrate estradiol modulatory influences on cerebellar-based eye movement patterns.

Estradiol and Visual Perceptual Functioning

In the last decade, changes in the female sex steroid estradiol have been found to be associated with changes in visual perceptual functioning, particularly with regards to short-wavelength light processing. For example, Eisner, Burke, and Toomey (2004) demonstrated that short-wavelength sensitive cone (S-cone) sensitivity might be related to estradiol changes during the menstrual cycle. Two groups of women were tested: one group containing four women not on oral contraceptives or any other medicinal hormonal

substitute, and the other made up of three women using the oral contraceptive Ortho Tri-Cyclen™. One participant belonged to both groups at two different times. In this study, participants were tested five days a week using a psychophysical method that collected threshold versus illuminance (tvi) data. A tvi function plots difference thresholds as a function of adapting field illuminance and chromaticity. Tvi effects were assessed with background illuminances ranging from 1.2 log troland (td) to 4.4 log td in 0.4 log td unit steps. Test wavelengths of 440-, 460-, 490-, 510-, 580-, and 640- nm were also used. Of the seven participants, one showed evidence of visual sensitivity changes that occurred around day 14 of her menstrual cycle. This was in line with her time of ovulation. Of particular interest was the fact that this participant fully participated in both the control and the experimental test groups due to her starting time on an Othro Tri Cyclen™ regimen midway through the study. In this case, the S-cone based tvi curves were found to be steeper during the late luteal (LL) phase than during the late follicular (LF) phase especially for the 440-nm tests on 4.0 log td backgrounds. Thus, her S-cone mediated vision was most sensitive during the LF phase, and these changes were detected prior to her use of oral contraceptives, (i.e., changes were not detected when the oral contraceptive use was initiated). Seeing as the significant results were found prior to contraceptive use, and due to the fact that non-contraceptive users should show greater estradiol cyclicity, this, and other studies detailed below, supports the theory that naturally cycling estradiol alters visual functioning. It is important to note that these findings reflect the results of one participant; therefore, this study required replication in order to garner more confidence in the findings.

Several other studies have examined wavelength sensitivity with hormone medications (see Eisner et al., 2004; Eisner, O'Malley, Incognito & Toomey, 2006; Eisner, Toomey, Incognito, O'Malley & Samples, 2006). One of the main focuses of these studies has been the selective-estrogen-receptor-modulator (SERM) tamoxifen, which is used to treat women with breast cancer. SERMs are compounds that have similar effects to receptor agonists and antagonists, however SERMs have differing effects on estrogen receptors depending on where the tissue is located. For example, Tamoxifen acts as an agonist at bone and as an antongist at breast and uterus. Tamoxifen has been found to affect colour discrimination while apparently not affecting the general integrity of the lens, cornea, or macula (Gorin, Day, Costantino, Fisher & Redmond, 1998). Eisner et al. (2004) demonstrated that tamoxifen affects S-cone mediated visual sensitivity, likely because of the localized presence of estradiol receptors in the retina. The reason S-cones were examined over the other photoreceptors is because of their susceptibility to environmental toxins and other exogenous agents (e.g., Haug, Kolle, Trenkwalder, Oertel, & Paulus, 1995; Heron, Adams, & Husted, 1988; Pacheco & Edgar, 1999). Eisner, Austin, and Samples (2004) used the Short-Wavelength Automated Perimetry (SWAP) test to assess S-cone sensitivities with SERM use. The SWAP measures S-cone retinal sensitivity across four concentric rings at 3°, 9°, 15°, and 21° eccentricity, in which short-wavelength "blue" spots of light superimposed on a bright middle wavelength "yellow" background are presented along different spatial positions within each of the rings.

Eisner et al. (2004) showed that the duration of tamoxifen use (in years) was negatively correlated with S-cone mediated visual sensitivity. That is, there was less

retinal S-cone sensitivity with the use of the SERM compared to non-tamoxifen users. Interestingly, SWAP sensitivities were most dependent on treatment duration when examining the visual field periphery (>21°). In other words, those participants who had lower levels of sensitivity to the peripheral stimuli often had a history of using tamoxifen for longer periods of time. The fact that differences in S-cone sensitivity are putatively dependent on duration of tamoxifen use exemplifies the potential importance of retinal estradiol on S-cone specific functionality. We argue that if duration of tamoxifen use can have this effect on retinal sensitivity, then naturally occurring changes in estradiol levels associated with the female reproductive cycle may also have an effect. In fact, Gupta, Johar, Napgal and Vasavada (2005) observed, through *in situ* hybridization, retinal estrogen receptor mRNA. This implicates the retina as a potential primary target for endogenous estradiol modulation. As gonadotropins fluctuate during different phases of the reproductive cycle, the retinally-based estrogen receptors may be affected as much as other, more traditional receptor sites, and consequently visual sensitivity changes comparable to those indirectly observed with the tamoxifen studies may result. Ultimately, retinal sensitivity may be increased during the late follicular phase of the menstrual cycle when there are heightened levels of estradiol present.

The Oculomotor System Overview

Several oculomotor systems enable the primate visual system to have high foveal acuity even when an image is in motion. Such systems include optokinetic movement, vergence, vestibular ocular movement, saccadic movement, and SPEM (e.g., Krauzlis, R. J., 2005; Ono & Mustari, 2008; Sharpe, 2008). The last two systems are prominent as compensatory mechanisms for restricted spatial resolution and kinetic image change.

Due to the fact that the number of axons in the optic nerve is limited, the visual system has relatively high spatial resolution representation located in a very small area of the fovea. Thus, the nervous system has to adapt to this spatially restricted area with fast acting saccadic precision and foveal-tracking SPEM.

SPEM facilitates volitional eye movements that allow for the close examination of an image located on or near the fovea (i.e., the central retina that falls in line with the 0° visual axis and is responsible for central visual acuity necessary for detail-demanding activities such as reading or driving). Several cortical regions known as the cortical pursuit system, support SPEM. These regions are interconnected and include the middle temporal (MT), frontal eye field (FEF), lateral intraparietal, supplementary eye fields (SEFs), and medial superior temporal (MST) cortex (Ilg & Their, 2008; Ono & Mustari, 2008). Evidently, the cortical pursuit system is intricately separated into subdivisions, with each subdivision being responsible for different aspects of SPEM signal processing. For example, the MT region is involved in detecting retinal-based motion signals and serves as a closed-loop signal necessary for SPEM execution (Ilg & Their, 2008).

The other type of eye movement that compensates for spatial resolution is the saccadic system, which is a two-step process that begins with a high velocity gaze shift shadowed by visual fixation. Although saccades are useful for initially establishing image fixation on the fovea, SPEMs are necessary for stabilizing foveated retinal images of moving objects (Ono & Mustari, 2008). Essentially, saccades are used to place the desired image on the fovea, and SPEM is used to keep the moving image stable on the fovea. Although SPEM and saccades are functionally disparate, their coordination is

suggestive of a neural link across pathways (Ilg & Thier, 2008; Missal et al. 2002; Sharpe, 2008).

A substantial amount of research has been done on the brain regions involved with SPEM and saccadic eye movements in rhesus monkeys *(Macaca mulatta)* because of the many similarities found between humans and macaques. Methods of studying smooth pursuit in the macaque typically include micro-stimulation and lesion work in order to locate areas of the brain that are involved in processing SPEM information, such as the junction of parietal, temporal and occipital cortex at area V5, and their respective connections with the parietal and FEFs. Projections from these areas are then sent to the brainstem and the cerebellum (Krauzlis, 2005; Sharpe, 2008). Of most interest to the present study are the projections to the cerebellum and brainstem regions of the cerebrum including nuclei in the following areas: paramedian pontine reticular formation, rostral interstitial nucleus of the medial longitudinal fasciculus, and the central mesencephalic reticular formation. The ventral paraflocculus and lobules VI and VII of the vermis are the cerebellar regions that are involved in SPEM, and they project to the vestibular nuclei, which then connect to ocular motor nuclei in the brainstem (for a review, see Sharpe, 2008). Portions of the SPEM pathways that will be referred to in this study are schematically outlined in Figure 5.

Studies have shown that inhibition plays a key role in SPEM. For example, there are omnipause neurons in the pons that are responsible for inhibiting excitatory burst neurons known to elicit saccades (Krauzlis, 2005; Missel & Kellar, 2002; Sharpe, 2008). Omnipause neurons, therefore, are involved in controlling the temporal course of pursuit by inhibiting saccades. The GABAergic system is a key inhibitory transmitter system in

the CNS (e.g., Rewal, Jung, Brun-Zinkernagel, & Simpkins, 2003; for a review, see Walker, 1983). In addition to the above, it is also important to note that estradiol has been found to inhibit GABAergic systems (e.g., Hu, Watson, Kennedy, & Becker, 2006; Noriega et al. 2010; Velisek & Veliskova, 2002; Wagner, Ronnekleiv, Bosch, & Martin, 2001). One notable recent study revealed that estradiol deprivation results in enhanced GABA receptor activity in the arcuate nucleus (Daendee, Thongson, & Kalandakanond-Thongsong, 2013). Consequently, SPEM, by virtue of its dependence on active inhibition by the omnipause neurons to the excitatory burst neurons, may be a behavioral expression that is influenced by cycling estradiol during the menstrual cycle. These points will be further discussed in the following section.

The transduction of the target movement/image motion to the retinal image is what elicits SPEM responses. Deviations in eye velocity from the target velocity are corrected for by retinal slip signals that become active when these deviations are detected (Krauzlis & Lisberger, 1989). Retinal slip signals activate what is referred to as "the controller" (Krauzlis & Lisberger, 1989; Lisberger, Morris, & Tychsen, 1987). The controller works on error signals derived from comparisons between kinesthetic gaze movement (the sum of eyeball and head movements) and target movement in space (retinal image movement) (Krauzlis & Lisberger, 1989; Lisberger, Morris, & Tychsen, 1987). The initiation of SPEM in the natural environment lags actual target movement by about 100 ms, mainly due to visual system delays (Sharpe, 2008). However, if target movement is anticipated, then the pursuit delay is reduced or even eliminated (Jarret & Barnes, 2002). In our study, we plan to focus on predictive horizontal SPEM because primates, including humans, perform better at eye tracking tests that include a horizontally moving stimulus rather

than a vertical one, and also because there is less noise associated with a horizontally moving stimulus due to the lessening of catch-up saccades required of this directional track (Rottach et al., 1996).

Estradiol and Oculomotor Activity

As noted above, not only does estradiol putatively affect cone sensitivities, but it also can influence oculomotor activity (e.g., Andreescu et al., 2007; Gyenes, Hoyk, Csakvari, Siklos, & Parducz, 2010). Oculomotor dysfunction has been related to Premenstrual Syndrome (PMS), a condition in which symptoms increase during the late luteal phase of the menstrual cycle and include depressed mood, anxiety, irritability, affective lability, difficulty concentrating, changes in appetite, low energy, decreased interest in usual activities, changes in sleep, headaches, a sense of being overwhelmed or out of control, breast tenderness or swelling, abdominal bloating, and joint or muscle pain (Pearlstein & Steiner, 2007). Interestingly, individuals with PMS-type symptoms demonstrate an increased likelihood to engage in a phenomenon referred to as the attention-startle-response (Kaviani, Gray, Checkley, Raven, Wilson & Kumari. 2004). The attention-startle-response is an involuntary response to fixate on a newly presented stimulus. The fact that PMS-type individuals have a propensity for startle further highlights the possible connection between oculomotor functioning and estradiol. Because of the unique hormonal dynamics that are characteristic of PMS, as well as the presence of estradiol receptors throughout the rhombencephalon, it may be that the oculomotor substrates that mediate automated reflexive startle response effects are modulated by the same unique hormonal patterns that are present in women with PMS.

In addition to the attention-startle-response, mood disorders such as clinical and seasonal depression have also been shown to alter oculomotor behaviors such as saccadic movement, electrooculographys (EOGs) and SPEM (e.g., Flechtner, Steinacher, Sauer, & Mackert, 1997; Kathmann, Hochrein, Uwer, & Bondy, 2003; Lam, Beattie, Buchanan, Remick, & Zis, 1991; Mahlberg, Steinacher, Mackert, & Flechtner, 2001; Ozaki, Rosenthal, Myers, Schwartz, & Oren, 1995; Pardo, Pardo, Humes, & M, 2006; Seggie, et al.,1991; Wichniak, Riemann, Kiemen, Voderholzer, & Jernajczyk, 2000).

Pardo et al. (2006) used a task developed by Posner et al. (1984) referred to as the "Posner Task" to assess visuospatial attention in depressed patients. They used nine participants who were clinically depressed and who were not receiving any treatment, and fourteen non-depressed participants. The Posner Task involved the participant sitting in front of a video monitor where they were told to fixate on a mark in the center of the screen. The participants were instructed to press a key as quickly as possible with their index finger when the target (an asterisk) was presented in the center of either the left or right visual field target region. Participants were cued as to the location of the target on some of the trials with the use of the previous target becoming brighter 1 s following the beginning of the trial. The probability of the subsequent target being presented in that same brightened region was 80%. The target was presented either 100 or 800 ms after the onset of the cue (stimulus onset asynchrony—SOA). Depressed patients performed significantly slower compared to the controls for all of the conditions demonstrating that patients with depression experienced attentional deficits during relatively complex tasks, which in this case caused them to experience psychomotor slowing. Pardo et al.'s depressive psychomotor slowing phenomenon may also be evident in SPEM persistence.

SPEM persistence is the continuation of tracking following stimulus or target offset. SPEM is volitional which inherently requires attention; therefore SPEM persistence probably reflects a vulnerability to attentional deficits like those expressed by depressed participants.

Wichniak et al. (2000) investigated eye movement properties using 21 randomly selected polysomnograms collected from patients with major depressive disorders. The polysomnograms were collected from two consecutive nights in a sleep laboratory. Rapid eye movement (REM) latency (LREM), REM density (RD), latencies of eye movement (LEM), and mean latency of eye movements (M-LEM) were measured. The depressed participants demonstrated higher RD, shortened M-LEM and shortened LEM in the 1st and 4th REM sleep periods. Again, these findings suggest that depression affects oculomotor functioning, with eye movement latency being related to depression. Due to the fact that mood alteration is also a symptomatic expression of PMS, it is logical to hypothesize that the modulated oculomotor pathways associated with mood disorders have convergent pathways in individuals with PMS.

As previously mentioned, SPEM persistence is a visual phenomenon in which tracked SPEMs continue to be elicited following the termination of a repetitiously moving target. The persistence of an eye-movement is an example of an automatic learned motoric pattern that is controlled by the cerebellum and other lower brain stem regions where estradiol receptors have been found (Andreescu et al. 2007; Kawakami & Ohno, 1981; Robinson & Fuchs, 2001). The present study investigated the significance of estradiol in relation to oculomotor functioning with the specific intention of localizing potential SPEM pathways involved with tracking persistence. In order to isolate the role

of estradiol, testing was done across three groups of participants during two opposing stages of the female menstrual cycle: the late follicular (LF) and the late luteal (LL) phases, where estradiol levels are known to be high and low respectively. A control group, consisting of women who have not been using hormonal contraception for at least three months and who were not categorized as PMS-type, was included as a means to assess the cyclicity of estradiol throughout the menstrual cycle compared to women who were categorized into the PMS-type or Alesse™ user group; both of which were expected to demonstrate estradiol stability throughout the menstrual cycle. It is important to note that including the PMS-type group was not an effort to better understand these symptoms or treat them. There is evidence that women with PMS-type symptoms experience estradiol stability across the menstrual cycle (Wesner & Richards, 2011; Wesner, Currie, Richards & Oinonen, 2014). Therefore, including the latter group as well as monophasic oral contraceptive users in this study allowed us to compare naturally cycling estradiol to stabilized estradiol, as Alesse™ users take a standard dose of 20 µg of ethinyl estradiol and 0.10 mg of progestin levonorgestrel daily for the duration of the 28 pill package. We expected to see changes in estradiol and progesterone across the menstrual cycle that is in agreement with Table 1 (see page 57).

Menstrual Cycle Overview

The first day of the menstrual cycle is marked by the first day of menstruation. At this time, estrogen and progesterone levels are low, and gonoadotropin-releasing hormone (GnRH) is secreted from the hypothalamus. The secretion of GnRH stimulates the release of Follicle Stimulating Hormone (FSH) from the pituitary. This marks the beginning of the follicular phase. The secretion of estrogen from the ovarian follicles is

stimulated by the release of FSH, which results in the cellular proliferation of the uterine lining. Negative feedback is exerted by estrogen on FSH release from the pituitary and one follicle becomes dominant and secretes increasing amounts of estrogen. This marks the middle follicular phase as well as the end of the first week of the menstrual cycle. The second week of the menstrual cycle involves an increase in the pulsatile release of GnRH leading to the secretion of the luteinizing hormone (LH). This is the beginning of the luteinizing phase. The secretion of LH triggers ovulation (the expulsion of the egg from the follicle). Ovulation coincides with a rising surge of estrogen levels, and ovulation represents the end of the follicular phase (Parry et al., 2006). Post ovulation, the corpus luteum releases large amounts of progesterone, as well subtle amounts of estrogen compared to progesterone. If the egg is not fertilized atrophy of the corpus luteum occurs. Here, progesterone levels decline and the initiation of menstruation takes place in which shedding of the uterine lining occurs within 12-16 days of ovulation (Reed, Levin, & Evans, 2008). There are declining estradiol levels during the last few days of the luteal phase of the menstrual cycle which signals a release of the negative feedback on the FSH secretion, and a rise in FSH levels leads to the next menstrual cycle (see Figure 1).

Horizontal SPEM Neural Circuitry

Research on the neural circuitry involved in horizontal SPEM is a relatively burgeoning area, however the recent focus on this subject has pointed to several brain regions of importance to this study. Although intricate, the neural circuitry involved in horizontal SPEM has been isolated in several areas of the brain. The projection pathways involved in horizontal SPEM include the medial superior temporal (MST), middle

temporal (MT), and the frontal eye fields (FEF) to ipsilateral pontine nuclei to contralateral cerebellar (dorsal) vermis (lobules VI and VII) then through vestibular and abducens cranial nuclei (Fukushima, 2003; Sharpe, 2008). Sharpe (2008) discovered that lobule IX of the cerebellar vermis is involved in regulating bidirectional lowering of horizontal pursuit gain. Another finding involves the neural integrator circuit that regulates horizontal SPEM. This circuit is made up of the nucleus prepositus hypoglossi (NPH) and the middle vestibular nucleus (MVN). Together, the NPH and the MVN transform eye-velocity signals to eye position signals in order to integrate conjugate eye movements (Sharpe, 2008). Lastly, second order vestibular neurons excite lateral rectus motorneurons and rectus internuclear neurons in the contralateral abuducens nucleus to produce smooth eye motion. This pathway creates a double decussation of the horizontal pursuit pathway wherein the first decussation occurs at the ponto-cerebellar projections through contralateral middle peduncle to vermis and paraflocculus and the second decussation is from the second order vestibular neurons in the MVN to the contralateral abducens (Sharpe, 2008). Again, pathways that are the focus of this study are outlined in Figure 5.

Horizontal SPEM Neural Circuitry and Estradiol

Cerebellum and brainstem. Several connections can be made between estradiol receptor activity and the rhombencephalon. For example, the floccular lobe of the cerebellum is involved in automated horizontal SPEM. Horizontal P-Cells found in the floccular lobe seem to play a role in SPEM maintenance because they discharge after SPEM onset (Fukushima, 2003). Fukushima notes that lesions to the floccular lobe and the dorsal vermis (lobues VI and VII) reduce SPEM amplitude change and that

Horizontal P-cells from the floccular lobe send projections to the brainstem, an area noted for its involvement in SPEM. According to Sharpe (2008), the cerebellum is responsible for executing motor commands that are involved in SPEM and the flocullus and the dorsal parafloculus are involved in amplitude change.

There are functional differences that should be noted regarding the flocullar lobe and the dorsal vermis. Firstly, the floccular lobe controls slow eye movements, whereas the dorsal vermis controls eye velocity in conjunction with eye and head movements (Fukishima, 2003). A notable finding by Andreescu et al. (2007) and colleagues is that estradiol receptors are present in the cerebellum (specifically the vermis and floccular lobe) and that estradiol has been shown to affect motoric learning. Given that estradiol has been shown to exert its effects in the cerebellum with respect to motoric learning, it is likely that estradiol may also play a role in the SPEM properties that are regulated by the cerebellum. Further, elevated levels of aromatase have been found in the cerebellum. Aromatase is an important metabolic enzyme in that catalyzes the last step in biosynthesis of estrogen. In a study by Biegon et al. (2010) Positron Emission Tomography (PET) scans revealed that estradiol regulates neurogenesis in Purkinje cells and interneurons in the cerebellum. Other evidence that implicates the role of estradiol in SPEM functioning comes from Tsutsi (2012) who noted several morphological changes in the cerebellum based on estradiol levels. He found that estradiol deficiency in mice decreases dendritic growth, spinogenesis, and synaptogenesis in Purkinje cells. All of this evidence is suggestive that fluctuations in estradiol levels (such as the respective peak-to-trough changes in estradiol during the menstrual cycle) may affect SPEM in the cerebellum.

Although there are several areas of the brainstem that are known to be tuned to SPEM including the dorsolateral pontine nuclei (DLPN) and the nucleus reticularis tegmenti pontis (NRTP), experimental research that demonstrates the impact of estradiol levels in the brainstem have focused primarily on one brain area: the MVN (Sharpe, 2008) (See Fig. 4). The MVN is involved in integrating conjugate eye movements. In a study by Scarduzio et al. (2013) they tested the impact of estradiol on mice MVN. When E2 is blocked in mice they found that long-term potentiation (LTP) of the neurons in the MVN was abolished while the opposing long-term depression systems went unaffected. Also, when E2 is present, the MVN is more active. By virtue of the increased activity of the MVN, the integration of conjugate eye movements should be enhanced; perhaps leading to greater amplitude gain at peaked levels of estradiol such as during the LF phase of the menstrual cycle.

Estradiol and Brain Plasticity

The effect of estradiol on memory, through neuroplasticity and mechanisms of synaptic enhancement, is an area that needs to be further examined, especially with respect to the cerebellum. Andreescu et al. (2007) posit that, like the hippocampus (see Scarduzio et al 2013; Shiroma, Yamaguchi, & Kometani, 2004; Vouimba, Foy, Foy, & Thompson, 2000), there is a strong presence of estradiol receptors in the cerebellum. Therefore, it is not unreasonable to predict that similar, albeit less pronounced, estradiol-based morphological changes related to learning and memory detected in the hippocampus may also occur in the cerebellum (Hedges, Ebner, Meisel, & Mermelstein, 2012; Rhodes & Frye, 2004). In fact, Andreescu and colleagues were interested in motor memory formation, and expected to see motoric behavioural changes correlate with

physiological cerebellar changes that relate to changes in estradiol levels during the reproductive cycle. Specifically, based on the morphological impact of E2 on hippocampal memory formation, Andreescu and colleagues speculated what impact estradiol would have on the plasticity of the vestibulo-ocular reflex (VOR) following a visuovestibular training paradigm that included the pairing of head rotation with 50 minutes of rotation of the visual environment. The type of plasticity that Andreescu and colleagues investigated was long-term depression (LTD) and long-term potentiation (LTP), molecular events that were studied at the parallel-fiber to Purkinje-cell synapse. Interestingly, LTP has been found to have a negative effect on neural gain in the hippocampus, whereas LTD has a positive effect (Cordoba & Carrier, 1997; Hansel, Linden, & D'Angelo, 2001). The authors focused on cerebellar LTP because it has been shown to decrease VOR gain which is defined as the change in eye angle divided by the change in the head angle during a head turn (Boyden & Raymond, 2003). Horizontal and vertical VOR gain should be about 1.0—anything different and image motion on the retina potentially causes blurred vision. More accurate eye movements can be achieved with motor learning to adjust for VOR gain.

The cerebellum is vital to motor learning, and given estradiol presence in the cerebellum, estradiol may mitigate LTP in the cerebellum (similar to what happens in the hippocampus) ultimately enhancing cerebellar plasticity. The Andreescu (2007) study was designed to investigate the effects of E2 on LTP at the parallel fiber to Purkinje cell synapse on gain-decrease training of the VOR. The researchers predicted that estradiol modulation of LTP would decrease VOR gain, which would correct retinal image motion and enhance motor learning. Seventy, 6 to 8 week old female C57BL/6 mice, 17 male

C57BL/6 mice, and 8 Purkinje cell-specific ER- β knock-out female mice were subjected to the tests. Mice, like humans, are mammals and, therefore, share many similarities in structure and function throughout the CNS making most results found in rodent research potentially generalizable to the human population. In one group of female mice (Eovx mice), E2 was at a constant high level caused by ovariectomy (OVX) as well as daily subcutaneous injections of 5 μg of estradiol benzoate dissolved in 0.1 ml sesame oil. In the other group (Covx mice), E2 levels were stabilized at low levels by OVX with subsequent daily subcutaneous injections of only 0.1 ml of sesame oil. Intact female and male mice were used as controls. Daily vaginal smears were taken, as well as blood and uterus collections on the day of testing to ensure correct hormonal status. The mice received daily treatments of estradiol or saline between days 0 and 28 and were kept in complete darkness between the training (days 19-20) and testing (days 26-27) days. VOR measurements were taken pre-and-post training using a visual-vestibular interactive training paradigm that consisted of the presentation of phase sinusoidal vestibular and visual stimuli (±5°, 0.2-1 Hz).

Post-mortem sagittal slices were collected from the cerebellar vermis of the Eovx, Covx and the two groups of control mice. The slices were stored in artificial CSF and whole-cell patch-clamp recordings were performed at room temperature. The presynaptic and postsynaptic levels of LTP and LTD were estimated by measuring a paired-pulse facilitation (PPF) ratio, which is found by stimulating the parallel fibers with two pulses delivered 50 ms apart, and examining the voltage-clamp recording responses. The second pulse has a corresponding facilitated response because of short-term enhancement in synaptic efficacy that is caused by residual presynaptic Ca^{2+}, permitting more

transmitter release. Parallel fibers were investigated alone in order to assess LTP, and LTD was examined according to the stimulation of the parallel fibers being affected by other fibers. Again, it was essential to differentiate LTP from LTD so that the effects of VOR gain (as a means to stabilize images on the retina) via LTP could be assessed in order to determine whether estradiol affected VOR gain in the cerebellum. LTP was hypothesized to decrease VOR gain and enhance motor learning, whereas LTD was predicted to increase VOR gain.

The flocculus (from OVX females that received the daily estradiol benzoate injections) was processed for electronic microscopy in order to determine the synaptic density at the parallel fiber and climbing fiber to Purkinje cell. The number of synapses was counted from each brain and the total postsynaptic density area (PSD) and the PSD perimeter were measured.

The results showed that when LTP was induced in the Eovx mice, the excitatory postsynaptic current (EPSC) amplitude was higher compared to the Covx mice. These findings were replicated when comparisons were made between the Eovx mice and the male control group. There were no significant findings for LTD. Estradiol did, however, improve VOR motor learning as defined by VOR gain decreases assessed by before and after visuovestibular training. The Eovx mice showed a 60% decrease in VOR gain whereas the Covx mice had a significantly smaller learning effect. These motor learning advantages were attributed to the initiation of LTP at the parallel fiber to Purkinje cell synapse. Further, the post-mortem analyses of the flocculus showed that ERß was localized in the cell soma and dendrites in the Purkinje cells in both the flocculus and paraflocculus along with their terminals and in the vestibular nuclei. This study confirms

the existence of estradiol receptors in the cerebellum and that estradiol can affects motoric learning via the vermis and the flocculus in the cerebellum. The Andreescu et al. study also sheds lights not only on the effects of estradiol on the EPSC during motoric learning, but also on sexually dimorphic traits that may be implicated by estradiol. The observed sexually dimorphic disparity (Eovx group compared to the male mice) on the visuovestibular task performance could be related to cerebellar motor learning that affects the proestrus needs of females to find and attract an appropriate male. Such a finding may explain other sexually dimorphic traits in humans where females out-perform males, including verbal ability and fine motor skills (Epting & Overman, 1998).

Another interesting theory relating visuovestibular control regulation by estradiol involves pregnancy. Like the estrous cycle, changing estradiol levels during pregnancy may influence the Purkinje cell activation in the cerebellum. Andreescu et al. (2007) suggest that during pregnancy, changes in abdominal size inevitably changes the center of gravity leading to distorted balance. This makes it necessary for the vestibulocerebellum to reset for balance maintenance. If the VOR has to adjust for a new center of gravity, then enhancement related to estradiol may speed up the motor learning required of such an adjustment. Therefore, estradiol may play an influential role in ensuring that postural balance is maintained and eye movement position is not interrupted, potentially leading to parturition success.

In a related way, estradiol has also been found to play a role in spatial reference memory (important for navigation), various forms of visual memory, as well as problem solving controlled by the cerebral cortex (Frick et al., 2002; Leuner et al., 2004) all of which implicate hippocampal-type plasticity with coordinated oculomotor spatial activity.

While estradiol's primary role may be predominately reproductive in nature, it appears that it also plays other homeostatic roles through the modulations of cerebellar, cerebral brainstem and associative cortical neural circuits related to oculomotor activity.

Estradiol, PMS/PMDD, and Eye Movement Effects

According to the Diagnostic and Statistical Manual of Mental Disorders (5th ed.; DSM-V; American Psychiatric Association, 2013), severe PMS, known as Premenstrual Dysphoric Disorder (PMDD), is operationalized as having mood-related symptoms experienced prior to menses. Some of these symptoms include: depressed mood, anxiety, irritability, affective lability, difficulty concentrating, changes in appetite, low energy, decreased interest in usual activities, changes in sleep, headaches, a sense of being overwhelmed or out of control, breast tenderness or swelling, abdominal bloating, and joint or muscle pain (Pearlstein & Steiner, 2007). In order to be diagnosed with PMDD, the luteal phase must be associated with the experience of impairment of social and/or work functioning caused by the premenstrual symptoms. Interestingly, this hormonal disorder PMDD has also been found to affect eye movements (Nyberg et al., 2004).

Nyberg et al. (2004) conducted a study that measured eye movements in women with PMDD who were subjected to alcohol infusions during the laboratory sessions. Time of testing occurred in the mid follicular and late follicular phases. Fourteen women who met the criteria for PMDD participated in the study, and 12 women without significant dysphoric symptoms participated as part of the control group. The participants were tested on four occasions, twice during each testing phase. Within each menstrual cycle phase participants from both groups were randomly selected to receive a low dose of alcohol or a placebo infusion within an interval of 48 hours. Baseline levels of

estradiol and progesterone plasma were taken with the use of an intraveneous cannula inserted in each forearm. The researchers also took three sets of saccadic eye velocity (SEV) measurements and visual analogue ratings to determine the participants' baseline SEV. EOG was used to measure SEV. The stimuli included an array of light-emitting diodes (LED) at eye level, 67 cm from the glabella. Subjects were instructed to focus on the LED and then move their eyes to the subsequent LED displays following the offset of the previous LED. Apparent target movements as induced by the phi phenomenon occurred at 1.5-sec intervals. A fixed, non-random sequence of 4 x 20 targets producing target steps of 10°, 20°, 30°, and 40°, was displayed. The EOG was DC amplified and low–pass filtered. The 80 individual EOGS were analyzed by processing the data from each target displacement to locate the saccades. The saccades were analyzed to determine the saccade in degrees, the peak saccadic acceleration, deceleration, and latency from target movement to onset of saccade. A velocity-saccade size curve was used to process the saccadic parameters. The results showed that PMDD participants that received the low dose of alcohol had reduced SEV and saccade deceleration during the late luteal phase compared to the mid-follicular phase. While under the alcohol condition the control group did not show a significant change in SEV or saccade deceleration. The authors concluded that women with PMDD were more susceptible to changes in eye movements, specifically SEV, compared to the control group when they were exposed to low doses of alcohol. It is interesting to note that alcohol is known to interact with several neurotransmitter systems, particularly GABAergic and serotonergic, two systems that interact with estradiol (Rapkin, 1999). The latter study provides indirect evidence that differences in estradiol activity between women with PMDD and control women

may have an effect on saccadic eye movement properties. Given that saccadic and SPEM are two eye movements that work in opposition to one another, one might expect similar findings from the latter study to be found with respect to SPEM in women with PMDD versus controls.

Altered levels of progesterone is a factor related to PMS-type symptoms. Progesterone is produced by the corpus luteum, which is also involved in implementing the cyclicity of PMDD. This is important to note because, although the present study focuses on the effects of estradiol on SPEM functioning due to the presence of estradiol receptors in the retina and throughout the CNS, progesterone is likely to have indirect effects on SPEM by virtue of its antagonistic interaction with estradiol throughout the menstrual cycle. Reduced sensitivity to GABAergic substances have been found in PMDD patients during the late luteal phase (Sunstrom et al., 1998). Once again, the cyclicity of PMDD is implemented by the corpus luteum, and progesterone is the major steroid that is produced by it. This is important because endogenous progesterone metabolites, called neurosteroids, are involved in the allosteric modification of $GABA_A$-receptors increasing receptor affinity (Majewska, Harrison, Schwartz, Barker, & Paul, 1986). Thus, progesterone modulation may lead to GABAergic system enhancement of inhibition. If the production of progesterone is affected by the corpus luteums interaction with PMDD, and if the exocytotic release of GABA is affected by progesterone, which by its very nature is interactive with estradiol levels, then it is reasonable to hypothesize that other areas that are driven by GABA may be affected by the inhibitory-implications caused by PMDD, such as SPEM. In fact, the GABA agonist muscimol has been shown to cause reduced velocity of saccades via the superior colliculus (Hikosaka & Wurtz,

1985). Therefore, PMDD may have indirect implications for eye movements via the GABAergic system -- possibly through the omnipause system that inhibits excitatory burst neurons known to elicit saccades. Again, while estradiol is known to inhibit GABA, progesterone is antagonistic to estradiol throughout the menstrual cycle, therefore progesterone may enhance the inhibition of omnipause neurons ultimately inhibiting saccades via excitatory burst neurons.

