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Visuo-perceptual Task Performance Across the Menstrual Cycle in Women With and Without Premenstrual Symptoms: Potential Influences of Estradiol and Estradiol Sensitivity on Retinogeniculostriate, Extrastriate, and Elementary Retinal-Based Smooth Pursuit Pathways

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Abstract

The present study examined whether women with premenstrual symptoms (PMS) exhibit a different pattern of performance on psychophysical tasks across the menstrual cycle in comparison to control women. Research has shown that a related and more extreme presentation of this phenomenon, Premenstrual Dysphoric Disorder (PMDD) may be experienced as the result of a heightened sensitivity to normal phasic changes in sex steroid concentrations. In addition, research suggests that performance on certain measures of visuo-perceptual ability is associated with changing levels of estradiol. Thus, women who are more sensitive to changing hormonal levels (e.g., women with PMS) may exhibit different performance on such tasks when compared to controls; and as a function of the menstrual cycle. Control women (N = 18) and women with PMS symptoms (N = 16) performed a series of four psychophysical tasks during laboratory sessions at both the late-follicular (LF) and late-luteal (LL) phases of the menstrual cycle. Women provided salivary estradiol samples and participated in: two chromatic contrast sensitivity (CCS) measurements, a test of short-wavelength automated perimetry (SWAP), a texture discrimination task (TDT), and an eye tracking procedure. Results indicated that control women experienced a significant shift in salivary estradiol concentrations between menstrual cycle phases, while women with PMS symptoms did not. A significant overall effect of phase was observed for the CCS procedure. With respect to the SWAP procedure, higher levels of estradiol in the LL phase were predictive of higher S-cone sensitivity in the LF phase. As well, an inverse relationship was found between SWAP performance and changes in estradiol levels between the LF and LL phases. Significant phase-order effects were observed with respect to texture learning;

with the most robust improvements noted in women who trained in the LL phase and tested in the LF phase. No significant effects were noted for eye tracking. For all four tasks, trends were found involving group or phase effects or interactions. Findings are discussed with reference to relevant neurological mechanisms, and the potential influence exerted on these systems by changing levels of reproductive hormones across the menstrual cycle in women with and without PMS symptoms.

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Decades of research have provided support for the observation that women are more likely than men to experience episodes of mood-related disturbance following adolescence. The epidemiological evidence suggests that after adolescence women exhibit a greater lifetime risk for major depressive disorders (MDD) (Weissman, Leaf, & Holzer, 1984), the depressive subtype of bipolar illness (Arnold, 2003), and cyclical forms of affective disruption such as rapid cycling (Schneck, Miklowitz, Calabrese, Allen, Thomas et al., 2004) and seasonal affective disorder or SAD (Rosenthal, Sack, Gillin, Lewy, Godwin, Davenport et al., 1984). In addition to sex differences in these disorders, many mood episodes experienced by women are often associated with known changes in ovarian steroid levels and the reproductive axis (e.g., mood changes during the premenstrual, post-partum, and menopausal periods) (Noble, 2005).

In recent years, the elucidation of effects exerted by gonadal steroids, outside of simple reproductive function, has begun to influence our conceptualization of such things as learning, memory, and perception (e.g., Spencer, Waters, Romeo, Wood, Milner & McEwan, 2008). Research has demonstrated that gonadal hormone receptors are located in diffuse regions throughout the mammalian brain, thereby implicating candidates such as estradiol and progesterone in a variety of nonreproductive cognitive operations never before considered (McEwan, 2001; Spencer et al., 2008). In addition to functional modulation, ovarian hormones are thought to exert their effects on mood either directly or indirectly through interactions with neurotransmitter, neuroendocrine, and/or circadian systems (e.g., McEwan, 2001; Parry &

Newton, 2001). Given the cyclic fluctuations of ovarian steroids throughout the different phases of the reproductive cycle, the discovery of hormonal receptors in the brain offers unique opportunities to investigate the neurological and circadian functioning of both normal and potentially dysrhythmic endocrine systems throughout the CNS.

Mood disturbance associated with the premenstrual portion of the menstrual cycle has been referred to historically as premenstrual syndrome (PMS). Clinically significant versions of such mood disturbances have been defined more rigorously as late luteal phase dysphoric disorder (LLPDD) in the DSM-III-R; and as premenstrual dysphoric disorder (PMDD) in the DSM-IV (American Psychiatric Association 1994).

PMS is a reproductive phenomenon in women that is, in general, poorly understood. It is a syndrome characterized by somatic and mood complaints in the late luteal phase of the menstrual cycle. In women, premenstrual symptoms are conceptualized along a continuum. While some women experience few symptoms, up to 8% have been found to experience them to an extent that is debilitating. This more severe form of the presentation is commonly referred to as PMDD (Halbreich, Backstrom, Eriksson, O'Brien, Calil, Ceskova et al., 2007). Although symptoms of PMS are endorsed by a large number of women, and have been estimated to parallel a level of impairment comparable to that of the major dysphoric disorders (Halbreich, Borenstein, Pearlstein, & Kahn, 2003), the World Health Organization (WHO) failed to include either PMDD or PMS in its comprehensive report on disease control priorities related to mental, neurological, developmental, and substance abuse disorders (World Health Organization, 2006). In addition, it remains as an entry in the appendices (i.e., Appendix B) of the DSM-IV-TR (APA, 2000) among several other conditions that require further research validation.

There isn't any strong evidence to suggest that women with PMDD exhibit disparate concentrations of ovarian hormones (Schmidt, Nieman, Danaceau, Adams, & Rubinow, 1998). Several studies have demonstrated that women with PMS or PMDD do not possess dissimilar levels of estradiol or progesterone compared to women without such symptoms (Payne, 2003). Unlike non-PMDD women, however, women with PMDD do seem to respond favorably to bright light and wake therapy, and to Selective Serotonin Reuptake Inhibitor (SSRI) pharmacotherapy, implicating PMDD as a mood-related disorder influenced by the endocrine system (Lam, Carter, Misri, Kuan, Yatham, & Zis, 1999; Terman & Terman, 2005). The apparent lack of steroid concentration differences between PMDD and non-PMDD women, and the efficacious nature of light and wake therapy suggests that the symptoms of PMDD are caused by either (1) deficits in biorhythmic functioning (i.e. circadian behaviours or phenomena, such as sleep, that are regulated by internal pacemakers or neuroendocrine systems), or (2) increased sensitivity to hormone levels or hormonal change. Over the course of the past two decades, research findings with respect to chronobiological factors such as sleep and melatonin rhythms in women with PMDD have been inconsistent (for a review see Moline, Broch, Zak, & Gross, 2003), indicating that much work remains in understanding the influence reproductive hormones exert on circadian systems, the spectrum of phenotypic expression resulting from this relationship, and the neurological mechanisms that underlie it.

Based upon observations that women with PMDD have been found to exhibit differences in sleep parameters, including melatonin rhythms (Parry, Berga, Mostofi, Klauber, & Resnick, 1997); and colour perception (Eisner, Burke, & Toomy, 2004), the purpose of the present study was to determine whether women with symptoms of PMS can be differentiated from healthy control women on performance measures obtained at two distinct points in the menstrual cycle.

To assess such periodic functionality, we used noninvasive psychophysical measures designed to assess visual functionality that target specific parallel processing streams making up the visual pathway: the parvocellular (PC), and more notably the koniocellular (KC) visual stream. Preliminary evidence has suggested that the KC stream may respond differentially to changes in levels of estradiol (Eisner, et al., 2004a; 2004b). The KC stream is involved with colour perception with specific responsive properties to the blue-yellow opponency channel (e.g., Calkins, 2001). In addition to examining direct, bottom-up chromatic processing streams, we examined visual learning phenomena. Across animal studies, research has demonstrated that estrogen enhances synaptic plasticity, and improves performance on hippocampal-dependent cognitive behaviours (see review in Spencer et al., 2008). To determine whether similar estradiol-based modulations exist in cortical vision pathways, we investigated visual learning in women with and without PMS symptoms based on the performance measures of a texture discrimination task (Karni & Sagi, 1993; Stickgold, Whidbee, Schirmer, Patel, & Hobson, 2000). These measures have been shown to demonstrate rapid learning in initial sessions, resistance to decay (e.g., 100% retention after 5 weeks) (Karni & Sagi, 1991), and support the localization of texture learning to long-term experience dependent changes in V1 (plasticity). In other words, performance improvement may be solely based on V1 modifications analogous to those that have been observed in comparative hippocampus studies. For the present study, the hippocampus was used as a model for estradiol-based plasticity in V1 operations. Finally, the potential effects of estradiol-induced alteration of GABAergic systems on motor control and inhibition were assessed with the use of an eye tracking procedure. Pursuit-related neural activity is observed in the rostral regions of the superior colliculus (rSC), which contain cells that

respond to both smooth pursuit eye movements and project via the cerebellum to extra-ocular muscles in the brainstem (Krauzlis, Basso, & Wurtz, 2000).

Overall, it is our intent to better understand the potential effects of ovarian steroid disruptions and periodicity in women with and without PMS symptoms by assessing performance across a series of psychophysical tasks designed to probe sensory systems implicated in rhythmic, reproductive steroid functioning. Given the large proportion of women reporting symptoms of PMS, and the fact that self-report constitutes the basis for diagnosis, the establishment of physiological or perceptual parameters along which those with PMS or PMDD differ could serve to provide a more concrete marker for its detection, thereby contributing to the validity of the category. In addition to the potential to establish a marker for symptoms of hormonally mediated somatic and affective dysregulation, this study furthers our understanding of how hormonal-based neural modulation may impose functional changes and plasticity not only in hippocampus, but also in visual sensory and perceptual systems along the entire neuro-visual hierarchy.

Premenstrual Syndrome (PMS) and Premenstrual Dysphoric Disorder (PMDD)

Premenstrual syndrome (PMS) is prevalent among women of reproductive age. Dysphoric symptoms are among the most prevalent and bothersome premenstrual symptoms and are often the reason for treatment seeking (Halbreich et al., 2003). The hallmark feature of PMS is the predictable cyclic nature of symptoms that begin in the late luteal phase of the menstrual cycle and remits shortly after the onset of menstruation (Steiner, Pearlstein, Cohen, Endicott, Kornstein, Roberts et al., 2006).