Also related to the latter, some studies have shown that individuals with PMS-type symptoms have slowed psychomotoric responses. For example, Kaviani et al. (2004) conducted a study that demonstrates that participants with Major Depressive Disorder (MDD) display what is known as the attention-startle-response in response to emotionally positive, neutral and negative short films. The attention-startle-response, as pointed out earlier, occurs when an involuntary reaction is made to a precipitous sensory stimulus onset. Such psychomotoric slowing was previously found with respect to affective limbic responses (Kaviani et al., 2004) and oculomotor fixation (Donaghy et al., 2009). Given that many behaviours that are regulated by various and diffuse areas of the CNS are affected by the attention-startle-response, it is not surprising that Kaviani and colleagues investigated the effects of the later phenomenon on oculomotor behaviour in order to see if visual cues have implications for oculomotor reactivity. Kaviani and colleagues used a 50 dB white burst-tone as their startle stimulus. The startle stimulus was presented three times during each of the 6 short films. Startle amplitude, baseline electromagnography (EMG), latency to response onset, and affective responses were included as the dependent variables. The results showed a main effect of valence in which systematic increases in startle amplitude were found with increases in

unpleasantness. Therefore, all of the participants displayed more intense startle responses to the unpleasant clips. However, a significant main effect for group was found wherein the highly depressed group showed slower startle reflexes relative to the low depressed group and the control group. Participants with affective disorder-symptoms have more difficulty dissociating from the invoking stimulus compared to a low depression group and control participants. In other words, Kaviani et al. showed that MDD individuals took longer to redirect the gaze off of a precipitous stimulus onset.

Evidently, the hormonal implications for affective disorders such as depression, PMS and PMDD are related to eye movements insofar as women with PMS-type symptoms may have difficulty dissociating from visual stimuli. Therefore, SPEMs and SPEM persistence, in which eye tracking continues even after termination or offset of a target stimulus, may also be affected by this psychomotor slowing, and this slowing may be partially attributed to GABAergic modulation mentioned above.

Finally, dysregulated eye movements have been related to PMDD, depression, and sleep, and these oculomotor dysregulations have also been linked to estrogen. For example, Hadjimarkou, Benham, Schwarz, Holder, and Mong (2008) investigated estradiol influences on sleep physiology by measuring electroencephalograhic recordings on gonadally intact and ovariectomized female rats. The researchers discovered that rapid eye movement sleep phases (REM) were blunted when estradiol levels peaked in the intact mice compared to the other days of the estrous cycle. Also, the amount of sleep activity that occurred in the intact mice was reduced during the times of peaked estradiol compared to when there was minimal estradiol in the cycle. These changes related to eye movements, estradiol, and sleep were not found in the ovariectomized rats, and as such,

these findings suggest that estradiol has a regulatory effect on sleep. This result is especially interesting with respect to REM in that REM is defined by prominent oculomotor activity; again, supporting the hypothesis that estradiol may play a role in the control of eye movements.

In a polysomnography experiment (a multi-parametric sleep test) Shechter, Lesperance, Kin, and Boivin (2012) examined the sleep patterns of PMDD and non-PMDD control women. In this study, women spent every third night of their menstrual cycle sleeping in the lab. A significant finding was discovered as PMDD women experienced higher amounts of slow wave sleep (SWS) compared to the control participants across the menstrual cycle. With the use of urine samples they found that nocturnal melatonin secretion was characteristically lower in PMDD women compared to non-PMDD women, and melatonin has been found to reduce SWS via $GABA_A$-receptors, which inhibit SWS (Aeschbach, Lockyer, Dijk, Lockley, Nuwayser, et al., 2009). Assays showed that progesterone was higher during the LL phase compared to the LF phase. Therefore, these findings suggest that women with PMDD may be experiencing altered levels of SWS due to the blunted level of melatonin concentrations and melatonin's interaction with $GABA_A$-receptors.

REM sleep disruptions have been associated with affective disorders such as seasonal affective disorder (SAD; Koorengevel, Beersma, Den Boer, & Hoofdakker, 2002) and clinical depression (Palagini, Baglioni, Ciapparelli, Gemignani, & Riemann, 2013). The REM symptoms in these disorders include short REM latency, increase of REM density and shortening of mean latency of eye movements (Wichniak, Riemann, Kiemen, Voderholzer, & Jenajcyk, 2000). Once again, these findings support the theory

that estradiol affects oculomotor functioning via the brainstem and the cerebellum because SAD and clinical depression may both be characterized by hormonal sensitivity. Given that both of these disorders have been related to oculomotor changes in REM sleep, one may speculate that the relationship between these disorders and REM variations are related to synaptic plasticity changes caused by estradiol.

The present study investigated the effect of cyclic female estradiol levels on visual systems governed by brainstem and cerebellar pathways of the oculomotor systems. This was done with the use of an eye tracking devise in order to assess estradiol's involvement with eye movements, more specifically, the most straightforward closed-loop system: retinal-based horizontal SPEM.

In summary, there is converging evidence of estradiol effects on SPEM functioning including SPEM persistence as follows: (1) modulation of estradiol on visual functioning including oculomotor activity, due to estradiol receptors having been found in the areas of the brain that are involved with SPEM (i.e., the cerebellum and the brainstem), (2) that eye movement disruptions (such as the attention-startle-reflex) are present as a symptom of PMS-type individuals and there is some evidence that these women may have stable estradiol levels throughout their cycle, (3) estradiol is a known mediator of synaptic plasticity in select CNS regions, and estradiol has been reported to modulate REM oculomotor activity, particularly with groups that are sensitive to hormonal changes such as women with PMDD (4) that progesterone (an antagonist to estradiol during the cycle) can alter the sensitivities of $GABA_A$ receptors (an action known to be involved with neural plasticity in a number of CNS systems), and (5) similar GABAergic systems under the influence of female steroids have been implicated in cerebellar and other brainstem

regions known to be involved with saccadic eye movements and SPEM. Real-time eye tracking measurements of SPEM along with SPEM persistence (i.e., eye movements that continue to track after the stimulus has disappeared) was considered in the present study to reveal any possible influences of estradiol on one of the more prominent retinal-based oculomotor functions, horizontal SPEM. The role of cycling estradiol influences in females was examined by using non-PMS-type non-hormonal contraceptive users as controls, PMS-type individuals, and Alesse™ users. We hypothesized that estradiol cyclicity would be associated with SPEM persistence duration, amplitudes, and oculomotor tracking efficiency as defined by target lead (eyes in front of moving target) and target lag (eyes trailing behind moving target). We also predict that there will be group differences in which the estradiol cyclicity present in the control group will lead to changes in eye movements compared to the PMS-type and Alesse™-user groups who have putatively different estradiol cyclicity throughout the menstrual cycle.

Methods

Participants

Of the 423 women from Introductory and upper-level Psychology classes at Lakehead University screened by the online questionnaire, 66 participated in the laboratory portion of the study. The study was described as an investigation of women's health and visual functioning. The participants received between one and three bonus marks toward their grade in research-participation eligible psychology courses. The total points that the participants were awarded depended on the participants' length of involvement in the study, in which the screening phase and both laboratory sessions were worth one point each. All of the participants were females between the ages of eighteen and forty-four years (M = 22.42, $SD = 0.79$), and they were either free-cycling (i.e., not

taking hormonal contraceptives, $n = 47$) or taking the monophasic oral contraceptive Alesse™ or the generic form Aviane™ ($n = 19$). The Alesse™ users were taking a standard quotidian dose of 20 µg of ethinyl estradiol and 0.10 mg of progestin levonorgestrel for the duration of their 28-pill package. The mean LF menstrual cycle day for the control ($M = -14.23$, $SD = 1.7$), PMS-type ($M = -14.2$, $SD = 1.15$), and Alesse™ user ($M = -14.14$, $SD = 1.35$) groups were almost identical. For the LL menstrual cycle phase, the mean cycle day for the control ($M = -4.39$, $SD = 1.59$), PMS-type ($M = -4.15$, $SD = 1.89$), and Alesse™ user ($M = -4$, $SD = 1.5$) also showed little deviation from each other. All participants had normal or corrected-to-normal vision, and were screened for a history of ophthalmological, and neurological. Further exclusion criteria included: age, whether they had been diagnosed with a psychiatric disorder, whether they are/were being treated with pharmaceutical agents, and reproductive health. Each participant was given a ten-dollar gift certificate to either Chapters or Tim Horton's in addition to course bonus marks for qualifying student participants

Apparatus

Menstrual cycle phase measures.

Ovulation test strips. The participants used ovulation test strips that indicated the presence or absence of the luteinizing hormone (LH) surge. The ovulation test strips were a part of a kit called the Home Pregnancy Test™ and were designed to detect as little as 5 mIU/mL of LH in urine, with a specificity of 99.8%. The strips were used to confirm the correspondence of the free-cycling participant's position in their menstrual cycle with the proper scheduling of the laboratory sessions. All of the participants used the test strips, regardless of their experimental group inclusion (see Laboratory Screening below for the group categorization of Controls, PMS-Type, and Alesse™ Users), in order

to ensure standardization of the procedures. The proper use of the strips was explained thoroughly, and each participant received five strips and five small Dixie cups (to collect the urine sample). It is easiest to detect LH in urine between 10:00 and 20:00, so the participants were instructed and asked to use the strips during these times for five consecutive days beginning on day 11 of the menstrual cycle. The strips were used by inserting the end of the strips into the urine sample for up to ten seconds, followed by a ten-minute waiting period before considering the result. Either a negative or positive result for LH occurred, indicated by the presence (positive result) or absence (negative result) of a test line adjacent to the control line. The test line needed to be as dark or darker than the control line. A positive result was indicative of an LH surge occurring within forty-eight hours. The participants' late-follicular session was booked to take place within 24 hours after they reported a positive result on the test strips, which occurred between days 11 and 16 of the menstrual cycle. LL laboratory sessions were scheduled between and including days 25 to 27 (or days -2 to -4 using reverse counting – see below).

Reverse counting strategy. A reverse counting strategy or "backwards counting" strategy was used to book the LL session on or between -2 to -4 based on a 28-day cycle (Haselton & Gangestad, 2006). This method of counting backward from the first day of the next estimated menstruation is an easy and effective way to estimate menstrual cycle day because *(1)* you estimate the beginning of the next menstruation based on the first day of the last menstruation and the individuals reported cycle length, *(2)* estimate the participant's onset of their next menstruation and count back from there, and *(3)* confirm the day of testing by counting backward from the participants true first day of menses.

Further justification for using the reverse counting strategy is that the LL phase duration (days) has been reported as including less variability compared to the LF phase (Jöchle, 1973; Pillsworth, Haselton, & Buss, 2004). Therefore, counting backwards from the onset of menstruation rather than pinpointing the LL phase prospectively is advantageous for this study.

Salivary collection for estradiol and progesterone assays. As an extra method to confirm menstrual cycle position, estradiol and progesterone measurements were attained via salivary samples. Participants were asked to passively drool through an approximately 5 cm in length straw into a cryovial. As a precaution, participants were instructed to refrain from using chewing gum, lemon drops, granulated sugar, drink crystals, brushing their teeth, and eating a large meal for at least an hour prior to their laboratory session. The participants were asked not to consume alcohol for a minimum of 12 hours prior and not eat acidic or high sugar foods for at least 20 minutes before their laboratory session. Salivary sample collection occurred three times during each laboratory session until a total of approximately 2 ml of saliva was collected. Each laboratory session lasted approximately an hour. The first salivary sample was collected after the visual screening procedures (see page 42), followed by the second sample after the laboratory questionnaire was completed, and the final sample was taken after the completion of the eye tracker test. Collecting the samples three times rather than once compensates for the pulsatile release of hormones in order to avoid collecting a sample at a trough or peak in levels. Samples were frozen at -21° until salivary analysis (see page 46).

Laboratory Screening.

Online screening questionnaire. The screening questionnaire was used to categorize the participants into their respective groups which are broken down as follows: (1) a control group of women reporting low levels or an absence of PMS symptoms; none of whom report the use of hormonal contraceptives, (2) women reporting high levels of PMS symptoms, and (3) women reporting the use of the hormonal contraceptive Alesse™ or the generic form Aviane™. The screening questionnaire was also used to determine whether the participants met the exclusion criteria (such as age, hormonal contraceptive use, whether they had been diagnosed with a psychiatric disorder, whether they are/were being treated with pharmaceutical agents, and reproductive health) to qualify for the study.

Several questionnaires were used to screen the participants for PMS symptoms, including the Menstrual Distress Questionnaire (MDQ) (Moos, 1968) and the Lakehead Inventory of Premenstrual Symptoms (LIPS), which is a 33-item measure of PMS severity that was created for Meghan Richard's dissertation (Richards, 2011). Confounding of other affective disorders was also screened for using the Hamilton Rating Scale for Depression (Hamilton, 1960) to screen for depression, the Seasonal Assessment Pattern Questionnaire (SAPQ) (Rosenthal et al., 1984) to collect information on seasonal patterns of mood disturbance, and finally the Beck Anxiety Inventory (BAI) (Beck, 1990) was used as a post-hoc measure to ensure that the participants did not differ based on level of anxiety symptoms.

Menstrual distress questionnaire (MDQ). The MDQ (Moos, 1968) is a 47-item questionnaire that is rated on a five-point scale ranging from "0" *(no experience of the*

symptom) to "4" *(present/severe)*. The items assess current symptom experience within the last 24 hours (MDQ-T) as well as current presence or experience of symptoms during the following three phases of the most recent cycle: menstrual, intermenstrual, and premenstrual (MDQ-C). Eight scales on the MDQ are used to assess pain, concentration, water retention, behaviour change, negative affect, autonomic reactions, arousal, and control. The MDQ-T and the MDQ-C were used to select participants to their respective groups. The MDQ-T was used in the lab as a prospective measure of pms-type symptoms, while the MDQ-C was used in the screening questionnaire as a retrospective measure.

Lakehead inventory of premenstrual symptoms (LIPS). The LIPS is a 33-item scale developed as part of an unpublished dissertation (Richards, 2011). It is a retrospective self-report measure of the extent to which participants meet the criteria for PMDD. It can also be used, as it is here, to assess the severity of one's typical level of pms-type symptoms. This questionnaire includes three questions that meet each of the eleven criteria listed in the DSM-IV. The three questions include: (1) the frequency with which each set of symptoms is experienced, (2) the degree to which each symptom impairs work, school, or interpersonal performance/functioning, and (3) the severity with which each symptom is experienced. The questions are rated on a seven-point Likert-type scale anchored by "0" (*not at all*) on one end and "6" (*extremely*) for the latter two categories of questions and "6" (frequently) for the first category. The women were also asked to report if the symptoms from the questions occur in more menstrual cycles than not over the previous 12 months. Possible scores on this questionnaire range from 0 to 198, and higher scores are indicative of greater severity of symptoms as well as higher degree of impairment.

Hamilton rating scale for depression (HRSD). The HRSD (Hamilton, 1960) assesses current symptoms of depression. The scale includes a likert-type rating scale that ranges from "0" *(not at all)* to "4" *(marked or severely)* for 28 items that assess patterns of diurnal variation, depersonalization, obessions/compulsions, paranoia, and atypical/vegetative symptoms. Participants whose scores meet or exceed 20 were excluded from the study as this was the criteria outlined in a study that screened for Seasonal Depression (a disorder that has several overlapping symptoms with PMS) conducted by Wesner and Tan (2006). O'Hara and Rehm (1983) report that the intraclass correlation for HRSD was .91 ($p < .0001$) when rated by expert raters and .76 ($p < .0001$) by novice raters. The criterion validity estimates were determined using comparisons between the means of the novice and expert raters. The mean of the expert ratings ($M = 14.50$; $SD = 7.42$) was not significantly different, $t(19) = 1.81$, $p > .05$, from the mean of the novice raters ($M = 13.72$; $SD = 6.82$).

Beck anxiety inventory (BAI). The BAI is a measure of self-reported anxiety (Beck & Steer, 1990). It includes a four-point scale where "0" indicates a response of not at all, "1" is mild, "2" is moderate and "3" is severe. The four-point scale is used to rate 21 descriptive anxiety symptoms. According to Beck et al. (1988), the BAI had high internal consistency (Cronbach coefficient alpha = .92) and a high test retest reliability of .75.

Seasonal assessment pattern questionnaire (SAPQ). The SAPQ (Rosenthal et al., 1984) asks participants about their seasonal variations in mood, weight, appetite, sleep length, social activity, concentration, and energy. The SAPQ has been shown to have high reliability and validity (Magnusson, Friis, & Opjordsmoen, 1997). The SAPQ

was included as a means to assess PMS in future studies, but was not used in the present study.

Laboratory questionnaire. During each laboratory session the participants were asked to complete a brief questionnaire (Richards, 2011; see Appendix F) asking whether or not they were taking pain medication, or if they had consumed alcohol or caffeine in the last twenty-four hours. Participants were also asked to indicate how much sleep they had attained prior to attending the lab for testing, and they were asked to answer questions regarding their current affective state. The Positive and Negative Affect Scale (PANAS) (Watson, Clark, & Tellegen, 1988) as well as the Menstrual Distress Questionnaire (MDQ) were included in the Laboratory Questionnaire. The PANAS includes two scales: positive affect (PA) and negative affect (NA). High scores on PA were interpreted as reflecting high energy, concentration and engagement. Low scores on PA were interpreted as sadness and lethargy. High scores on NA reflect greater degrees of subjective distress and unpleasant mood states such as anger, contempt, and disgust, whereas low scores were interpreted as calm and secure. Each item was rated on a five-point scale from 1 (*very slightly or not at all*) to 5 (*extremely*). Like the SAPQ, these ratings were not assessed in the present study, but were included for future analyses.

Three scales from the Menstrual Distress Questionnaire (MDQ)-T was included in order to asses menstrual cycle related pain and/ or discomfort. The items in the laboratory questionnaire reflected the following three scales: pain (six questions), water retention (four questions), and negative affect (8 questions). These scales were used to prospectively confirm the presence of PMS symptoms during group classification.

Screening for visual acuity. A Freiberg visual acuity test (FrACT; Bach, 1996; 2007;) was used in order to determine whether each participant had the necessary visual acuity to qualify for the study. During the FrACT participants were presented with Landolt "C" stimuli on a 75 cm distanced, 24° high x 18° wide CRT monitor. The Landolt "C" was presented at various orientations and participants were asked to determine which of 8 directional orientations the "C"-gap was pointing to. The gap of the Landolt C varied from 1° to 5' visual angle. The size of the gap that was properly detected 75 percent of the time determined threshold. Acuity estimates were attained using a PEST (Parameter Estimation by Sequential Testing) procedure in which the delta iteration in gap size was based on the participants prior test response. Participants were asked to use a chin-rest to ensure that they viewed the stimuli from the proper distance. Gap direction responses were made on a keyboard number pad. The information acquired from the FrACT was used to ensure that the participants had the near visual acuity required to participate in the study. An example of the FrACT stimuli and keyboard configuration is shown in Figure 2.

Visual screening for colour vision. The Ishihara pseudoisochromatic plate test, 24-plate edition (Ishihara, 1993) was used to determine whether the participants met the criteria for normal trichromatic color discrimination. The test included 24 plates (see Figure 3) that assess whether or not the participants showed deviations on the protan, deutan, or tritan lines. Each plate showed number patterns within an array of equiluminant colored dots to determine whether participants showed signs of dichromatic colour blindness. The plates were viewed under a D-65 standard illuminant lamp. Participants who could not read at least one of the plates were asked to discontinue their

participation without bonus mark penalty. Colour blindness screening was required because of the chromatic "yellowness" of the SPEM target that was designed to ensure L- and M-cone activity over an S-cone system that is influenced by estradiol (Eisner et al., 2004).

Eye tracking calibration. The eye tracking procedure was an adaptation of Smyrnis et al. (2007). We used a Cambridge Systems, infrared eyetracker [Cambridge Research Systems (CRS), Rochester, UK]. The participant dark-adapted for seven minutes before performing the calibration phase of the eye tracker test. During the calibration, the participant was instructed to first look at the fixation target and then to focus on the target where-and-when it was presented. Calibration values were used to determine saccade patterns. The moving stimuli (the targets) were presented on a 22-inch Mitsubishi Diamond Pro 2070 monitor powered by a CRS ViSaGe stimulus generator with 14-bit resolution per color channel at a 200 Hz frame. A Dell Precision Workstation with a Pentium 4 processor running at 3.6 GHz Calibration and gamma-correction of the software provided with the ViSaGe system was the source for driving the monitor.

The eyetracking calibration phase began with the participants being presented with a target ("white" square 0.5 x 0.5°) that appeared at the center of the monitor. After starting at the fixed center position, the target moved from right to left at constant intervals (2 seconds) in either 5° or 10° distances, and then back to the center. Both the 5° and 10° movement-cycles were repeated twice so that there were a total of four saccades for each of four positions (left/right, 5/10°) recorded. When necessary, left/right differences in amplitude were corrected with a manual adjustment and the calibration

procedure was repeated. Following the calibration phase, the experimental procedure began (see Procedure below).

Procedure

Participant orientation to menstrual cycle scheduling. Participants were provided with a link to the online questionnaire (see Appendix A) that included the consent form, a brief review of the study (Richards, 2011), and a screening questionnaire during the recruitment phase of the study. Those who were qualified according to the participation criteria (see page 33) were contacted by telephone or e-mail and asked to meet with the researcher for an orientation session. The orientation consisted of the researchers describing each of the psychophysical tests, the laboratory questionnaire, the salivary sampling procedures, and the ovulation test strip procedures, so that the participant knew what to expect during their laboratory sessions. During this time, the researchers made a preliminary appointment (to allow for adjustments) to book the participant's first laboratory session based on her self-report of her last first day of menses (as well as the length of her menstrual cycle) as indicated by her Alesse™ package for the oral contraceptive users, or by the reverse counting strategy for the non-users. LF sessions for the non-oral contraceptive users were scheduled based on the same self-reports described above and confirmed using the LH test strips (see page 35). For the Alesse™ users, day one of the menstrual cycle typically occurs on or around pill 21 in a 28-day package. Therefore, the LF session was scheduled between the daily pills 8-11, and LL session was scheduled between pills 18-21. The rest of the participants were scheduled for the LF session on days 11-14 and the LL session was scheduled

between and including days 25-28. Confirmation of next menses from each subject was used to ensure that the laboratory sessions were correctly scheduled.

Laboratory session procedures. Following the completion of the consent form (see Appendix D), the participants completed the laboratory questionnaire, the first portion of the salivary sample was collected, and then the FrACT (see Figure 2) and the Ishihara pseudoisochromatic plate test (see Figure 3) were conducted to ensure the participants' eligibility. One error on the Ishihara and a near acuity of 0.3 minimum angle resolution or greater on the FrACT were the qualifiers for participation. The researcher then collected the second portion of the salivary sample and then the eye tracking calibration was done. The final portion of the salivary sample was collected after the eye tracking test; therefore it took approximately an hour to complete the full sample.

Laboratory testing.

Eye tracking experiment. During the testing phase (after successful track calibration), the participants were first instructed to focus on a fixation target. They were then asked to follow a horizontally moving "yellow" round target (80 cd/m^2) with a subtending visual angle range of ±13° from fixation center (see Figure 4). The stimulus was superimposed on a "gray" background (mean luminance of 23.1 cd/m^2). Again, the target was "yellow" and not "white" because we wanted to ensure primarily parvocellular L and M cone involvement with little koniocellular responsivity (see Eisner et al., 2004). Target frequencies (cycles/sec or Hertz, Hz) were presented at 0.25, 0.5, and 1.0 Hz averaged over 10 cycles per frequency. Infrared Purkjinje-image positions were acquired as x,y, ASCI files and downloaded to a large memory Apple storage facility (RAID system) that was linked to a network server in order to back-up the data and prevent

excessive memory demands on the Dell hard drive. To measure tracking persistence, we used the data that was collected during the same frequencies described above (0.25, 0.5, and 1.0 Hz); however, instead of simply looking at the repeated 10 cycles we assessed the data that was collected after the stimulus disappeared wherein the eye movements were timed for 2 seconds.

Following the completion of the test, the final portion of the salivary sample was taken. Lastly, the participant's next laboratory session was scheduled. If the next session was during the LF phase, then the participant was re-instructed on the proper use of the hormone test-strips.

Second laboratory session. With the exception of signing the consent form, and completing the FrACT and the Ishihara Plate Test, all of the procedures from the first laboratory session were repeated again for the second session. The participants were debriefed following their completion of the study and given the ten-dollar gift certificate to either Chapters or Tim Horton's.

Salivary assay for estradiol and progesterone. Enzyme Linked ImmunoSorbent Assay (ELISA) was the method used to analyze the samples. Analysis occurred in the Lakehead University Center for Biological Timing & Cognition Bioassay Lab (BAL). The analysis followed a standardized Salimetrics™ protocol (see kit description in Appendices G and H).

The plates were arranged *a priori*, where samples were thawed, vortexed, and centrifuged at 1500 *g* for 15 minutes. Five microcentrifuge tubes were filled with 300-mL assay diluent and labeled 2 through 6, respectively. The 32 pg/mL standards (tube 1) was then serially diluted 2x by removing 300 ml and adding it to tube 2. This procedure

was repeated for tubes 3 through 6 creating final standard concentrations of 32 pg/ml, 16 pg/mL, 8 pg/mL, 4pg/mL, 2 pg/mL, and 1 pg/mL for tubes 1 through 6 respectively. Adhering to the *a priori* plate layout, 100 ml of standards, unknowns, and controls were pipetted into corresponding wells. All samples were assayed in duplicate. One-hundred ml of assay diluent was used in each non-specific binding (NSB) well. Using a 1:800 dilution, 100 ml of enzyme conjugate, (15 ml of enzyme conjugate and 12 mL estradiol assay diluent) was added to each well using a multi-channel pipette. The plate was then covered with adhesive covering and mixed on a Bokel® Scientific Model 130000 plate rotator for five minutes at 500 rpm and incubated at room temperature for 115 minutes. Following incubation the plate was washed with a Molecular Devices Skan Washer 400 plate-washer four times with 1x wash buffer. Two-hundred mL of tetramethylbenzidiene (TMB) solution was then added to each well using a multichannel pipette. The plate was then mixed on a plate rotator for five minutes at 500 rpm and incubated in the dark at room temperature for 25 minutes. Fifty mL of stop solution was then added using a multichannel pipette. Samples were subsequently mixed on a plate rotator for three minutes at 500 rpm. Finally, samples were read in a Molecular Devices SPECTRA max 384 plus plate reader at a 450 nm setting for concentration count and 660 nm setting for zero optics calibration. Final calculations were computed by subtracting the average optical density (OD) for the NSB wells from the average OD of the zero, standards, controls, and unknowns. Percent bound for standards, unknowns and controls was calculated by dividing the average OD by the average OD for the zero. Hormone levels were examined for group and menstrual cycle phase differences.

Data management.

Screening for group inclusion. Three exclusion criteria were used to classify the participants to either of the Control, PMS, or Alesse™ users (oral contraceptive user) groups both before and after the laboratory sessions. The first criterion (used prior to any laboratory involvement in the study) was the retrospective mean premenstrual phase MDQ-C scores collected from the online screening questionnaire ($N = 423$). Participants who had scores above the mean were assigned a "1" (PMS-type) and those with scores below the mean were assigned a "0" (Control or Alesse™ users). The second criterion was a prospective measure of PMS symptoms taken from the laboratory MDQ-C scales that were tested twice--once during the LF phases and once during the LL phases. Those who scored higher during the LF phase were coded as a "1" while women whose scores remained the same or were lower during the LL phase were assigned a "0". The final step was to calculate the mean score on the LIPS for the entire sample of participants who completed the online screening questionnaire ($N = 423$). In line with the previous coding system, "1"s and "0"s were assigned to participants who scored higher than the entire sample mean and lower than the sample mean, respectively. Participants who were not taking oral contraceptives and were assigned two or more "1"s were selected for the PMS-type group. Non oral-contraceptive users that received less than two "1"s were included in the Control group. Using the three criteria, 23 participants were classified as non- users without PMS-type symptoms (Control group), 22 as nonusers with PMS-type symptoms, and 21 as Alesse™ and/or Avianne™-users. .

Salivary assay for estradiol and progesterone. Concentrations of controls and unknowns were determined using SoftMax Pro® software through interpolation with a 4-

parameter sigmoid minus curve fit. We determined averages of all repeats for estradiol and progesterone as well as the coefficients of variability (CV). We confirmed all values to percent Inter-Assay CV and Intra-Assay CV limits. CVs were calculated from the calculated concentrations, Inter-assay % CVs of less than 15 were acceptable and Intra-assay % CVs less than 10 were included. The calculated concentrations (x) were determined by a four-parameter descending equation fitted to the standard known concentrations:

Eq 1. $$x = c\,[\,(-a + y) / d - y)\,]^{1/b}\;;$$

where b is the slope, a is the low x-value asymptote and d is the high x-value and d is the high x-value asymptote, and c is the midpoint parameter between a and d.

Real-target (stimulus present) database. Participant eye positions (amplitudes) for every corresponding discrete 20 msec time stamp was converted from millimeters (mm) to degrees visual angle by multiplying the amplitude by a subtending angle of 0.075. Next, each participant's eye movements were averaged across ten full cycles to calculate the average amplitude-per-time-stamp. Noise reduction was achieved through signal averaging with the original ten repeated cycles over 40,000, 20,000, and 10,000 msec for the 0.25, 0.5, and 1.0 Hz frequencies, respectively. A cutoff amplitude of ±20 visual angle was used to filter out saccadic and eye-blink responses.

Average position (real target amplitude). The average across 10 full cycles of the real-target was calculated for each participant by menstrual phase and by frequency. The averages were used to examine the average eye position within the -13° to 13° range. Findings from the average position were used to corroborate findings from the lead and lag as well as to determine any directional eye position biases.

Lead and lag (real target). Data from the real-target averaged across 10 full cycles were used to assess whether the participants eye movements were ahead of (lead) or behind (lag) the target stimulus. Only eye movements recorded after 90° and before 270° phases were coded as lead or lag in order to assess one part of the sine wave where the eye movements were moving at the fastest velocity (through center position) and did not have the opportunity to catch up to either extreme excursion point on the screen (-13˚ or 13˚). While the eye was moving from -13˚ to center all positive amplitudes were coded as leads and negative amplitudes were coded as lags, and while the eye was moving center to 13˚ all positive amplitudes were coded as lags and the negative amplitudes were coded as leads. A nonparametric count was made to sum the total count of leads for averaged groups, menstrual cycle, and frequency. The lead count was divided by the total number of 20 msec time bins for one averaged real-target cycle examined from 90˚, passing through 180˚, and ending at 270˚ to determine the percentage of average lead. This percent average lead was used to assess SPEM latency with > 50% representing eye movements that were ahead of the target.

Terminated target database (extrapolated trajectory). Eye movements following the termination of the target was collected for 2 seconds as a means to assess eye movement persistence. Persistence data was also converted to amplitude degrees and filtered for eye blinks and saccades using the same criterion as the real-target data. Stimulus offset was programmed to be randomized to begin at one of four cardinal phase positions within its current cycle; either 0°, 90°, 180°, or 270°. The participants' cycle phase termination positions were recorded manually for both phases of the menstrual cycle and for all three frequencies using their real-target database. The onset of

termination was flagged by the Matlab programming by a double "2" beside the original time-stamped data. Sinusoidal cycle phase points were manually marked throughout each participant's dataset to determine the exact sinusoidal cycle phase where the target terminated and where persistence began.

The *extrapolated trajectory* (i.e., where the participants eye movements would be based on the ten-cycle average of each participants eye positions with a real target) was calculated for all frequencies and menstrual phases by using the following steps.

The real-target ten-cycle tracking averages (per-participant, per-menstrual phase and per-frequency) were entered into a Matlab dataset as a function of time; where time in msec was converted to seconds. The following equation was a Matlab curve fitting procedure used to fit a Fourier transform of the data into sinusoidal fundamentals:

Eq 2. $f(x) = a_0 + a_1 \bullet \cos(x \bullet \omega) + b \bullet \sin(x \bullet \omega)$

where the parameters a_0 was the x-intercept; a_1 was the amplitude, b was the range of excursion from center (i.e., ±13° visual angle from center screen; and ω was the temporal frequency). The goodness of fit was determined using R^2 and any menstrual phase and/or frequency-per participant model fit below a moderate R^2 (0.4 or lower) was eliminated from future analysis (see Hu, 2014).

The coefficient values from the Fourier transform fits were then entered into an Excel™ spreadsheet where the optimized fitted equation was used for each participant's menstrual phase and frequency data. These calculations provided a theoretical trajectory of where the eye movements would have been (again, in amplitude degrees visual angle) if the stimulus had not been terminated; therefore these calculations were subsequently referred to as the theoretical *extrapolated trajectory* after target offset. The extrapolated

trajectories were aligned with relative time stamps beginning at 0 (start point after target termination) to the ending time of 2 seconds (i.e., the full recording epoch after offset). Note that the theoretical trajectory time stamp was normalized so that the 0 time stamp always factored in the randomized offset for each participant. Again, randomized offsets were either at the 0°, 90°, 180°, or 270° phase of all frequency cycles; therefore, I will subsequently refer to the onset of termination as the *relative onset of termination* and time as *relative time*.

Our next step was to calculate the difference in amplitude between the extrapolated trajectory and the real eye movements in order to assess a statistical level of persistence (i.e., how long the participant's eye movement was following the extrapolated trajectory after termination). A two-tailed, 95 percent confidence interval was calculated for every discrete time stamp as well as overall confidence interval calculations (per menstrual phase and frequency) from the 10 full cycles obtained from the real-target database. Both confidence interval measures (individual time-stamp and overall) were relatively consistent in indicating when the participant's eye-movements went off-track from the extrapolated trajectory, so we calculated the individual deviations as the "delta" indicator for *when* (in msec) the participants' no longer showed persistence movement. In these calculations, we filtered out "0"s and amplitudes outside of the excursion range of -13° to 13° and then took the absolute value of the participants amplitude subtracted from the extrapolated trajectory (i.e., |theoretical amplitude| - |actual eye movements|). That value provided the difference score for each discrete time-stamp. When the participant's eye position (amplitude) deviations exceeded the respective time-stamp confidence interval, that time stamp was selected as the point in time when the participants no longer

persisted with their tracking movements. From this point in time to the time of target offset defined persistence duration (see Figure 8). Further, we plotted eye position (amplitude in deg visual angle) as a function of time was for every participant as a non-statistical graphical means of qualifying and confirming the persistence duration and extinction. Sample plots of individual tracks for the control, PMS-type and Alesse™-user group by menstrual phase and frequency are shown in Figures 9-11, respectively.

Amplitude decline of eye movements. In order to compare the difference in persistence properties between groups by menstrual cycle phase and frequency, we converted the delta calculations (see above) to a percentage of eye movement accuracy with respect to the *relative* theoretical extrapolated trajectory using the formula:

Eq. 3. $(1- [|x-A| /R]) \bullet 100$;

where x is the theoretical position of where the target would be in degrees visual angle if it were not terminated (i.e., extrapolated trajectory), A is the eye position (in deg visual angle amplitude), and R is the full amplitude range from center screen (which we defined as 20° instead of 13° to avoid negative values). This formula allowed the calculation of the percent tracking accuracy for each derived delta score per relative time stamp. From there, the averages were calculated for every relative time-stamp for each group, across menstrual phase (LL, LF) and across frequency (0.25, 0.5, 1.0 Hz). The total cross averages were plotted as a function of relative time for the control, PMS-type, and Alesse™ user groups (see Figures 14-18, Column A). A second set of plots (Figures 14-18, Column B) show the same cross averages in Column A excluding data that fell within the relative extrapolated target position on the return leg towards center (0°) from the peak left (90° phase) or peak right (270° phase), which is referred to here as the *rebound*

limb. These rebounds cause artificial inflations in percent accuracy such that even if the participants' eye positions were to steadily stare at center screen, the resultant returning trajectory would impose a smaller delta, and thus a recalculation of "on-track" accuracy. Figures 12 and 13 highlight in red which of the limbs of the 0.5 and 1.0 Hz trajectory were isolated to eliminate this rebound effect. The red time ranges outlined at the bottom of each respective plot also indicate those intervals that were kept to better estimate the decay. The slowest 0.25 Hz trajectory was not used in the analysis due to a sizeable noise factor. The statistically derived persistence termination is indicated on the plot with a black arrowhead. A decay function was fit to both Column A and B data sets to better delineate the percent accuracy declines in amplitude:

Eq. 4. $y = b \bullet e^{(-m \bullet x)}$;

where x is relative time, b is the y-intercept and m defines the slope starting at the onset of consistent decay (i.e., following the onset of the statistically derived offset of persistence when the eye movements were no longer within the 95% confidence interval range previously described).

Results

Our hypotheses predicted that estradiol would have differential effects on SPEM and therefore we expected variation in SPEM across our groups (Control, PMS-type, and Alesse™ users), target frequencies (0.25, 0.5, and 1 Hz), and across menstrual cycle phase (LF, LL). The SPEM-tracking variables included (*1)* persistence time, (*2)* amplitude decline of eye movements, (3) lead and lag, and (*4)* average amplitude (position) differences. The null hypothesis predicted that persistence time, lead and lag, and average position would not vary according to the previously stated factors. Linear

Mixed Models (LMMs) were performed to analyze the data with group classified as a mixed-fixed factor and menstrual cycle phase and frequency specified as both mixed-fixed and mixed-repeated factors. LMM was selected as opposed to Repeated Measures Analysis of Variance (rmANOVA) because LMM allows for full factorial analysis for both between and within subjects variables without excluding pairwise missing data. The dependent variables were persistence time after target termination, lead and lag, and eye-movement position. This research design was determined *a priori* and consisted of a mixed 3 X 2 X 5 Between (Group: [Control, PMS-type, and Alesse™ users]) X Within: (Menstrual Cycle Phase [MF, LL]) X Frequency (0.25, 0.5, 1.0, 1.25, 2.0 Hz) using Bonferonni adjusted alpha levels. Alpha was set at $p < 0.05$.

Salivary Assay for Estradiol and Progesterone

There are several findings to note from the estradiol and progesterone assays. Beginning with estradiol, there was a main effect for group $F(2, 51.97) = 4.22$, $p = 0.02$. The PMS-type group ($M = 5.09$, $SD = 0.56$) was significantly different from the Alesse™ users ($M = 3.76$, $SD = 0.21$) ($p = .05$) (see Figures 6 and 7). In fact, the PMS-type group had the highest overall concentration of estradiol across the menstrual cycle compared to the other groups. Although the group x menstrual phase interaction was only approaching significance $F(2, 51.97) = 2.64$, $p = 0.08$, a pairwise comparison across all groups revealed that the mean differences in estradiol concentration between the LF phase ($M = 4.94$, $SD = .32$) and the LL phase ($M = 3.94$, $SD = .25$) was significantly different ($p = .02$) for the controls. Interestingly, neither the PMS-type nor the Alesse™ user groups showed significant differences in estradiol concentration between menstrual cycle phases.

The results for progesterone also revealed several noteworthy findings. There was a main effect for menstrual cycle phase, $F(1, 66.59) = 5.52$, $p = .02$). The participants had higher progesterone concentrations during the LL phase ($M = 162.27$, $SD = 12.85$) compared to the LF menstrual cycle phase ($M = 125.91$, $SD = 8.93$). Although there was not a significant interaction effect for group x menstrual phase, an analysis of the pairwise comparisons revealed that both the control group ($p = .03$) and the PMS-type group ($p = .05$) had significant progesterone concentration differences between the LF and the LL phases. For the control group, progesterone was significantly higher during the LL phase ($M = 166.58$, $SD = 13.53$) compared to the LF phase ($M = 124.19$, $SD = 13.66$). With regards to the PMS-type group, the LL phase ($M = 202.15$, $SD = 31.68$) also showed significantly higher progesterone concentrations relative to the LF phase ($M = 131.26$, $SD = 14.01$). The Alesse™ user group did not show significant shifts between the menstrual cycle phases.

Follow-up paired samples t-tests confirmed the above findings. For estradiol, the control group had significant differences between menstrual phases $t(21) = 3.46$, $p = .002$, whereas the PMS-type and Alesse™ user groups did not show significant differences. For progesterone, both the control group, $t(16) = -2.20$, $p = .034$, and the PMS-type group, $t(18) = -2.16$, $p = .045$, had significant differences between menstrual phases while the Alesse™ user group did not (see Figures 6 and 7). The salivary assay results demonstrate that, for estradiol, the control group showed a change across the menstrual cycle phases. The PMS-type and Alesse™ user group did not show a change across the menstrual cycle for estradiol. Therefore, if there are group differences in SPEM functioning and persistence that differentiate the control group from the PMS-type and

Alesse™ users then this suggests that estradiol is involved. However, the results for progesterone showed a menstrual cycle phase effect for the control group and the PMS-type groups. There was not a change in progesterone between menstrual phases for the Alesse™ user group. Therefore, if significantly different oculomotor effects are found for the control and PMS-type groups compared to the Alesse™ user group, then this suggests that progesterone is involved (see Table 1).

Table 1

Sex steroid levels at Late Follicular and Late Luteal for estradiol and progesterone for the control, PMS-type, and Alesse™ user groups. For estradiol, the control group showed a significant change across the menstrual cycle but the PMS-type and Alesse™ users did not. For progesterone, there was a significant main effect for menstrual cycle phase for the control and PMS-type groups, but not the Alesse™ user group.

Group	*Estradiol*	*Progesterone*
Control	change	change
PMS-Type	no change	change
Alesse™ Users	no change	no change

Eye-Movement Position (Real Target Amplitudes)

Unfortunately, there were no significant menstrual cycle phase effects found for eye position. There was a significant group main effect that was observed between the control group ($M = 0.08$, $SD = 0.11$) and the Alesse™ users ($M = 0.56$, $SD = 0.12$) for average amplitude. The PMS-type group also had higher average amplitudes compared to the control group ($M = 0.39$, $SD = .10$). Finally, a group by frequency interaction effect was found with the 0.5 Hz trials, where the Alesse™ users ($M = 0.55$, $SD = 0.20$) and the PMS-type ($M = 0.027$, $SD = 0.19$) showed a left-to-right bias evident by their pattern of positive positional eye-movements overall compared to the Control group ($M =$-0.03, $SD = 0.18$) who had negative overall positional eye-movements (see Figure 19 and Table 2).

Table 2

Linear Mixed Model analysis of real target amplitude (position) showing a group main effect where the Alesse™ user group had higher amplitudes compared to the control group and a group by frequency interaction where the PMS-type and Alesse™ user groups showed a left-to-right excursion bias whereas the control group remained closer to the center of the screen.

Variable	*df*	*F*	*p*
Group	2	4.776	.009
Phase	1	0.223	.637
Frequency	2	1.170	.313
Group x Frequency	8	2.499	.014
Group x Phase	2	2.063	.129
Phase x Frequency	2	0.191	.827
Group x Phase x Frequency	4	0.230	.921

Lead and Lag (Real Target)

No menstrual cycle phase effects were found. There was a frequency main effect observed for lead and lag, $F(2, 166.50) = 6.09$, $p = 0.003$ (see Figure 20 and Table 3). A consistent pattern for the frequencies was found where the participants' eye-movements lagged behind the target more than they were leading the target. Therefore, although we are using the "percentage of lead" metric, the results showed that the participants' eye-movements were in fact lagging behind the target (i.e., under 50%). There was not a higher percentage of lead for 1.0 Hz ($M = 49.52$, $SD = 1.12$), compared to the 0.25 Hz target ($M = 46.23$, $SD = 0.78$) and the 0.5 Hz target ($M = 44.12$, $SD = 1.10$). There were no significant interaction effects observed for lead and lag; however, groups did approach a main effect, $F(2, 236.08) = 2.69$. $p = 0.07$. The PMS-type group ($M = 45.81$, $SD = 0.96$) and the Alesse™ user group ($M = 45.54$, $SD = 1.07$) showed more eye-movement-to-target lag compared to the control group ($M = 48.53$, $SD = 1.00$) (see Figure 21).

Table 3

Linear Mixed analysis of real target lead and lag showing a frequency main effect where there was an average lag for eye movements at all of the frequencies and there was a trend found for the groups in which the PMS-type and Alesse™ user groups had more lag compared to the control group.

Variable	*df*	*F*	*p*
Group	2	2.691	[t].070
Phase	1	2.682	.103
Frequency	2	6.094	.003
Group x Frequency	4	0.481	.750
Group x Phase	2	1.693	.186
Phase x Frequency	2	0.029	.971
Group x Phase x Frequency	4	0.074	.990

[t] approaching significance

Persistence Time after Target Termination (Extrapolated Trajectory)

There were no significant menstrual cycle phase effects for persistence time after target termination; however, there was a frequency main effect, as expected given that the speed of a target at a higher frequency should cause greater off-target variations in SPEM (see Figure 22). Participants had longer durations of persistence after target termination when tested with the 0.25 Hz oscillating target ($M = 0.38$, $SD = 0.02$) compared to the 0.5 Hz target ($M = 0.27$, $SD = 0.02$) and the 1.0 Hz oscillating target ($M = 0.28$, $SD = 0.02$). A group by frequency interaction effect was observed in which the PMS-type group had a significantly different persistent duration compared to the controls during the 1.0 Hz frequency trial, where the controls ($M = 0.32$, $SD = 0.03$) persisted longer than the PMS-type group ($M = 0.21$, $SD = 0.03$) (see Figure 23). These effects can be seen in Table 4.

Table 4

Linear Mixed Model analysis of duration of persistence after target termination showing a frequency main effect in which there was longer persistence durations during 0.25 Hz compared to 0.5 and 1.5 Hz. A group by frequency interaction effect revealed that the PMS-type group persisted for shorter durations compared to the control group during at 1.0 Hz.

Variable	*df*	*F*	*p*
Group	2	0.644	.526
Phase	1	0.011	.916
Frequency	2	8.104	<.001
Group x Phase	2	0.402	.669
Group x Cycle			
Phase x Frequency	2	0.132	.876
Group x Phase x Frequency	4	0.803	.525

Amplitude Decline of Eye Movements (Extrapolated Trajectory)

The results of the decay functions were not assessed statistically because logistically we could not run multiple repeating trials for sufficient signal averaging and still keep within a reasonable time frame for experiment participation. Instead, we analyzed the fitted decay function slopes for group, menstrual cycle phase, and with frequencies to look for trends. Using the fitted slope parameters averaged across groups and across menstrual cycle phases for both 0.5 and 1.0 Hz we compared the slopes calculated in Part B (fitted decays separated by sinusoidal limbs; see Figures 14-18). Column A (total decay; see Table 5 and 6 showing the slope parameters) reveals sufficient noise compared to the data where the rebounds were isolated and removed (Column B). Therefore, the slopes derived for Column B were further analyzed and shown in Figures 14-18.

Table 5

Slope parameters of the fitted decay function to the calculated percent accuracy amplitudes (see eq. 2) for the 0.5 Hz condition. The limbs are defined as those amplitudes on the return leg towards center (0°) from peak left (90° phase) or peak right (270° phase). NOTE: The number of limbs indicates the number of times the participants kept from reaching 0% accuracy with each rebounding extrapolated trajectory.

Group	*Limb*	*Fitted Slope*	R^2
Control	1	1.3978	0.948
Control	2	3.1257	0.921
PMS-type	1	1.4823	0.910
PMS-type	2	2.4849	0.961
Alesse	1	1.1349	0.945
Alesse	2	1.4424	0.781
Late Follicular	1	1.4951	0.954
Late Follicular	2	3.041	0.928
Late Luteal	1	1.056	0.974
Late Luteal	2	2.1134	0.945

$R^2 > .5$ (see Hu, 2014).

Table 6

Slope parameters of the fitted decay function to the calculated percent accuracy amplitudes (see eq. 2) for the 1.0 Hz condition. The limbs are defined as those amplitudes on the return leg towards center (0°) from peak left (90° phase) or peak right (270° phase). NOTE: The number of limbs indicates the number of times the participants kept from reaching 0% accuracy with each rebounding extrapolated trajectory.

Group	*Limb*	*Fitted Slope*	R^2
Control	1	1.401	0.89
Control	2	3.7601	0.96
Control	3	1.0211	0.93
PMS-type	1	2.6775	0.97
PMS-type	2	3.1098	0.94
PMS-type	3	2.3256	0.55
Alesse	1	2.2573	0.91
Alesse	2	5.9769	0.91
Alesse	3	1.2014	0.71
Late Follicular	1	2.1588	0.96
Late Follicular	2	2.7053	0.89
Late Follicular	3	2.1253	0.73
Late Luteal	1	1.2275	0.9
Late Luteal	2	3.9629	0.9
Late Luteal	3	0.96528	0.84

$R^2 > .5$

Comparisons between the two frequencies showed steeper fitted slopes with the 0.5 Hz overall (across the menstrual phase condition compared to the 1.0-Hz condition). For the 0.5-Hz condition, the PMS-type and Alesse™ user groups decayed earlier as evidenced by the analysis of the slopes in which the control group did not provide a reasonable fit until the third limb (see Figure 14 and Table 7 for slope references to the limbs) while the other groups decayed within the first declining limb. These results suggest that it took longer for the control group to reach decay asymptote.

Fitted decay functions to percent accuracy amplitude declines averaged across menstrual cycle phase showed trends at the 0.5 Hz condition as well. The fitted decays for both the LF and LL phases started at roughly the same time; however, the LF data

stayed closer to the extrapolated trajectory even when their amplitude was on decline compared to the LL phase (see limbs 3 in Figures 17 and 18 and in Table 7).

Table 7

Percent Amplitude Accuracy decay with respect to the Theoretical Extrapolated Target Position for the 0.5 Hz condition. NOTE: The number of limbs indicates the number of times the participants kept from reaching 0% accuracy with each rebounding extrapolated trajectory. R^2 values less than 0.4 were not used in defining decay slopes. See text for details.

Group	*Limb*	*Slope*	R^2
Control	3	2.1213	0.872
PMS-type	1	2.9943	0.865
PMS-type	3	1.7284	0.974
Alesse	1	2.6507	0.887
Alesse	3	2.2171	0.566
Late Follicular	1	1.2077	0.913
Late Follicular	3	2.7799	0.878
Late Luteal	1	0.95145	0.53
Late Luteal	3	1.3191	0.8717
Late Luteal	4	2.3387	0.60753

$R^2 > .5$

At the 1.0 Hz frequency the control group did not show decay until the second rebound (i.e., decay function fits were viable only the second and third limb (see Figure 14 and Table 8). The control group continued to decay at the end of 2 seconds (never reaching 0%) whereas the PMS-type group was moving into higher percentages of accuracy during the latter portion of the 2 seconds suggesting that they were initially distracted by the abrupt offset of the stimulus but later began to look for the stimulus where it would have been if it had not been terminated.

Comparisons between the two menstrual cycle phases at 1.0 Hz showed that there were steeper decays in the first limb during the LL compared to the LF phase.

Table 8

Percent Amplitude Accuracy decay with respect to the theoretical Extrapolated Target Position for the 1.0 Hz condition. Details as in Table 7.

Group	*Limb*	*Slope*	R^2
Control	2	3.4751	0.94
Control	3	0.73281	0.63
PMS-type	1	1.5752	0.8
PMS-type	2	2.9355	0.9
Alesse	1	2.7346	0.93
Alesse	2	3.3555	0.74
Alesse	3	0.8936	0.74
Late Follicular	1	2.7688	0.96
Late Follicular	2	3.0975	0.91
Late Luteal	1	0.90064	0.71
Late Luteal	2	3.5753	0.85

$R^2 > .5$

Discussion

For the salivary assays for estradiol there was a group main effect where the PMS-type was significantly different from the Alesse™ user group. There was a significant change in estradiol across the menstrual cycle phases for the control group. The results for progesterone revealed a menstrual cycle phase effect in which progesterone concentrations were higher during the LL phase compared to the LF phase. Pairwise comparisons for group by menstrual cycle phase revealed significant differences between the control and PMS-type groups between the LF and LL phases. The control group as well as the PMS-type group had higher progesterone concentration levels during the LL compared to the LF menstrual cycle phase. The Alesse™ user group did not show progesterone concentration differences between menstrual cycle phases.

For eye movement position (real target amplitude) there was a group main effect where the control group had lower overall amplitudes compared to the PMS-type and Alesse™ user groups. There was also a group by frequency interaction that revealed a

left-to-right eye movement bias for the PMS-type and Alesse™ users groups, but not the control group. For lead and lag there was an overall lag for all frequencies and the group main effect was approaching significance wherein the PMS-type and Alesse™ user groups had more eye movement lag compared to the control group. For persistence time after target termination (extrapolated trajectory) the participants had longer durations of persistence at 0.25 Hz, and the PMS-type group showed significantly less persistence duration than the control group. Finally, when assessing the amplitude decline of eye movements it was found that there were steeper slopes at 0.5 Hz, the PMS-type and Alesse™ user groups decayed earlier than the control group, and at 1.0 Hz there were steeper slopes during the LL compared to the LF menstrual cycle phase in the first limb.

We did not find menstrual cycle phase effects outside of the salivary assays; however our group findings suggest that there is something characteristic about the groups that is non-humoral that is showing SPEM functioning effects. This could be a result of neurological differences between the groups. The pattern of cycling and non-cycling hormones may be a manifestation of these neural circuits. Therefore, all of our interpretations of the results, with the exception of the salivary assays could be related to CNS estradiol rather than ovarian peripheral hormone cycles related to LF and LL.

While some of the effects we observed were expected (i.e., frequency main effects) many of our results require further replication. For example, SPEM persistence duration differentiated the control group from the PMS-type group at the 1.0 Hz frequency in which the control group persisted for longer durations than the PMS-type group. This was not the case for the Alesse™ users, pointing to CNS estradiol mediation in the length of persistence duration. Although this PMS-type behaviour may seem contradictory at

first with respect to many of the perseveration-type symptoms characteristic of depressed groups, it may also be consistent with PMS-type symptoms in that disruptions in attention and concentration may have been amplified by the abrupt action of target termination.

Salivary Assay for Estradiol and Progesterone

The salivary assay results (see page 54) are an integral part of our findings as we expanded on the previous study by Richards (2011) and discovered group differences in which the control group, but not the PMS-type and Alesse™ users, showed significantly higher estradiol concentrations across menstrual cycle phases; however for progesterone both the control and PMS-type groups showed significant shifts where progesterone concentrations were highest during the LL phase compared to the LF phase. For progesterone, the results did not show significant concentration differences between the menstrual cycle phases. The latter finding should be expected given the consistent dosage across the cycle and the inhibition of ovulation which stops the luteal phase from occurring. Further, the control group showed shifts in estradiol and progesterone between menstrual phases; however the PMS-type and Alesse™ user groups showed stability for estradiol. For progesterone, the control and PMS-type groups, but not the Alesse™ user group, showed significant shifts across menstrual phases. These findings suggest that estradiol may be the modulating agent (possibly with progesterone as a secondary influence) for the behavioral findings, a point that highlights horizontal SPEM neural circuitry and its susceptibility to estradiol influences (see Figure 5). If estradiol inhibits GABA, which in turn inhibits omnipause neurons responsible for inhibiting excitatory saccadic-burst neurons (Krauzlis, 2005), then elevated CNS estradiol levels

should lead to increased saccades, which would diminish the integrity of SPEM.

Real Target Data

Directional Bias in Position. Our real target results were an extended replication of Richards (2011) results**.** Not only did the control group positionally demonstrate less percent lag to target, but the average position results also provided insight into the pattern of eye movement differences across the groups. The control group had lower amplitudes in 10 averaged cycles compared to the other groups. This means that the control group stayed closer to the center of the test screen whereas the PMS-type and Alesse™ user participants showed greater excursion values. The frequency by group interaction effect for amplitudes demonstrated that, at least with the 0.5 Hz trials, the Alesse™ users had the highest excursions, but there was a directional difference in that the control group had overall negative average amplitudes whereas the PMS-type group and the Alesse™ users had overall positive average amplitudes. These results support findings from an earlier analysis from a separate block of data that also assessed real target amplitudes in sets of 10 repeating cycles over more frequencies where the results did show greater overall excursions in the positive direction for the PMS-types (Wesner, et al., 2014). Ultimately, the PMS-type and Alesse™ user groups had overall average positive excursions. This means that their eye-movements were biased from left-to-right and the control group did not show this bias. The left-to-right SPEM bias may be due to group-related dysregulated CNS estradiol. For example, CNS estradiol-mediated cerebellar modifications may modulate activity of the FEF known for its involvement with steady left-to-right SPEM (e.g., Swenson, 2006). Our findings, therefore, suggest that ipsiversive SPEM might be modulated by estradiol at the FEF, which would have

modulatory effects throughout other oculomotor neural circuits such as the cerebro-cerebellar circuits. All of these group differences support our hypothesis that SPEM would vary based on hormonal group.

With respect to the research on schizophrenia (see page 67; Mahlberg, Steinacher, Mackert, & Flechtner, 2001; Robinson, 1975), along with depression (Posner et al., 2004), and the results of the present study, one might argue that our findings are supportive of specific oculomotor pathways being involved in SPEM functioning (see Figure 5). The effects exerted on several oculomotor pathways are different depending on the hormonal patterns of the groups. Progesterone itself has been found to decrease saccadic eye velocity (van Broekhoven, Backstrom, & Verkes, 2006). As previously discussed, progesterone systems enhance GABAergic systems. The findings that the PMS-type (see page 57) and Alesse™ user groups had greater real-target amplitude excursions from the center position and a directional bias demonstrates two possible lines of SPEM deficits with respect to latency and direction. Similar to PMS and PMDD, the neurobiology of SPEM in bipolar I is a vaguely researched area; however, Martin et al., (2011), using functional magnetic resonance imaging, recently discovered that bipolar disordered individuals (compared to controls) had increased cerebellar vermis activity during a SPEM task where they followed a white horizontally moving target at a velocity of 16.7º per second. As already mentioned, the cerebellar vermis is a known area involved in horizontal SPEM; however, the finding from the Martin et al. study suggests a similar rhombencephalic pathway to SPEM for affective disorders including PMS and PMDD. The cerebellar vermis is involved in the maintenance of SPEM (i.e., persistence, amplitude position, lead and leg) as well as regulating saccades, and if it is more active in

individuals with PMS-type symptoms, like the effect found for participants with bipolar disorder, then this could help explain the group by menstrual phase and frequency effects found in the present study.

Lower Lag in Controls Versus PMS-Types and Alesse™ Users. For lead and lag, there were overall lag patterns found across the groups for all of the frequencies. As to be expected, when the target was fastest (1.0 Hz) there was less lag compared to 0.25- and 0.5-Hz conditions as shown in Figure 20. Although there were no significant interactions found for menstrual cycle phase or group there was a trend found within the groups. The control group did not lag behind the target to the same degree as the PMS-type and Alesse™ user groups ($p = 0.070$). This suggests that SPEM efficiency is enhanced (i.e., is more "on target") in the normal free-cycling control group compared to the more hormonally-stable groups (i.e., Alesse and PMS-type). As previously noted, estradiol inhibits $GABA_A$, and GABA inhibits omnipause neurons which inhibit excitatory burst neurons that are known to elicit saccades. CNS estradiol's indirect effects on saccades could explain why the control group was more "on target" compared to the PMS-type and Alesse™ user groups. Given that saccades disrupt SPEM and that estradiol may be responsible for the inhibition of the mechanism responsible for initiating saccades, the stability of estradiol in the PMS-type and Alesse™ user groups may explain why they were not as "on target" compared to the control group because a lack of change in estradiol concentration across the menstrual cycle may be caused by modulation related to affective disorders such as PMS and PMDD.

Extrapolated Trajectory Data

Shorter Durations of Persistence for PMS-type and Alesse™ users. The main effect found for frequency where the persistence duration was longest at 0.25 compared to 0.5 and 1.0 Hz is most likely attributable to the fact that the eye movements take the longest amount of time to get to either the extreme left (-13˚) or extreme right (13˚) following stimulus termination during the 0.25 Hz trial. This means that the eye movements took longer to change direction, which would have made it easier for the eye movements to stay "on track". Our other findings are not as simply interpreted, however. Increased saccadic eye-movements countermand SPEM and they might explain the shorter durations of persistence for the PMS-type group relative to the controls. As previously noted (see pages 5 and 22), affective symptoms such as PMS are also related to the attention-startle-response phenomenon. Kaviani and colleagues (2004) found that their participants who had depression were more distracted by a startling stimulus as evidenced by their expression of psychomotor slowing and it took the depressives longer to dissociate from it. Such findings may explain our measured persistence time differences where the control group persisted longer than the PMS-type group at 1.0 Hz [as well as the trends in our decay slopes (see page 62) in which the PMS-type group had limbs that fit decays earlier than the control group]. The decreased persistence duration present in the PMS-type group relative to the control group may have occurred at 1.0 Hz rather than 0.25 or 0.5 Hz because it would not take as long to get to an extreme position (-13˚ or 13˚) where the persistence would be most likely to end because the eye movements have to change direction. Therefore, the distractibility in the PMS-type group may have been exacerbated by the change in target direction that occurs earlier in the 1.0

Hz compared to the slower target movements with the 0.25- and 0.5-Hz conditions.

Research has found that schizophrenics, have higher levels of peak velocity of saccadic eye movements that seems to be regulated by the saccadic-excitatory burst neurons in the brainstem (Mahlberg, Steinacher, Mackert, & Flechtner, 2001; Robinson, 1975). Once again, the PMS-type group may have displayed CNS estradiol modulated inhibition of the neural circuitry involved in the inhibition of saccades that caused elevated saccadic intrusion which then would have lead to less persistence immediately following target offset, much like the oculomotor behaviour observed in the schizophrenic group in the Mahlberg and colleagues study. Given that drugs that inhibit GABA are used to treat schizophrenia symptoms along with the research that demonstrates eye movement dysfunctions in this group. Majewska and colleagues (1986) pointed out that inhibitory effects of $GABA_A$ were potentiated in cultured rat hippocampus, and this effect was mediated by higher levels of luteal progesterone. Further, Backstrom et al. (2011), based on their investigation of progesterone during the luteal phase of the menstrual cycle, found enhanced sensitivity to GABAergic substrates have been found during the LL phase in women with PMDD. If the GABAergic system is affected during the LL phase, and if saccadic eye movements are interrupted in people with schizophrenia due to changes in GABA, then similar eye movement changes may be found in people with PMS-type symptoms, which are marked by progesterone dysregulation. Progesterone seems to enhance inhibition, and given that SPEM is in fact regulated by inhibition and that PMDD symptoms have been associated with the above finding, the next logical step is to investigate the progesterone affinity to key receptors involved in SPEM circuitry. As already mentioned, estradiol and progesterone are

antagonistic to one another during the menstrual cycle in that progesterone seems to mitigate the inhibitory effects of estradiol. Therefore, CNS estradiol systems in PMS-types (like the Alesse™ users) may be causing the menstrual cycle stability found in ovarian estradiol, which may have an indirect effect that causes estradiol's inhibitory effects to be mitigated, thereby enhancing inhibitory systems. Our group differences support this logic because the control group (which has shifts in estradiol between menstrual phases) persisted longer than the hormonally stable PMS-type group. The hormonal stability may be a product of something characteristic about the PMS-type (as well as the Alesse™ users) that is not actually causing estradiol to inhibit $GABA_A$ ultimately leading to more saccadic intrusions and shorter persistence durations. CNS estradiol differences between the groups may have such effects on saccades.

Amplitude decline of eye movements. One of our interesting findings from the decay slopes is that while the PMS-type group dissociated faster than the control group (possibly related to the attention-startle-response; see page 60), the accuracy of the PMS-type groups' eye movements did increase toward the end of the two-second recordings even though the rebound limbs were removed. If the rebound limbs were not removed then we could postulate that the eyes were static at center position and the extrapolated trajectory rebounds would have created an artificially inflated accuracy; however, the fact that the rebound limbs were removed suggests that the PMS-type group continued to follow the extrapolated trajectory and did not aymptote to 0 percent. Therefore, although the PMS-type group did not persist as long as the control group, they may have had higher accuracy of eye movements near the end of the two seconds, suggesting that the PMS-type group is more easily distracted by the termination of a stimulus, however their

eye-movements continued to move in the fashion of the sinusoid. Estradiol has been found to inhibit GABA which is responsible for inhibiting omnipause neurons which then inhibit excitatory burst neurons that go on to elicit saccades. Changes in CNS estradiol between groups could, therefore, indirectly impact the excitatory burst neurons ability to elicit saccades. If the PMS-type group has characteristic estradiol compared to the control group then such neurological differences may cause less saccades in the control group causing them to have longer durations of persistence.

Overall, the findings from the present study suggest that there estradiol seems to exhert its effects on the neural circuitry involved in SPEM. Again, such findings (especially with our salivary assay results) implicates estradiol stability as the common factor with the possibly of progesterone as secondary antagonistic operator, in horizontal SPEM functioning.

Limitations and Further Directions

There were several limitations to the present study. Firstly, the number of eligible participants was limited due to our exclusion of males as well as women taking oral contraception other than Alesse™ and Avianne™. Not only was the participant pool restricted, several data files collected for the different frequency trials had to be eliminated from the study due to calibration issues with the eye-tracker.

Also, based on our short persistence durations, it probably would have been beneficial to use an eye-tracker with a resolution greater than 60 Hz to better observe the subtle variations that may have occurred within the msec time frame of persistence. Another limitation includes the fact that the target termination was not completely randomized and, in effect, the termination points for 0.5 and 1.0 Hz began only at 0 degrees or 180

degrees. Therefore, the persistence data from these frequencies all began at the center of the screen. This is potentially confounding as subjects could develop a spatial expectation regarding where their stimulus may terminate during their second session. Having said this, however, it turned out that these serendipitous center screen offsets proved to be beneficial when it came to normalizing the relative time stamps for the percent amplitude decay functions; otherwise a secondary confound would have developed based on velocity changes at peak and trough excursions.

For future research, one could include measurement of the saccades rather than filtering them out. As discussed several times throughout this paper, saccades include a high velocity gaze shift toward the target in order to focus the target on the fovea. Perhaps a future direction could be to focus on the nature of the saccades within SPEM to investigate the maintenance and regulation between menstrual cycle phase and group. Also, while our intention was to discover any potential effects of estradiol in SPEM, we discovered cyclic patterns of progesterone in our salivary assays where progesterone was elevated during the LF phase in the PMS-type as well as the control group.

Our salivary assay results showed a heightened and cyclic presence of progesterone in the PMS-type and control participants, but not the Alesse™ users (which is expected of the Alesse™-user group because oral contraceptive suppress hormones across the cycle. Estradiol was not cyclic in the PMS-type women whereas it was in the controls. Due to the latter, as well as the fact that elevated levels of progesterone during the LL menstrual cycle phase is characteristic of PMS-type symptoms, the metabolic interaction of estradiol and progesterone might be important for understanding the differences in SPEM functioning found in women with PMS-type symptoms. Efforts should be made to

replicate these findings to better understand the hormonal modulation of SPEM across the menstrual cycle. One means to further investigate this research could be to include a group of women receiving hormone replacement therapy as several oculomotor dysfunctions have been associated with the latter (see Baum, 2005; Eisner, Toomey, Incognito, O'Malley, & Samples, 2006b; Joffe, Hennen, Soares, Carleson, & Cohen, 2002). Also, given that testosterone is aromatized into estradiol, the presence of testosterone throughout the cerebellum and brainstem should be further investigated. Overall, the present study has garnered several findings that provide further insight into estradiol influences on SPEM and these findings point to several avenues of future research to further understand potential affective and neurobiological mediation of SPEM.

References

American Psychiatric Association (2013). *Diagnostic and statistical manual of mental disorders (5th ed.)*. Washington, DC: Author.

Andreescu, C. E., Bogdan, A. M., Haasdijk, E. D., Kramer, P., De Jong, F. H., Krust, A., De Zeeuq, C. I., & De Jeu, M. T. (2007). Estradiol improved cerebellar memory formation by activating estrogen receptor β. *The Journal of Neuroscience, 27*(4), 10832-10839. doi:10.1523/JNEUROSCI.2588-07.2007

Bach, M. (1996). The Freiburg visual acuity test-automatic measurement of visual acuity. *Optometry & Vision Science, 73*(1), 49-53. doi:10.1097/00006324-199601000-00008

Bach, M. (2007). The Frieburg Visual Acuity Test-Variability unchanged by post-hoc reanalysis. *Graefe's Archive of Clinical Experimental Opthalmology*. doi: 10.1007/s00417-006-0474-4

Backstrom, T., Haage, D., Lofgren, M., Johansson, I.M., Stromber, J., et al. (2011). Paradoxical effects of GABA-A modulators may explain sex steroid induced negative mood symptoms in some persons. *Neuroscience*, *191*, 46-54. doi: 10.1016/j.neuroscience.2011.03.061

Baum, M. (2005). Adjuvent endocrine therapy in postmenopausal women with early breast cancer: Where are we now? *European Journal of Cancer*, 41, 1667-1667, doi: 10.1016/j.ejca.2005.05.006

Beck, A. T., Epstein, N., Brown, G. & Steer, R. A. (1988). An inventory for measuring clinical anxiety: Psychometric properties. *Journal of Consulting and Clinical Psychology, 56*, 893-897. doi: 10.1037/0022-006X.56.6.893

Beck, A. T., & Steer, R. A. (1990). Beck Anxiety Inventory manual. New York: The Psychological Corporation Harcourt Brace Jovanovich Inc.

Biegon, A. et al. (2010). Unique distribution of aromatase in the human brain: in vivo studies with PET and [N-methyl-11C]vorozole. *Synapse 64*, 801–807.

Boyden, E. S. & Raymond, J. L. (2003). Active reversal of motor memories reveals rules governing memory encoding. *Neuron, 39*:1031–1042. doi:10.1016/S0896-6273(03)00562-2

Cordoba Montoya, D. A., & Carrer, H. F. (1997). Estrogen facilitates induction of long term potentiation in the hippocampus of awake rats. *Brain Research, 778*, 430 – 438.