The constellation of symptoms experienced premenstrually by many women occurs along a continuum of severity. The symptoms associated with PMS may be somatic, emotional, or

behavioral in nature, and may or may not impair daily functioning. On the far end of the spectrum is PMDD, which is differentiated from PMS by its increased severity of symptoms, predominance of mood disturbances, and significant functional impairment. Past epidemiological studies on the prevalence of PMDD have been difficult to conduct given the loosely defined criteria for the disorder, as well as the fact that many women endorse meeting, or having met at least one of the criteria (e.g., Wittchen, Becker, Lieb, & Krause, 2002). The currently accepted definition for the disorder, as defined by the American Psychiatric Association (1994), stipulates that women with PMDD must experience marked disruptions in their relationships, work, or social activities at levels similar to those with major depression. Differentiating PMDD from major depression is the cyclic nature, or timing, of the symptoms, which, for PMDD, must occur premenstrually and abate after the termination of the menstrual period. As previously described, symptoms of PMDD are representative of three distinct classes and can manifest in the form of physical, behavioural, or emotional complaints. The DSM IV-TR (APA, 1994) stipulates that over the course of a year, five (or more) of the following symptoms must be entrained in more menstrual cycles than not: (1) markedly depressed mood, feelings of hopelessness, or self-deprecating thoughts; (2) marked anxiety, tension, feelings of being "keyed up," or "on edge"; (3) marked 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 in concentrating; (7) lethargy, easy fatigability, (8) or marked lack of energy; marked change in appetite, overeating, or specific food cravings; (9) hypersomnia or insomnia; (10) a subjective sense of being overwhelmed or out of control; and finally, (11) other physical symptoms, such as breast tenderness or swelling, headaches, joint or muscle pain, a sensation of

"bloating," and/or weight gain. These criteria, particularly the requirement of five symptoms, each with a severity that disrupts functioning, has resulted in a more restrictive diagnosis of PMDD over the past decade (Freeman, 2003). Despite the fact that the strict application of symptom criteria has served to strengthen the reliability, and increase the homogeneity of groups involved in studies examining PMDD, controversy still exists with respect to the arbitrary nature of the number of selection criteria required for the diagnosis (Freeman, 2003). As well, there continues to be disagreement surrounding the categories of symptoms that should be included in the diagnosis. For example, the under-representation of physiological symptoms in the consideration of a diagnosis of PMDD may prohibit a thorough conceptualization of the true pathophysiology of the disorder (Freeman, 2003). Freeman also notes that the World Health Organization classified premenstrual tension within a category designated for gynecological disorders, rather than psychological disorders, in its International Classification of Diseases (ICD) 10^{th} edition. Classified in this context, the cyclical nature of physiological symptoms such as migraine headaches are emphasized in order to establish the nature of a somatic syndrome named “premenstrual tension”. This emphasis on physiological complaints may be an attempt by the authors of the ICD to more firmly establish the validity of a category that to date has been dependent on self-reports of vaguely defined mood-related symptoms. This classification system lacks other specific criteria as well, such as symptom severity, degree of symptom change in the cycle, or relationship with other disorders, and therefore makes it difficult to differentiate the distinct clinically significant diagnosis of premenstrual related distress from other physiological symptoms expressed by psychological disorders such as anxiety, depression and SAD. Additionally, the APA definition of PMDD does not provide a means of quantifying the magnitude of the distress of the symptoms, nor the level of impairment in female functioning. A

factor analytic study supported the existence of a two-factor model of PMDD, with dysphoric-somatic complaints represented as a primary factor, and functional/behavioural impairment constituting a second order factor (e.g., Wang, Teng, Vieira-Filho, Gorenstein, & Andrade, 2007). These findings suggest that both symptoms and impairment level should be investigated in studies of PMS and/or PMDD.

In an effort to examine the symptoms, duration, subjective distress, social impairment, patterns of comorbidity and personality features associated with PMS, Angst, Sellaro, Stolar, Merikangas, and Endicott (2001) conducted a prospective longitudinal study of a representative community cohort of women who were interviewed five times between the ages of 21 and 35 years. The results of their study suggest that irritability, nervousness, and tension constitute the core elements of PMS. More importantly, they state that, despite the findings of other researchers describing high levels of comorbidity with depression and anxiety, there are also a substantial proportion of women who manifest the core features of PMS without depressed mood. This data suggests that premenstrual symptoms are expressed as a unique constellation of symptoms that can occur with or without depressed mood.

Epidemiology of PMDD

Although few comprehensive epidemiological studies are available, the research of Wittchun et al. (2002) suggests that the 12-month prevalence of DSM-IV PMDD is 5.8%. Application of the diagnostic exclusion rules with regard to concurrent major depression and dysthymia decreased this rate only slightly to 5.3%. The authors classified an additional 18.6% of these participants as “near-threshold” cases, predominately because they failed to meet the mandatory impairment criteria. Lifetime incidence was cited as 7.4%, and PMDD was found to be stable across 48 months, with a 10% rate of remission among baseline PMDD cases. Of note,

and potentially problematic in conducting studies with pure samples was the fact that 12-month and lifetime co-morbidity rates with depression, anxiety, and somatoform disorders were high, with incidence rates of 47.4%, 22.9%, and 28.4% respectively. Only 26.5% of women diagnosed with PMDD did not exhibit characteristics of another mental disorder. These findings suggest that studies comparing women with and without PMDD need to either use pure PMDD samples, or include control groups of women with similar rates of depression, anxiety, and other somatoform disorders.

Estrogen and Mood

Major Depressive Disorder (MDD) is the most commonly diagnosed psychiatric disorder among adults, with U.S. lifetime prevalence rates of 20 to 25% for women and 9 to 12% for men; point prevalence rates are approximately 6% and 3% for women and men respectively (APA, 2000). Thus, in terms of MDD, there appears to be a sex bias in that rates of MDD in women are approximately twice those of men.

Although the reason for this gender difference is most likely due to a combination of biological, social, and environmental factors, it has been suggested that estrogen may play a key role in this phenomenon (Steiner, Dunn, & Born, 2003). For example, women are known to experience depressive episodes at times of hormonal change in their reproductive years. Affective disorders such as premenstrual syndrome (PMS), postnatal depression, and post-menopausal depression all occur at times of low serum levels of estrogen (see review by Pearlstein, Rosen, & Stone, 1997). Furthermore, many women experience negative physiological and mood-related side effects when taking hormonal contraceptives, which also lead to dramatically reduced levels of endogenous gonadal hormone levels (Rapkin & Sonalkar, 2011). Such side effects include headaches, fatigue, negative mood change, weight gain, irregular

bleeding, and loss of sexual interest or pleasure (Oinonen, 2009). Many symptoms of PMS are similar to oral contraceptive side effects and are also associated with a number of aversive physical and mood complaints. Consistently reported in epidemiological studies are the symptoms of irritability, tension, depression, bloating, mastalgia, and headache. Dysphoric symptoms are among the most prevalent and bothersome premenstrual symptoms and are often the reason for treatment seeking (Freeman, 2003). Currently, it is repeatedly cited that 3 to 9% of women report having dysphoric PMS that is severe enough to seek and warrant treatment (e.g., Halbreich et al., 2003). Thus, reductions in endogenous gonadal hormone levels, especially estrogen, are associated with mood-related complaints, and PMS is one example of this.

The effects of estrogen on mood are most notable in studies examining the treatment of women in the peri- and post-menopausal periods. Several studies have demonstrated the efficacy of estrogen administration for depression during these periods (see review by Zwifel & O'Brien, 1997). As well, a 2002 review examining estrogen as a treatment for depression concluded that it is superior to placebo in treating reproductive –related mood disorders including those in the peri and post-menopausal periods (Grigoriadis & Sidney, 2002). These authors cite difficulties in the interpretation of the literature, however, due to several different methodological considerations such as the combining of women of different ages together into groups, failure to confirm life stage, the use of different types of estrogens, the inclusion of women with a range of mood disturbances, and the enrollment of women with concurrent psychiatric illness.

Despite the rigorous methodological considerations required to investigate the effects of estrogen on mood, Soares, Almeida, Joffe, and Cohen (2001) investigated the efficacy of 17-β estradiol for the treatment of depressive disorders in endocrinologically-confirmed perimenopausal women. Of 50 women enrolled in the study, 26 met DSM-IV criteria for major

depressive disorder, 11 for dysthymic disorder, and 13 for minor depressive disorder. Through random assignment, women were introduced to either a treatment condition consisting of transdermal patches of 17-β estradiol (100 μg) or a placebo condition, which employed physiologically inert patches. After 12 weeks, remission of depression was observed in 68% women treated with 17-β estradiol, compared with 20% in the placebo group ($P = .001$).

Similarly, Schmidt, Nieman, Danaceau, Tobin, Roca and Murphy (2000) examined the efficacy of estrogen in the treatment of depression in perimenopausal women with and without hot flashes. Women with perimenopause-related depression were randomized in a double blind parallel design to receive either 17β-estradiol or placebo administered via an active or inert patch changed every three days for three weeks. Subsequently, women receiving estradiol during the first three weeks continued receiving estradiol for an additional three weeks, whereas women who had received placebo crossed over to estradiol for three weeks. This extra three weeks of treatment was included to permit the comparison of effects of estradiol on mood at three and six weeks as well as to determine the proportion of women who would respond to both placebo and estradiol. Outcome measures included standardized mood rating scales and a visual analog scale self-report instrument. Of 34 female participants, 16 received estradiol first and 18 received placebo first. After three weeks of estradiol treatment, standardized mood rating scale scores and visual analog scale symptom scores (e.g., sadness, anhedonia, and social isolation) were significantly decreased compared with baseline scores and were significantly lower than scores in women receiving placebo, who showed no significant improvement. Neither the presence of hot flushes nor the duration of treatment (three weeks or six weeks) influenced outcome. A full or partial therapeutic response was seen in 80% of participants receiving estradiol and 22% of those receiving placebo. The results of the above studies indicate that estradiol administration

may aid in the amelioration of depressive symptoms, and provide evidence for the role of ovarian steroids in the regulation of mood and affect.

Results similar to those observed with estradiol administration in women with perimenopausal depression have also been documented in women with post partum depressive depression (PPD). Although additional safety and efficacy data are required prior to estradiol being considered as a first-line treatment for PPD, a preliminary review suggests that few side effects and minimal passage to infants through breast milk makes it a promising candidate for treatment (Moses-Kolko, Berga, Kalro, Sit & Wisner, 2009). Very few clinical trials have been published in this area, however, in one such study Ahokas, Kaukoranta, Wahlbeck, and Aito (2001) administered sublingual estradiol to 23 women fulfilling ICD-10 criteria for major depression with postpartum onset for eight weeks. Serum estradiol concentrations were measured at baseline and weekly for the duration of the study. The treatment effect was assessed using the Montgomery-Asberg Depression Rating Scale (MADRS), a clinician-rated depression symptom scale. Baseline assessment indicated that all patients were severely depressed at the inception of the study and had low serum estradiol concentrations. Remarkably, in 69% of patients, estradiol concentrations were lower than the threshold value for gonadal failure. Results indicated that during the first week of estradiol treatment, depressive symptoms diminished significantly, and serum estradiol concentrations approached those of the follicular phase. At the end of the second week of treatment, the MADRS scores were compatible with clinical recovery in 83% of patients.

While very few studies have documented the effect of estradiol administration as a treatment for PMDD, Rapkin, McDonald, and Winer (2007) reported that a combined oral contraceptive pill containing 20 μg of ethinyl estradiol and 3 mg of the progestin drospirenone in

a novel dose regimen (24 active pills followed by 4 placebo pills) demonstrated efficacy for the treatment of symptoms of premenstrual dysphoric disorder. Similarly, Smith, Studd, Zamblera, and Holland (1995) reported significant symptom improvement in a group of women with severe PMS symptoms who were administered twice weekly doses of either 100μg or 200μg of estradiol transdermally over an eight-month period.

The results of these studies indicate that already low or declining levels of estradiol may contribute to mood disturbances in women who are sensitive to changes in hormonal concentrations, and that supplementation or administration of exogenous estradiol can aid in the amelioration of these negative mood side effects. These data also provide evidence for the supposition that that there is a subgroup of women who appear to be vulnerable to hormonally triggered negative mood changes. This effect may be exacerbated in women with pre-existing mood conditions such as depression or bipolar disorder, indicating that women who are vulnerable to one type of hormonally triggered mood disturbance may also be vulnerable to other types (Payne, 2003).

Overview of the Menstrual Cycle

The adult human menstrual cycle can be divided into follicular and luteal phases, with ovulation occurring between the two. Neuroendocrine control of this recurring reproductive event requires the pulsatile secretion of gonadotropin releasing hormone released into the pituitary portal system to stimulate the synthesis and secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the gonadotropes of the anterior pituitary (Hall, 2004). The follicular phase begins on day one of the cycle, the first day of menstrual bleeding. At this time, the growth of ovarian follicles is stimulated by a rise in follicle stimulating hormone (FSH) released by the anterior pituitary gland. Generally, only one follicle of several will

become dominant and continue to grow. As it grows, the dominant follicle produces increasing amounts of estradiol, thereby initiating the breakdown of smaller competing follicles. Coinciding with the peak in serum estradiol concentrations is the maturation of the growing follicle, and consequently the release of (LH) from the anterior pituitary. The release of LH from the adenohypophysis stimulates the release of the immature ovum, or secondary oocyte from the follicle. The rupturing of the follicle and release of the secondary oocyte constitutes ovulation, which occurs approximately 13 to14 days into the cycle, marking the end of the follicular phase. Ovulation lasts between 16 and 32 hours and is terminated upon the release of the egg. In the final steps of ovulation, the secondary oocyte matures into an ovum, at which point it is swept into the fallopian tube where it is absorbed by the body if left unfertilized. The luteal phase begins following ovulation, at which point the ruptured follicle closes and forms a body known as the corpus luteum, which produces increasing amounts of progesterone. It is the increase in progesterone produced by the corpus luteum that causes the uterine lining to develop for ovum maintenance in case of successful fertilization. It also causes body temperature to increase slightly during the luteal phase and remain elevated until the onset of menses. This increase in temperature can be used to estimate whether ovulation has occurred. If fertilization does not occur, the corpus luteum degenerates, thereby leading to a cessation of secreted progesterone and the sloughing off of the blood-enriched uterine lining. It is the fall in progesterone levels that trigger the re-release of FSH and the beginning of a new menstrual cycle (Hampson & Young, 2008). Figure 1 illustrates an overview of hormonal fluctuations across the menstrual cycle.

To study specific shifts in hormone concentrations, studies have divided the menstrual cycle into finer time periods, in order to more thoroughly examine discrete events such as ovulation. For example, both the follicular and luteal phases can be subdivided into early, mid,

and late periods. As well, some authors define the period around ovulation as the periovulatory phase (e.g. Oinonen & Mazmanian, 2007). The early portion of the follicular phase is marked by menses, typically lasting from one to five days (Hall, 2004), while the mid-follicular phase has been defined as days 6 to10 days where day 1 is the first day of menses (Parry et al., 1997; Parry, Meliska, Martinez, Lopez, Sorenson, Hauger et al., 2008; Parry, Mostofi, LeVeau, Cover-Nahum, Golshan, Laughlin, & Gillin, 1999). It is during the late follicular phase (LF; days 11 to 13) that levels of estradiol begin to rise as the dominant follicle reaches maturity. Both the ovulatory period as well as the phase of the cycle immediately following ovulation in which levels of estradiol and LH are falling warrant investigation in women with premenstrual symptoms as they are associated with changing hormone levels. If women with PMS and/or PMDD are indeed more sensitive to normal fluctuations of ovarian steroids, differential performance may be observed between women with and without such symptoms during the LF and LL phases on tasks that are putatively influenced by estradiol. In contrast to the follicular phase, the luteal phase is marked by decreases in estradiol, LH, and FSH, and increases in levels of progesterone. Research has shown that the length of the luteal phase is relatively fixed between 13 and 15 days (Hampson & Young, 2008). Thus, much of the variation in cycle length between women is attributable to differences in the length of the follicular phase (Hampson & Young, 2008). Based on the mid-cycle luteinizing hormone surge and assuming a 14-day luteal phase, the mid luteal phase corresponds to approximately days five or six following the LH surge (Baker, Waner, Viera, Taylor, Driver, & Mitchell, 2001) and is characterized by elevated levels of progesterone, and low levels of LH and FSH. At this point, levels of estradiol are intermittent between levels seen at ovulation (high) and levels seen at menses (low). The late luteal (LL) phase has been defined as 12 ± 2 days following the LH surge (Parry et al., 1997). It is at this

juncture that both levels of estradiol and progesterone begin to fall and women are most likely to experience symptoms of PMDD characterized by mood-related and somatic disturbance (Rubinow & Schmidt, 2006; Schmidt et al., 1998).

Origins of PMDD

The absence of objective indicators to substantiate the presence of PMDD speaks to the likelihood of an interaction of several different factors in its origin and development. Despite the absence of such markers, theories abound as to the cause and maintenance of dysphoric and somatic complaints associated with the syndrome. Abnormal levels of estrogen and progesterone have been suggested as causal factors in PMDD, however, it has been shown that women who exhibit symptoms of PMDD do not have abnormally high or low concentrations of circulating estradiol or progesterone (e.g., Schmidt et al., 1998). This has led researchers such as Schmidt and colleagues (1998) to propose that normal plasma concentrations of gonadal steroids can trigger abnormal behavioural responses (e.g., change in mood state or somatic complaints) in women who may be susceptible. Evidence supporting this hypothesis was obtained from an elegant experiment in which the symptoms of PMDD were eliminated via ovarian suppression with the administration of leuprolide, a gonadotropin releasing hormone (GnRH) agonist (Rubinow & Schmidt, 2006). GnRH agonists bind to gonadotropin receptors in the pituitary gland. When GnRH agonists are administered early in the menstrual cycle, an initial stimulatory "flare" effect causes a release of gonadotropins from the pituitary gland for 1 to 2 weeks. As compared to endogenous GnRH, exogenous agonists such as leuprolide occupy gonadotropin receptors for a longer period of time, and through negative feedback, reduce secretion of endogenous gonadotropin which in turn decreases the amount of released FSH, consequently

preventing ovarian stimulation. Thus, the inhibition of follicular stimulation at later stages of leuprolide treatment prevents the estradiol surge brought about by the mature ovum.

In a double-blind, placebo crossover design, women with PMDD were treated with leuprolide, which led to a significant reduction in self-reported premenstrual symptoms. In a subsequent phase of the study, both control women and women with PMDD were administered both estrogen and progesterone, or placebo following leuprolide administration. With respect to mood, women with PMDD had significant increases in symptoms during treatment with leuprolide plus hormone replacement, as compared to those administered leuprolide treatment alone. Interestingly, normal women remained asymptomatic following administration of estrogen and progesterone. Specifically, the symptoms that increased in women with PMDD included sadness, anxiety, impaired functioning, and irritability. Despite the fact that all women in the study were screened for normal pituitary-gonadal functioning, non-PMDD women did not experience changes in mood as a result of either the leuprolide or hormone replacement treatments. The results of this study provide evidence for the hypothesis that in contrast to women without PMDD, women with PMDD exhibit aberrant physiological and psychological reactions to normal concentrations of circulating ovarian steroids.

It has been suggested that the pathophysiology of severe PMS and PMDD may be closely linked to an overactive hypothalamic-pituitary-gonadal (HPG) axis (Steiner et al., 2006). The menstrual cyclicity of the ovarian hormones is most likely the trigger for the psychological as well as the somatic premenstrual symptoms. As previously described, however, there seems to be no demonstrable hormonal imbalance in women with severe PMS or PMDD. Rather, it is assumed that normal ovarian function triggers biochemical events both in the central and peripheral nervous systems, which in turn precipitate premenstrual symptoms in vulnerable or

predisposed women (Steiner et al., 2006). In a recent study using the estrogen challenge test, Eriksson, Backstrom, Stridsberg, Hammarlund-Udenaes, and Naessen (2006) tested the hypothesis that brain responsiveness to normal hormonal fluctuations is increased in women with premenstrual dysphoria. The estrogen challenge test consists of the administration of intramuscular injections of estradiol, and the subsequent monitoring of the rates of change of concentrations of hormones contingent upon the presence of the injected estrogen. The aim of the Eriksson et al. (2006) study was to test whether the sensitivity of the brain to a standardized gonadal steroid challenge differed between women with and without severe premenstrual mood symptoms. The authors hypothesized that the brains of women with PMDD (n=13) would be more sensitive to the estradiol challenge than those of non-PMDD controls (n=12), as determined by faster and stronger feedback effects involving the release of LH and FSH. They also hypothesized that the strength of the feedback response would correlate with the severity of self-reported mood symptoms during menstrual cycles before the challenge. Estradiol (estradiol benzoate 0.04 mg/kg), was administered as an intramuscular gluteal injection in the morning on day three or four of the menstrual cycle for all participants, and blood samples were collected at eleven intervals ranging from 0 to 144 hrs post injection. For all samples, levels of estradiol, FSH and LH were measured. Results indicated a significantly different LH response to estrogen in women with PMDD as compared to controls. Specifically, compared to controls, women with PMDD demonstrated a stronger negative feedback response to the point of nadir LH levels, higher LH levels at the nadir, more LH surge-like reactions, and showed LH concentrations over time that were 50% higher than the controls. With respect to self-report measures taken before the experimental estradiol challenge, the authors further discovered that an association between changes in LH and luteal-phase-self-reported irritability differed significantly between women

with and without PMDD. Specifically, the mean visual analog score for premenstrual irritability (days 1-10 before menstrual onset) in the pre-experimental cycle positively correlated with the area under the curve for LH in the negative feedback phase, and with the area under the curve for LH during the negative feedback plateau. The LH response during negative feedback also correlated with the self-rated perception of the physiological symptom of bloating in a pre-challenge menstrual cycle. The FSH response in women with PMDD was not statistically different from that of the asymptomatic controls. In accordance with the work of Schmidt et al. (1998) the authors of the above study postulate that women with PMDD may exhibit a variable response to normal concentrations of gonadal steroids, which implies different neuroendocrine feedback regulation in women with PMDD from that in asymptomatic, non-PMDD women.