Donaghy, C., Pinnock, R., Abrahams, S., Cardwell, C., Hardiman, O., Patterson, V., McGinern, C. R., & Gibson, J. M. (2009). Ocular fixation instabilities in motor neuron disease: A marker of frontal lobe dysfunction? *Journal of Neurology, 256*, 420-426. doi:10.1007/s00415-009-0109-x

Eisner, A., Austin, D. F., & Samples, J., R. (2004). Short wavelength automated perimetry and tamoxifen use. *British Journal of Ophthalmology*, *88*, 125-130.

Eisner, A., Burke, S. N., & Toomey, M., D. (2004). Visual sensitivity across the menstrual cycle. *Visual Neuroscience*, *21*, 513-531. doi:10.1017/S0952523804044037

Eisner, A., Incognito, L. J., Toomey, M. D., O'Malley, J. P., & Samples, J. P. (2005). Tamoxifen induced optic-nerve-head swelling. *Investigative Ophthalmology and Vision Science* (ARVO abstract # 2547).

Eisner, A., O'Malley, J. P., Incognio, L. J., & Toomey, M. D. (2006a). Small optic cup sizes among women using tamoxifen: Assessment with scanning laser ophthalmoscopy. *Current Eye Research*, 31, 367-379. doi:10.1018/02713680600602547

Eisner, A., Samples, J. R., Campbell, H. M., & Cioffi, G. A. (1995). Foveal adaptation abnormalities in early glaucoma. *Journal of Optical Society of America A*, 12, 2318.

Eisner, A., Toomey, M. D., Falardeau, J., Samples, J. R., & Vetto, J. T. (2007). Differental effects of breast cancer survivors. *Breast Cancer Research and Treatment*, *106*(2):161-70.

Eisner, A., Toomey, M. D., Incognito, L. J., O'Malley, J. P., & Samples, J. R. (2006b). Contrasting blue-on-yellow with white-on-white visual fields: roles of visual adaptation for healthy peri-or postmenopausal women younger than 70 years of age. *Investigative Ophthalmology & Vision Science*, *47*(12), 5605-5614.

Eisner, A. (1993). Longitudinal changes of visual function over 18 months: evaluation of eyes with high-and low-risk macular degeneration characteristics. *Documenta Ophthalmologica Proceedings Series*, 56, 175-187.

Flechtner, K. M., Steinacher, B., Sauer, R., & Mackert, A. (1997). Smooth pursuit eye movements in schizophrenia and affective disorder. *Psychological Medicine*, 27, 1411-1419

Frick K. M., Fernandez S. M., & Bulinski S. C. (2002). Estrogen replacement improves spatial reference memory and increases hippocampal synaptophy-sin in aged female mice. *Neuroscience, 115*, 547–558.

Fukushima, K. (2003). Roles of the cerebellum in pursuit-vestibular interactions. *The Cerebellum, 2*, 223-232. doi:10.1080/14734220310016178

Gyenes, A., Hoyk, Z., Csakvari, E., Siklos, L., & Parducz, A. (2010). 17β-estradiol attenuates injury-induced microglia activation in the oculomotor nucleus. *Neuroscience, 171*(3), 677-682. doi:10.1016/j.neuroscience.2010.09.033

Gorin, M. B., Day, R., Costantino, J. P., Fisher, B., Redmond, C. K., Wickerham, L., Gomolin, J. E., Margolese, R. G., Mathen, M. K., Bowman, D. M., Kaufman, D. I., Dimitrov, N. V., Singerman, L. J., Bornstein, R., & Wolmark, N. (1998). Long-term tamoxifen citrate use and potential ocular toxicity. *American Journal of Ophthalmology*, 125, 493-501.

Gupta, P. D., Johar, K., Napgal, M. S., & Vasavada, A. R. (2005). Sex hormone receptors in the human eye. *Survey of Ophthalmology*, *50*(3), 274-284. doi:10.1016/j.survophthal.2005.02.005.

Hakim, O. M., Gaber El-Hag, Y., & Maher, H. (2008). Persistence of eye movement following disinsertion of extraocular muscle. *Journal of AAPOS, 12*(1), 62-65.

Hansel, C, Linden, D. J., & D'Angelo, E. (2001). Beyond parallel fiber LTD: the diversity of synaptic and non-synaptic plasticity in the cerebellum. *Natural Neuroscience, 4*, 467– 475.

Hadjimarkou, M. M., Benham, R., Schwarz, J. M., Holder, M. K., & Mong, J. A. (2008). Estradiol suppresses rapid eye movement sleep and activation of sleep-active neurons in the ventrolateral pre-optic area. *European Journal of Neuroscience, 27*, 1780-1792. doi:10.1111/j.1460-9568.2008.06142.x

Hakosaka, O., & Wurtz, R. H. (1985). Modification of saccadic eye movements by GABA-related substances. I. Effect of muscimol and bibubulline in money superior colliculus. *Journal of Neurphysiology*, *52*(1), 266-291.

Hamilton, M. (1960). A rating scale for depression. Journal of Neurology, Neurosurgery and Psychiatry, 23(1), 56-62.

Hartlage, S. A., Arduino, K. E., & Gehlert, S. (2001). Premenstrual dysphoric disorder and risk for major depressive disorder: A preliminary study. *Journal of Clinical Psychology, 57*(12), 1571-1578.

Haselton, M. G., & Gangestad, S. W. (2006). Conditional expression of women's desires and men's mate guarding across the ovulatory cycle. *Hormones and Behavior, 49*, 509-518. doi:10.1016/j.yhbeh.2005.10.006

Haug, B. A., Kolle, R. U., Trenkwalder, C., Oertel, W. H., & Paulus, W. (1995). Predominant affection of the blue cone pathway in Parkinson's disease. *Brain*, 118, 771-778.

Hedges, V. L., Ebner, T. J., Meisel, R. L. & Mermelstein, P. G. (2012). The cerebellum as a target for estrogen action. *Frontiers in Neuroendocrinology, 33*, 403-411. doi: 10.1016/j.frne.2012.08.005

Heron, G., Adams, A. J., Husted, R. (1988). Central visual fields for short wavelength sensitive pathways in glaucoma and ocular hypertension. *Investigative Opthalmology & Visual Science, 29*(1), 64-72.

Howell, A. (2005). New developments in the treatment of postmenopausal breast cancer. *Trends Endocrinology Metabolism Journal*, 16, 420-428. doi:10.1016/j.tem.2005.09.003

Hu, M. (2014, March 31). *What does it mean to have a low R-squared? A warning about misleading interpretation.* Retrieved from: http://humanvarieties.org/2014/03/31/what-does-it-mean-to-have-a-low-r-squared-a-warning-about-misleading-interpretation/

Hu, M., Watson, C.J., Kennedy, R.T., & Becker, J. B. (2006). Estradiol attenuates the K+-induced increase in extracellular GABA in rat striatum. *Synapse, 59*(2), 122-124.

Ilg U. J. & Thier, P. (2008). The neural basis of smooth pursuit eye movements in the rhesus monkey brain. *Brain and Cognition*, 68, 229-240. doi:10.1016/j.bandc.2008.08.014

Inaba, N., Shinomoto, S., Yamane, S., Takemura, A., & Kawano, K. (2007). MST neurons code for visual motion in space independent of pursuit eye movements. *Journal of Neurophysiology, 97,* 3473-3483. doi: 10.1152/jn.01054.2006

Jarrett, C. B., & Barnes, G. (2002). Volitional scaling of anticipatory ocular pursuit velocity using precues. *Brain Research Cognitive Brain Research, 14,* 383-388. doi:10.1016/S0926-6410(02)00140-4

Jöchle, W. (1973). Coitus induced ovulation. *Contraception*, *7*(6), 523564.

Joffe, H., Hennen, J., Soares, C. N., Carlson, K., & Cohen, L. (2002). Hot flashes associated with depression in perimenopausal women seeking primary care. *Menopause, 9*(6), 392-398.

Kaja S., Yang, S. H., Wei, J., Fujitani, K., Liu, R., Brun-Zinkernagel, A. M., Simkins, J. W., Inokuchi, K., & Koulen, P. (2003). Estrogen protects the inner retina fron apoptosis and ischema-induced loss of Vesl-1L/Homer 1c immunoreactive synaptic connections. *Investigative Opthalmology & Visual Science, 44,* 3155-3162.

Kanayama, R., Nakamura, T., Sano, R., Ohki, M., Okuyama, T., Kimura, Y., et al. (1994). Effect of aging on smooth pursuit eye movement. Acta Otolaryngology (Stockh), Suppl 511, 131-134.

Kathmann, N., Hochrein, A., Uwer, R., & Bondy, B. (2003). Deficits in gain of smooth pursuit eye movements in schizophrenia and affective disorder patients and their unaffected relatives. *American Journal of Psychiatry, 160*, 696-702.

Kaviani, H., Gray, J. A., Checkley, S. A., Raven, P. W., Wilson, G. D., & Kumari, V. (2004). Affective modulation of the startle response in depression: influence of the severity of depression, anhedonia, and anxiety. *Journal of Affective Disorders, 83*, 21-31. doi:10.1016/j.jad.2004.04.007

Kawakami, M., & Ohno, S. (1981). Estrogen-sensitive neurons with preoptic projections in the lower brain stem of the female rat. *Endocrinology Journal, 28*(5), 677-684.

Khakpay, R., Semnanian, S., Java, M., & Janahmadi, M. (2010). The effect of inta-locus coeruleus injection of 17β-estradiol on inflammatory pain modulation in male rat. *Behavioural Brain Research*, *214*(2), 409-416. doi:10.1016/j.bbr.2010.06.012

Koorengevel, K.M., Beersma, D.G., Den Boer, J.A., & Van Den Hoofdakker, R.H. (2002). Sleep in seasonal affective disorder patients in forced desynchrony: an explorative study. *Journal of Sleep Research, 11*(4), 347-356. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12464103

Krauzlis, R. J., & Lisberger, S. G. (1989). A control systems model of smooth pursuit eye movements with realistic emergent properties. *Neural Computation, 1,* 116-122.

Krauzlis, R.J. (2005). The control of voluntary eye movements: New Perspectives. *The Neuroscientist, 11*(2), 124-137.

Lam, R. W., Beattie, C. W., Buchanan, A., Remick, R. A., & Zis, A. P. (1991). Low electrooculographic ratios in patients with seasonal affective disorder. American Journal of Psychiatry, 148(11), 1526-1529.

Leuner B., Mendolia-Loffredo S., & Shors T. J. (2004). High levels of estrogen enhance associative memory formation in ovariectomized females. *Psychoneuroendocrinology, 29*, 883– 890. doi:10.1016/j/psyneuen.2003.08.001

Lisberger, S. G., Morris, E. J., & Tychsen, L. (1987). Visual motion processing and sensory-motor integration for smooth pursuit eye movements. *Annual Review of Neuroscience, 10*, 97-129. doi:10.1146/annurev.ne.10.030187.000525

Lisberger, S. G., & Movshon, J. A. (1999). Visual motion analysis for pursuit eye movements in area mt of macaque monkeys. *The Journal of Neuroscience, 19,* 2224-2246.

Majewska, M. D., Harrison, N. L., Schwartz, R. D., Barker, J. L., & Paul, S. M. (1986). Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science 232*, 1004-1007.

Mahlberg, R., Steinacher, B., Mackert, A., & Flechtner, K. M. (2001). Basic parameters of saccadic eye movements – differences between unmedicated schizophrenia and affective disorder patients. *European Archives of Psychiatry and Clinical Neuroscience*, 251, 205–210.

Manns, I. D., Mainville, L., & Jones, B. E. (2001). Evidence for glutamate, in addition to acetylcholine and GABA, neurotransmitter synthesis in basal forebrain neurons projecting to entorhinal cortex. *Neuroscience*, *107*(2), 249-263.

Martin, L. F., Olincy, A., Ross, R. G., Du, Y. P., Singel, D., Shatti, S., & Tregellas, J. R. (2011). *Journal of Psychiatric Research, 45*; 670-677.

McEwen, B. S., & Milner, T. A. (2007). Hippocampal formation: shedding light on the influence of sex and stress on the brain. *Brain Research Reviews*, 55, 343-355. doi:10.1016/j.brainresrev.2007.02.006

McEwen, B. S. (2001). Invited review: estrogens effects on the brain: Multiple sites and molecular mechanisms. Journal of Applied Physiology, 91(6), 2785-801.

Medina, J. F., & Lisberger, S. G. (2007). Variation, signal, and noise in cerebellar sensory-motor processing for smooth-pursuit eye movements. *The Journal of Neuroscience, 27,* 6832-6842. doi:10.1523/JNEUROSCI.1323-07.2007

Missal, M., & Keller, E. L. (2002). Common inhibitory mechanism for saccades and smooth-pursuit eye movements. Journal of Neurophysiology, 88, 1880-1892.

Miura, K., Kobayashi, Y., & Kawano, K. (2009). Ocular responses to brief motion of textured backgrounds during smooth pursuit in humans. *Journal of Neurophysiology*, 102, 1736-1747.

Moos, R. H. (1968). The development of a menstrual distress questionnaire. *Psychosomatic Medicine*, *30*, 853-867. Retrieved from: http://www.psychosomaticmedicine.org/content/30/6/853.full.pdf+html

Munaut, C., Lambert, V., Noel, A., Frankenne, F., Deprez, M., Foidart, J.M., Rakic, J.M. (2001). Presence of oestrogen receptor type beta in human retina. *British Journal of Ophthalmology*, 85, 877-882. doi:10.1136/bjo.85.7.877

Noriega, N. C., Eghlidi, D. H., Garyfallou, V. T., Kohama, S. G., Kryger, S. G., & Urbanski, H. K. (2010). Influence of 17 β-estradiol and progesterone on GABAergic gene expression in the arcuate nucleus, amygdala and hippocampus of the rhesus macaque. *Brain Research, 1307*, 28-42.

Nyberg, S., Wahlstrom, G., Backstrom, T., & Poromaa, I. G. (2004). Altered sensitivity to alcohol in the late luteal phase among patients with premenstrual dysphoric disorder. *Psychoneuroendocrinology*, *29*, 767-777. Doi:10.1016/jpsyneuen.2011.02.011

Ogueta, S. B., Schwartz, S. D., Yamashita, C. K., & Farber, D. B. (1999). Estrogen receptor in the human eye: Influence of gender and age on gene expression. *Investigative Ophthalmology & Vision Science*, 40, 1906-1911.

Ono, S., & Mustari, M. J. (2008). Smooth Pursuit-Related Information Processing in Frontal Eye Field Neurons That Project to the NRTP. *Cerebral Cortex*, 19, 1186-1197. doi:10.1093/cercor/bhn166

O'Hara, M. W., & Rehm, L. P. (1983). Hamilton Rating Scale for Depression: reliability and validity in judgments of novice raters. *Journal of Consulting and Clinical Psychology*, *51*(2), 318-319.

Ozaki, N., Rosenthal, N. E., Myers, F., Schwartz, P. J., & Oren, D. A. (1995). Effects of season on electro-oculographic ratio in winter seasonal affective disorder. *Psychiatry Research*, 59, 151-155.

Pacheco-Cutillas, M. & Edgar, F. D. (1999). Acquired colour vision defects in glaucoma—their detection and clinical significance. *British Journal of Optholmology,83*, 1396-1402. doi:10.1136/bjo.83.12.1396

Pack, C. C., & Born, R. T. (2001). Temporal Dynamics of a Neural Solution to the Aperture Problem in Visual Area MT of Macaque Brain. *Nature, 409,* 1040-1042. doi:10.1038/35059085

Palagini, L., Baglioni, C., Ciapparelli, A., Gemignani, A., & Riemann, D. (2013). REM sleep dysregulation in depression: State of the art. *Sleep Medicine Reviews.* Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23391633

Pardo, J. V., Pardo, P. J., Humes, S. W., & M, I. P. (2006). Neurocognitive dysfunction in antidepressant-free, non-elderly patients with unipolar depression: alerting and covert orienting of visuospatial attention. *Journal of Affective Disorders*, *92*(1), 71-78.

Parry, B., L., Cover, H., Mostofi, N., LeVeau, B., Sependa, P. A., Resnick, A., & Gillin, C. (1995). Early vesus late partial sleep deprivation in patients with premenstrual dysphoric disorder and normal comparison subjects. *The American Journal of Psychiatry, 152*(3), 404-412. doi:10.1016.j.psychres.2007.11.017

Parry, B. L., Hauger, R., LeVeau, B., Mostofi, N., Cover, H., Clopton, J., & Gillin, C. (1996). Circadian rhythms of prolactin and thyroid-stimulating hormone during the menstrual cycle and early versus late sleep deprivation in premenstrual dysphoric disorder. *Psychiatry Research, 62*, 147-160.

Pearlstein, R. & Steiner, M. (2008). Premenstraul dysphoric disorder: burden of illness and treatment update. *Journal of Psychiatry Neuroscience, 33*(4), 291-301. doi:10.1007/s00737-012-0294-y

Pillsworth, E. G., Haselton, M. G., & Buss., D. M. (2004) Ovulatory shifts in female sexual desire. Journal of Sex Research, 41, 55-65. doi:10.1080/00224490409552213

Posner, M. I., Walker, J. A., Friedrich, F. J., Rafal, R. D. (1984). Effects of parietal injury on covert orienting of attention. *Journal of Neuroscience 4*, 1863-1874.

Raffi, M., Squatrito, S., & Maioli, M. G. (2007). Gaze and Smooth Pursuit Signals Interact in Parietal Area 7m of the Behaving Monkey. *Experimental Brain Research*, 182, 35-46. doi:10.1007/s00221-007-0967-3

Rapkin, A. J. 1999. Progesterone, GABA and mood disorder in women. *Archives of Women's Mental Health, 2*, 97–105. doi:101007/s007370050041

Rapkin, A.J., McDonald, M., & Winer, S. A. (2007). Ethinyl estradiol/drospirenone for the treatment of the emotional and physical symptoms of premenstrual dysphoric disorder. *Women's Health, 3*(4), 395-408. doi:10.2217/17455057.3.4.395

Reed, S. C., Levin, F. R., & Evans, S. M. (2008). Changes in mood, cognitive performance and appetite in the late luteal and follicular phases of menstrual cycle in women with and without PMDD (premenstrual dysphoric disorder). *Hormones and Behaviour, 54*, 185-193. doi: 10.1016/j/yhbeh.2008.02.018.

Rewal, M., Jung, M.E., Wen, Y., Brun-Zinkernagel, A.M., & Simpkins, J.W. (2003). Role of the $GABA_A$ system in behavioral, motoric, and cerebellar protection by estrogen during ethanol withdrawal. *Alcohol, 31*, 49-61.

Rhodes, M. E., & Frye, C. A. (2004). Estrogen has mnemonic-enhancing effects in the inhibitory avoidance task. *Pharmacology and Biochemistry of Behaviour,* 78, 551-558.

Richards, M. A. (2011). *Visual and Hormonal Indicators of Premenstrual Dysphoric Disorder (PMDD)*. (Unpublished doctoral dissertation). Lakehead University, Thunder Bay, ON.).

Robinson, D.A. (1975). Oculomotor control signals. In: Bach-y-Rita P, Lennerstrand G (eds) Basic Mechanisms of Ocular Motility and Their Clinical Implications. Pergamon, Oxford, pp 337-374.

Robinson, F. R., & Fuchs, A. F. (2001). The Role of the Cerebellum in Voluntary Eye Movements. *Annual Review of Neuroscience, 24*, 981-1004.

Rosenthal, N. E., Sack, D. A., Gillin, J. C., Lewy, A. J., Goodwyn, F. K., Davenport, P. S. et al. (1984). Seasonal affective disorder. A description of the syndrome and estradiol, visual functioning, and pms 218 preliminary findings with light therapy. Archives of General Psychiatry, 41(1), 72-80. Retrieved from: http://blog.ecu.edu/sites/penderst/files/2011/01/SAD-original-Rosenthal-report.pdf

Rottach, K. G., Zivotofsku, A. Z., Das, V. E., Averbuch-Heller, L., Discenna, A. O., Poonyathalang, A., & Leigh, R. J. (1996). Comparison of horizontal, vertical and diagonal smooth pursuit eye movements in normal human subjects. *Vision Research, 36*(14), 2189-2195.

Scarduzio, M., Panichi, R., Pettorossi, V. E. & Grassi, S. (2013). Synaptic Long-Term Potentiation and Depression in the Rat Medial Vestibular Nuclei Depend on Neural Activation of Estrogenic and Androgenic Signals. *PLOS ONE*, *8*(11), 1-10.

Schlack, A., Hoffmann, K. P., & Bremmer, F. (2003). Selectivity of macaque ventral intraparietal area (area VIP) for Smooth Pursuit Eye Movements. *Journal of Physiology, 551,* 551-561. doi:10.1113/jphysiol.2003.042994

Seggie, J., MacMillan, H., Griffith, L., Shannon, H. S., Martin, J., Simplson, J., et al. (1991). Retinal pigment epithelium response and the use of the EOG and Arden ratio in depression. *Psychiatry Research*, 36, 175-185.

Sharpe, J. A. (2008). Neurophysiology and neuroanatomy of smooth pursuit: Lesion studies. *Brain and Cognition*, 68, 241–254.

Shechter, A., Lesperance, P., Ying Kin, N. M., & Boivin, D. B. (2012). Nocturnal polysomnographic sleep across the menstrual cycle in premenstrual dysphoric disorder. *Sleep Medicine, 13*, 1071-1079. doi:10.1016/j.sleep.2012.05.012

Shiroma, S., Yamaguchi, T., & Kometani, K. (2005). Effects of 17beta-estradiol on chemically induced long-term depression. *Neuropharmacology, 49*(1), 97-102. doi:10.1084/jem.20102665

Smith, R. N., Studd, J. W., Zamblera, D., & Holland, E. F. (1995). A randomized comparison over 8 months of 100 micrograms and 200 micrograms twice weekly doses of transdermal estradiol in the treatment of severe premenstrual syndrome. *British Journal of Obstetrics and Gynaecology, 102*(6), 475-484.

Smyrnis, N., Evdokimidis, I., Mantas, A., Kattoulas, E., Stefanis, N., & Constantinidis, T. S. (2007). Smooth pursuit eye movements in 1,087 men: effects of schizotypy, anxiety, and depression. *Experimental Brain Research, 179*, 397-404. doi:10.1007/s00221-006-0797-8

Soares, C. N., Almeida, D. D., Joffe, H., & Cohen, L. S. (2001). Efficacy of estradiol for the treatment of depressive disorders in perimenopausal women: a double-blind, randomized, placebo-controlled trial. *Archives of General Psychology*, 58, 529-534.

Sparks, D. L. (2002). The brainstem control of saccadic eye movements. *Nature Reviews Neuroscience, 3,* 952-964.

Stuphorn, V., & Schall, J. D. (2006). Executive control of countermanding saccades by the Supplementary Eye Field. *Nature Neuroscience, 9*, 925-931. doi:10.1038/nn1714

Sundström, I., Andersson, A., Nyberg, S., Ashbrook, D., Purdy, R., & Bäckström T. (1998). Patients with premenstrual syndrome have a different sensitivity to a neuroactive steroid during the menstrual cycle compared to control subjects. *Neuroendocrinology 67*(2), 126–138. doi: 10.1016/S0893133X(97)00086-9

Sundström, P, Smith, S., & Gulinello, M. (2003). GABA receptors, progesterone, and premenstrual dysphoric disorder. *Archive of Women's Mental Health, 6*, 23-41. doi: 10.1007/s00737-002-0147-1

Swenson, R. D. (2006). Review of Clinical and Functional Neuroscience. Retrieved from http://www.dartmouth.edu/~rswenson/NeuroSci/index.html

Their, P., & Erickson, R. G. (1992). Responses of Visual-Tracking Neurons from Cortical Area MST To Visual, Eye and Head Motion. *The European Journal of Neuroscience, 4*, 539-553.

Tsutsi, K. (2012). Neurosteroid Biosynthesis and Action During Cerebellar Development. *Cerebellum*, *11*, 414-415. doi:10.1007/s12311-011-0341-7

van Broekhoven, F., Backstrom, T., & Verkes, R. J. (2006). Oral Progesterone Decreases Saccadic Eye Velocity and Increases Sedation in Women. *Psychoneuroendocrinology*, *31*; 1190-9.

Velisek, L., & Veliskova, J. (2002). Estrogen treatment protects GABA(B) inhibition in the dentate gyrus of female rate after kainic acid-induced status epilepticus. *Epilepsia, 43*(5), 146-151.

Vouimba, R. M., Foy, M. R., Foy, J. G., & Thompson, R. F. (2000). 17ß-estradiol suppresses expression of long-term depression in aged rats. *Brain Research Bulletin, 53*(6), 783-787.

Wagner, E. J., Ronnekleiv, O. K., Bosch, M. A., & Martin, K. J. (2001). Estrogen biphasically modifies hypothalamic GABAergic function concomitantly with negative and positive control luteinizing hormone release. *The Journal of Neuroscience, 21*(6), 2085-2093

Walker, J. E. (1983). Glutamate, GABA, and CNS disease: A Review. *Neurochemical Research, 8(4)* 521-550.

Wallace, J. M., Stone, L. S., & Masson, G. S. (2005). Object motion computation for the initiation of Smooth Pursuit Eye Movements in humans. *Journal of Neurophysiology, 93,* 2279-2293. doi:10.1152/jn.01042.2004

Watson, N. R., Studd, J. W., Savvas, M., Garnett, T., & Baber, R. J. Treatment of severe premenstrual syndrome with oestradiol patches and cyclical oral norethisterone. *Lancet, 2*(8665), 730-732. doi:10.1002/14651858

Wesner, M.F., Currie, E., Richards, M. & Oinonen, K. (2014). Women With Premenstrual Syndrome (PMS) Symptoms and Monophasic Contraceptive Users, Compared to Non-Symptomatic Controls, Show Asymmetric Horizontal Smooth Pursuit Amplitudes During Their Late Luteal Menstrual Phase, *Journal of Vision, Vision Science Society.*

Wesner, M.F. & Richards, M.A. (2011). Women with PMS show unilateral bias in horizontal smooth pursuit eye movements during their late luteal (low estradiol) menstrual cycle phase. 41st Annual Meeting of the Society for Neuroscience, Washington, DC.

Wesner, M. F. & Tan, J. (2006). Contrast sensitivity in seasonal and nonseasonal depression. *Journal of Affective Disorders, 95*, 19-28.

Wichniak, A., Riemann, D., Kiemen, A., Voderholzer, U., & Jernajczyk, W. (2000). Comparison between eye movement latency and REM sleep parameters in major depression. *European Archives of Psychiatry and Clinical Neuroscience, 250*, 48–52.

Wickham, L. A., Gao, J., Toda, T, Rocha, E. M., Ono, M., & Sullivan, D. A. (2000). Identification of androgen, estrogen and progesterone receptor mRNA's in the eye. *Acta Ophthalmologic Scandinavica,* 78, 146-153.

Appendices

Appendix A

1. Women's Eye Movements and the Menstrual Cycle Introductory Statement

Dear Potential Participant,

Thank you for your interest in our study titled "Women's Eye Movements and the Menstrual Cycle". My name is Emily Currie and I am a Master's of Science student at Lakehead University. My supervisors are Drs. Michael Wesner and Kirsten Oinonen. We are currently conducting a study that is investigating health and visual functioning in women. This study was sponsored by the Canada Foundation for Innovation. If you are a student in an eligible psychology course you can receive up to three points toward your course credit for participating in this study.

Following completion of a screening questionnaire (containing personal questions about demographic information, health and reproductive history, emotions and personality, caffeine intake, and morning/evening preference) some participants will be selected to participate in two approximately half-hour laboratory sessions. Dates for laboratory testing will be scheduled based upon the results of a simple hormonal test, completion of a brief questionnaire, and two visual tests. These tests are based on standard computer-based psychophysical procedures. In the visual tasks, participants will be presented various images on a computer display and will subsequently be asked to make judgments based on the appearance of the images. Following completion of the laboratory sessions, all participants will be fully debriefed, and provided with a list of project references and resources.

An extensive history of research has demonstrated that all tasks involved in this study constitute minimal risk to participants. Your participation is voluntary, and if at any time you feel uncomfortable with any of the experimental tasks or procedures, or note any negative effects, you are free to withdraw your consent at any time. Participants may choose not to answer any questions of which they are uncertain or material which causes them discomfort. Early termination of the study will not affect the award of bonus points. The benefits that you may expect to derive from this study include (a) an appreciation of research on health, (b) an opportunity to contribute to scientific research, and (c) course credit (up to three bonus points).

All information collected during the course of this project will be held in strict confidence. At no time will any identifying information be divulged to individuals outside of the research team. Anonymity and confidentiality will be maintained at all times throughout the study and in the dissemination of results for publication and appropriate conference presentations. After data is collected there will be no way to connect any data with any particular participant. All information will be stored in the laboratories of Drs. Wesner and Oinonen at Lakehead University for a period of at least five years. Findings from the projects will be made available to interested participants upon completion of the project.

If you have any questions or concerns regarding the experiment, please do not hesitate to contact us directly. I can be reached through e-mail (evcurrie@lakeheadu.ca). You may also contact Meghan Richards (mrichar4@lakeheadu.ca), Dr. Kirsten Oinonen (koinonen@lakeheadu.ca), Dr. Michael Wesner (mwesner@lakeheadu.ca), or Lakehead University's Research Ethics Board at (807) 343-8283.

Thank you for your interest in the project.

Sincerely,

Emily Currie, BA (Hons),
MSc Candidate,
Department of Psychology,
Lakehead University

2. Consent Form A

Dear Potential Participant,

The purpose of the present study, titled "Women's Eye Movements and the Menstrual Cycle", conducted by Emily Currie, Dr. Meghan Richards, Dr. Michael Wesner, and Dr. Kirsten Oinonen of the Department of Psychology at Lakehead University, is to examine health and visual functioning in women. This study is sponsored by the Canada Foundation for Innovation. If you are currently enrolled in an eligible Psychology course, one bonus mark will be awarded for the completion of this screening questionnaire. Information collected from the screening questionnaire will be used to select some participants for subsequent laboratory sessions. Individuals who are selected for these sessions and who participate further in the study will receive an additional one to three bonus points (depending on the number of laboratory sessions attended) towards their final mark in their Psychology course.

Your participation in the screening will involve the completion of a questionnaire that will take approximately 30-40 minutes. The questionnaire includes personal questions designed to collect information on: demographics, reproductive history, the menstrual cycle, emotion and personality, caffeine intake, and morningness-eveningness. You may choose not to respond to any questions that cause you discomfort.

Participation in this study is voluntary and you may withdraw at any time without explanation or penalty. Records of your participation will be kept in strict confidence. As well, no identifying information will be disclosed in the dissemination of the results of this study. As per university requirements, all data will be stored for at least five years in research laboratories at Lakehead University and remain anonymous and confidential. Individuals who meet specific criteria will be asked to participate in subsequent laboratory sessions. Therefore, we have asked for your name and telephone number here. Once we have determined who will be asked to participate in subsequent sessions, this information will be removed and information from your questionnaire will remain both anonymous and confidential. The benefits you may expect from the study are (a) an appreciation of research on health, (b) an opportunity to contribute to scientific research and (c) course credit (up to three additional bonus points). Throughout the study, participant information will be coded using a numbering system. There is no way that your name can be connected to your responses. There are no known physical or psychological risks associated with participating in this study. If you have any questions or concerns regarding this study please contact the principal investigator, Emily Currie (evcurrie@lakeheadu.ca), or Dr. Meghan Richards (mrichar4@lakeheadu.ca), or Dr. Michael Wesner (343-8457), or Dr. Kirsten Oinonen (343-8096) directly.

1. Name

2. Phone Number

3. Email Address

4. Student Number

5. Course Name, Number and Instructor

6. Date

	Month	Day	Year
Date			

7. I have read and understand the information provided in this Information Letter and understand what is involved in participating in this research study. I agree with the information provided and by completing the questionnaire indicate my consent to participate.

☐ yes

☐ no

3. Default Section

1. Age

2. Sex

- ◯ Female
- ◯ Male

3. Please indicate the highest level of education you have completed.

- ◯ some elementary school
- ◯ completed grade 8
- ◯ some high school
- ◯ completed high school
- ◯ some college
- ◯ completed college
- ◯ some university
- ◯ completed a university degree
- ◯ some graduate studies
- ◯ completed a graduate degree

4. Date

	Month	Day	Year
Date			

5. Please indicate your height (e.g. 5 feet, 7 inches) :

feet

inches

6. Please indicate your weight (in either pounds or kilograms)

- ◯ pounds
- ◯ kilograms

weight

7. Are you currently taking any medications?

- ◯ yes
- ◯ no

If yes, please list:

8. Please list any medical or psychological conditions that you have been diagnosed with (e.g. hypothyroidism, depression, asthma, cancer, diabetes, etc.)

9. Have you ever been diagnosed with or treated for depression?

- ◯ yes
- ◯ no
- ◯ maybe

10. Have you ever been diagnosed with or treated for bipolar disorder or manic depression?

- ◯ yes
- ◯ no
- ◯ maybe

11. Have you ever been diagnosed with or treated for an anxiety disorder?

- ◯ yes
- ◯ no
- ◯ maybe

12. Do you think any of your biological relatives (i.e. parents, siblings, children, grandparents) have had any mental health problems (i.e. depression, anxiety, schizophrenia, alcoholism, eating disorders)?

- ◯ yes
- ◯ no
- ◯ maybe

13. For each of the following, please check the box if you think that one of your biological relatives has been diagnosed with or treated for this problem. Also, on the line beside each mental health problem, please indicate the relationship of the family member(s) to you (e.g., mother, father, sister, grandmother, uncle).

	Depression	Personality Disorder	Schizophrenia	Anxiety	Bipolar Disorder/Manic Depression	Diabetes	Eating Disorder	Alcoholism	Drug Abuse	Thyroid Disorder
Mother	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Father	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sister	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Brother	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Grandmother	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Grandfather	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Aunt	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Uncle	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cousin	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Other (please specify)

14. Please indicate the statement that best describes you:

- ◯ I feel happiest and most productive in the morning hours of the day.
- ◯ I feel happiest and most productive in the evening hours of the day.
- ◯ I feel equally happy and productive in the morning and evening.
- ◯ I feel that none of the above statements apply to me.

4. Section II: Menstrual Cycle Related Questions

1. The following list shows common symptoms and feelings associated with menstruation. For each item, choose the descriptive category from the box below that best describes your experience during each of three time periods indicated. That is, for each item, decide whether you have "no experience of the symptom, " or whether your experience is "present mild," "present moderate," "present strong," or "present severe." Then indicate the number in the space provided. If none of the categories exactly describes your experience, choose the one that most closely matches what you feel. Be sure to rate every item.