Additional studies have implicated concentrations of luteal phase estradiol and LH in the severity of premenstrual symptoms. Seippel and Backstrom (1998) found a relationship between high serum concentrations of estradiol during the luteal phase, and the severity of premenstrual symptoms defined by five negative mood symptoms (depression, anxiety, tension, fatigue, and irritability) and three somatic signs (breast tenderness, swelling/water retention, headache), as well as the severity of menstrual bleeding. A similar relationship was seen between high LH levels and symptom severity (14 ± 0.7 IU/L across 10 premenstrual days). This implies that estradiol might exert effects indirectly by influencing the LH response that in turn increases subjective symptoms of PMDD.

Elucidating the mechanisms responsible for symptoms of PMS and PMDD has the potential to offer much in the way of understanding affective dysregulation in both menstrual-related disorders and in other conditions as well. This will not be accomplished, however, by subscribing to simple cause and effect, or hormone deficiency models (Rubninow & Schmidt,

2006). As suggested by previously described studies, a complex interplay between the normal fluctuation of ovarian steroid levels, and a sensitivity at the physiological/molecular level in predisposed individuals likely accounts for the observed differences between those who experience symptoms of PMDD and those who do not. The understanding of PMDD requires a model that accounts for the timing of symptoms, their emergence over the course of the lifespan, as well as their minimal expression in the overall population of women (Rubinow & Schmidt, 2006). A more in depth understanding of the role played by estradiol, including its influence on brain structure and function, as well as its interactions with other neurotransmitter and endocrine systems can only further serve to enhance our knowledge of reproductive disorders in women. Complex targets for estradiol may include the serotonergic system, as well as candidate neural signaling systems such as particular elements of the cyclic adenosine monophospate (cAMP) response element binding protein (CREB) system and Protein Kinase C (PKC) pathways. These are some examples of mechanisms through which estradiol is capable of exerting its effects indirectly (Payne, 2003). Given the proliferative quality of the above systems, and their abilities to influence cellular activity at the nuclear level, a more in depth examination of estradiol in the nervous system is warranted.

Where Does Estrogen Exert its Effects in the Brain?

Estrogens have most commonly been examined in the context of reproductive behaviour. For example, early animal studies focused on estrogenic actions on the hypothalamus affecting ovulation and reproductive behavior (Boiling & Blandeau, 1939). Since this time, estrogen receptors have been located throughout the brain and have been implicated in such complex functions as learning and behaviour, emotion and affect, motor coordination, pain sensitivity and

neuroprotective function (see review by McEwan & Alves, 1999). Recent work has also detected the presence of estradiol receptors in the retina (Gupta, Johar, Nagpal, & Vasvada, 2005)

Indeed, the effects of estrogens on the brain are diffuse and widespread, influencing several neurotransmitter systems including serotonin or 5-hydroxytryptamine (5-HT), norepinephrine (NE), dopamine (DA), acetylcholine (ACh), γ-amino butyric acid (GABA), and glutamate (GLU) (Rubinow & Schmidt, 2006). In addition, the hippocampus, a structure important for declarative and episodic memory, as well as spatial learning, is responsive to estrogens (McEwen, 2002). Cerebral blood vessels and glial cells have also been shown to respond to the actions of estrogens (see review by McEwan & Alves, 1999).

With respect to learning and memory, Heikkinen, Puolivali, Liu, Rissanen, and Tanila (2002) examined the effects of long-term estrogen treatment on learning in both radial arm (RAM) and T-maze tasks. In addition, they correlated choline-acetyl-transferase (ChAT) and hippocampal monoamine levels at the end of the study to task performance. Both sham-operated (gonadally intact) and ovariectomized (OVX) female mice (n=95) were treated with estradiol for either 7 or 40 days prior to behavioral testing (maze performance). Heikkinen et al. (2002) reported that ovariectomies impaired and estrogen administration improved performance in the RAM task in both sham operated and OVX mice. Seven-day estrogen treatment also improved the acquisition of the T-maze task, however, only in OVX mice. The improvement induced by the estrogen treatment was even more pronounced when the treatment for these mice was extended to 40 days. In addition, the authors found decreased concentrations of several neurotransmitters, associated enzymes, and enzyme-metabolites in the OVX mice including: hippocampal noradrenalin (NA), ChAT, 5-hydroxyindoleacetic acid (5-HIAA), and dihydroxyphenylacetic acid (DOPAC). Furthermore, the effects of estrogen treatment on

hippocampal levels of 5-HT neurotransmitter and its metabolite 5-HIAA were different in sham-operated mice compared to OVX mice. Among hippocampal neurotransmitters, the serotonergic system was most affected by the estrogen treatment. The concentrations of hippocampal 5-HT and 5-HIAA appeared to depend on the duration of the estrogen treatment and ovarian status. Although the 7-day estrogen treatment led to an increased 5-HIAA/5-HT ratio in sham-operated mice, the 5-HIAA/5-HT ratio was significantly increased after the 40-day treatment. In contrast, OVX mice in the 7-day estrogen treatment showed notable, though nonsignificant, decreases in their hippocampal 5-HIAA/5-HT ratios after 7 and 40 days. Thus, changing levels of estradiol or sensitivity to changes in estradiol may lead to alterations in serotonergic functioning. This relationship between estrogen and serotonin, although found in mice, may contribute to the affective symptoms associated with PMS in women.

Although several studies have implicated estrogen in the regulation of the serotonergic system (Bethea, Gundlah, & Mirkes, 2000; Bethea, Pecins-Thompson, Schutzer, Gundlah, & Lu, 1998; Huttner & Shepherd, 2003; Joffe & Cohen, 1998; Rubinow, Schmidt, & Roca, 1998), the mechanisms behind this action are poorly understood and speculative at best. Many studies to date have focused extensively on animal models (Amin, Canli, & Epperson, 2005) and current research findings are in need of integration and translation to human populations (Amin et al., 2005). At the biochemical level, estrogen has been found to increase the production of tryptophan hydroxylase, the key enzyme responsible for 5-HT synthesis, and to suppress the expression of the serotonin transporter gene (5-HTT) in macaque raphé nuclei (see review by McEwan & Alves, 1999). Additionally, estrogen and progesterone treatment has been found to alter the expression of several genes responsible for serotonergic transmission within the dorsal raphé nucleus of the rat including the expression of the postsynaptic 5-HT_{2A} receptor (Cavus &

Duman, 2003), the presynaptic 5-HT reuptake transporter (SERT) gene (McQueen, Wilson, & Fink, 1998), and the vesicular monoamine transporter. These findings suggest that estradiol acts as a modulator, capable of enhancing serotonergic transmission at the cellular level within the dorsal raphé nucleus either through enzymatic biosynthesis of 5-HT, or through nuclear functional genomics of receptor and transporter systems.

Serotonin is known to exert widespread effects on mood, and thus it is possible that cycling ovarian hormones in women may affect mood and behaviour through the regulation of a multiplicity of pre- and post-synaptic serotonergic expressions (Moses, Drevets, Smith, Mathis, Kalro, Butters, et al., 2000). In a recent study examining the effect of the absence of estrogen β-isoform receptors (ERβ) in mice, Imwalle, Gustafsson, and Rissman (2005) found that female knockout (ERβKO) mice exhibited enhanced anxiety as measured by level of exploration in the elevated plus maze as well as decreased concentrations of 5-HT and DA in several brain regions. ERβKO females exhibited reduced serotonin content in the bed nucleus of stria terminalis, preoptic area (POA), and hippocampus. The authors hypothesized that ERβ is required during development to modulate the effects of estrogen on anxiety and catecholamine concentrations in female mouse brains.

In keeping with the findings of Imwalle et al. (2005), additional studies have found that treatment with estradiol alone or ER receptor agonists increase the availability of 5-HT metabolites and/or 5-HT precursors, providing additional evidence for the augmentation of serotonergic systems by estrogens. Lubbers, Zafian, Gautreaux, Gordon, Alves, Correa et al. (2010) found that the administration of 17-β estradiol to OVX rats increased levels of the 5-HT metabolite 5-HIAA by 38% in the striatum, ventral hippocampus, and ventral tegmenatal area. Availability of the metabolite may be indicative of increased metabolism of 5-HT to 5-HIAA as

the result of enhanced uptake of 5-HT to cells via the serotonin transporter (SERT) or increased activity of monoamine oxidase (MAO). With respect to the influence of ERβ agonists on 5-HT precursors, Donner and Handa (2009) found that subcutaneous injections of the selective ERβ agonist diarylpropionitrile (DPN) to OVX rats (*N*= 8) daily for a period of eight days, elevated the expression of tryptophan hydroxyase-2 (*tph2*) mRNA in the caudal and mid-dorsal dorsal raphe nucleus. Behaviourally, this effect manifested in decreased anxiety in open field tests and elevated plus maze performance. Similar results were obtained by Hiroi, McDevitt, and Neumaier (2006) who noted increased levels of *tph2* mRNA in the mid-ventromedial and caudal subregions of the dorsal and medial raphé nuclei of OVX rats treated with 17-β estradiol for a two week period.

The results of these studies may have implications for women who experience premenstrual symptoms. The above studies indicate that estradiol exerts an influence on the serotonergic system which may affect the release, metabolism, reuptake, and/or synthesis of 5-HT. Thus, a predisposition to negative mood and affective symptoms in women with PMS symptoms could be the result: of 1) a potentially blunted response to the estradiol-induced release and regulation of 5-HT at points in the menstrual cycle when estradiol is high, such as in the periovulatory period, or 2) A heightened sensitivity of the serotonergic system to decreasing levels of estradiol from the periovulatory phase to the LL phase of the menstrual cycle resulting in more rapid decreases in or metabolism of 5-HT.

As reviewed above, the administration of estrogens has been found to exert antidepressant effects in times of affective dysregulation (Ahokas et al., 2001; Soares et al., 2001), and estrogen treatment has been found to influence the response to SSRI treatment in postmenopausal women with depression (Lee, Yang, Ko, & Joe, 2008). These findings are not

surprising given that many areas of the brain that have been implicated in mood disturbances such as depression, including the prefrontal cortex (PFC), amygdala, hippocampus, striatum, and thalamus (Drevets, 2000; Drevets, 2003; Posener, Wang, Price, Gado, Province, Miller, et al., 2003). These regions have also been found to contain estrogen receptors indicating that their functionality and the neurological processes governed by them may be modulated by the presence of estradiol, and these functions may be differentially affected in men and women (see review by McEwan & Alves, 1999). For example, in a study using positron emission tomography to examine cortical activity in women performing the Wisconsin Card Sorting Test (WCST), Berman, Schmidt, Rubinow, Danaceau, Van Horn, Esposito et al. (1997) demonstrated that regional cerebral blood flow (rCBF) was reduced in the PFC of women who had been administered the GnRH-agonist leuprolide, (which results in ovarian suppression). When either progesterone or estrogen was added to the leuprolide regimen, a reversal to typical rCBF activation patterns observed during WCST performance was observed (e.g. higher activation in the PFC). These findings suggest that ovarian steroids modulate cognition-related gross neural activity in humans, and consequently may be implicated in information processing deficits at higher cortical levels. This is of particular interest because processing deficits have been demonstrated in individuals with mood-related disturbances such as major depressive and bipolar disorders (Clark, Chamberlain, & Sahakian, 2009). Given that estradiol exerts its effects at so many areas of the brain, and has been linked with a multiplicity of effects within the serotonergic system, it is likely that it also contributes to mood regulation. Many women are susceptible to changes in mood and affect during the LL phase of the menstrual cycle as well as at other times of altered estrogen levels across the lifespan. It is therefore possible that both estrogen and serotonin play a role in the development and/or maintenance of PMS symptoms.

Evidence for the interaction of Estrogen and Serotonin in PMDD

The observation that women with PMDD respond favorably to SSRI treatment and less well to other categories of antidepressants (Rubinow, Schmidt, & Roca, 1998) suggests that 5-HT may play a greater role than other neurotransmitters in the pathophysiology of the illness. In addition to these behavioral observations, a review by Noble (2005) reports that several biological markers believed to reflect brain serotonergic functioning have been found to differ in women with PMDD compared to healthy controls. These include platelet monoamine oxidase activity; density of 5-HT transporters in platelets; serotonin-mediated release of prolactin; and the ratios between the DA metabolite, homovanillic acid; and the 5-HT metabolite 5-hydroxyindoleacetic acid in cerebrospinal fluid. By binding to intracellular receptors, estrogen mediates a broad range of cellular effects, including the transcription of genes that encode enzymes that regulate numerous pathways involved in the synthesis and metabolism of neurotransmitters, neuropeptides and their receptors, neurotransmitter transporters, nerve growth factors, and signal transduction proteins (Amin et al., 2005; McEwan & Alves, 1999; Rubinow et al., 1998). Although estrogen has not been demonstrated to interact with all 5-HT receptors, manipulation of estrogen levels has been shown to affect 5-HT_{2A} (Sumner & Fink, 1995) and 5-HT_1 (Biegon & McEwen, 1982) receptors.

Much of the research examining the effects of serotonergic activity in response to manipulations of estrogen to date has been conducted in animals, but the behavioral findings in these comparative studies demonstrate the need for further human study. An example of this can be seen in a study by Krezel, Dupont, Krust, Chambon, and Chapman (2001), who showed a relationship between estrogen, serotonin receptor function, changes in electrophysiology, and affective behavioral response, when comparing $ER\alpha$ knockouts, $ER\beta$ knockouts, and wild-type

mice. In females, there was increased anxiety in ER_{β} mutants according to the open field test and elevated plus maze, whereas the rotarod test of motor function showed no differences. This agrees with the Imwalle et al's (2005) study described earlier, which showed greater maze-running anxiety with concomitant decreases in 5-HT and DA brain concentrations in female knockout ($ER_{\beta}KO$) mice. Furthermore, with electrophysiological stimulation of the amygdala and hippocampus, Krezel et al. (2001) showed a reduced threshold for induction of synaptic plasticity in the basolateral amygdala in these knockouts. In addition, there was a local increase in 5-HT_{1A} receptor expression in the medial amygdala but not in the basolateral amygdala or ventral posterolateral thalamus, structures adjacent to and in close proximity to the amygdala respectively. These findings suggest that ER_{β} dysfunction results in changes in emotional behavior (i.e., anxiety), increased amygdala sensitivity, and increased 5-HT_{1A} receptor expression in the amygdala; mechanisms that may be implicated in the phenotypic expression of severe premenstrual symptoms.

As described previously, Steiner et al. (2006) reported that antidepressant treatment with SSRIs works as an effective first line treatment of PMDD. The authors state that: "SSRIs can be administered continuously throughout the entire month, intermittently from ovulation to the onset of menstruation, or semi-intermittently with dosage increases during the late luteal phase" (Steiner et al. 2006, p. 57). These authors further state that SSRIs have been found to ameliorate both emotional and physical symptoms of PMDD including irritability, depressed mood, dysphoria, as well as bloating, breast tenderness, and appetite changes. These results have been confirmed in several studies and reiterated in a review by Freeman (2004), who states that sertraline, fluoxetine and paroxetine (as an extended-release formulation) are approved by the Unites States Food and Drug Administration for use continuously or in the luteal phase alone.

Among these studies was a double blind, placebo controlled study conducted by Freeman, Rickels, Sondheimer, Polanski, and Xiao (2004). They found that two sertraline-treated groups improved significantly more than a placebo group as assessed by total premenstrual Daily Symptom Rating Form scores over three treatment months. The Daily Symptom Rating Form factors that showed significant improvement were reflective of mood and physical symptoms. A history of major depression was not associated with treatment response. In addition, a greater number of sertraline-treated women reported improved functioning in the domains of family relationships, social activities, and sexual activity. The observation of symptom improvement in women with PMDD when treated with SSRIs further supports serotonin's role in the maintenance and/or development of PMDD.

Additional Roles of Estradiol in the Nervous System

Recently, it has been shown that steroid receptors, including estradiol receptors, exist in the human eye. These receptors have been found in several locations including the cornea, lens, iris and ciliary body, retina, lacrimal gland, meibomian gland, and conjunctiva (Gupta, Johar, Nagpal, & Vasavada, 2005). In addition, estradiol receptors have been located in the suprachiasmatic nucleus (SCN) (Kruijver & Swaab, 2002), an area associated with vegetative visual processing and circadian rhythms. Aromatase, the enzyme responsible for catalyzing estradiol synthesis, also has been mapped in regions of basal forebrain, cerebral cortex, hippocampus, thalamus, cerebellum and brainstem (Azcoltia, Yague, & Garcia-Segura, 2011). These observations suggest that the actions of sex steroid hormones may modulate functioning at the sensory/perceptual level, and that, in addition, hormonally mediated events such as age, menopause, menarche, pregnancy, and menstrual cycles may affect visual processes and the circadian rhythms that are entrained by them.

In 2001, using immunohistochemical techniques and reverse transcription-polymerase chain reaction (RT-PCR), Munaut, Lambert, Noel, Frankenne, Deprez, Foidart et al. (2001), discovered estrogen receptor (ER) mRNA in macular and extramacular regions of the retina as well as in the choroids of male and female eyes. Additionally, ER protein was localized in the ganglion cell layer and in the choroid. At the transcriptional level, mRNA for both ER_{α} and ER_{β} receptor subtypes was present. Local differences in the expression level were observed, however, suggesting the possibility of variation in the ratio between the two different subtypes. The presence of two ER subtypes in the human ocular posterior segment raises questions about their potential physiological role and the potential role of estrogen in regulating and/or entraining various visual systems and their associated pathways.

Recent hypotheses regarding mood disturbance are concerned with deficits in neuroplasticity and neuronal/glial survival in the CNS (Rubinow & Schmidt, 2006) and this fact may have bearing on long-term estradiol influences. For example, it has been shown that cell survival proteins (cytoprotective proteins) like B-cell lymphoma 2 (Bcl-2) and brain derived neurotrophic factor (BDNF) are altered under conditions of stress and depression (e.g., Charney & Manji, 2004; Manji, Drevets, & Charney, 2001). This alteration may take place through a variety of mechanisms, including the facilitation of glutamatergic transmission via NMDA and non-NMDA receptors, and through the reduction of the cell's energy capacity via the disruption of mitochondrial calcium uptake. One of the major mechanisms by which BDNF promotes cell survival is by increasing the expression of Bcl-2. Bcl-2 attenuates cell death via a variety of mechanisms, including impairing the release of calcium and cytochrome c, sequestering proforms of death-inducing caspase enzymes, and enhancing mitochondrial calcium uptake. The chronic administration of a variety of antidepressants increases the expression of BDNF, and its

receptor. For example, lithium and VPA are known to robustly upregulate Bcl-2 (Charney & Manji, 2004). Of importance to our study is the fact that the long-term administration of estradiol also increases BDNF activity (Cavus & Dumon, 2003), indicating that estradiol may act on pathways similar to that of the traditional antidepressants. This suggests that women who are more sensitive to changing concentrations of ovarian steroids may be vulnerable to deficits in neuroprotective mechanisms, and consequently alterations in mood symptoms.

Melatonin, the Circadian and Menstrual Cycles, and Mood

Melatonin is a ubiquitous substance found in several plant and animal species, including humans (Hardeland & Fuhrberg, 1996). Widely distributed in nature, its presence has been documented in unicellular organisms, plants, fungi, and animals. In many vertebrates including humans, melatonin is synthesized primarily in the pineal gland and is regulated by the environmental light/dark cycle via the SCN (e.g., Pandi-Perumal, Srinivasan, Maestroni, Cardinali, Poeggeler, & Hardeland, 2006). The synthesis and release of melatonin are stimulated by darkness and inhibited by light (Brezezinski, 1997). Similar to 5-HT, melatonin is derived from the amino acid precursor tryptophan. In the brain, tryptophan is converted from 5–hydroxytryptophan to 5-HT. Serotonin is then acetylated to form N-acetylserotonin by arylakylamine N-acetyltranferase (AA-NAT). Finally, N-acetylserotonin is converted into melatonin by hydroxyindole-*O*-methytransferase (Pandi-Perumal et al., 2006).

The production and secretion of melatonin are mediated largely by hypothalamic-paraventricular postganglionic retinal nerve fibers of the retinohypothalamic tract, which are responsible for the transmission of information regarding the daily pattern of light and darkness. More specifically, the light/dark cycle information is passed through the retinohypothalamic tract to the SCN, then to the superior cervical ganglion, and finally to the pineal gland (Brzezinski,

1997). Humans seem to require light of considerably greater intensity for melatonin suppression than do other mammals (Lewy, Wehr, Goodwin, Newsome, & Markey, 1980). As both the pineal gland and the retinohypothalamic tract are implicated in the production of melatonin, in the latter case via projection to the SCN, it is not surprising that melatonin has been associated with the regulation of sleep/wake cycles. These cycles are known to be disrupted in those with mood disorders, most specifically those with SAD. However, as described later in this review, melatonin levels and sleep architecture have also been found to differ across the menstrual cycle and in those who are hormonally sensitive.

The observation that women with PMDD have been found to exhibit biorhythmic disruptions similar to those individuals with SAD suggests that they may share a common, underlying, neurological mechanism that is influenced by ovarian steroids. Therefore, the examination of sleep-stage and circadian profiles in women with PMDD may offer clues as to underlying neurological mechanisms, and suggest possible targets for investigation at the perceptual level.

It is well documented that individuals suffering from mood disturbances experience alterations in their sleep patterns. In fact, disrupted sleep represents a core criterion for nearly all major mood disorders within the DSM IV-TR (APA, 1994). Impairment in sleep quality is pervasive in those diagnosed with depression (Hayashino, Yamazaki, Takegami, Nakayama, Sokejima, & Fukuhara, 2010; Kupfer, 2006; Peterson & Benca, 2008). Typically, patients suffer from difficulties falling asleep, frequent nocturnal awakenings, and early morning awakening (Riemann, Berger, & Voderholzer, 2001). Measurements of melatonin either in saliva or plasma, or of its main metabolite 6-sulfatoxymelaton in urine, have shown decreased amplitudes in some depressed patients during the acute phase of illness (Srinivasan, Smits, Spence, Lowe, Kayumov,

Pandi-Perumal, et al., 2006). As will be discussed below, this pattern has also been observed in women with PMDD.