	Most recent menstrual period	Four days prior to menstrual period	Remainder of cycle
Muscle Stiffness			
Weight Gain			
Dizziness, faintness			
Loneliness			
Headache			
Skin Blemish or Disorder			
Cold Sweats			
Anxiety			
Mood Swings			
Cramps			
Painful or tender breasts			
Nausea, vomiting			
Crying			
Backache			
Swelling (breasts/abdomen)			
Hot flashes			
Irritability			
Tension			
Fatigue			
Feeling guilty, sad, or blue			
General aches or pains			
Restlessness			
Insomnia			
Poor school or work performance			
Affectionate			

Feelings of suffocation			
Forgetfulness			
Take naps, stay in bed			
Orderliness			
Chest pains			
Confusion			
Poor judgment			
Stay at home			
Excitement			
Ringing in the ears			
Difficulty concentrating			
Avoid social activities			
Feelings of well-being			
Heart pounding			
Distractible			
Decreased efficiency			
Bursts of energy, activity			
Numbness, tingling			
Minor accidents			
Blind spots, fuzzy vision			
Poor motor coordination			
Increased appetite			

2. Was your last menstrual cycle in any way unusual?

◯ yes

◯ no

If yes, please indicate the reason

3. Some women experience changes in mood and physical functioning during the week prior to their menstrual period. As best as you can, please indicate the frequency, severity, and level of impairment encountered for the following 11 symptoms during your PREMENSTRUAL PHASE over the past year.

	Number of menstrual cycles in which the above symptom(s) have been experienced over the past 12 months	To what extent do these symptoms cause impairment in work, school, or occupational functioning	Please rate the degree of severity of these symptoms
1. Markedly depressed mood, feelings of hopelessness, or self-deprecating thoughts?			
2. Marked anxiety, tension, tension, or feelings of being "keyed up," or "on edge?"			
3. Affective lability (e.g. feeling suddenly sad or tearful, or increased sensitivity to rejection)?			
4. Persistent and marked anger or irritability, or increased interpersonal conflicts?			
5. Decreased interest in usual activities (e.g. work, school, friends, hobbies)?			
6. A subjective sense of difficulty concentrating?			
7. Lethargy, easy fatigability, or marked lack of energy?			
8. Marked change in appetite, overeating, or specific food cravings?			
9. Sleeping too much or too little?			
10. A subjective sense of being overwhelmed or out of control?			
11. Other physical symptoms, such as breast tenderness or swelling, headaches, joint or muscle pain, or sensations of "bloating" or weight gain?			

4. Which statement best describes your menstrual cycle

- ◯ I never have my period.
- ◯ Some months I get my period and some months I don't.
- ◯ I usually get my period every month, but it is irregular and I cannot predict when it will start.
- ◯ I usually get my period within two or three days of when I expect it.
- ◯ My period is like clockwork and the same number of days elapse between periods each month.

5. How old were you when you first started menstruating (started your period)?

6. As a teenager and young adult, how did/does you acne/pimples compare to your same-age peers? I had _____ acne compared to most girls/women my age (circle the best response).

- ◯ Significantly Less
- ◯ Slightly Less
- ◯ About the same
- ◯ Slightly More
- ◯ Significantly More

***7. Using the calendar provided below, please indicate the first day of your LAST menstrual period. If you are not completely certain, please estimate the day that you believe your LAST menstrual period began.**

	Month	Day
Date		

***8. Using the calendar provided below, please estimate the first day of your NEXT menstrual period.**

	Month	Day
Date		

2014 CALENDAR

JANUARY

M	T	W	T	F	S	S
		1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30	31		

FEBRUARY

M	T	W	T	F	S	S
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28		

MARCH

M	T	W	T	F	S	S
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24 31	25	26	27	28	29	30

APRIL

M	T	W	T	F	S	S
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30				

MAY

M	T	W	T	F	S	S
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30	31	

JUNE

M	T	W	T	F	S	S
						1
2	3	4	5	6	7	8
9	10	11	12	13	14	15
16	17	18	19	20	21	22
23 30	24	25	26	27	28	29

JULY

M	T	W	T	F	S	S
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30	31			

AUGUST

M	T	W	T	F	S	S
				1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	20	21	22	23	24
25	26	27	28	29	30	31

SEPTEMBER

M	T	W	T	F	S	S
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30					

OCTOBER

M	T	W	T	F	S	S
		1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30	31		

NOVEMBER

M	T	W	T	F	S	S
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30

DECEMBER

M	T	W	T	F	S	S
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

9. How confident are you that the above indicated day was the first day of your LAST menstrual period?

	0 (0% confident)	1	2 (25% confident)	3	4 (50% confident)	5	6 (75% confident)	7	8 (100% confident)
Confidence	◯	◯	◯	◯	◯	◯	◯	◯	◯

10. How confident are you that the above indicated day will be the first day of your NEXT menstrual period?

	0 (0% confident)	1	2 (25% confident)	3	4 (50% confident)	5	6 (75% confident)	7	8 (100% confident)
Confidence	◯	◯	◯	◯	◯	◯	◯	◯	◯

11. Are you are currently menstruating? If yes, please indicate day of bleeding below.

◯ yes

◯ no

Day of bleeding (if yes to above)

_____

***12. What is the average length of your menstrual cycle right now (how many days are there from the first day of one period to the day before the first day of your next period)? Most people range between 25 and 35 days. Women on standard birth control pills have a 28-day cycle.**

***13. What is your average length of your menstrual period/bleeding when you are not taking hormonal contraceptives (birth control pill, injections)? In other words, how many days does your period last? Most people's periods last between 1 and 10 days.**

14. Do you think that you have started to go through menopause?

◯ yes

◯ no

◯ maybe

15. Are you currently pregnant?

◯ yes

◯ no

◯ maybe

5. Section III: Emotions and Personality

1. Please read each of the following statements carefully and indicate the answer that best corresponds to your agreement or disagreement. Indicate "strongly disagree (sd)" if the statement is definitely false or if you strongly disagree. Indicate "disagree (d)" if the statement is mostly false or if you disagree. Indicate "neutral (n)" if your opinion is neutral. Indicate "agree (a)" if the statement is mostly true or if you agree. Indicate "strongly agree (sa)" if the statement is definitely true or if you strongly agree. There are no right or wrong answers, and you need not be an "expert" to complete this questionnaire. Describe yourself honestly and state your opinions as accurately as possible.

	strongly disagree	disagree	neutral	agree	strongly agree
1. I am known for my prudence and common sense.	◯	◯	◯	◯	◯
2. I don't take civic duties like voting very seriously.	◯	◯	◯	◯	◯
3. I keep myself informed and usually make intelligent decisions.	◯	◯	◯	◯	◯
4. I often come into situations without being fully prepared.	◯	◯	◯	◯	◯
5. I pride myself on my sound judgment.	◯	◯	◯	◯	◯
6. I don't seem to be completely successful at anything.	◯	◯	◯	◯	◯
7. I'm a very competent person.	◯	◯	◯	◯	◯
8. I am efficient and effective at my work.	◯	◯	◯	◯	◯
9. I would rather keep my options open than plan everything in advance.	◯	◯	◯	◯	◯
10. I keep my belongings neat and clean.	◯	◯	◯	◯	◯
11. I am not a very methodical person.	◯	◯	◯	◯	◯
12. I like to keep everything in its place so I know just where it is.	◯	◯	◯	◯	◯
13. I never seem to be able to get organized.	◯	◯	◯	◯	◯
14. I tend to be somewhat fastidious or exacting.	◯	◯	◯	◯	◯
15. I'm not compulsive about cleaning.	◯	◯	◯	◯	◯
16. I spend a lot of time	◯	◯	◯	◯	◯

looking for things I've misplaced.					
17. I try to perform all of the tasks that have been assigned to me conscientiously.	○	○	○	○	○
18. Sometimes I am not as dependable or reliable as I should be.	○	○	○	○	○
19. I pay my debts promptly and in full.	○	○	○	○	○
20. Sometimes I cheat when I play solitaire.	○	○	○	○	○
21. When I make a commitment, I can always be counted on to follow through.	○	○	○	○	○
22. I adhere strictly to my ethical principles.	○	○	○	○	○
23. I try to do jobs carefully so they won't have to be done again.	○	○	○	○	○
24. I'd really have to be sick before I'd miss a day of work.	○	○	○	○	○
25. I am easy going and lackadaisical.	○	○	○	○	○
26. I have a clear set of goals and work toward them in an orderly fashion.	○	○	○	○	○
27. When I start a self improvement project, I usually let it slide after a few days.	○	○	○	○	○
28. I work hard to accomplish my goals.	○	○	○	○	○
29. I don't feel like I'm driven to get ahead.	○	○	○	○	○
30. I strive to achieve all I can.	○	○	○	○	○
31. I strive for excellence in everything I do.	○	○	○	○	○
32. I'm something of a "workaholic".	○	○	○	○	○
33. I'm pretty good about pacing myself so as to get things done on time.	○	○	○	○	○
34. I waste a lot of time before settling down to work.	○	○	○	○	○
35. I am a productive person who always gets the job done.	○	○	○	○	○

36. I have trouble making myself do what I should.	○	○	○	○	○
37. Once I start a project, I almost always finish it.	○	○	○	○	○
38. When a project gets too difficult, I am inclined to start a new one.	○	○	○	○	○
39. There are so many little jobs that need to be done that I sometimes just ignore them all.	○	○	○	○	○
40. I have a lot of self-discipline.	○	○	○	○	○
41. Over the years I have done some pretty stupid things.	○	○	○	○	○
42. I think things through before coming to a decision.	○	○	○	○	○
43. Occasionally I act first and then think later.	○	○	○	○	○
44. I always consider the consequences before I take action.	○	○	○	○	○
45. I often do things on the spur of the moment.	○	○	○	○	○
46. I rarely make hasty decisions.	○	○	○	○	○
47. I plan ahead carefully when I go on a trip.	○	○	○	○	○
48. I think twice before I answer a question.	○	○	○	○	○

2. Below is a list of common symptoms of anxiety. Please read each item in the list carefully and indicate how much you have been bothered by each symptom DURING THE PAST 7 DAYS INCLUDING TODAY by choosing the best answer among the choices presented in the columns.

	Not at all	Mildly (I am not bothered much)	Moderately (I feel very unpleasant but I can handle it)	Severely (I can barely stand it)
1. Numbness or tingling.	◯	◯	◯	◯
2. Feeling hot.	◯	◯	◯	◯
3. Wobbliness in legs.	◯	◯	◯	◯
4. Unable to relax.	◯	◯	◯	◯
5. Fear of the worst happening.	◯	◯	◯	◯
6. Dizzy or lightheaded.	◯	◯	◯	◯
7. Heart pounding or racing.	◯	◯	◯	◯
8. Unsteady.	◯	◯	◯	◯
9. Terrified.	◯	◯	◯	◯
10. Nervous.	◯	◯	◯	◯
11. Feelings of choking.	◯	◯	◯	◯
12. Hands trembling.	◯	◯	◯	◯
13. Shaky.	◯	◯	◯	◯
14. Fear of losing control.	◯	◯	◯	◯
15. Difficulty breathing.	◯	◯	◯	◯
16. Fear of dying.	◯	◯	◯	◯
17. Scared.	◯	◯	◯	◯
18. Indigestion or discomfort in abdomen.	◯	◯	◯	◯
19. Faint.	◯	◯	◯	◯
20. Face Flushed.	◯	◯	◯	◯
21. Sweating (due to heat).	◯	◯	◯	◯

3. Compared to how you feel when you are in an even or normal mood state, how would you rate yourself on the following items during the PAST 2 WEEKS?

	Not at all (0)	Just a little (1)	More than just a little (2)	Quite a bit/moderately (3)	Marked or severely (4)
1. down and depressed	◯	◯	◯	◯	◯
2. less interested in doing things	◯	◯	◯	◯	◯
3. less interested in sex	◯	◯	◯	◯	◯
4. less interested in eating	◯	◯	◯	◯	◯
5. that I have lost some weight	◯	◯	◯	◯	◯
6. that I can't fall asleep at night	◯	◯	◯	◯	◯
7. That my sleep is restless	◯	◯	◯	◯	◯
8. that I wake up too early	◯	◯	◯	◯	◯
9. heavy in my limbs or aches in back, muscles, or head, more tired than usual	◯	◯	◯	◯	◯
10. guilty or like a failure	◯	◯	◯	◯	◯
11. wishing for death or suicidal	◯	◯	◯	◯	◯
12. tense, irritable, or worried	◯	◯	◯	◯	◯
13. sure I'm ill or have a disease	◯	◯	◯	◯	◯
14. that my speech and thoughts are slow	◯	◯	◯	◯	◯
15. fidgety, restless or antsy	◯	◯	◯	◯	◯
16. that morning is worse than evening.	◯	◯	◯	◯	◯
17. that evening is worse than morning	◯	◯	◯	◯	◯
18. unreal or in a dream state	◯	◯	◯	◯	◯
19. suspicious of people/paranoid	◯	◯	◯	◯	◯
20. preoccupied/obsessed that I must check things a lot	◯	◯	◯	◯	◯
21. physical symptoms when worried	◯	◯	◯	◯	◯
22. like socializing less	◯	◯	◯	◯	◯
23. that I have gained weight	◯	◯	◯	◯	◯
24. that I want to eat more than usual	◯	◯	◯	◯	◯
25. that I HAVE eaten more	◯	◯	◯	◯	◯

than usual					
26. that I crave sweets and starches	◯	◯	◯	◯	◯
27. that I sleep more than usual	◯	◯	◯	◯	◯
28. that my mood slumps in the afternoons/evenings	◯	◯	◯	◯	◯

6. Section IV: Reproductive Questions

***1. Have you ever taken a hormonal contraceptive?**

- ◯ yes
- ◯ no

***2. Are you currently taking any form of hormonal contraception?**

- ◯ yes
- ◯ no

3. If yes to #2 above, please indicate the type of contraception you are currently taking:

- ◯ IUD
- ◯ Oral contraceptive pill
- ◯ Norplant
- ◯ Depo-provera
- ◯ Nuva Ring
- ◯ Birth Control Patch

4. If you are currently taking ORAL contraceptives (e.g "the pill"), please indicate what brand you are taking:

	Brand
Oral Contraceptive	

Other (please specify) _____

5. If you are NOT currently taking oral contraceptives, but have previously, how many years and months has it been since you stopped taking your most recent oral contraceptive?

	Years	Months
Time since oral contraceptive use was discontinued:		

6. If you have ever taken oral contraceptives, did you experience any negative side effects?

- ◯ yes
- ◯ no

7. If you indicated YES to the above question, did these effects cause you to discontinue use of your contraceptive?

- ◯ yes
- ◯ no
- ◯ n/a

8. If you have ever taken oral contraceptives, please look at the following list of side effects that some people experience while taking oral contraceptives. For each of the following PHYSICAL effects please indicate yes if you have experienced the symptom when taking oral contraceptives, and no if you have not. If you indicate yes for any of the listed side effects, please indicate what brand of oral contraceptive you were taking (e.g. Alesse, Ortho Tri-Cyclen 28, Marvelon) when you experienced the side effect and the strength/severity of the symptom using the key below. Please check all that apply.

	Effect Experienced	Brand 1	Brand 2 (if experienced more than once)	Severity
Nausea/vomiting				
Breast size increase				
Decreased ability to orgasm				
Weight gain				
Increased sex drive				
Fewer menstrual cramps				
Positive mood change				
Tiredness/Fatigue				
High blood pressure				
Irregular heartbeat				
Clearer complexion				
Complete loss of periods				
Lighter periods				
Breakthrough Bleeding				
Headaches				
Breast size decrease				
Increased ability to orgasm				
Weight loss				
Decreased sex drive/arousal				
More menstrual cramps				
Negative mood change				
Dizziness/faintness				
Painful or tender breasts				

Swelling of breasts or abdomen				
Complexion problems				
Heavier periods				
Fibrocystic breasts				
Fainting/Seizure				

9. If you have ever taken oral contraceptives, please look at the following list of side effects that some people experience while taking oral contraceptives. For each of the following MOOD effects please indicate yes if you have experienced the symptom when taking oral contraceptives, and no if you have not. If you indicate yes for any of the listed side effects, please indicate what brand of oral contraceptive (e.g. Alesse, Ortho Tri-Cyclen 28, Marvelon) you were taking when you experienced the side effect and the severity of the symptom using the key below. Please check all that apply.

	Effect Experienced	Brand 1	Brand 2 (if experienced more than once)	Severity
Slept more than usual				
Slept less than usual				
Depression				
More Pessimistic				
More irritable				
Feelings of inferiority				
Disrupted sleep				
More aggressive				
More jealous				
Less moody				
Lower self-esteem				
Cried more than usual				
More self critical				
More moody				
Less trust in partner				
More sensitive to criticism				
Sadness				
Less jealous				

10. Do you have a biological mother or sister who has experienced negative mood effects while taking oral contraceptives?

- ◯ yes
- ◯ no
- ◯ we have not discussed

7. Section V: Caffeine Consumption

1. Do you engage in the regular consumption of caffeine?

- ◯ yes
- ◯ no

2. How many servings of caffeine do you typically consume over the course of one day? (please note Tim Horton's Large and Starbucks grande +venti sizes = 2 servings)

	1	2	3	4	5	6	7	8	9	10
Coffee	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Tea	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Canned Soda (355 mL)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Bottled Soda (710 ml)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Energy Drinks (Rock Star, Red Bull etc...)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯

3. Please answer the following questions based on your past experience with caffeine. Below is a list of side effects. Please indicate: (a) the extent to which you believe that you would experience each of these effects if you were to drink 3 cups of coffee over several hours, and (b) the extent to which you have experienced each of these effects during any of your experiences with caffeine. Please also indicate the severity of the expected or experienced effects.

	Effect expected with three cups of coffee?	Severity	Effect actually experienced after drinking coffee?	Severity
Affects mood positively				
Affects mood negatively				
Irritability				
Anger				
Increased Alertness				
Increased Heart Rate				
Racing thoughts				
Jumpiness				
Anxiety				
Increased productivity				
Increased Self-Confidence				
Increased Focus				
Panic Attacks				
Digestive Problems				
Heart Burn				
Ulcers				
Nausea				
Vomiting				
Diarrhea				
Increased urination				
Increased creativity				
Increased social interaction				
Difficulty concentrating				
Migraines/headaches				
Fibrocystic breasts				
Problems Sleeping				
Fast and Frequent Speech				
Dizziness				

8. Section VI: Morningness-Eveningness

1. Considering your own feelings, at what time would you GET UP if you were entirely free to plan your day?

- ◯ am
- ◯ pm

Time

2. Considering your own feelings, at what time would you GO TO BED if you were entirely free to plan your day?

- ◯ am
- ◯ pm

Time

3. If there is a specific time you have to wake up in the morning, to what extend do you depend on using an alarm clock?

- ◯ Not at all dependent
- ◯ Slightly dependent
- ◯ Fairly dependent
- ◯ Very dependent

4. Assuming adequate environmental conditions, how easy do you find getting up in the morning?

- ◯ Not at all easy
- ◯ Slightly easy
- ◯ Fairly easy
- ◯ Very easy

5. How alert do you feel during the first half hour after having woken in the morning?

- ◯ Not at all alert
- ◯ Slightly alert
- ◯ Fairly alert
- ◯ Very alert

6. How is your appetite during the first half hour after having woken in the morning?

- ◯ Not at all good
- ◯ Slightly good
- ◯ Fairly good
- ◯ Very good

7. During the first half hour after having woken in the morning, how tired do you feel?

- ◯ Very tired
- ◯ Slightly tired
- ◯ Fairly refreshed
- ◯ Very refreshed

8. When you have no commitments the next day, at what time do you go to bed compared to your usual bedtime?

- ◯ Seldom or never later
- ◯ Less than one hour later
- ◯ 1-2 hours later
- ◯ More than 2 hours later

9. You have decided to engage in some physical exercise. A friend suggests that you do this one hour twice a week and the best time for him is between 0700 and 0800h. Bearing in mind nothing else but your own inclinations, how do you think you would perform?

- ◯ Would be on good form
- ◯ Would be on reasonable form
- ◯ Would find it difficult
- ◯ Would find it very difficult

10. At what time in the evening do you feel tired and in need of sleep?

- ◯ early pm
- ◯ late pm

Time _____

11. You wish to be at your peak for a test which you know is going to be mentally exhausting and lasting for two hours. You are entirely free to plan your day, when would you do this task?

- ◯ 0800 – 1000
- ◯ 1100 – 1300
- ◯ 1500 – 1700
- ◯ 1900 – 2100

12. If you went to bed at 11:00 p.m at what level of tiredness would you be at that time?

- ◯ Not at all tired
- ◯ A little tired
- ◯ Fairly tired
- ◯ Very tired

13. For some reason you have gone to bed several hours later than usual, but there is no need to get up at any particular time the next morning. Will you:

- ◯ Wake up at the usual time and not go back to sleep
- ◯ Wake up at the usual time and doze
- ◯ Wake up at the usual time and go back to sleep
- ◯ Wake up later than usual

14. One night you have to remain awake between 0400 and 0600h. You have no commitments the next day. Which suits you best:

- ◯ Not to go to bed until 0600h
- ◯ Nap before 0400h and sleep after 0600h
- ◯ Sleep before 0400h and nap after 0600h
- ◯ Sleep before 0400h and remain awake after 0600h

15. You have to do 2 hours physical work. Which hours would you prefer to do it between:

- ◯ 0800 – 1000
- ◯ 1100 – 1300
- ◯ 1500 – 1700
- ◯ 1900 – 2100

16. You have decided to engage in some physical exercise. A friend suggests that you do this between 10:00p.m and 11:00p.m twice a week. How do you think you would perform:

- ◯ Would be on good form
- ◯ Would be on reasonable form
- ◯ Would find it difficult
- ◯ Would find it very difficult

17. One hears of "morning" and "evening" types. Which do you consider yourself to be?

- ◯ Morning type
- ◯ More morning than evening
- ◯ More evening than morning
- ◯ Evening type

9. Section VII: Seasonality

Section VII: Seasonality

1. In the following questions, fill in boxes for all applicable months. This may be a single month, a cluster of months, or any other grouping.

At what time of year do you....

	January	February	March	April	May	June	July	August	September	October	November	December
a. Feel Best	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
b. Tend to gain most weight	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
c. Socialize Most	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
d. Sleep least	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
e. eat most	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
f. lose most weight	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
g. socialize least	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
h. feel worst	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
I. eat least	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
j. sleep most	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

2. To what degree do the following change with the seasons?

	No Change	Slight Change	Moderate Change	Marked Change	Extremely Marked Change
a. Sleep length	◯	◯	◯	◯	◯
b. Social activity	◯	◯	◯	◯	◯
c. Mood (overall feeling of well-being)	◯	◯	◯	◯	◯
d. Weight	◯	◯	◯	◯	◯
e. Appetite	◯	◯	◯	◯	◯
f. Energy level	◯	◯	◯	◯	◯

3. If you experience changes with the seasons, do you feel that these are a problem for you?

◯ yes

◯ no

4. If you indicated yes to the above, is this problem:

- ◯ mild
- ◯ moderate
- ◯ marked
- ◯ severe
- ◯ disabling

5. By how much does your weight fluctuate during the course of the year?

- ◯ 0-3 lbs.
- ◯ 4-7 lbs.
- ◯ 8-11 lbs.
- ◯ 12-15 lbs.
- ◯ 16-20 lbs.
- ◯ Over 20 lbs

6. Approximately how many hours of each 24-hour day do you sleep each season? (includes naps)

	Number of Hours
Winter (Dec 21 -Mar 20)	
Spring (Mar 21 -June 20)	
Summer (June 21 - Sept 20)	
Fall (Sept 21- Dec 20)	

7. Do you notice a change in food preference during the different seasons?

- ◯ yes
- ◯ no

10. DEBRIEFING FORM A

Thank you for participating in the screening phase of our study. The study is being conducted by Ms. Emily Currie, Dr. M. Richards, Dr. M. Wesner and Dr. K. Oinonen. If you are selected to participate in the second part of the study, you will be contacted by one of the researchers in the next three weeks. Participants in the next phases of the study will receive between one and three additional points towards their final mark (if they are Psychology 1100 students). If you are chosen to participate in subsequent laboratory sessions, you will be asked to complete several measures of visual functioning.

Please be assured that once participants have been selected for the study, the consent forms will be dissociated from all identifying information. To conceal your identity, all of your responses will be coded using a numbering system, thus assuring that all data will remain anonymous. If you have any questions, please feel free to contact any of the researchers using the contact information listed below.

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Appendix B

Dear Potential Participant,

Thank you for your interest in our research. We are currently conducting a study that is investigating health and visual functioning in women. Individuals who participate in this study will receive up to four points toward their credit in the Introductory Psychology course.

Following completion of a screening questionnaire (containing personal questions about demographic information, health and reproductive history, emotions and personality, caffeine intake, and morning/evening preference) some participants will be selected to participate in two approximately one-and-a-half-hour laboratory sessions. Dates for laboratory testing will be scheduled based upon the results of a simple hormonal test and will involve the collection of a salivary sample, completion of a brief questionnaire, and 4 visual tests. These tests are based on standard computer-based psychophysical procedures. In all visual tests, participants will be presented various images on a computer display and will subsequently be asked to make judgments based on the appearance of the images. A sub-sample of participants will be asked to collect salivary samples at home and return them to the lab the following morning. Following completion of the laboratory sessions, all participants will be fully debriefed, and provided with a list of project references and resources.

An extensive history of research has demonstrated that all tasks involved in this study constitute minimal risk to participants. If at any time you feel uncomfortable with any of the experimental tasks or procedures, or note any negative effects, you are free to withdraw your consent at any time. Participants may choose not to answer any questions of which they are uncertain or material that cause them discomfort. Early termination of the study will not affect the award of bonus points. The benefits that you may expect to derive from this study include (a) an appreciation of research on health, (b) an opportunity to contribute to scientific research, and (c) course credit (up to four bonus points).

All information collected during the course of this project will be held in strict confidence. At no time will any identifying information be divulged to individuals outside of the research team. Anonymity and confidentiality will be maintained at all times throughout the study and in the dissemination of results for publication and appropriate conference presentations. After data is collected there will be no way to connect any data with any particular participant. All information will be stored in the laboratories of Dr. Wesner and Dr. Oinonen at Lakehead University for a period of at least five years. Findings from the projects will be made available to interested participants upon completion of the project.

If you have any questions or concerns regarding the experiment, please do not hesitate to contact us directly. I can be reached by phone (807) 252-1412 or through e-mail (evcurrie@lakeheadu.ca). Dr. Michael Wesner (mwesner@lakeheadu.ca), or Lakehead University's Research Ethics Board at (807) 343-8283.

Thank you for your interest in the project. Sincerely,

Emily Currie, Department of Psychology, Lakehead University

Appendix C

The purpose of the present study, conducted by Emily Currie and Dr. Michael Wesner of the Department of Psychology at Lakehead University, is to examine health and visual functioning in women. If you are currently enrolled in an Introductory Psychology section, one bonus point will be awarded for the completion of this screening questionnaire. Information collected from the screening questionnaire will be used to select some participants for subsequent laboratory sessions. Individuals who are selected for these sessions and who participate further in the study will receive an additional one to three bonus points (depending on the number of laboratory sessions attended) towards their final mark in Introductory Psychology.

Your participation in the screening will involve the completion of a questionnaire that will take approximately 30-40 minutes. The questionnaire includes personal questions designed to collect information on: demographics, reproductive history, the menstrual cycle, emotion and personality, caffeine intake, and morning/evening preference. You may choose not to respond to any questions that cause you discomfort.

Participation in this study is voluntary and you may withdraw at any time without explanation or penalty. Records of your participation will be kept in strict confidence. As well, no identifying information will be disclosed in the dissemination of the results of this study. As per university requirements, all data will be stored for at least five years in research laboratories at Lakehead University and remain anonymous and confidential. Individuals who meet specific criteria will be asked to participate in subsequent laboratory sessions. Therefore, we have asked for your name and telephone number here. Once we have determined who will be asked to participate in subsequent sessions, this information will be removed and information from your questionnaire will remain both anonymous and confidential. The benefits you may expect from the study are (a) an appreciation of research on health, (b) an opportunity to contribute to scientific research and (c) course credit (up to four bonus points). Throughout the study, participant information will be coded using a numbering system. There is no way that your name can be connected to your responses. There are no known physical or psychological risks associated with participating in this study. If you have any questions or concerns regarding this study please contact the principal investigators, Emily Currie (252-1412) or Dr. Michael Wesner (343-8457) directly.

Appendix D

I agree to participate in the present study that is investigating health and visual functioning in women. I understand that my participation is entirely voluntary: I may withdraw my consent from this experiment at any time, and this act will have no bearing on any remuneration I will receive, nor will it have any undesirable consequences.
The following points have been explained to me:

1. The purpose of this research is to identify what factors are related to women's health and visual functioning. The benefits I may expect from the study are (a) an appreciation of research on health, (b) an opportunity to contribute to scientific research and (c) course credit (up to three additional bonus points).
2. The procedure will be as follows: During a single session, researchers will obtain a salivary sample (via the technique of passive drool). Following a brief visual screening test, I will then be required to complete a total of four tests of visual functioning. These tests of visual functioning will be repeated in a subsequent lab session scheduled following the completion of the first lab session. A subset of participants will also be given the necessary materials, and asked to collect salivary samples at home to be returned to the lab the following day.
3. There are no known serious risks involved in participating in this study.
4. All of the data collected as well as my salivary samples will remain strictly confidential. My responses will not be associated with my name. Instead, my data will be associated with a code number when the researchers store the data.
5. The experimenter(s) will answer any other questions about the research either now or during the course of the experiment (other than specific questions about the hypotheses). If I have any other questions or concerns, I can address them to the principal experimenter, Emily Currie (evcurrie@lakeheadu.ca) or Dr. M. Wesner 343-8457 (mwesner@lakeheadu.ca)
6. Upon completion of my participation, I will receive a more detailed written explanation about the rationale underlying this experiment.
7. I am interested in receiving a summary of the results upon completion of the study:

☐ yes ☐ no

If yes, please indicate your email address: _____

I have read and understood the consent form, and I agree to participate in this study under these conditions.

_____ _____
Participant's Printed Name Signature

_____ _____
Date Experimenter Name

Appendix E

We appreciate your participation in our study, and thank you for spending your time to help us with our research. When you arrived here you were told that the purpose of this study was to investigate health and visual functioning in women. One of the factors in which we are interested is how hormonal sensitivity may affect perception within the visual system. In order to test this hypothesis, we have selected groups of women that the literature suggests are hormonally sensitive. These include women who experience symptoms of PMS, as well as women who report a history of experiencing oral contraceptive side effects. We also selected women without any of these symptoms.

Given that this study involves some aspects of which you were not fully informed at the outset, it is very important that you not discuss your experiences with other students until the end of the term. If participants have prior knowledge of our specific predictions it may influence their results, and the data we collect would be not be reliable. Because you will be given a copy of this feedback to take home, please do not make it available to other students. If you do not keep this form, please dispose of it rather than leaving it somewhere where other students might read it. Please feel free to discuss with the experimenter any feelings you have about the study right away. Should you have further questions, do not hesitate to contact Emily Currie, Brandi Person, or Dr. Michael Wesner, using the information listed below.

In addition to examining visual functioning, we will also be using your salivary samples to obtain a measure of estradiol. We have included three references on the following page in case you are interested in doing further reading relating to the study topics.

We hope that you have enjoyed participating in our study, and thank you very much for your assistance. As noted on the consent form, you will receive a summary of the results of the study at its completion if you have indicated an interest.

Principal Investigators:

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Dr. M. Wesner
Associate Professor
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Mental Health Resource Sheet

Sometimes people can feel upset when thinking about their mood. If you feel as though you would like to talk to a mental health practitioner for any reason please consider the resources listed below:

- *Lakehead University Health and Counseling Centre: 343-8361*
- *Thunder Bay Counseling Centre : 626-1880*
- *Catholic Family Development Centre: 345-7323*
- *Thunder Bay Crisis Response (24 hours): 346-8282*
- *Emergency services are available at the Thunder Bay Health Sciences Centre*

If you are interested in doing further reading that is related to this study, here are three relevant journal articles that you might want to obtain.

Oinonen, K., & Mazmanian, D. (2002). To what extent do oral contraceptives Influence mood and affect? Journal of Affective Disorders, 70, 229-240.

Stickgold, R., Whidbee, D., Schirmer, B., Patel, V., & Hobson, J.A. (2000). Visual discrimination task improvement: A multi-step process occurring during sleep. Journal of Cognitive Neuroscience, 12(2), 246-254.

Appendix F

Laboratory Questionnaire

Date_____
Time_____
Participant number_____

Cycle Phase LL MF

Are you currently taking Oral or Hormonal Contraceptives YES NO

1) This scale consists of a number of words that describe different feelings and emotions. Read each item and then mark the appropriate answer in the space next to that word. Indicate to what extent you have felt this way *today*. Use the following scale to record your answers.

	Very Slightly or not at all (1)	A little (2)	Moderately (3)	Quite a bit (4)	Extremely (5)
Interested	1	2	3	4	5
Distressed	1	2	3	4	5
Excited	1	2	3	4	5
Upset	1	2	3	4	5
Strong	1	2	3	4	5
Guilty	1	2	3	4	5
Scared	1	2	3	4	5
Hostile	1	2	3	4	5
Enthusiastic	1	2	3	4	5
Proud	1	2	3	4	5
Irritable	1	2	3	4	5
Alert	1	2	3	4	5
Ashamed	1	2	3	4	5
Inspired	1	2	3	4	5
Nervous	1	2	3	4	5
Determined	1	2	3	4	5
Attentive	1	2	3	4	5
Jittery	1	2	3	4	5
Active	1	2	3	4	5
Afraid	1	2	3	4	5

2) The following list shows common symptoms and feelings. For each item, choose the descriptive category from the box below that best describes what you have been feeling for the ***past 24 hours***. That is, for each item, decide whether you have “no experience of

the symptom, ” or whether your experience is “present mild,” “present moderate,” “present strong,” or present severe.” Then indicate your response by circling the corresponding number.