As with the light/dark and seasonal cycle, the pineal gland has been found to play a role in the neuroendocrine control of reproductive physiology as evidenced in seasonally breeding animals. Although humans are not seasonal breeders, research has shown that seasonal fluctuations have been found in human reproduction patterns. In a review, Aleandri, Spina, and Morini, (1996) report that in northern countries, the conception rate is reported to be higher in summer than in winter, and as a consequence, the birth rate reaches a maximum in the spring season. This is in contrast to areas with warmer climates, in which peaks in conception rates have been found during winter months. The authors further report that in northern countries, where there are greater seasonal fluctuations in levels of light, the photoperiod is more likely to affect the activity of the human reproductive axis. This has been corroborated by observations of reduced activity of the anterior pituitary–ovarian axis and increases in serum melatonin during winter months (Rojanski, Brzezinski, & Schenker, 1992). In addition, Kivela, Kauppila, Ylostalo, Vakkuri, and Leppaluoto, (1988) report increased levels of darkness affecting plasma concentrations of melatonin and LH significantly in those living in northern countries in winter months; higher nocturnal plasma melatonin concentrations on day 10 of the menstrual cycle in winter than in summer; and higher nocturnal plasma LH levels in summer than in winter. All of these data are consistent with the above findings regarding conception and birth rates in northern countries. It has therefore been suggested that seasonal changes in daylight, through melatonin secretion, may affect female reproductive function. This involvement of melatonin was demonstrated by Voordouw, Euser, Verdonk, Alberda, de Jong, Drogendijk et al. (1992) in which the long-term daily administration of 300 mg of melatonin (n = 8) or combinations of 300

mg and 75 mg melatonin with 0.75 mg norethisterone (synthetic progestin), induced in the fourth month of treatment, a significant decrease in LH (with absence of the mid-cycle peak), estradiol, and progesterone plasma concentrations. Furthermore, a combination of 300 mg of melatonin with 0.15 mg of norethisterone or 75 mg of melatonin with 0.3 mg of norethisterone, administered for 21 days, was also reported to inhibit ovulation, suggesting an additive, or synergic effect of the two hormones.

Melatonin has also been reported to play a role in menstrual cyclicity (Aleandri et al., 1996) and menstrual-cycle related mood disturbances (Parry, et al., 1997). This suggests possible ovarian steroid modulation of CNS circuitry responsible for affect, and suggests that circadian profiles warrant further investigation in those who experience menstrual-related somatic and/or mood disturbance such as women with PMDD.

Response to Light Therapy in SAD

Rosenthal, et al. (1984) were among the first to document the phenomenon of SAD, an illness characterized by a unique set of sleep anomalies and vegetative-like depressive symptoms occurring annually at the same time each year. In their study, Rosenthal et al. (1984) described 29 patients who exhibited depressive symptoms conforming to a specific seasonal pattern. Although many of these patients had a bipolar affective disorder, potentially confounding the purity of the sample, their depressive episodes were generally characterized by hypersomnia, overeating, and carbohydrate cravings. The researchers noted that preliminary studies extending the photoperiod of these individuals with bright artificial light in the morning had an antidepressant effect in 11 of 29. This research led to the phase shift hypothesis proposed by Lewy, Sack, Singer, White, and Hoban (1988). According to this hypothesis, endogenous circadian rhythms in individuals with chronobiological mood disorders can be abnormally phase

advanced or phase delayed with respect to real time and real sleep (Lewy et al., 1988). If bright light is shown to individuals two hours before bedtime, it will delay their circadian phase, causing later sleep onset and later wake-up time. This delaying effect will persist until light exposure is given approximately five hours after usual bedtime. At this exposure time, the phase response will shift and the effect of bright light will no longer serve to delay the circadian phase, but rather advance it, initiating earlier wake up and sleep onset (Revell, Burgess, Gazda, Smith, Fogg, & Eastman, 2007). Thus, the insomnia associated with many mood episodes can be said to mimic the effect of a phase delay, and bright light therapy in the morning has been shown to be an effective treatment for many individuals with mood disorders, as it serves to advance their circadian phases, thereby preventing them from sleeping late into the morning and allowing them to fall asleep at an earlier time in the evening. An increase in the effect of phase advance has been shown if the bright light treatment is augmented with afternoon melatonin administration (Revell et al., 2007). Although several studies have documented the beneficial effects of time of day exposure as effective in the treatment of mood disturbance, others have found time of day exposure to be irrelevant. Lee, Blashko, Janzen, Paterson, and Chan (1997) propose that it is the duration of time to which patients are exposed to light, rather than the time of day that accounts for the observed effects of morning-evening light treatments. They advocate a photon-count hypothesis that stems from the observation that a shorter winter photoperiod deprives susceptible individuals of sufficient quanta of light to maintain euthymia. In accord with this hypothesis, Lee et al. (1997), report that increases in the duration of phototherapy equivalent to two daily sessions (e.g. morning and evening) lead to a longer total period of light exposure that may account for the antidepressant effects of light therapy in SAD.

Although employed as a treatment strategy less frequently than either cognitive or pharmaceutical techniques, sleep deprivation has been demonstrated to be effective in the amelioration of depressive symptoms. In a 2006 review, Peterson and Benca report that preventing sleep altogether during the night, can quickly reduce depressive symptoms within hours in 30 to 60% of patients reporting symptoms of a major depressive episode. Symptom improvement was defined as a 50% reduction of Hamilton Depression Rating Scale scores. The authors also state that similar levels of improvement have been observed using the technique of partial sleep deprivation, particularly during the latter part of the night.

The effects of sleep deprivation on symptom improvement have also been observed in women with PMDD. Without discriminating between sleep stages, Parry et al. (2008) found that both early and late wake therapies produced improvements in mood symptoms in women with PMDD. Each type of sleep therapy, however, produced different effects on melatonin timing parameters. In addition to measuring mood changes during LL phase interventions with early wake therapy and late wake therapy, Parry et al. (2008) measured plasma melatonin levels every 30 minutes between 18:00 and 09:00 h in 19 women with PMDD and 18 normal control women during the MF phase (days 6 to10 after the start of menses) and the LL phase (2 to 4 days prior to menses). During the treatment phase of early wake therapy, participants slept between 03:00 and 07:00 h, while during late wake therapy, participants slept between 21:00 and 01:00 h. With respect to menstrual cycle phase, results indicated that melatonin offset was delayed (equivalent to that which would be observed during a phase delay), and duration of secretion was longer in the LL phase compared to the MF phase in both control and PMDD subjects. As well, late wake therapy, but not early wake therapy advanced melatonin offset, and shortened its duration. Both treatments were found to improve mood symptoms. Melatonin offset was associated with more

depressed mood in PMDD patients. Also, longer melatonin duration in the MF phase predicted greater mood improvement following late wake therapy. Parry et al. (2008) hypothesize that in women predisposed to depression, melatonin-timing alterations might exacerbate luteal phase depressive symptoms. The fact that melatonin duration also increased significantly in control subjects without worsening mood suggests that delayed luteal phase melatonin offset does not invariably lead to symptoms of depression in these women, however, PMDD patients may be more vulnerable to depression due to greater increases in melatonin offset duration in the LL phase. These findings suggest that sleep deprivation can alter sleep/wake cycles and melatonin patterns, and cause changes in mood in women with PMDD. The links between the above variables suggest that sleep architecture and the sensory parameters that mediate them may differ in women with PMDD.

A longer duration of melatonin secretion also occurs in primarily female patients with SAD (Wehr, Duncan , Sher, Aeschbach, Schwartz, Turner, et al., 2001), postmenopausal depression (Tuunainen, Kripke, Elliott, Assmus, Rex, Klauber, et al., 2002) and other major depressive disorders (Blaicher, Speck, Imhof, Gruber, Schneeberger, Sator, et al., 2000), and there is high comorbidity in patients with PMDD and SAD (Praschak-Rieder, Willeit, Neumeister, Hilger, Stastny, Thierry et al., 2001; Portella, Haaga, & Rohan., 2006). As in animal models, depressed patients may be more sensitive to changes in melatonin duration, rather than to absolute levels.

Shared deficiencies between SAD and Premenstrual Symptoms

In a 2001 study, Praschak-Rieder, et al. compared the point prevalence rates of PMDD in a sample of 46 premenopausal female patients suffering from SAD to a group of 46 healthy female controls. All participants completed semi-structured clinical interviews based on DSM-IV

criteria to ensure diagnosis of PMDD and also completed the Seasonal Pattern Assessment Questionnaire (SPAQ) (Rosenthal et al., 1984) to assess the presence of SAD. To verify the diagnosis of PMDD, all patients were followed up in stable summer remission using daily self-rating scales for two full menstrual cycles. Results indicated that women with SAD met the diagnostic criteria for PMDD significantly more often than healthy controls (46% vs. 2%, respectively). These results indicate that those with SAD and PMDD may share a common underlying neurological mechanism.

Operating under the suspicion that a positive correlation between measures of seasonal and premenstrual symptoms could reflect an overlap in symptoms or a common depression history rather than the co-occurrence of two separate problems, Portella et al. (2006) conducted a study examining the comorbidity of SAD and PMDD symptoms in a general sample of 91 female college students. The correlation between seasonality as measured by the SPAQ and premenstrual symptoms as measured by the Menstrual Distress Questionnaire (MDQ) (Moos, 1968) was reduced when scores on a depressive symptom measure were statistically controlled, but remained positive and significant ($r = .21$; $p = 0.045$). This relationship persisted in a subsample of individuals with subsyndromal SAD on a screening measure ($r = .48$; $p = 0.007$). Although this correlation was reduced when depressive symptom severity was statistically controlled, it remained positive and significant. Each of these relationships suggests that the association between seasonal and premenstrual symptoms is not solely due to their overlap in depressive symptoms. That is, women who experience physical PMDD symptoms appear more likely to experience physical symptoms of SAD.

Given that the symptoms of atypical and seasonal depression are similar in nature to those of PMDD (irritability, tension, loss of concentration, increased appetite, hypersomnia, low

self-esteem and sensitivity to social rejection in the premenstrual week), Haffmans, Richmond, Landman, and Bloom (2008) examined whether women with PMDD respond to light therapy. They applied light therapy periodically to a small sample of women diagnosed with PMDD at periodic intervals during the late luteal phase. Symptom improvement was noted in all ten female participants as measured by two self-report measures of PMDD. However, in order to maintain this level of improvement, light therapy administration was required during all menstrual cycles.