No experience of symptom (0)	Present Mild (1)	Present Moderate (2)	Present Strong (3)	Present Severe (4)	
Muscle stiffness	0	1	2	3	4
Headaches	0	1	2	3	4
Cramps	0	1	2	3	4
Backache	0	1	2	3	4
Fatigue	0	1	2	3	4
General aches and Pains	0	1	2	3	
Weight gain	0	1	2	3	4
Skin Blemish or disorder	0	1	2	3	4
Painful or tender breasts	0	1	2	3	4
Swelling	0	1	2	3	4
Loneliness	0	1	2	3	4
Anxiety	0	1	2	3	4
Mood Swings	0	1	2	3	4
Crying	0	1	2	3	4
Irritability	0	1	2	3	4
Tension	0	1	2	3	4
Feeling sad or Blue	0	1	2	3	4

Restlessness 0 1 2 3 4

3) Please rate your current level of physical pain by circling the best answer.

1- none at all 2- mild 3- moderate 4-severe 5-extremely severe/debilitating

4) Please rate your current level of discomfort by circling the best answer.

1- none at all 2- mild 3- moderate 4-severe 5-extremely severe/debilitating

5) Please rate your level of fatigue by circling the best answer

1- none at all 2- mild 3- moderate 4-severe 5-extremely severe/debilitating

6a) Have you consumed any alcohol in the past 24 hours? YES NO

b) If yes, please indicate the number of drinks _____

7a) Have you consumed any caffeine prior to coming to the lab this morning? YES NO

b) If yes, please indicate the number of servings _____

8. Have you taken any medication to alleviate symptoms of pain in the past 24 hours (Tylenol, Advil, Midol, etc…) YES NO

9. How many hours of sleep did you receive last night? _____

10. What time did you wake up this morning? _____

11. Are you currently menstruating? YES NO

12. If day 1 is the day that you started menstruating, what day of the menstrual cycle are you on? _____

Appendix G

SALIMETRICS

101 Innovation Blvd., Suite 302
State College, PA 16803 USA
(T) 814-234-7748, (F) 814-234-1608
800-790-2258 (USA & Canada only)

www.salimetrics.com
support@salimetrics.com

High Sensitivity
SALIVARY 17β-ESTRADIOL ENZYME IMMUNOASSAY KIT

Item No. 1-3702, (Single) 96-Well Kit;
1-3702-5, (5-Pack) 480 Wells

For Research Use Only

Intended Use

The Salimetrics™ estradiol kit is a competitive immunoassay specifically designed and validated for the quantitative measurement of salivary estradiol. It is not intended for diagnostic use. It is intended only for research use in humans and some animals. Please read the complete kit insert before performing this assay. For further information about this kit, its application, or the procedures in this insert, please contact the technical service team at Salimetrics, or your local sales representative.

Introduction

Estradiol (17β-estradiol, E_2, 1,3,5(10)-estratriene-3, 17β-diol), a steroid hormone, is produced primarily by the ovarian follicles from testosterone (1,2). Estradiol is the most active naturally secreted estrogen (1). In men, estradiol originates in the testes and from extraglandular conversion of androgens (1).

Circulating estradiol levels are relatively high at birth in both males and females, but decrease postnatally (2). In prepubertal children and men, levels are non-cyclic and low. During puberty, there are gradual increases in estradiol levels in both males and females. Interactions between luteinizing hormone (LH) and follicle-stimulating hormone (FSH) cause the release of estradiol from the ovaries in premenopausal women. Estradiol secretion is low in postmenopausal women.

Research concerning estradiol has focused predominantly on reproductive issues such as conception, ovulation, infertility, and menopause (3,4,5). Yet, estradiol affects a diversity of biological processes involved with pubertal and reproductive capacity, establishment and maintenance of pregnancy, infant care, coronary artery disease, immunocompetence, and cancer susceptibility (6,7,8). Estradiol is also believed to affect individual differences in cognitive and socioemotional processes as well as psychopathology (9,10).

Estrogens have been measured by many immunoassay methods. Studies suggest that estradiol can be accurately measured in saliva (3,4,11,12).

Test Principle

A microtitre plate is coated with rabbit antibodies to estradiol. Estradiol in standards and unknowns competes with estradiol linked to horseradish peroxidase for the antibody binding sites. After incubation, unbound components are washed away. Bound estradiol peroxidase is measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction with 2-molar sulfuric acid. Optical density is read on a standard plate reader at 450 nm. The amount of estradiol peroxidase detected is inversely proportional to the amount of estradiol present (13).

pH Indicator

A pH indicator in the estradiol assay diluent alerts the user to samples with high or low pH values. Acidic samples will turn the diluent yellow. Alkaline samples will turn the diluent purple. Dark yellow or purple wells indicate that a pH value for that sample should be obtained using pH strips. Estradiol values from samples with a pH ≤ 5 or ≥ 9 may be artificially inflated or lowered. Samples with a pH ≤ 5 or ≥ 9 should be recollected.

Precautions

1. Failure to follow kit procedure and recommendations for saliva collection and sample handling may result in false values.
2. Liquid stop solution is a 2-molar solution of sulfuric acid. This solution is caustic; use with care.
3. This kit uses break-apart microtitre strips. Unused wells must be stored at 2 - 8°C in the sealed foil pouch with desiccant and used in the frame provided.
4. Do not mix components from different lots of kits.
5. When using a multichannel pipette, reagents should be added to duplicate wells at the same time. Follow the same sequence when adding additional reagents so that incubation time with reagents is the same for all wells.
6. See "Material Safety Data" at the end of procedure.
7. We recommend that samples be screened for possible blood contamination (14,15) using a reliable screening tool such as the Salimetrics Blood Contamination EIA Kit (Item No.: 1-1302/1-1302-5). Do not use dipsticks, which result in false positive values due to salivary enzymes.
8. Routine calibration of pipettes is critical for the best possible assay performance.
9. When running multiple plates, or multiple sets of strips, a standard curve should be run with each individual plate and/or set of strips.
10. The temperature of the laboratory may affect assays. Salimetrics' kits have been validated at 68 - 74°F (20 - 23.3°C). Higher or lower temperatures will cause an increase or decrease in OD values, respectively. Salimetrics cannot guarantee test results outside of this temperature range.
11. The quantity of reagent provided with this kit is sufficient for three individual runs. The volume of diluent and conjugate used for assays using less than a full plate should be scaled down accordingly, keeping the same dilution ratio.
12. Avoid microbial contamination of opened reagents. Salimetrics recommends using opened reagents within one month.

Storage

All components of this kit are stable at 2 - 8°C until the kit's expiration date.

Reagents and Reagent Preparation

1. **Anti-Estradiol Coated Plate:** A ready-to-use, 96-well microtitre plate pre-coated with rabbit anti-estradiol antibodies in a resealable foil pouch.
2. **Estradiol Standard:** 1.6 mL of estradiol in a saliva-like matrix with a non-mercury preservative, at a concentration of 32 pg/mL.
3. **Estradiol Controls:** Two controls representing high and low levels of estradiol in a saliva-like matrix with a non-mercury preservative. Each vial contains 1 mL.
4. **Wash Buffer:** 100 mL of a 10X phosphate buffered solution containing detergents and a non-mercury preservative. Dilute only the amount needed for current day's use. Discard any leftover reagent. Dilute the wash buffer concentrate 10-fold with room temperature deionized water (100 mL of 10X wash buffer to 900 mL of deionized H_2O). *(**Note:** If precipitate has formed in the concentrated wash buffer, heat to 40°C for 15 minutes to dissolve crystals. Cool to room temperature before use in assay.)*
5. **Estradiol Assay Diluent:** 60 mL of a phosphate buffered solution containing a pH indicator and a non-mercury preservative.
6. **Enzyme Conjugate:** 50 µL of a solution of estradiol labeled with horseradish peroxidase. Dilute prior to use with estradiol assay diluent.
7. **Tetramethylbenzidine (TMB):** 25 mL of a non-toxic, ready-to-use solution.
8. **Stop Solution:** 12.5 mL of a solution of sulfuric acid.
9. **Non-specific Binding Wells (NSB):** One strip of wells that do not contain anti-estradiol antibody. They are located in the foil pouch. Wells may be broken off and inserted as blanks (optional) where needed.

Materials Needed But Not Supplied

- Precision pipette to deliver 15 µL, 100 µL, and 300 µL
- Precision multichannel pipette to deliver 50 µL, 100 µL, and 200 µL
- Vortex
- Plate rotator with 0.08-0.17" orbit
- Plate reader with a 450 nm filter
- Computer software for data reduction
- Deionized water
- Reagent reservoirs
- One 15 mL disposable tube
- Small disposable tubes for dilution of standard, controls, and samples
- Pipette tips
- Serological pipette to deliver 12 mL

Specimen Collection

Due to the episodic secretion pattern of steroid hormones, we can expect reproducible and reliable results only in cases of multiple sampling. Therefore, we recommend taking a minimum of 3 samples within at least a 2-hour period and pooling the samples before testing . Equal volumes from each of the samples should be pooled to create one sample that physically averages the fluctuations over that time period (16,17).

The preferred method of collecting whole saliva is by unstimulated passive drool. Collection protocols are available on request. **Do not use Salivettes, the Salimetrics Oral Swab (SOS), Sorbettes, cotton, or polyester materials to collect samples**. False readings will result (11). Do **not** add sodium azide to saliva samples as a preservative. Samples visibly contaminated with blood should be recollected. Avoid sample collection within 60 minutes after eating a major meal or within 12 hours after consuming alcohol. Acidic or high sugar foods can compromise assay performance by lowering sample pH and influencing bacterial growth. To minimize these factors, rinse mouth thoroughly with water 10 minutes before sample is collected. Record the time and date of specimen collection. After collection it is important to keep samples cold, in order to avoid bacterial growth in the specimen. Refrigerate sample within 30 minutes, and freeze at or below -20°C within 4 hours of collection. (Samples may be stored at -20°C or lower for long term storage.)

Freezing saliva samples will precipitate the mucins. On day of assay, thaw completely, vortex, and centrifuge at 1500 x g (@3000 rpm) for 15 minutes. It is important to avoid additional freeze-thaw cycles. However, if samples have been refrozen, centrifuge again prior to assaying. Samples should be at room temperature before adding to assay plate. Pipette clear sample into appropriate wells. Particulate matter may interfere with antibody binding, leading to falsely elevated results.

Procedure

Bring all reagents to room temperature. A minimum of 1.5 hours is necessary for the 12 mL of estradiol assay diluent used in Step 5 (conjugate dilution) to come to room temperature. **Note**: *It is important to keep the zip-lock pouch with the plate strips closed until warmed to room temperature as humidity may have an effect on the coated wells. Mix all reagents before use.*

Step 1: Determine your plate layout. Here is a suggested layout.

	1	2	3	4	5	6	7	8	9	10	11	12
A	32 Std	32 Std	C-H	C-H								
B	16 Std	16 Std	C-L	C-L								
C	8 Std	8 Std	Unk 1	Unk 1								
D	4 Std	4 Std	Unk 2	Unk 2								
E	2 Std	2 Std	Unk 3	Unk 3								
F	1 Std	1 Std	Unk 4	Unk 4								
G	Zero	Zero	Unk 5	Unk 5								
H	NSB	NSB	Unk 6	Unk 6								

Step 2: Keep the desired number of strips in the strip holder and place the remaining strips back in the foil pouch. If you choose to place non-specific binding wells in H-1, 2, remove strips 1 and 2 from the strip holder. Break off the bottom wells in each strip. Place the strips back into the strip holder leaving H-1, 2 blank. Break off 2 NSB wells from the strip of NSBs included in the foil pouch. Place in H-1, 2. Alternatively, NSBs may be placed wherever you choose on the plate. Reseal the zip-lock foil pouch with unused wells and desiccant. Store at 2 - 8°C.

Cautions: 1. Extra NSB wells should not be used for determination of standards, controls, or unknowns.
2. Do not insert wells from one plate into a different plate.

Step 3:

- Label five microcentrifuge tubes or other small tubes 2 through 6.
- Pipette 300 µL of estradiol assay diluent into tubes 2 through 6.

  Serially dilute the standard 2X by adding 300 µL of the 32 pg/mL standard (tube 1) to tube 2. Mix well. After changing pipette tips, remove 300 µL from tube 2 to tube 3. Mix well. Continue for tubes 4, 5, and 6. The final concentrations of standards for tubes 1 through 6, are 32 pg/mL, 16 pg/mL, 8 pg/mL, 4 pg/mL, 2 pg/mL, and 1 pg/mL, respectively. Standard concentrations in pmol/L are 117, 58.5, 29, 14.6, 7.3 and 3.65, respectively.
- Pipette 12 mL of estradiol assay diluent into a disposable tube. (Scale down proportionally if not using the entire plate.) Set aside for Step 5.

Step 4:

- Pipette 100 µL of standards, controls, and unknown samples into appropriate wells. Standards, controls, and unknown samples should be assayed in duplicate.
- Pipette 100 µL of estradiol assay diluent into 2 wells to serve as the zero.
- Pipette 100 µL of estradiol assay diluent into each NSB well.

  Note: *Pipetting of samples and reagents must be done as quickly as possible (without interruption) across the plate. Ideally, the process should be completed within 20 minutes or less.*

Step 5: Dilute the enzyme conjugate 1: 800 by adding 15 µL of the conjugate to the 12 mL of estradiol assay diluent prepared in Step 3. (Scale down proportionally if not using the entire plate.) Immediately mix the diluted conjugate solution and add 100 µL to each well using a multichannel pipette.

Step 6: Cover plate with adhesive cover provided. Mix plate on rotator for 5 minutes at 500 rpm (or tap to mix) and incubate at room temperature for an additional 115 minutes.

Step 7: Wash the plate 4 times with 1X wash buffer. A plate washer is recommended. However, washing may be done by gently squirting wash buffer into each well with a squirt bottle, or by pipetting 300 µL of wash buffer into each well and then decanting the liquid into a sink. After each wash, blot plate on paper towels before turning upright. *If using a plate washer, blotting is still recommended after the last wash.*

Step 8: Add 200 µL of TMB solution to each well with a multichannel pipette.

Step 9: Mix on a plate rotator for 5 minutes at 500 rpm (or tap to mix) and incubate the plate in the dark at room temperature for an additional 25 minutes.

Step 10: Add 50 µL of stop solution with a multichannel pipette.

Step 11:

- Mix on a plate rotator for 3 minutes at 500 rpm (or tap to mix). Be sure all wells have turned yellow. If green color remains, continue mixing until green color turns to yellow. **Caution:** *Do not mix at speeds over 600 rpm. Spillage may occur.*
- Wipe off bottom of plate with a water-moistened, lint-free cloth and wipe dry.
- Read in a plate reader at 450 nm. Read plate within 10 minutes of adding stop solution. (Correction at 630 is desirable.)

Calculations

1. Compute the average optical density (OD) for all duplicate wells.
2. Subtract the average OD for the NSB wells (if used) from the average OD of the zero, standards, controls, and unknowns.
3. Calculate the percent bound (B/Bo) for each standard, control, and unknown by dividing the average OD (B) by the average OD for the zero (Bo).
4. Determine the concentrations of the controls and unknowns by interpolation using software capable of logistics. We recommend using a 4-parameter sigmoid minus curve fit.

5. If a dilution of the sample is used, multiply the results by the dilution factor.

Quality Control

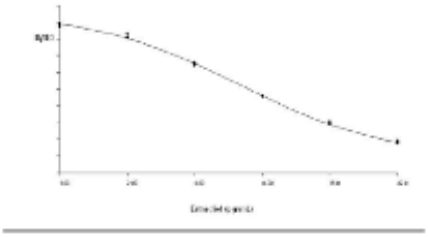
The Salimetrics' high and low salivary estradiol controls should be run with each assay. The control ranges established at Salimetrics are to be used as a guide. Each laboratory should establish its own range. Variations between laboratories may be caused by differences in techniques and instrumentation.

Typical Results

The following results are shown for illustration only and *should not* be used to calculate results from another assay.

Well	Standard	Average OD	B	B/Bo	Estradiol (pg/mL)
A1,A2	S1	0.183	0.174	0.185	32
B1,B2	S2	0.290	0.280	0.299	16
C1,C2	S3	0.438	0.429	0.457	8
D1,D2	S4	0.619	0.609	0.650	4
E1,E2	S5	0.773	0.764	0.814	2
F1,F2	S6	0.837	0.828	0.883	1
G1,G2	Bo	0.947	0.937	NA	NA
H1,H2	NSB	0.009	NA	NA	NA

Example: HS Estradiol 4-Parameter Sigmoid Minus Curve Fit



Material Safety Data*

Hazardous Ingredients
Liquid stop solution is caustic; use with care. We recommend the procedures listed below for all kit reagents. Specific kit component MSDS sheets are available from Salimetrics upon request.

Handling
Follow good laboratory procedures when handling kit reagents. Laboratory coats, gloves, and safety goggles are recommended. Wipe up spills using standard absorbent materials while wearing protective clothing. Follow local regulations for disposal.

Emergency Exposure Measures
In case of contact, immediately wash skin or flush eyes with water for 15 minutes. Remove contaminated clothing. If inhaled, remove individual to fresh air. If individual experiences difficulty breathing, give oxygen and call a physician.

*The above information is believed to be accurate but is not all-inclusive. This information should only be used as a guide. Salimetrics shall not be liable for accidents or damage resulting from contact with reagents.

Performance Characteristics

A. Sensitivity:

The lower limit of sensitivity was determined by interpolating the mean optical density minus 2 SDs of 10 sets of duplicates at the 0 pg/mL level. The minimal concentration of estradiol that can be distinguished from 0 is 0.1 pg/mL.

B. Precision:

The intra-assay precision was determined from the mean of 14 replicates each.

Sample	N	Mean (pg/ml)	Standard Deviation (pg/ml)	Coefficient of Variation (%)
High	14	20.26	1.42	7.0
Mid	14	7.24	0.45	6.3
Low	14	3.81	0.31	8.1

The inter-assay precision was determined from the mean of average duplicates for 10 separate runs.

Sample	N	Mean (pg/ml)	Standard Deviation (pg/ml)	Coefficient of Variation (%)
High	10	24.62	1.47	6.0
Low	10	4.76	0.42	8.9

C. Linearity of Dilution:

Four saliva samples were diluted with estradiol assay diluent and assayed.

Sample	Dilution	Expected (pg/ml)	Observed (pg/ml)	Recovery (%)
I			28.98	
	1:2	14.49	13.57	93.7
	1:4	7.25	7.24	99.9
	1:8	3.62	3.73	103.0
II			23.84	
	1:2	11.92	12.03	100.9
	1:4	5.96	5.56	93.3
	1:8	2.98	3.60	120.8
III			6.78	
	1:2	3.39	3.07	90.6
	1:4	1.70	1.70	100.0
IV			8.54	
	1:2	4.27	4.55	106.6
	1:4	2.14	1.93	90.2

D. Specificity of Antiserum

Compound	Spiked Concentration (ng/mL)	% Cross-reactivity in HS Salivary Estradiol EIA
Estriol	10	0.234
Estrone	1	1.276
Progesterone	100	ND
17 α-Hydroxyprogesterone	1000	ND
Testosterone	1000	ND
Coetisol	1000	ND
DHEA	1000	ND
Androstenedione	1000	ND
Aldosterone	1000	ND
Cortisone	1000	ND
11-Deoxycortisol	1000	ND
21-Deoxycortisol	1000	ND
Dexamethasone	1000	ND
Triamincinolone	1000	ND
Corticosterone	1000	ND
Prednisolone	1000	ND
Prednisone	100	0.016
Transferrin	1000	ND
Ethynodiol diacetate	1000	ND
Ethynylestradiol	10	0.189

ND = None detected (<0.004)

E. Recovery:

Five saliva samples was spiked with different levels of estradiol and assayed.

Sample	Endogenous (pg/ml)	Added (pg/ml)	Expected (pg/ml)	Observed (pg/ml)	Recovery (%)
I	2.92	20.48	23.40	23.84	101.9
II	4.68	13.65	18.33	17.91	97.7
III	3.80	3.20	7.00	6.78	96.9
IV	5.41	20.48	25.89	28.2	108.9
V	3.69	3.20	7.16	8.26	115.4

F. Correlation With Serum:

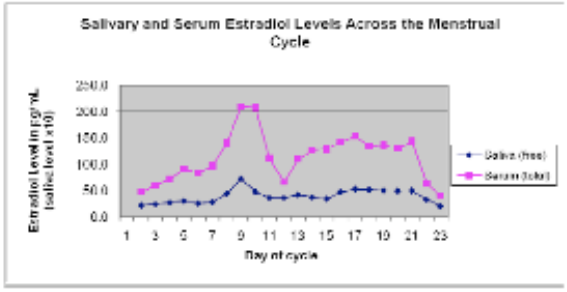
The correlation between saliva and serum estradiol in females was determined by assaying 11 matched samples. Samples were screened for pH and blood contamination. The magnitude of the saliva-serum correlation, $r(9) = 0.80$, $p = <0.001$, is consistent with the literature (4, 12, 18).

***Salivary Estradiol Expected Ranges:**

Pre-menopausal Adult Women	N	Mean (pg/mL)	Standard Deviation (pg/mL)
Follicular	20	1.35	0.80
Mid-Cycle	20	2.97	1.58
Luteal	20	2.56	0.84

*To be used as a guide only. Each laboratory should establish its own range.

Example of the variation of estradiol levels during the menstrual cycle of one woman:



Citations

1. Abraham, G.E. (1975). The applications of steroid radioimmunoassay to gynecologic endocrinology. In: Taymor, M.L. and Green, T.H. (eds.): *Progress in gynecology, Vol. 1,* 111-144. New York: Grune and Stratton.
2. Faiman, C., Winter, S. D., & Reyes, F.I. (1976). Patterns of gonadotropins and gonadol steroids throughout life. *Clin Obstet Gynecol, 3,* 467-483.
3. Lipson, S.F., & Ellison, P.T. (1996). Comparison of salivary steroid profiles in naturally occurring conception and non-conception cycles. *Hum Reprod, 11,* 2090-2096.
4. Choe, J.K., Khan-Dawood, F.S., & Dawood, M.Y. (1982). Progesterone and estradiol in saliva and plasma during the menstrual cycle. *Am J Obstet Gynecol, 46,* 557-562.
5. Belkien, L.D., Bordt, J., Moller, P., Hano, R.,& Nieschlag, E. (1985). Estradiol in saliva for monitoring follicular stimulation in an *in vitro* fertilization program. *Fertil Steril, 44,* 322-7.
6. McEwen, B.S. (1999). The molecular and neuroanatomical basis for estrogen effects in the central nervous system. *J Clin Endocrinol Metab 84,* 1790-1797.
7. Rodriguez, M.M., & Grossberg, G.T. (1998). Estrogen as a psychotherapeutic agent. *Clinics Geriatric Md, 14,* 177-189.
8. Zweifel, J., & O'Brien, W. (1997). A meta-analysis of the effects of hormone replacement therapy upon depressed mood. *Psychoneuroendocrinology, 22,* 189 - 212.
9. Uvnas-Moberg, K., Widstrom, A., Nissen, E., & Bjorvell, H. (1990). Personality traits in women 4 days postpartum and their correlation with plasma levels of oxytocin and prolactin. *Psychosom Obstet Gynaecol, 11,* 261-273.
10. Seeman, M.V. (1997). Psychopathology in women and men: Focus on female hormones. *Am J Psychiatry, 154,* 1641-1647.
11. Shirtcliff, E. A., Granger, D.A., Schwartz, E., & Curran, M.J. (2001). Use of salivary biomarkers in biobehavioral research: Cotton-based sample collection methods can interfere with salivary immunoassay results. *Psychoneuroendocrinology, 26,* 165-173.
12. Shirtcliff, E.A., Granger, D.A., Schwartz, E.B., Curran, M.J., Booth, A., & Overman, W.H. (2000). Assessing estradiol in biobehavioral studies using saliva and blood spots: Simple radioimmunoassay protocols, reliability, and comparative validity. *Hormones and Behavior, 38,* 137-147.
13. Chard, T. (1990). *An introduction to radioimmunoassay and related techniques* (4th ed.). Amsterdam: Elsevier.
14. Kivlighan, K.T., Granger, D.A., Schwartz, E.B., Nelson, V., & Curran, M. (2004). Quantifying blood leakage into the oral mucosa and its effects on the measurement of cortisol, dehydroepiandrosterone, and testosterone in saliva. *Hormones and Behavior, 46,* 39-46.
15. Schwartz, E., & Granger, D.A. (2004). Transferrin enzyme immunoassay for quantitative monitoring of blood contamination in saliva. *Clinical Chemistry, 50,* 654-656.
16. West, C.D., Mahajan, D.K., Chavre, V.J., Nabors, C.J. (1973). Simultaneous measurement of multiple plasma steroids by radioimmunoassay demonstrating episodic secretion. *Journal of Clinical Endocrinology & Metabolism, 36(6),* 1230-1236.
17. Brambilla, D.J., O'Donell, A.B., Matsumotot, A.M., & McKinlay, J.B. (2007). Intraindividual variation in levels of serum testosterone and other reproductive and adrenal hormones in men. *Clinical Endocrinology, 67,* 853-862.
18. Ellison, P.T. (1999). Salivary estradiol–A viable alternative? *Fertility and Sterility, 72(5),* 951-2.

Seller's Limited Warranty

"Seller warrants that all goods sold hereunder will be free from defects in material and workmanship. Upon prompt notice by Buyer of any claimed defect, which notice must be sent within thirty (30) days from date such defect is first discovered and within three months from the date of shipment, Seller shall, at its option, either repair or replace the product that is proved to Seller's satisfaction to be defective. All claims should be submitted in written form. This warranty does not cover any damage due to accident, misuse, negligence, or abnormal use. Liability, in all cases, will be limited to the purchased cost of the kit.

It is expressly agreed that this limited warranty shall be in lieu of all warranties of fitness and in lieu of the warranty of merchantability. Seller shall not be liable for any incidental or consequential damages that arise out of the installation, use or operation of Seller's product or out of the breach of any express or implied warranties."

Appendix H

Safety Precautions

Liquid stop is a 2-molar solution of sulfuric acid. This solution is caustic; use with care.

See 'Material Safety Information' at the end of procedure.

A safety data sheet is available upon request.

pH Indicator

A pH indicator in the assay diluent alerts the user to samples with high or low pH values. Acidic samples will turn the diluent yellow. Alkaline samples will turn the diluent purple. Dark yellow or purple wells indicate that a pH value for that sample should be obtained using pH strips. Progesterone values from samples with a pH < 4.0 or > 9.0 may be artificially inflated or lowered. (18)

Storage

All components of this kit are stable at 2-8°C until the kit's expiration date.

Materials Supplied with Single Kit

Item		Quantity/Size
1	**Microtitre Plate** Coated with rabbit anti-progesterone antibodies.	1/96-well
2	**Progesterone Standard** 2430 pg/mL, in a saliva-like matrix. Serially dilute before use according to Reagent Preparation. Contains: progesterone, buffer, preservative.	1 vial/1 mL
3	**Progesterone Controls** High, Low, in a saliva-like matrix. Ready to use. Contain: progesterone, buffer, preservative.	2 vials/500 µL each
4	**Wash Buffer Concentrate (10X)** Dilute before use according to Reagent Preparation. Contains: phosphate buffer, detergent, preservative.	1 bottle/100 mL
5	**Assay Diluent** Contains: phosphate buffer, pH indicator, preservative.	1 bottle/60 mL

6	**Progesterone Enzyme Conjugate** Concentrate. Dilute before use with assay diluent. (See step 5 of Procedure.) Contains: Progesterone conjugated to HRP, preservative.	1 vial/50 µL
7	**TMB Substrate Solution** Non-toxic, ready to use.	1 bottle/25 mL
8	**2 M Stop Solution** Contains: sulfuric acid.	1 bottle/12.5 mL
9	**Non-Specific Binding (NSB) Wells** Do not contain anti-progesterone antibody. Break off and insert as blanks (optional) where needed.	1 strip

Materials Needed But Not Supplied

Precision pipette to deliver 22.5 µL, 50 µL, 100 µL, and 200 µL
Precision multichannel pipette to deliver 50 µL, 150 µL, and 200 µL
Vortex
Plate rotator with 0.08-0.17 inch orbit capable of 500 rpm
Plate reader with a 450 nm filter
Log-linear graph paper or computer software for data reduction
Deionized water
Reagent reservoirs
One disposable polypropylene tube to hold at least 18 mL
Small disposable polypropylene tubes
Pipette tips and a serological pipette

Limitations

Samples with progesterone values greater than 2430 pg/mL should be further diluted with assay diluent and rerun for accurate results. To obtain the final progesterone concentration, multiply the concentration of the diluted sample by the dilution factor.

A pH value should be obtained on samples that appear yellow or purple after assay diluent is added and the plate is mixed (Step 6). Samples with pH values < 4.0 or > 9.0 should be recollected.

See “Specimen Collection” recommendations to insure proper collection of saliva specimens and to avoid interfering substances.

Samples collected with sodium azide are unsuitable for this assay.

Any quantitative results indicating abnormal progesterone levels should be followed by additional testing and evaluation.

Specimen Collection

Avoid sample collection within 60 minutes after eating a major meal or within 12 hours after consuming alcohol. Acidic or high sugar foods can compromise assay performance by lowering sample pH and influencing bacterial growth. To minimize these factors, rinse mouth thoroughly with water 10 minutes before sample is collected.

The preferred method for collecting whole saliva is by unstimulated passive drool. Donors may collect whole saliva by tilting the head forward, allowing the saliva to pool on the floor of the mouth, and then passing the saliva through the Saliva Collection Aid (SCA), Item No. 5016.02, into a polypropylene vial. Collection protocols are available on request or online at www.salimetrics.com.

Do not use Salivettes, the SalivaBio Oral Swab (SOS), Sorbettes, cotton, or polyester materials to collect samples. False readings will result. (19)

Samples visibly contaminated with blood should be recollected. We recommend that samples be screened for possible blood contamination (20,21) using a reliable screening tool such as the Salimetrics Blood Contamination EIA Kit (Item No. 1-1302/1-1302-5). Do not use dipsticks, which result in false positive values due to salivary enzymes.

Record the time and date of specimen collection.

Sample Handling and Preparation

After collection it is important to keep samples cold, in order to avoid bacterial growth in the specimen. Refrigerate sample within 30 minutes, and freeze at or below -20ºC within 4 hours of collection. (Samples may be stored at -20ºC or lower for long term storage.)

Do not add sodium azide to saliva samples as a preservative, as it may cause interference in the immunoassay.

Freezing saliva samples will precipitate mucins. On day of assay, thaw completely, vortex, and centrifuge at 1500 x g (@3000 rpm) for 15 minutes. Centrifuging removes mucins and other particulate matter which may interfere with antibody binding, leading to falsely elevated results. Samples should be at room temperature before adding to assay plate. Pipette clear sample into appropriate wells. Re-freeze saliva samples as soon as possible after adding to the assay plate. Centrifuge/re-centrifuge saliva samples each time that they are thawed. Avoid additional freeze-thaw cycles.

Reagent Preparation

•Bring all reagents to room temperature and mix before use. A minimum of 1.5 hours is recommended for the 18 mL of assay diluent used in Step 5 (conjugate dilution) to come to room temperature.

•Bring microtitre plate to room temperature before use. Note: It is important to keep the foil pouch with the plate strips closed until warmed to room temperature, as humidity may have an effect on the coated wells.

•Prepare 1X wash buffer by diluting wash buffer concentrate 10-fold with room-temperature deionized water (100 mL of 10X wash buffer to 900 mL of deionized water. Dilute only the amount needed for current day's use, and discard any leftover reagent. (If precipitate has formed in the concentrated wash buffer, heat to 40°C for 15 minutes to dissolve crystals. Cool to room temperature before use in assay.)

•Prepare serial dilutions of the progesterone standard as follows:

◦◦Label five polypropylene microcentrifuge tubes or other small tubes 2 through 6.

◦◦Pipette 200 µL of assay diluent in tubes 2 through 6.

◦◦Serially dilute the standard 3X by adding 100 µL of the 2430 pg/mL standard (tube 1) to tube 2. Mix well. After changing pipette tips, remove 100 µL from tube 2 to tube 3. Mix well. Continue for tubes 4, 5, and 6. The final concentrations of stan-dards for tubes 1 through 6 are, respectively, 2430 pg/mL, 810 pg/mL, 270 pg/mL, 90 pg/mL, 30 pg/mL, and 10 pg/mL. Values in nmol/L are 7.733, 2.576, 0.859, 0.286, 0.095 and 0.032 nmol/L, respectively.

General Kit Use Advice

•

This kit uses break-apart microtitre strips. You may run less than a full plate. Unused wells must be stored at 2-8°C in the sealed foil pouch with desiccant and used in the frame provided.

•The quantity of reagent provided with this kit is sufficient for three individual runs. The volume of diluent and conjugate used for assays using less than a full plate should be scaled down accordingly, keeping the same dilution ratio.
•Do not mix components from different lots of kits.
•When using a multichannel pipette, reagents should be added to duplicate wells at the same time. Follow the same sequence when adding additional reagents so that incubation time with reagents is the same for all wells.
•To ensure highest quality assay results, pipetting of samples and reagents must be done as quickly as possible (without interruption) across the plate. Ideally, the process should be completed within 20 minutes or less.
•When running multiple plates, or multiple sets of strips, a standard curve must be run with each individual plate and/or set of strips.
•The temperature of the laboratory may affect assays. Salimetrics' kits have been validated at 68-74ºF (20-23.3ºC). Higher or lower temperatures will cause an increase or decrease in OD values, respectively. Salimetrics cannot guarantee test results outside of this temperature range.
•Avoid microbial contamination of opened reagents. Salimetrics recommends using opened reagents within one month

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Avoid microbial contamination of opened reagents. Salimetrics recommends using opened reagents within one month.

Routine calibration of pipettes is critical for the best possible assay performance.

Use of progesterone-enhanced creams or supplements by the laboratory technician performing the analysis can adversely affect results.

Procedure

Step 1: Determine your plate layout. Here is a suggested layout.

	1	2	3	4	5	6	7	8	9	10	11	12
A				2430 Std		2430 Std			C-H		C-H	
B				810 Std		810 Std			C-L		C-L	
C				270 Std		270 Std			Unk-1		Unk-1	
D				90 Std		90 Std			Unk-2		Unk-2	
E				30 Std		30 Std			Unk-3		Unk-3	
F				10 Std		10 Std			Unk-4		Unk-4	
G				Zero		Zero			Unk-5		Unk-5	
H				NSB*		NSB*			Unk-6		Unk-6	

*NSB = Non-specific binding wells. These may serve as blanks. Use is optional.

Step 2: Keep the desired number of strips in the strip holder and place the remaining strips back in the foil pouch. If you choose to place non-specific binding wells in H-1, 2, remove strips 1 and 2 from the strip holder. Break off the bottom wells in each strip. Place the strips back into the strip holder leaving H-1, 2 blank. Break off 2 NSB wells from the strip of NSBs included in the foil pouch. Place in H-1, 2. Alternatively, NSBs may be placed wherever you choose on the plate. Reseal the zip-lock foil pouch with unused wells and desiccant. Store at 2-8°C.