In a randomized, double-blind, counter-balanced, crossover study spanning six menstrual cycles, Lam, Carter, Misri, Kuan, Yatham, and Zis (1999) examined the effect of dim versus bright light therapy in women with late luteal phase dysphoric disorder, now PMDD. Women collected baseline observations for two months, followed by two months of either a bright "white" light evening treatment condition, or dim "red" fluorescent light placebo condition. Both light conditions were administered through the use of lightboxes between 19:00h and 21:00h. In the bright "white" light condition, the light box consisted of cool-white fluorescent tubes rated at 10 000 lx at the level of the cornea, fitted with an ultraviolet filter. In the dim "red" light placebo condition, red gel filters were installed in identical light boxes, such that they emitted 500 lx at the level of the cornea. The authors stated that a 500-lx intensity was employed because "it has not had significant therapeutic effects in light box studies and does not appear to affect human circadian rhythms" (Lam et al., 1999, p.187). Although termed 'dim' light, use of a light box rated at 500 lx is still of greater illuminance than office lighting and thus is a plausible treatment for patients. To further enhance plausibility, the authors reported using the "red" gel filter because earlier studies have shown that long "red" wavelengths were less effective at reducing SAD symptoms compared to middle "green" or broadband "white" wavelengths. Following two months in their initial condition, women were immediately crossed over into the other

experimental condition. During each month of treatment, light therapy was administered for 30-minutes each day during the two-week luteal phase of the menstrual cycle. Outcome measures were assessed at the mid-follicular and luteal phases of each cycle using a variety of both self-report and observer-rating measures. Participants were assessed twice per month throughout the course of the study. One visit was scheduled during the mid-follicular phase (days 6-13), and the other visit during the symptomatic luteal phase of the cycle (days 21-28). Results indicated that both the active bright "white" light condition and red light placebo condition reduced depression and pre-menstrual tension scores during the symptomatic luteal phase, compared to baseline. Only the bright "white" light treatment condition produced significant results, however. These results indicate that bright, broad-band light therapy may be a viable treatment for women with PMDD in the luteal phase of their menstrual cycle.

In a recent review article addressing the efficacy, protocol, safety, and side effects of light therapy for seasonal and non-seasonal depression, Terman and Terman (2005) report that despite mixed findings in the literature, and the need for larger well-controlled trials, the treatment protocol administered by Lam et al. (1999) is a viable option for the treatment of PMDD and PMS, especially in instances where women have not responded to pharmacological interventions.

With respect to light therapy, Parry et al. (1997) attempted to replicate previous findings in which they discovered altered melatonin rhythms in PMDD subjects. In adults, dim light melatonin onset (DLMO) is defined as the time at which a salivary concentration of melatonin reaches a criterion of 4 pg/ml (Nagtegaal, Laurant, Kerkhof, Smits, van der Meer, & Coenen et al., 2002) and is considered to be normal when it occurs between 19:30 and 22:00 h. Phase response curves can be generated by using DLMO as a marker of circadian phase. Pandi-

Perumal, Smits, Spence, Srinivasan, Cardinali, Lowe et al. (2007) report that, by convention, circadian time (CT) zero is defined as the wake-up time in visually normal individuals. During daylight hours, melatonin levels are nearly undetectable until CT 13 (13 hours following normal morning wake time) when concentrations rise to approximately 2 pg/ml ($DLMO_2$), one hour later (CT 14) plasma concentrations are typically much higher, approximating 10 pg/ml ($DLMO_{10}$). During nighttime hours, human plasma melatonin levels normally exceed 40 pg/ml. The phase advance zone (corresponding to the time intervals during which melatonin administration alters circadian phase) for melatonin extends from CT 6 to CT 18 while the phase delay zone extends from CT 18 to CT 6 (Pandi-Perumal et. al., 2007). Thus, in humans with a typical sleep-wake cycle melatonin rhythms are circadian in nature and characterized by a 24-hour cycle in which levels remain minimal until evening hours after which they begin to increase rapidly with the decline or absence of daylight and onset of sleep.

Parry et al. (1997) sought to determine whether light therapy might exert its therapeutic effects by altering the phase, amplitude, or duration of the underlying circadian clock as reflected in plasma melatonin levels. In 21 women with PMDD and 11 control women, the authors measured the circadian profile of melatonin during both the mid-follicular (day 8 ± 2 following onset of menses) and late luteal (day 12 ± 2 days following the LH surge) menstrual cycle phases, and after one week of light therapy administered daily, in a randomized crossover design. During three separate luteal phases, the treatments consisted of either: bright white morning light (administered between 06:30 h and 08:30 h), bright white evening light (administered between 19:00 h and 21:00 h), or dim "red" evening light. With respect to melatonin rhythms alone, within group analyses revealed that in untreated PMDD women, melatonin onset time was delayed; duration was compressed (normal profiles are typically defined between 21:25 h ±

1:20h for $DLMO_{10}$ and 05:37h ± 1:23 h at termination of secretion); and area under the curve (AUC) (referring to the amount of melatonin produced), amplitude (maximum concentration at a given time point), and mean melatonin levels were decreased in the luteal phase compared to the follicular menstrual cycle phase. No such effects were found in control women suggesting that altered circadian rhythms may play a role in the development of somatic and vegetative symptoms in the LL phase of the menstrual cycle in women with PMS or PMDD.

After morning light in PMDD women, melatonin onset and offset times were advanced, and both duration and midpoint concentrations were decreased with broad-band light compared to the "red" light condition. After evening broad-band light in PMDD subjects, onset and offset times were delayed, midpoint concentration was increased, and duration was decreased as compared to the "red" light condition. By contrast, after light therapy in control subjects, duration did not change; but onset, offset, and midpoint concentration changed as they did in PMDD women. When onset and offset was compared, the authors found that in PMDD women, light shifted melatonin offset time more than onset time, and that morning light had a greater effect on shifting melatonin offset, whereas evening light had a greater effect in shifting melatonin onset time. These findings replicate the authors' previous observation that nocturnal melatonin concentrations are decreased in women with PMDD, and suggest specific effects of light therapy on melatonin circadian rhythms that are associated with mood changes in patient versus control groups. The different changes in melatonin onset and offset times during the menstrual cycle, and in response to morning and evening bright light compared with "red" light, support a two-oscillator (complex) model of melatonin regulation in humans in which an evening oscillator determines the rise of melatonin levels in evening hours, and a morning oscillator determines fall in melatonin in morning hours. This is in contrast to a one-oscillator model,

which predicts that onset and offset times shift together in response to photoperiodic changes. Parry et al. (1997) propose that the findings observed in PMDD women suggest tighter coupling between morning and evening oscillators, which increases resistance to photoperiodic changes in the late luteal phase of the menstrual cycle. These findings suggest that low or decreasing levels of reproductive hormones in the LL phase of the menstrual cycle affect the visuo-perceptual systems involved in the regulation of circadian rhythms, and that women susceptible to symptoms of PMDD may experience these effects to a greater extent than women who do not. Thus, re-stabilization of circadian rhythms over the menstrual cycle may decrease the vulnerability of PMDD women to the environmental and/or hormonal disturbances that are implicated in the experience of mood and somatic symptoms near the end of the cycle.

Sleep in women with premenstrual symptoms

Data from polysomnographic and survey studies indicate that definitive differences in sleep architecture across the menstrual cycles of normal women are lacking, and that many results are conflicting or inconclusive (Moline, et al., 2003). Despite age restrictions and small sample sizes, however, there is some evidence that suggests that women afflicted with PMDD exhibit variations in sleep architecture (see review by Parry, Martinez, Maurer, Lopez, Sorenson, & Meliska, 2006). Sleep complaints, either insomnia or hypersomnia, constitute one of the 11 criteria listed in the DSM-IV (APA, 1994) required to meet a diagnosis of PMDD. Although not all 11 criteria are required to qualify for diagnosis (APA, 2000) sleep disruption is a criterion that is supported by Mauri (1990) who reported that women with premenstrual symptoms tend to complain of insomnia, hypersomnia, tiredness, fatigue, disturbing nightmares or dreams, lethargy, and inability to concentrate.

Parry, Mendelson, Duncan, Sack, and Wehr (1989) provided initial direct EEG evidence of variations in sleep architecture between PMDD and non-PMDD women. The authors compared eight women with moderate to severe PMDD symptoms to eight non-PMDD women (controls) twice per week across one menstrual cycle and found that the PMDD subjects exhibited a greater percentage of stage two sleep with a corresponding lesser percentage of REM sleep than non-PMDD controls over the course of one four-week menstrual cycle. No between group effects were observed, however, with respect to cycle phase, or timing of temperature minima (based on normal cycle body temperature), all additional common physiological metrics associated with the menstrual cycle.

In a later study using a larger sample size, Parry, Mostofi, LeVeau, Nahum, Golshan, Laughlin et al. (1999) examined the differences in sleep architecture between those with (n=23) and without premenstrual symptoms (n=18). EEG recordings were taken during baseline MF and LL menstrual cycle phases (days 6-10 after menses and 2-4 days prior to the onset of menses, respectively) and after early (03.00 h to 07.00 h) and late (21.00 to 01.00 h) sleep deprivation. No differences were found on sleep EEG measures between PMDD and non-PMDD controls, however, for both groups, EEG measures differed significantly according to menstrual cycle phase. Both PMDD and non-PMDD women demonstrated significantly longer REM latencies (time to onset of REM) with decreased REM sleep (minutes and percent) in their luteal phases. Other studies have found significantly shorter REM latencies during the luteal versus the follicular phase, but still no significant differences between PMDD and non-PMDD women (Lee, Shaver, Giblin & Woods,1990). Lee et al. (1990), however, did report that PMDD individuals show significantly less delta sleep in both the follicular and luteal phases of the menstrual cycle. With respect to delta sleep, Shibui, Uchiyama, Masako, Kudo, Kim, Kamei et

al. (1999) reported that subjective daytime (09.00-16.30 h) sleepiness and the number of slow-wave sleep (SWS) containing naps significantly increases during the luteal phase suggesting that the increased number of daytime SWS-containing naps reflects a greater physiological demand for non-REM sleep during the luteal phase. The above results make it difficult to conclude that there are any consistent differences in sleep architecture between women with and without PMDD.

Given the inconsistencies in studies examining differences in sleep architecture between those with premenstrual affective symptoms and controls that do not have the affective symptoms, it is of interest to investigate methods that could more objectively delineate and distinguish substrate operations between groups. Many of the studies to date have been confounded by methodological flaws such as small sample size, variability in methods, an absence of hormonal markers to define menstrual cycle phase, subjective reports of symptoms, or failure to use standard diagnostic criteria (e.g., Parry et al., 2006).

Visual Learning, Memory Consolidation, and Sleep

During the past decade, research has supported a role for sleep in memory consolidation. In humans, there is evidence that sleep assists in the consolidation of memory with respect to procedural, non-declarative (implicit) task performance (Stickgold & Walker, 2005). Perceptual learning is no exception to this theory. Defined as a change in performance due to training (usually as a performance improvement), perceptual learning tends to persist over weeks to months, thereby distinguishing it from the transient performance characteristics of sensitization, habituation or priming (Fahle, 2005). Perceptual learning is often task specific, and does not lead to conscious insights that can be easily communicated. It is therefore considered to involve information retention of the procedural or implicit type (Fahle, 2005).