Cautions:
1. Extra NSB wells should not be used for determination of standards, controls, or unknowns.
2. Do not insert wells from one plate into a different plate.

Step 3: Pipette 18 mL of assay diluent into the disposable tube. (Scale down proportionally if not using the entire plate.) Set aside for Step 5.

Step 4:

•Pipette 50 µL of standards, controls, and unknowns into appropriate wells. Standards, controls, and unknowns should be assayed in duplicate.

•Pipette 50 µL of assay diluent into two wells to serve as the zero.

•Pipette 50 µL of assay diluent into each NSB well.

Step 5: Dilute the enzyme conjugate 1:800 by adding 22.5 µL of the conjugate to the 18 mL of assay diluent prepared in Step 3. (Scale down proportionally if not using the entire plate.) Conjugate tube may be centrifuged for a few minutes to bring the liquid down to the tube bottom. If using 6 or fewer strips, 12.5 µL of conjugate to 10 mL of assay diluent may be used. Immediately mix the diluted conjugate solution and add 150 µL to each well using a multichannel pipette.

Step 6: Cover plate with adhesive cover provided. Rotate the plate continuously at 500 rpm for 1 hr at room temperature.

Step 7: Wash the plate four times with 1X wash buffer. A plate washer is recommended. However, washing may be done by gently squirting wash buffer into each well with a squirt bottle, or by pipetting 300 µL of wash buffer into each well and then flipping the liquid into a sink. After each wash, the plate should be thoroughly blotted on paper towels before turning upright. If using a plate washer, blotting is still recommended after the last wash.

Step 8: Add 200 µL of TMB solution to each well with a multichannel pipette.

Step 9: Mix on a plate rotator for five minutes at 500 rpm and incubate the plate in the dark at room temperature for an additional 25 minutes.

Step 10: Add 50 µL of stop solution with a multichannel pipette.

Step 11:

•Mix on a plate rotator for three minutes at 500 rpm. Be sure all wells have turned yellow. If green color remains, continue mixing until green color turns to yellow.

Caution: Spillage may occur if mixing speed exceeds 600 rpm.

•Wipe off bottom of plate with a water-moistened, lint-free cloth and wipe dry.

•Read in a plate reader at 450 nm. Read plate within 10 minutes of adding stop solution. (Correction at 490 to 630 nm is desirable.)

Figures

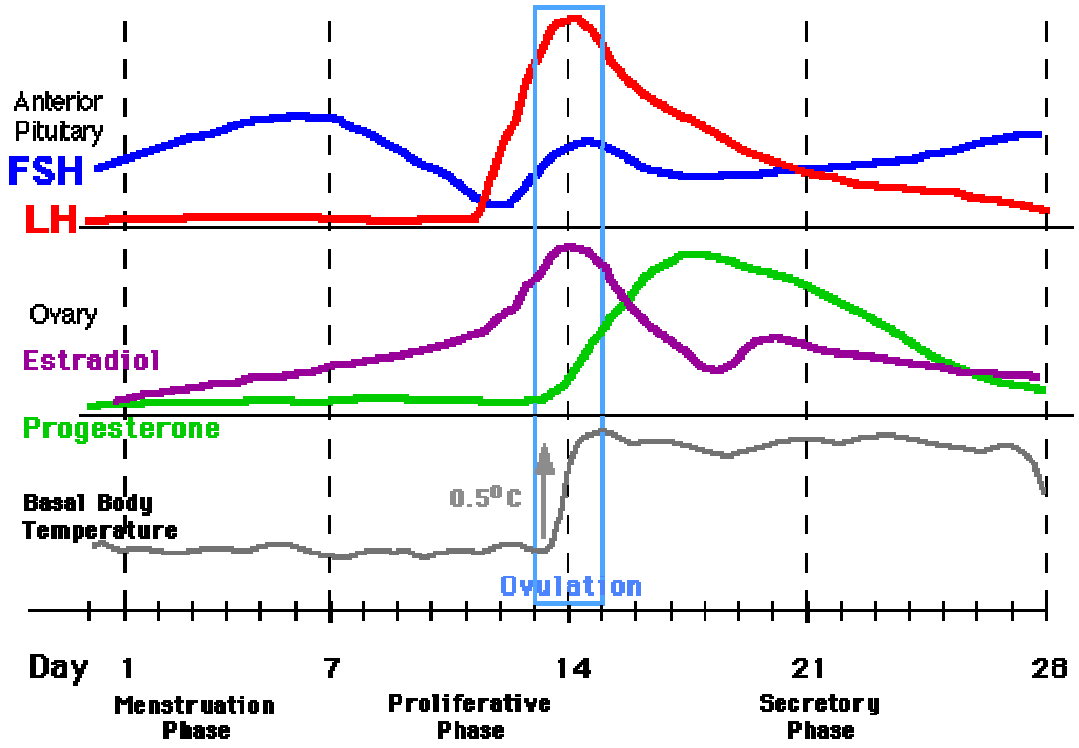

Figure 1. Hormones as a function of the menstrual cycle phases. Cyclic patterns of the follicle stimulating hormone (blue), the luteinizing hormone (red), estradiol (purple) and progesterone (green) are depicted. Figure obtained from: http://embryology.med.unsw.edu.au/Science/ANAT2341lecture03.htm

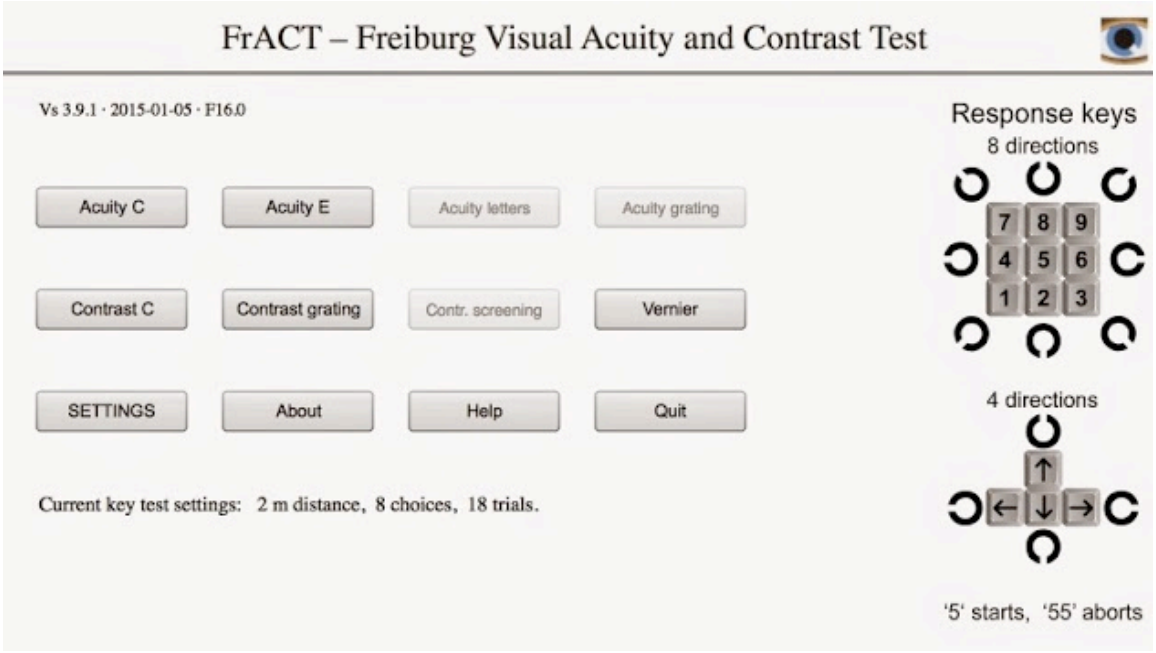

Figure 2. This figure depicts the screen that is used to set up the FrACT and explains the stimulus-response task. Landoldt 'C's (on the right) were displayed at 8 orientations with response keys mapped out to indicate where the observers perceived the opening of the 'C'. We used this display to explain to the participant that individual 'C's would be presented one-at-a-time and that the participant would have to individuate (using the number pad on the keyboard) where the opening of the 'C' was oriented. Figure obtained from Bach, 2007.

Figure 3. Above are examples of Ishihara plates used to assess colour blindness. The participant viewed the plates under a D65 daylight simulation light and had to read the number imbedded in the dots.

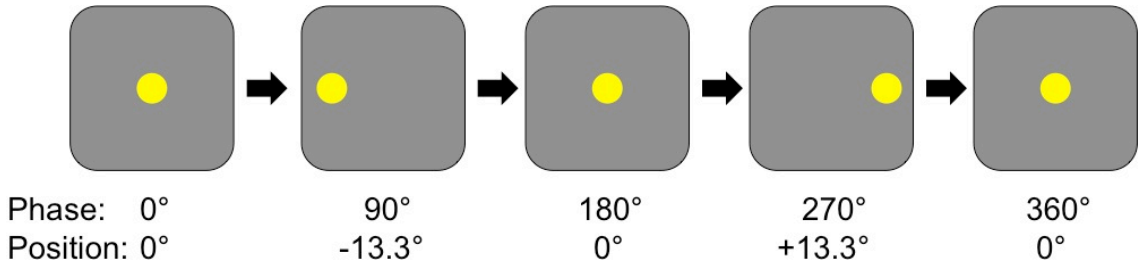

Figure 4. Horizontal, sinusoidal target amplitudes labeled as either degrees sine phase position (top row), or as degrees visual angle with respect to the screen (bottom row) in which negative defines leftward movement and positive defines rightward movement. The timing of these excursions is dependent on the temporal frequency (0.25, 0.5, 1.0 cycles/sec or Hz) of the oscillating spot target.

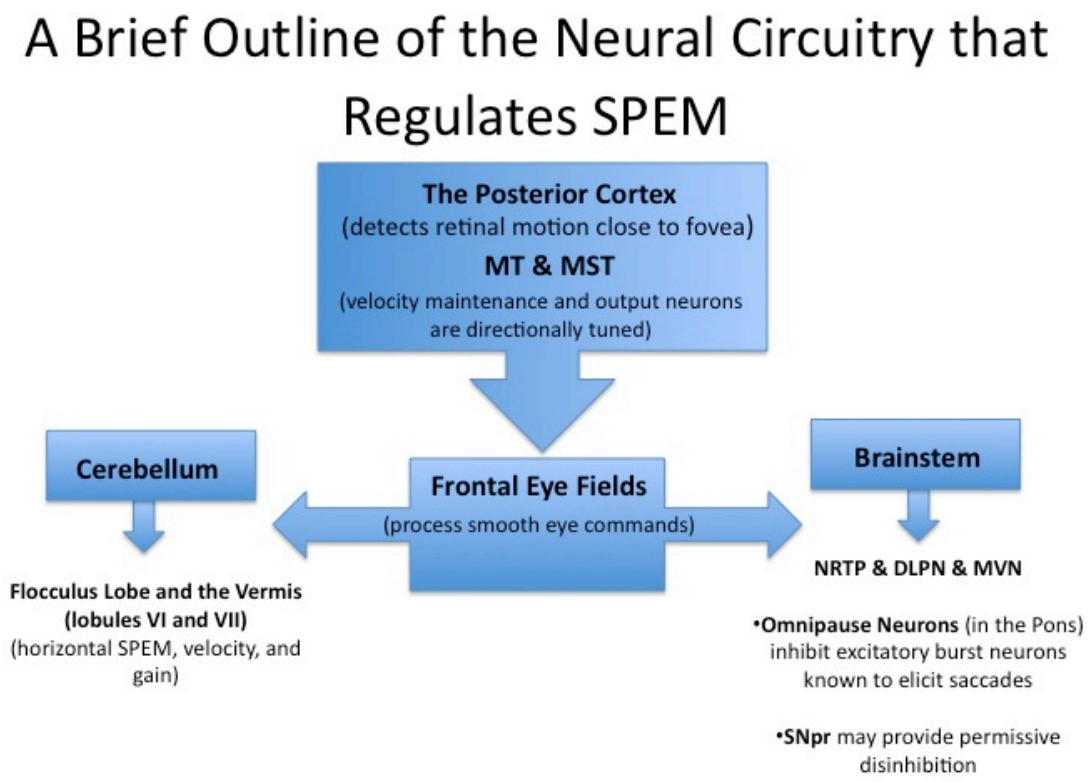

Figure 5. Neural circuitry involved in horizontal SPEM throughout the cerebral cortex, cortex, cerebellum, and brainstem. This overview is used to understand the underlying neural circuits where estradiol receptors have been found and/or where estradiol has putatively had effects on the maintenance and regulation of SPEM.

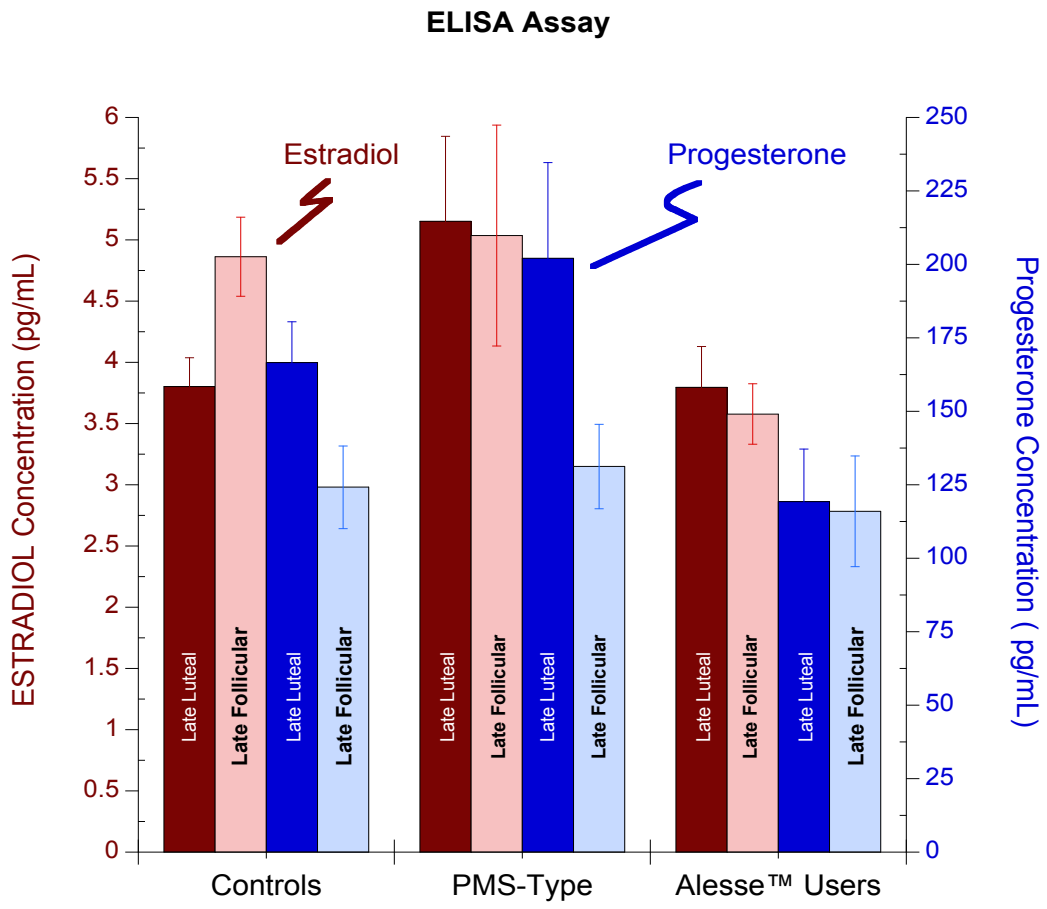

Figure 6. Enzyme-linked immunosorbent assay (ELISA). Estradiol (red) and progesterone (blue) concentrations *(± 1 SD)* taken during the Late Follicular (right*)* and Late Luteal (left) phases of the participant's menstrual cycle. Note PMS-type and Alesse™ users show no significant estradiol concentration changes across the cycle. In addition, Alesse™ users also show no progesterone shifts, which is to be expected based on the synthetic analogue effects on receptor sensitivities. Based on concentration, PMS-types appear to have the highest overall levels of estradiol of the three groups and the highest concentration of progesterone during LL phase. Of more interest, however, is in the temporal patterns: PMS and Alesse™ users have comparable stable estradiol levels, controls and PMS have comparable cycling progesterone levels, and Alesse™ users have stable progesterone levels similar to their stable estradiol levels across the cycle.

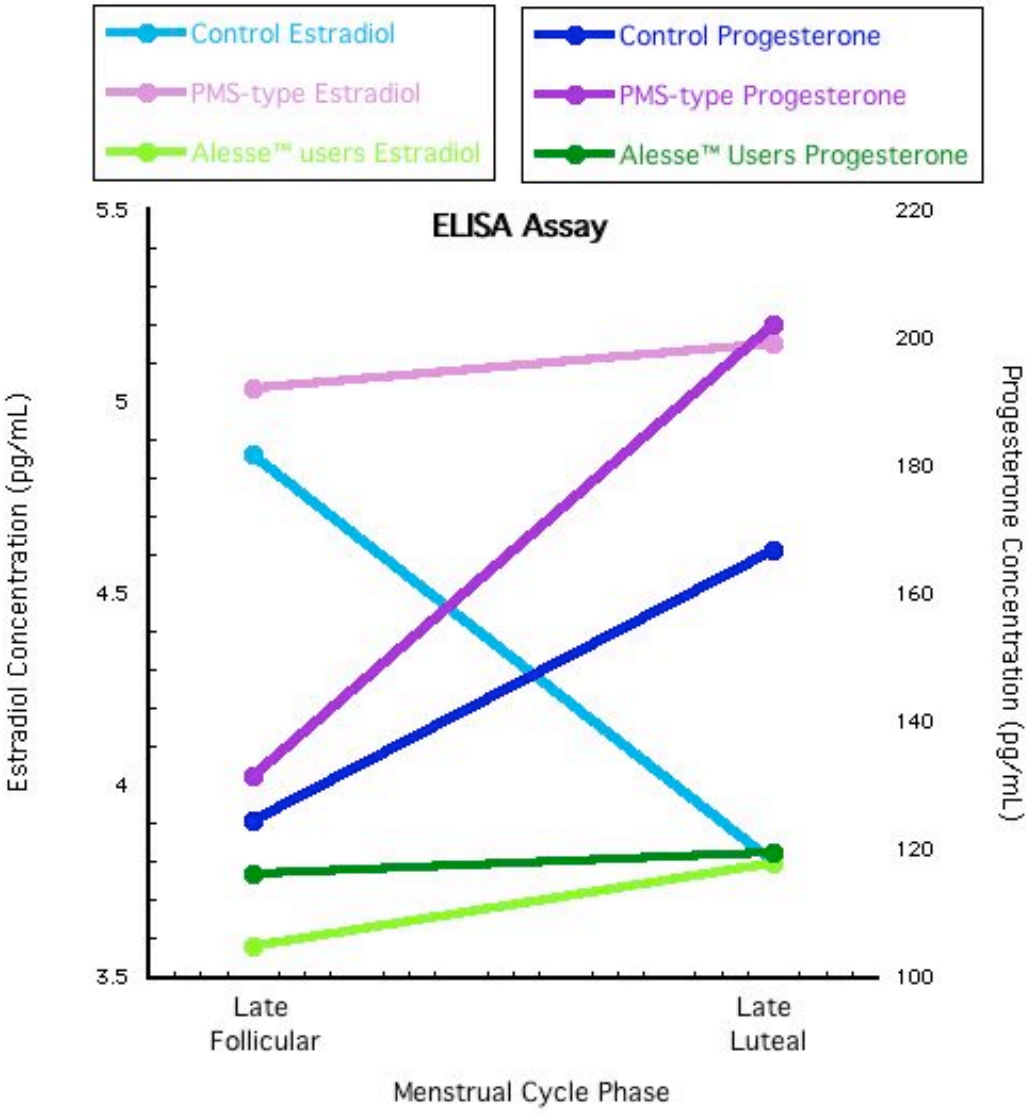

Figure 7. Enzyme-linked immunosorbent assay (ELISA). Estradiol (light colours) and progesterone (dark colours) concentrations *(± 1 SD)* taken during the Late Follicular (left*)* and Late Luteal (right) phases of the participant's menstrual cycle. Results as seen in Figure 6.

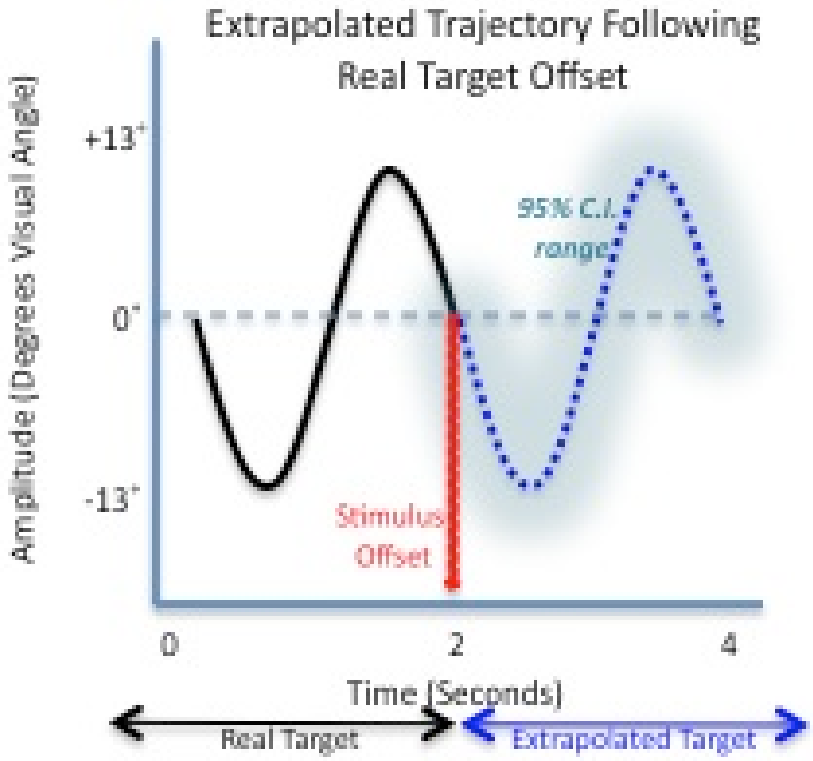

Figure 8. The extrapolated trajectory following real target offset with amplitude (degrees visual angle) as a function of time (sec). When the stimulus was terminated (red arrow) the real target eye movements (black) eye movements ended and the duration and accuracy of persistence was measured by comparing the participant's actual eye movements to where there eye movements would have been if the stimulus was still present (the blue extrapolated trajectory). If the participants eye movements exceeded the 2- sided 95% confidence interval range derived from their real target eye movements (highlighted by the light blue shading) then that deviation marked the completion of a continued track and the duration from offset to that deviation point defined persistence.

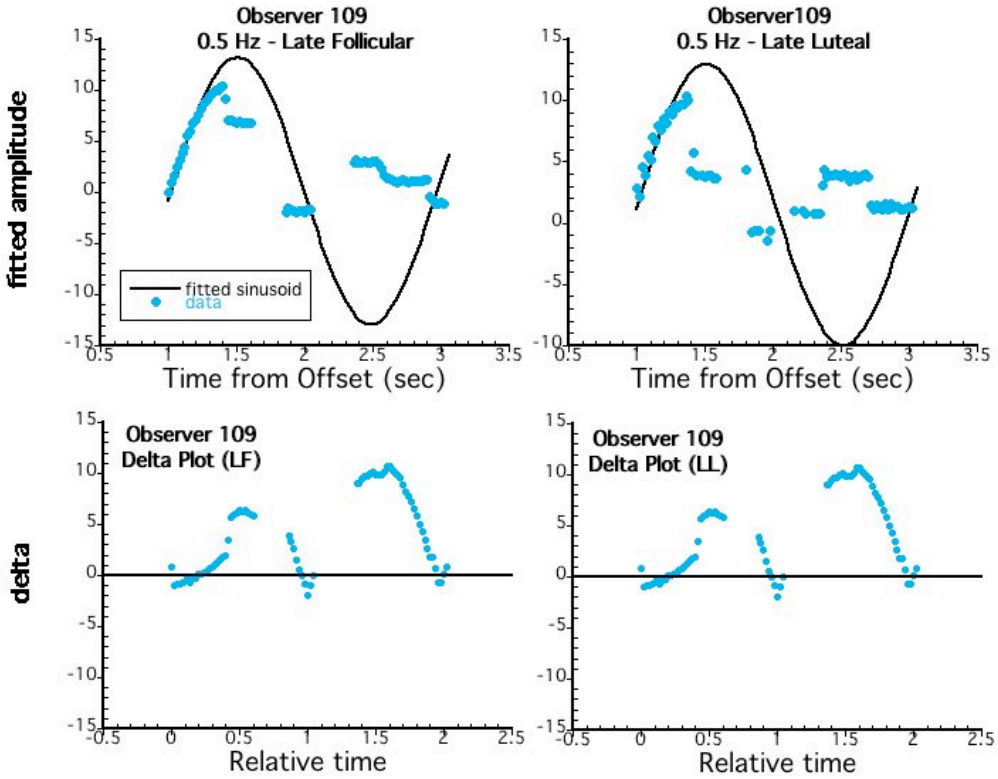

Figure 9. Individual observer plots of the participants eye movements following the offset of the stimulus for a control participant. The top row shows the participants eye movements (blue) relative to their own extrapolated trajectory (solid black). The fitted amplitude to the theoretical trajectory (see text) is plotted as a function of time (sec) from the stimulus termination onset for the 0.25 Hz frequency during the Late Follicular phase (left) and the Late Luteal Phase (right). The bottom row displays the same participant's errors between where their eyes should have been and where they actually were (deltas) as a function of relative time.

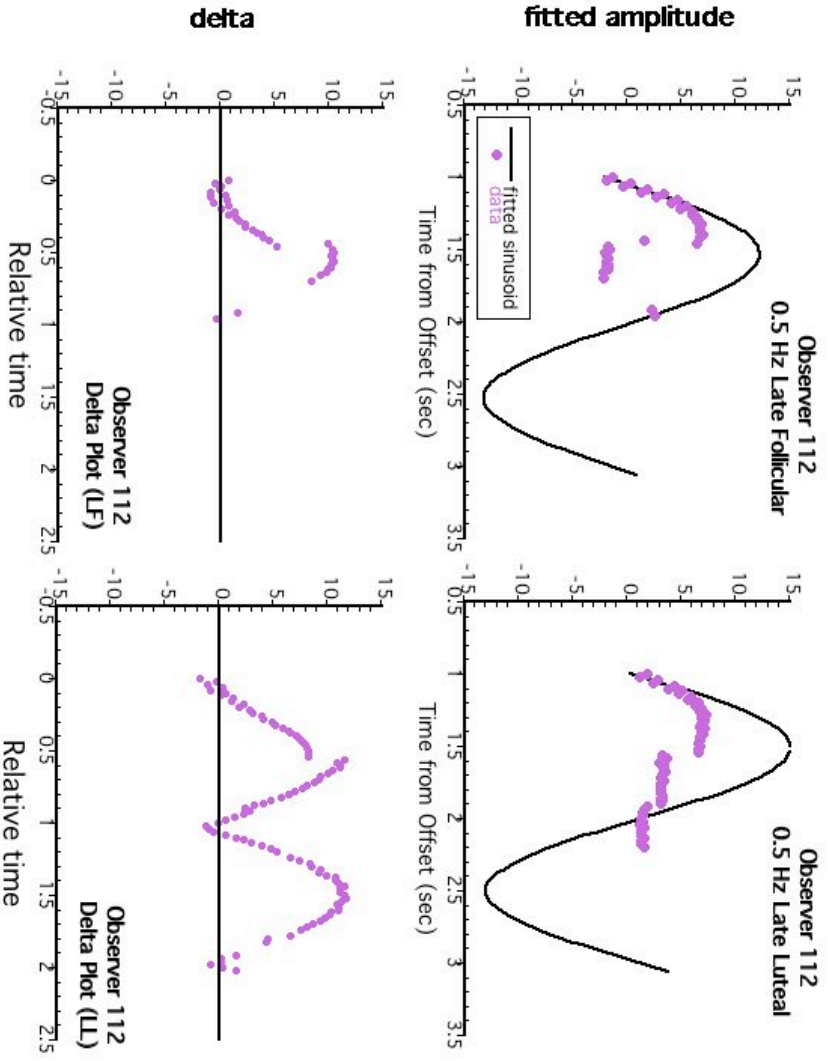

Figure 10. Fitted amplitude data and deltas as a function of time from offset and relative time, respectively, for a participant from the PMS-type group during a 0.5 Hz frequency trial. Details as in Figure 9 for extrapolated trajectory and menstrual phase.

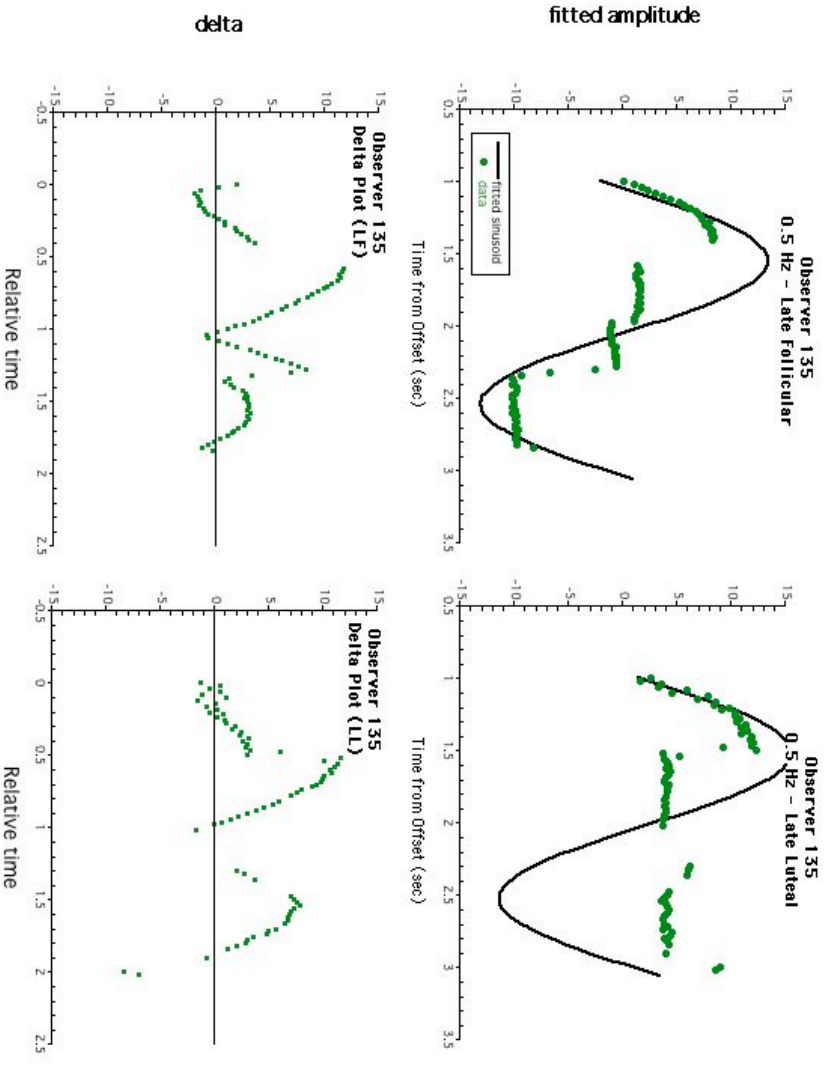

Figure 11. Fitted amplitude data and deltas as a function of time from offset and relative time, respectively, for a participant from the Alesse™ user group during a 0.5 Hz frequency trial. Details as in Fig.ure 9 for extrapolated trajectory and menstrual phase.

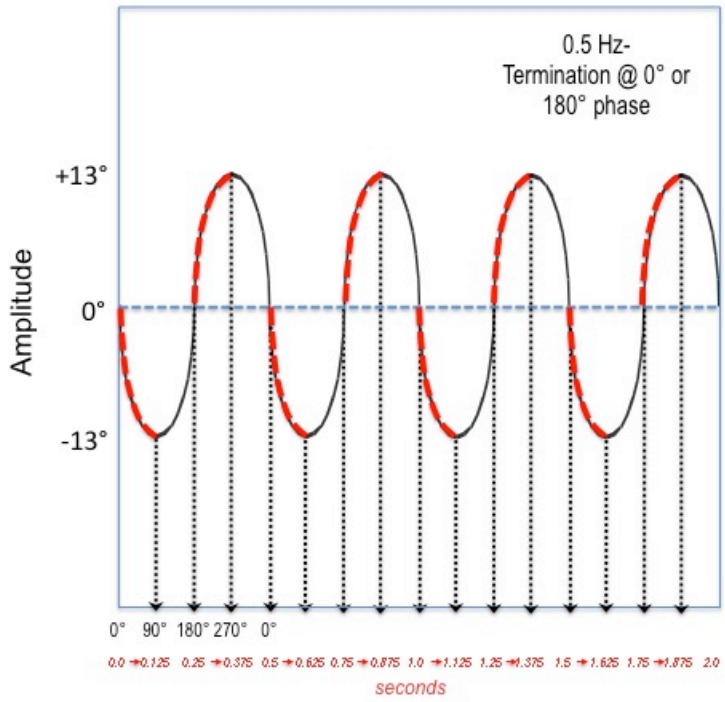

Figure 12. Sinusoidal cycle phase amplitude (position on the screen) ranging from -13° to 13° as a function of relative time (sec) for 0.5 Hz where the target was terminated at either 0° or 180° phase position. The sinusoid is partitioned by 0.125 second limbs to indicate what limbs of the sinusoid were used for further analysis (in red) and what were removed to avoid rebound effects. See text for further description of "rebounds".

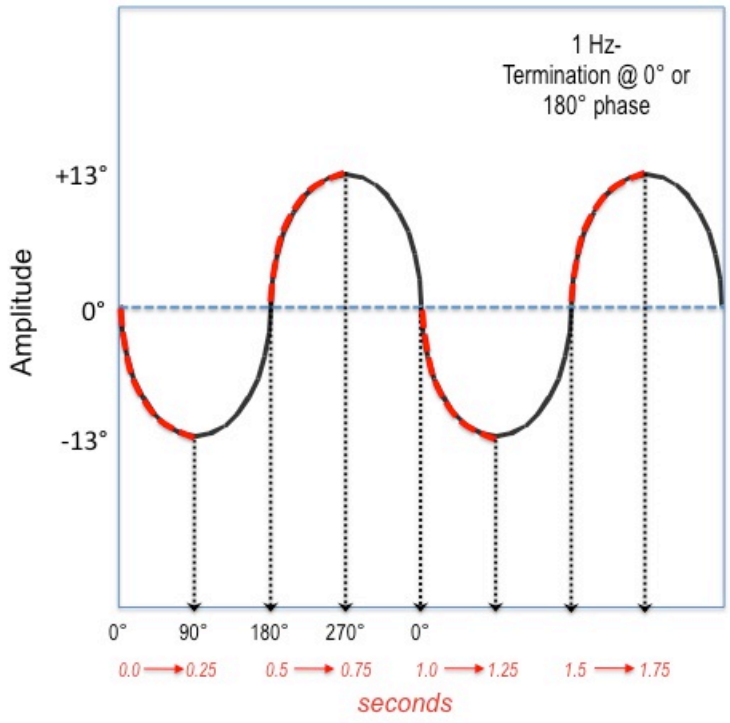

Figure 13. Same as Figure 12 except for a 1.0-Hz target. Limbs are separated by 0.25 second to indicate decay (in red) and rebound (in black) intervals.

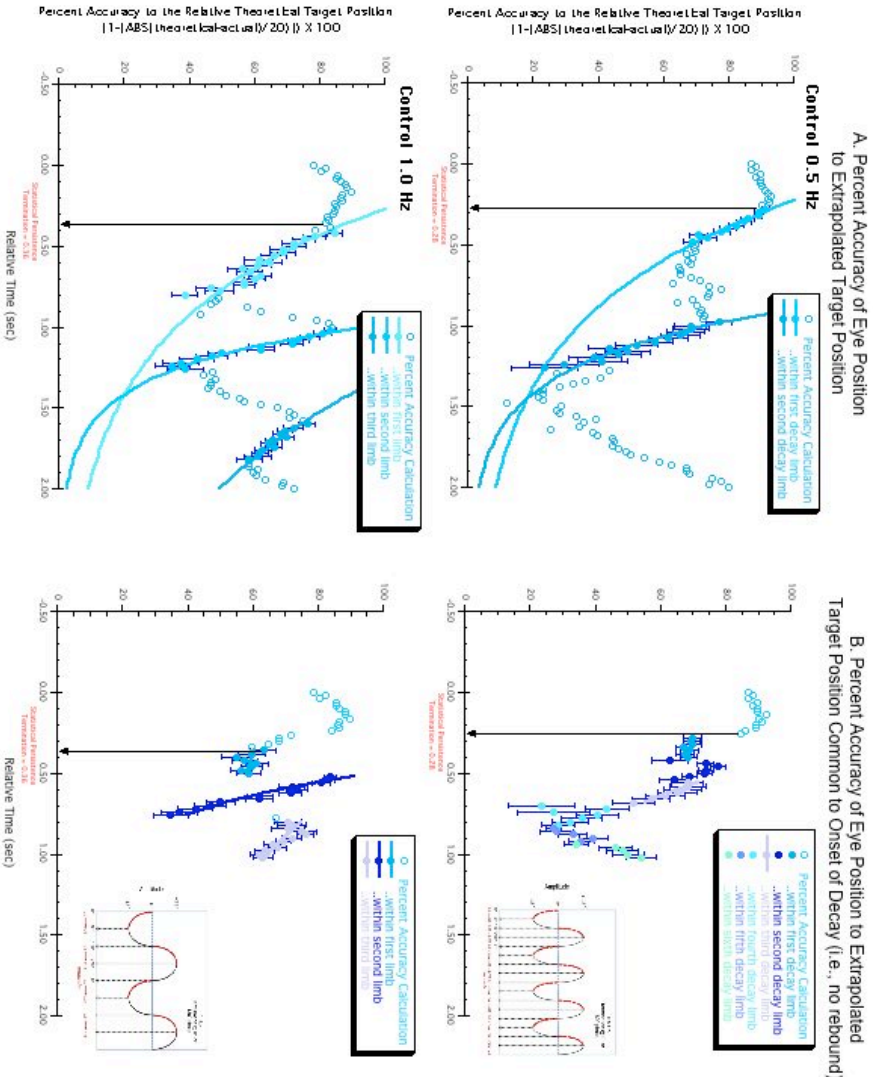

Figure 14. Averages taken from the control group collapsed across the menstrual cycles with 0.5 Hz (top row) and 1.0 Hz (bottom row) target cycles. Column A presents "percent accuracy of eye position to extrapolated target position" and Column B represents "percent accuracy of eye position to extrapolated target position common to onset of decay (i.e., no rebound limbs in analysis)". For column A, percent accuracy to the relative extrapolated trajectory as a function of relative time in seconds is presented via the blue hollow circles and the percent accuracy calculations (decay functions) are fitted with the solid blue lines. The statistically derived termination point in time is marked on the x-axis with an arrow. Column B is identical to Column A; however, sinusoidal rebounds are removed and the decay function is fitted to the percent accuracy calculations limb-by-limb as illustrated in Figures 12 and 13 (presented as an icon in the bottom right).

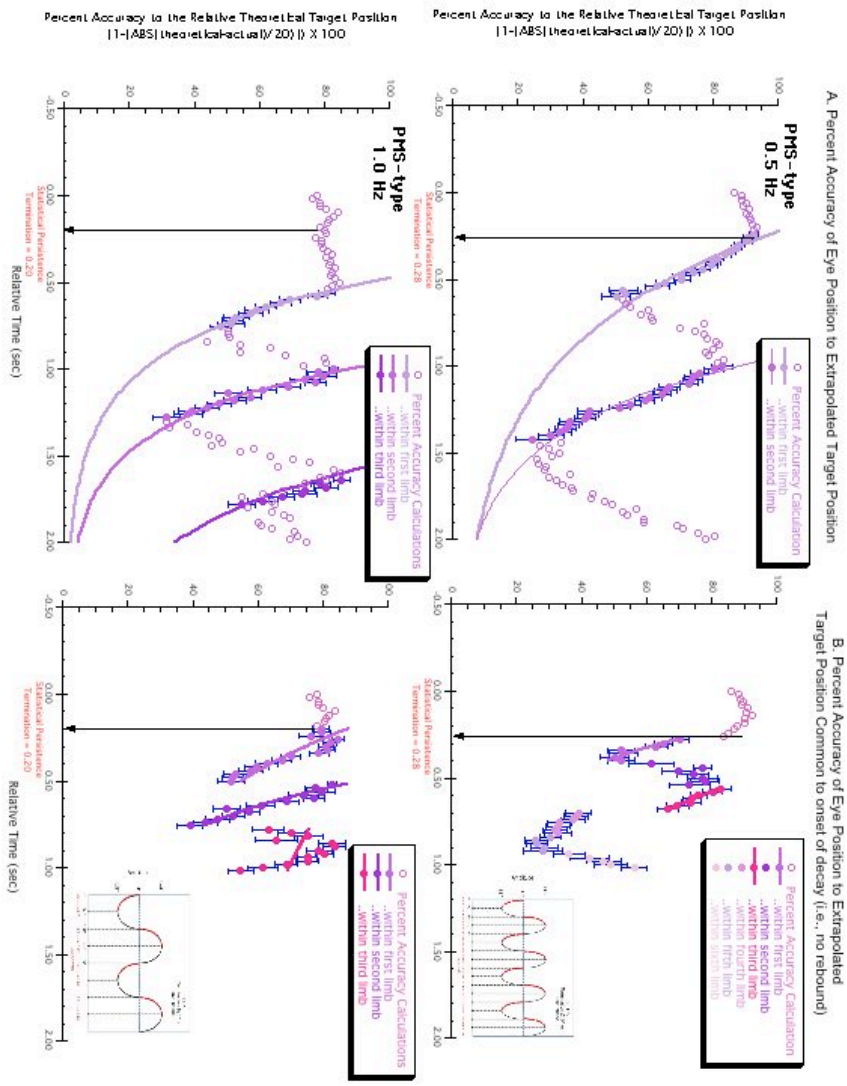

Figure 15. Cross averages with collapse of menstrual cycle for the PMS-type group at 0.5 Hz (top row) and 1.0 Hz (bottom row). Details as in Figure 14.

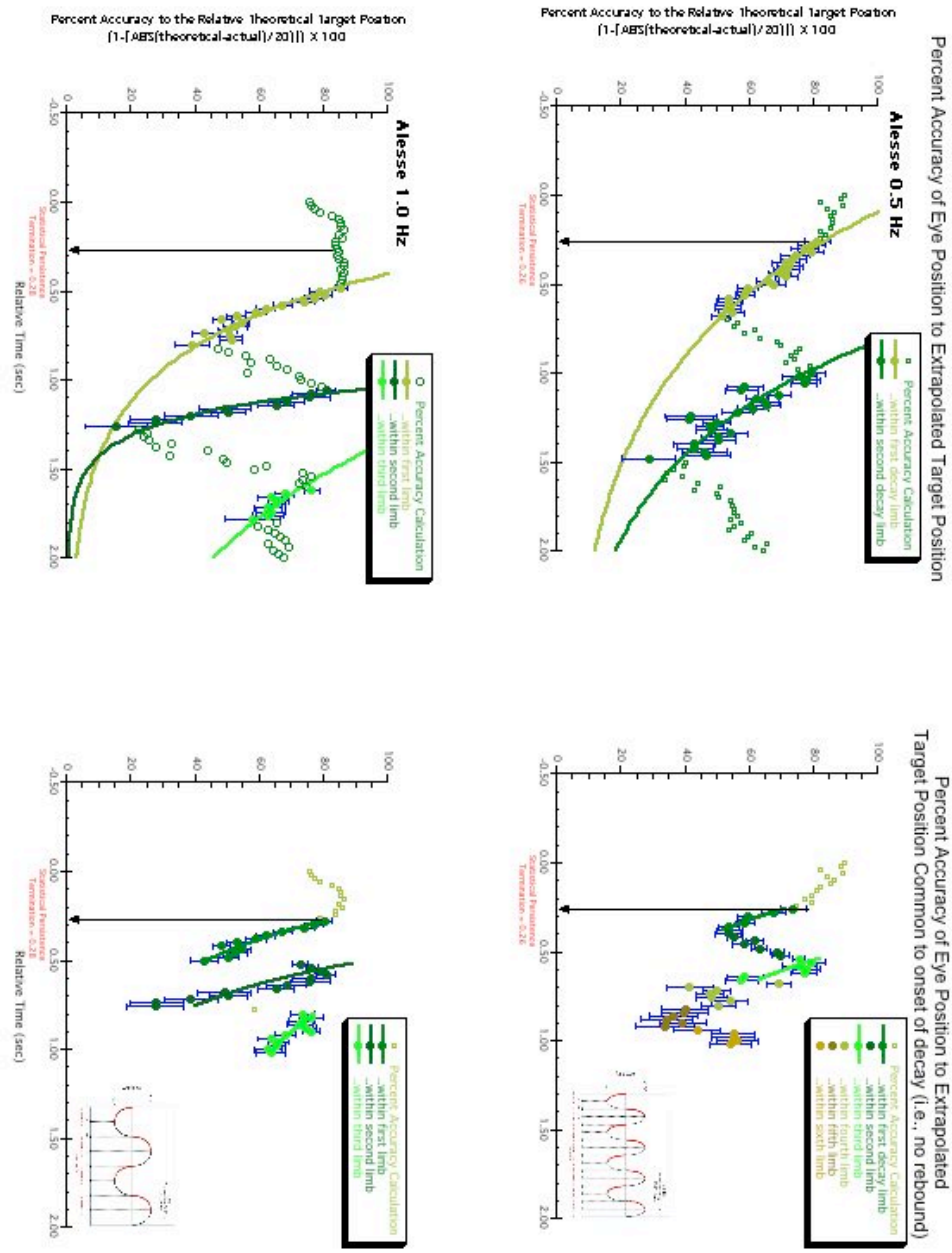

Figure 16. Cross averages with collapse of menstrual cycle for the Alesse™ user group at 0.5 (top) and 1.0 Hz (bottom). Details as in Figure 14.

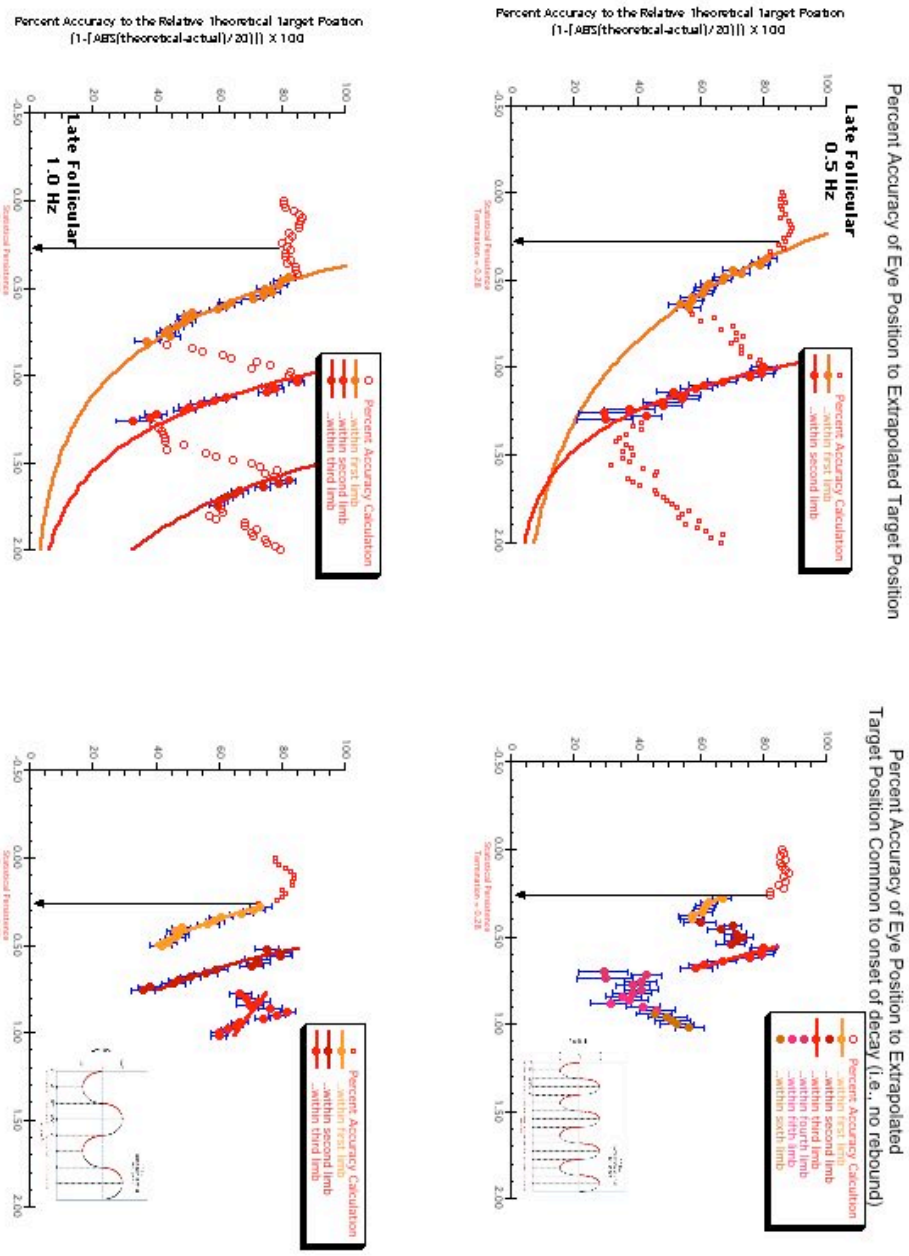

Figure 17. Cross averages with collapse of Group, with the Late Follicular menstrual phase data shown for 0.5 Hz target (top row) and 1.0 Hz target (bottom row). Details as in Figure 14.

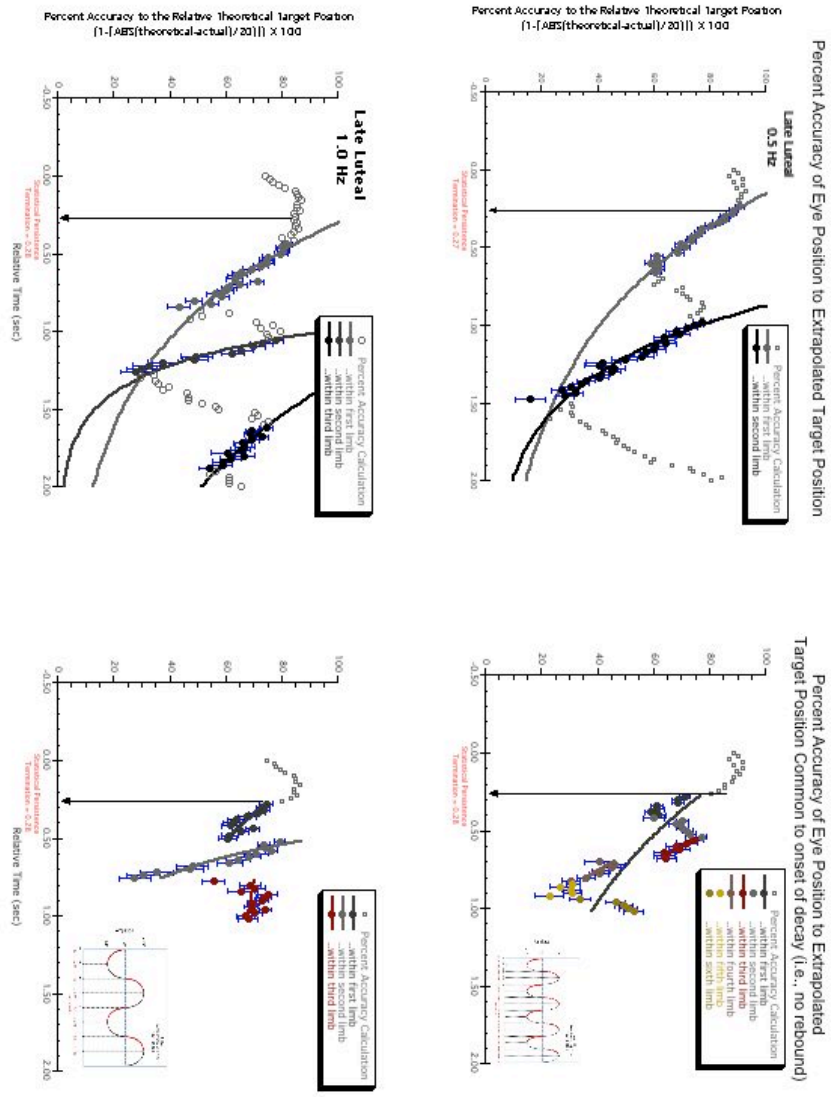

Figure 18. Cross averages with collapse of Group, with the Late Luteal menstrual phase data shown for 0.5 Hz target (top row) and 1.0 Hz target (bottom row). Details as in Figure 14.

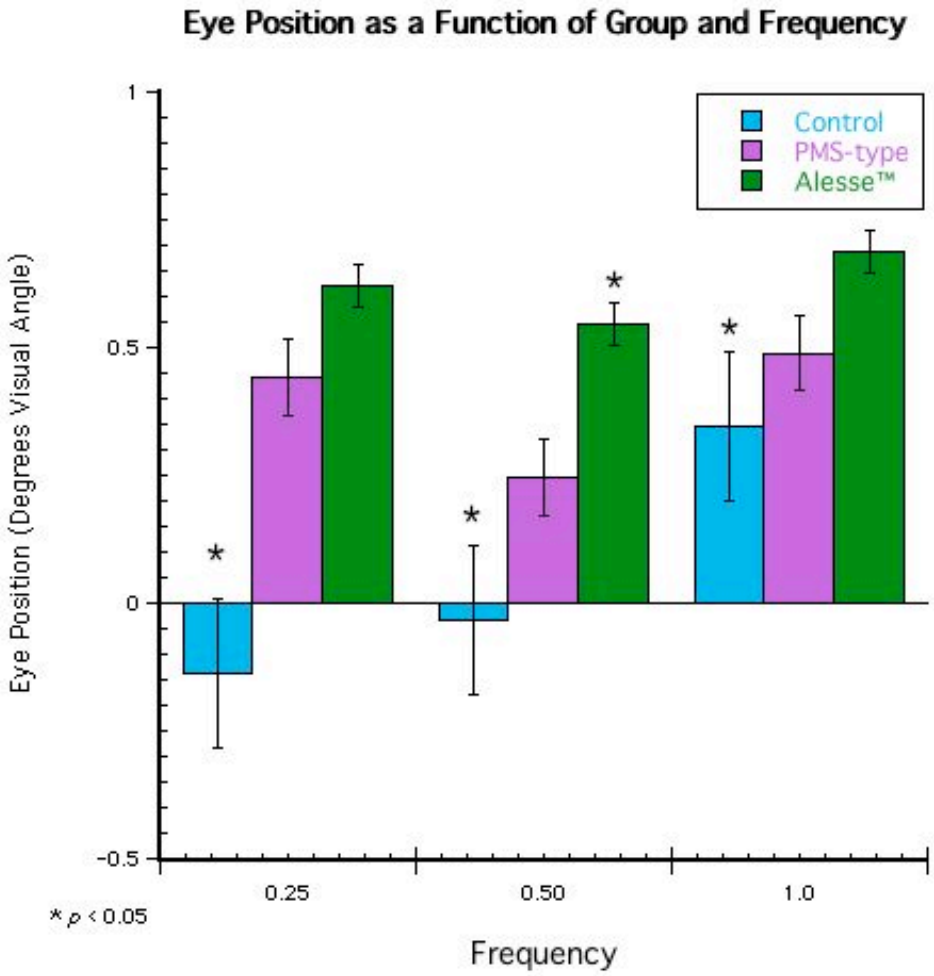

Figure 19. Mean average eye position on the eye tracker test collapsed across menstrual cycle phase with respect to real target measured in degrees visual angle as a function of frequency for the control (blue), PMS-type (purple), and Alesse™ users (green) groups *(± 1 SEM)*. Note the group difference between the controls and Alesse™ users with Alesse™ users showing greater real-target excursions as well as the group by frequency interaction between the Alesse™ users and controls at 0.5 Hz. Unfortunately, there were no menstrual cycle phase main effects (see text for more details).

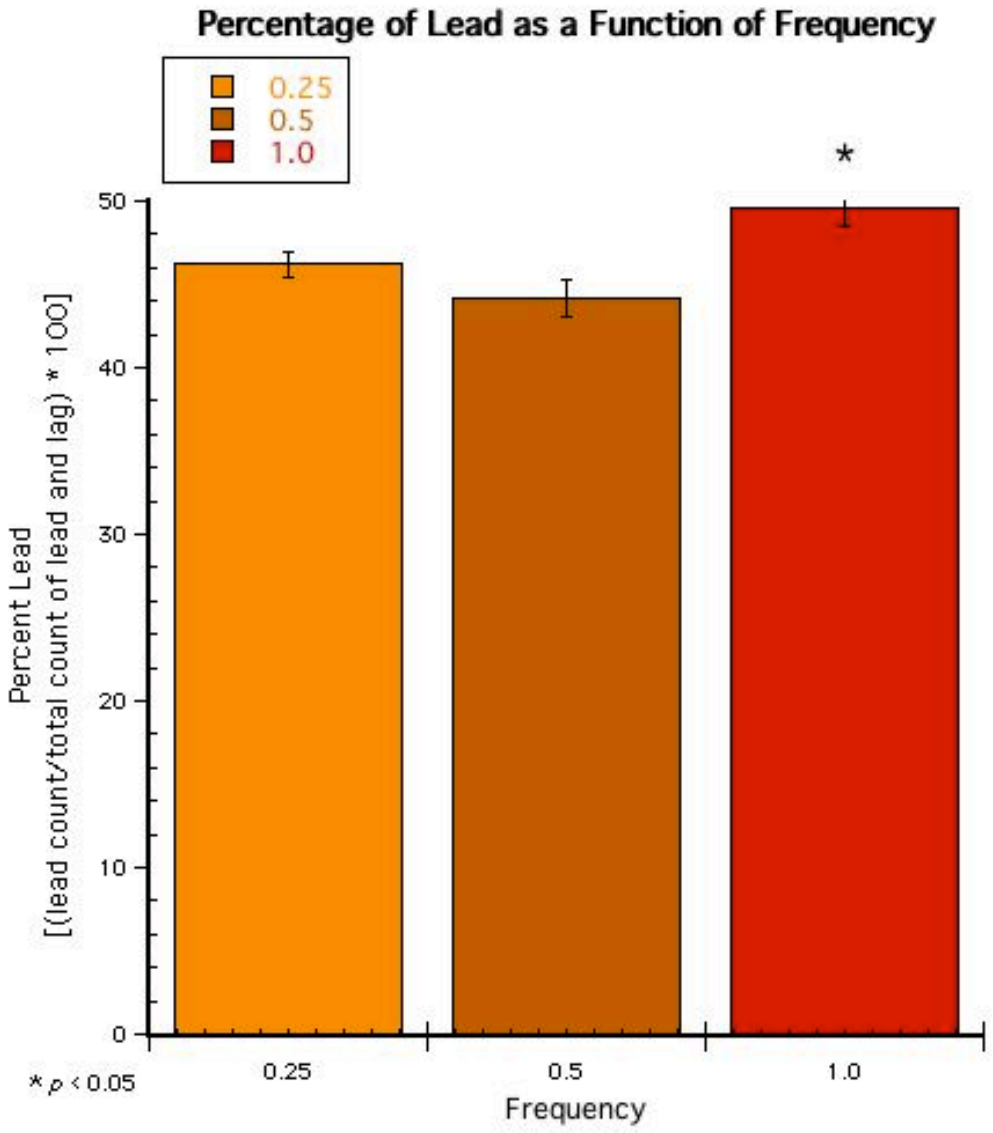

Figure 20. Percent lead averaged across group and menstrual phase based on lead count/total count of lead and lag • 100 as a function of frequency *(± 1 SEM)*. Note the main effect of frequency where the 1.0 Hz condition revealed significantly less lead than the 0.5 or 1.0 Hz frequencies.

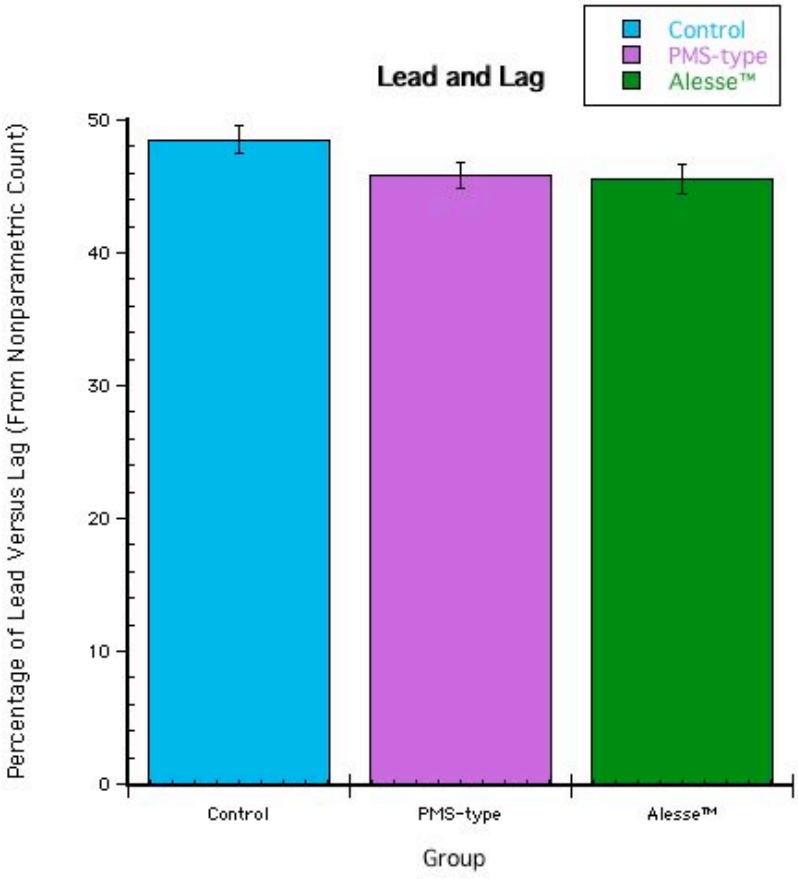

Figure 21. Percent of lead versus lag averaged across menstrual phase based on lead count/total count of lead and lag • 100 as a function of group *(± 1 SEM)*. Note there was a trend for a main effect where the control group lagged the target less than the PMS-type and Alesse™ users.

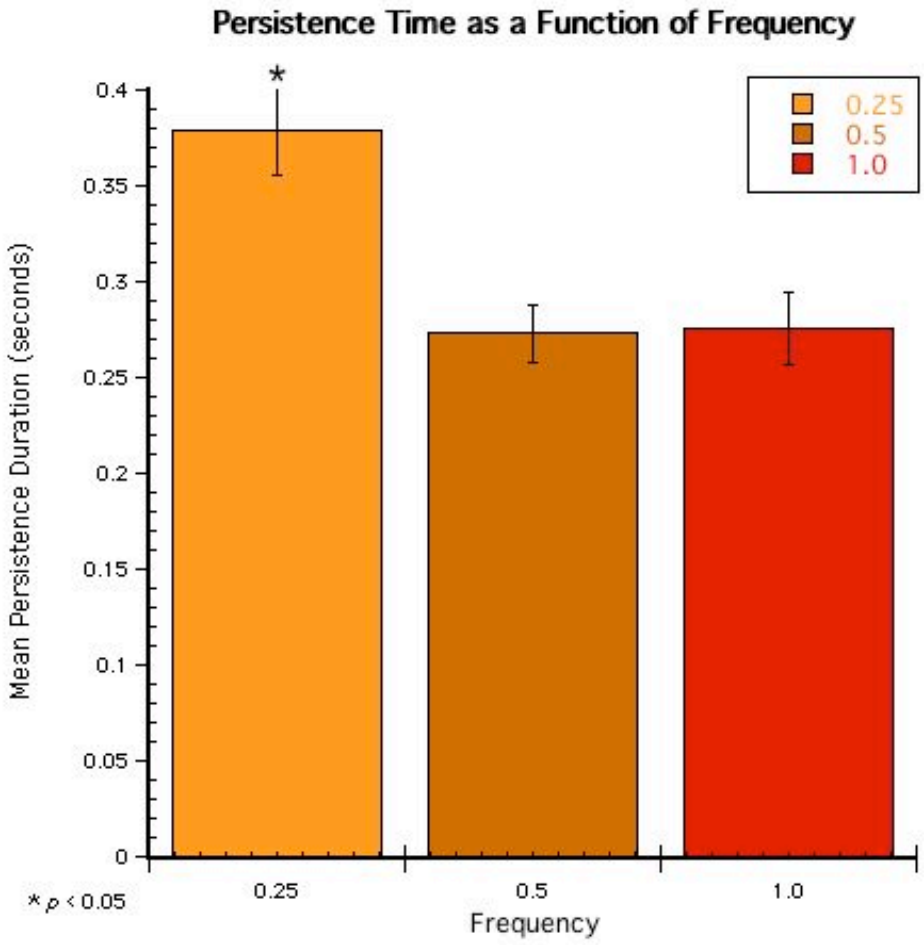

Figure 22. Mean persistence duration (in seconds) after target termination as a function of target frequency (in Hz) collapsed across group *(± 1 SEM)*. Note the main effect of frequency where the 0.25 Hz condition produced significantly longer persistence than the 0.5 or 1.0 Hz oscillating frequencies.

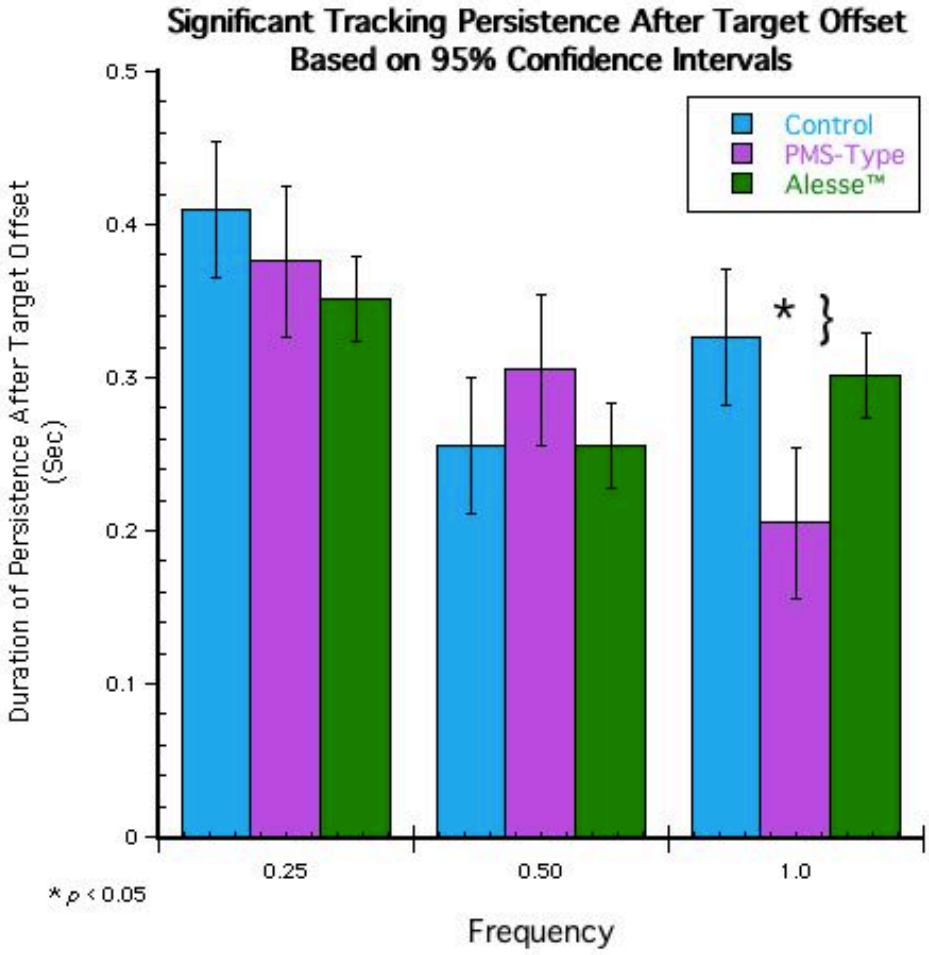

Figure 23. Mean persistence time measured in seconds with Persistence Duration after Target Offset plotted as a function of Frequency *(± 1 SEM)*. Duration was defined by 95% confidence intervals within real target tracking position (see text for details). Control (blue), PMS-type (purple), and Alesse™ (green) represent the mean persistence durations for the groups. Note the differences between frequencies as well as the interaction effect between the control and PMS-type groups at 1.0 Hz. Unfortunately there were no menstrual cycle phase main effects.