Performance on a visual texture discrimination task typically demonstrates marked improvement with practice (Karni & Sagi, 1991). It has also been shown to suffer as a result of both REM and SWS deprivation (Karni, Tanne, Rubenstein, Askenasy, & Sagi, 1994). If women with PMS symptoms exhibit differential sleep architecture across the menstrual cycle, their performance on a texture discrimination task that relies on overnight sleep might reflect this sleep architecture distinction. In the texture discrimination task developed by Karni and Sagi (1993) participants were presented with a small target texture consisting of three diagonal line elements (textons) embedded within a similar background of horizontal oriented textons, and asked to identify whether the target textons were aligned together in a horizontal or vertical direction. Performance was measured as the mean percent correct response for increasingly shorter time intervals between the briefly presented stimulus and a patterned mask (i.e., stimulus onset asynchronies, or SOAs, used to diminish iconic persistence). The patterened mask consisted of randomly oriented V-shaped micropatterns and a central compound pattern of randomly rotated 'F' characters used to mask the fixation of a 'T' or 'L' element. Psychometric curves (percent correct vs. SOA) are then plotted to monitor the effect of learning over time. A shift of this curve to the left is indicative of task improvement and learning. Karni and Sagi (1993) noted that in nine participants, a period of eight hours (latent phase) post learning was required before any gains in performance were noted. The following day after a normal night of sleep however, participants showed significant performance improvements. These performance gains were comparable to those who had been privy to additional practice sessions during the latent phase, indicating that additional training during this time between initial learning and subsequent testing offered no distinct advantage. What is more remarkable than the endurance of this learned task was that performance decay was not evident for a period of months following

REM consolidation. For example, one participant maintained performance gains for 32 months after initial training. Thus, it would appear that REM-dependent memory consolidation with respect to procedural, nondeclarative tasks such as texture discrimination is a robust phenomenon that resists decay months after initial learning.

Karni and Sagi (1993) suggested that the time required to demonstrate improvement (6 to 8 hours) upon the texture discrimination task might reflect an active process underlying the consolidation of experience-dependent plasticity within the adult visual cortex. If this was indeed the case, theirs would have been the first study to demonstrate a high degree of plasticity within the human primary visual cortex that is relevant to our ability to improve and develop perceptual skills (Stickgold & Walker, 2005). The model they proposed was Hebbian in nature, which specified synaptic activity enhancement in areas associated with texture perception. The purported mechanism for this action is believed to involve the local, retinal input-dependent modifications of neuronal connections between orientation selective cells and gradient sensitive cells in Brodmann area 17. This is particularly noteworthy if the post-primary V1 cortical areas that are responsive to texture discrimination involve the hippocampus—a structure that has been associated with spatial navigation and is well-known for Hebbian-type synaptic plasticity (e.g., Lange-Asschenfeldt, Lohmann, & Riepe, 2007).

Using the same task with slight modifications, Stickgold, Whidbee, Schirmer, Patel and Hobson (2000) evaluated task performance in two separate experiments. In the first experiment, participants were tested either on the same day of task performance training or on the day after. When testing occurred on the same day as training, no significant improvements in performance were noted. In addition, no improvements were observed as a function of time between training and test, which ranged from 3 to 12 hours. When testing was carried out on the subsequent day,

however, significant overnight improvement was observed for intervals of 9, 13, and 22.5 hours. Noteworthy is the fact that 12 hours of awake time was insufficient for producing a significant difference, but nine hours of sleep time was. No improvement was seen unless participants acquired at least 6 hours of sleep. These findings suggest that sleep plays a role in memory consolidation that affects learning and performance on basic perceptual tasks such as texture discrimination.

In a second experiment, 27 participants were brought back to the lab to examine the effect of sleep stage dependency on task improvement (Stickgold et al., 2000). Although task improvement was noted after an interval of sleep (as in experiment one), when the amount of improvement was associated with the amount of REM sleep acquired by participants in the lab, a strong correlation was observed. Specifically the amount of REM acquired in the last quartile of the night had the greatest effect on improvement in task performance. However, a positive trend was also observed between SWS and task improvement. The authors hypothesized that increases in performance following REM and SWS were not mutually exclusive. This is because participants deprived of either REM or SWS (but not both) did not exhibit as much improvement on the task as participants who acquired both stages of sleep. Given that the last quartile of REM appeared to exert the greatest influence on performance, these results led Stickgold et al. (2000) to propose a two-step model postulating that the amount of SWS acquired in the early evening, enhances REM-dependent memory consolidation in the last quartile of the night. Their two-step model of memory consolidation posits that a sequence of neurological events must occur that is mediated by REM and SWS.

Evidence derived from human and primate studies suggests that levels of circulating ovarian steroids can alter cognitive tasks. For example, in humans, several studies have reported

higher verbal fluency, but poorer spatial skills during periods of high estrogen levels, and improved spatial skills and lower verbal skills when estrogen levels are low (see review by Lacreuse, 2006). This seemingly contradicts previous rat and mice studies which showed that higher estradiol levels promote hippocampal dendritic arborization (e.g., Wooley, Weiland, McEwen. & Schwartzkroin,1997). However, compensatory or alternate mechanisms and/or interactions between estrogen and other neurotransmitter systems that exert their effects at higher levels within the brain (e.g. cortex) may account for these differences. It is also possible that subtle differences in the tasks used in the animal and human research may account for the differential findings.

In a recent study examining the effect of ovarian suppression on cognition, Craig, Fletcher, Daly, Picchioni, Brammer, Giampietro, et al. (2008) induced a pseudo-menopausal state in 17 premenopausal healthy women using a gonadotropin hormone releasing hormone agonist (GnRHa). They then used event-related fMRI to examine the effect of GnRHa on visual working memory (VWM) as measured by the Delayed Matching to Sample (DMTS) test adapted from the Cambridge Neuropsychological Test Automated Battery (CANTAB). This test requires that participants match a complex abstract pattern (encoding phase) to one of four possible choices presented simultaneously (recognition phase) following a delay. Neuroimaging outcomes were assessed in experimental participants and age-matched controls at baseline and eight weeks after GnRHa treatment. Although no significant effects were found for recognition time, group interaction results revealed that GnRHa treatment was associated with attenuation of left parahippocampal and middle temporal gyri activation during the DMTS task. The results of this study indicate that ovarian hormone withdrawal may lead to reduced activation in these brain circuits among which includes the hippocampus. Thus, compared to controls, women who are

more sensitive to normal ovarian hormone fluctuations may exhibit changes in task performance involving memory and/or spatial navigation at different points in the menstrual cycle.

Task Performance and Premenstrual Symptoms

Few studies have documented differences in task performance between women with and without PMDD or PMS, especially with respect to visuo-perceptual operations. With respect to cognitive performance, Reed, Levin, and Evans (2008) reported that compared to controls, women with PMDD showed impaired performance on the Immediate and Delayed Word Recall Task, the Immediate and Delayed Digit Recall Task, and the Digit Symbol Substitution Test in the luteal phase of the menstrual cycle. Other studies have found little difference between women with PMDD and normal controls on various neuropsychological tests (Resnick et al., 1998). However, subtle differences have been reported in the areas of psychomotor speed.

Resnick et al. (1998) evaluated neuropsychological functioning in women with and without PMDD across the domains of psychomotor speed, attention, and verbal learning and memory. The results of their study indicated that women meeting DSM-IV criteria for PMDD exhibit psychomotor slowing during the late luteal phase days of their menstrual cycles compared to their performances during the asymptomatic follicular phase (days 5 to 10 of the menstrual cycle). Psychomotor speed was assessed using five tests including the Digit Symbol Subtest of the Wechsler Adult Intelligence Scale Revised, the Grooved Pegboard Test , the Digit Vigilance Test, and the two-part (A and B) Trail Making Test. Furthermore, the trend of psychomotor slowing remained significant when the performance of women with PMDD was compared to that of women with subclinical (mild to moderate) PMS symptoms.

Attentionally, women with PMDD have recently been found to exhibit lower levels of prepulse inhibition (PPI). PPI occurs when a relatively weak sensory event, or prepulse, is

presented 30–500 ms before a strong startle inducing stimulus, and reduces the magnitude of the startle response (Braff, Geyer, & Swerdlow, 2001). In humans, PPI occurs in a robust, predictable manner when the prepulse and startling stimuli are presented using either the same or different modalities (i.e., acoustic, visual, or cutaneous) (Braff et al., 2001). Kask, Gulinello, Backstrom, Geyer, and Sundstrom-Poromaa (2008) compared PPI in women with and without PMDD using the eyeblink component of the acoustic startle response. Their experimental protocol consisted of a 115 decibel (db), 40 millisecond (ms) noise preceded at a 100 ms interval by 20 ms prepulses that were either 72, 74, 78, or 86 dB. Their results indicated that women with PMDD exhibited a significantly higher startle response (lower PPI) than controls during both phases of the menstrual cycle. PMDD patients exhibited lower levels of PPI with 78 dB and 86 dB prepulses compared to control subjects in the luteal, but not in the follicular, phase. Additionally, whereas non-PMDD participants displayed increased PPI during the late luteal phase compared to the follicular phase, PMDD women showed stable PPI magnitudes across their reproductive cycles. Relative to controls, PMDD patients displayed increased startle reactivity (lower PPI) across both menstrual cycle phases and significant deficits in prepulse inhibition of acoustic startle during the late luteal phase. PPI deficits have been associated with perceptual abnormalities, increased anxiety, and difficulty inhibiting intrusive thoughts (Braff et al., 2001). The authors state that the observed PPI response in women with PMDD could be attributed to the combined effects of estrogen and progesterone and their interactions in the CNS. For example, they report that progesterone can be metabolized to GABA-active neurosteroids, such as allopregnanolone (ALLO), which then bind to the $GABA_A$ receptor. Specifically, ALLO has been found to enhance inhibitory neurotransmission, consequently exerting anxiolytic, sedative, and antiepileptic effects (Kask et al., 2008). Thus, these results may reflect an aberrant

response in the GABA mediated inhibitory system in women with PMDD. This disruption could manifest itself in several different ways including: deficiencies in overall levels of ALLO, or the enzyme responsible for the metabolism of progesterone to ALLO; reduced sensitivity to ALLO, down-regulation of the $GABA_A$ receptor, or disrupted binding of ALLO to the $GABA_A$ receptor.

The results of Kask et al. (2008), are consistent with those of Epperson, Pittman, Czarkowski, Stiklus, Krystal, and Grillon (2007) who examined acoustic startle response (ASR) in women with the added condition of emotional valence, which has been found to either augment or diminish the magnitude of its effects. Thus, women, both with and without PMDD, were shown pleasant, neutral, or unpleasant pictures while undergoing the ASR procedure. Testing took place at the mid-follicular (days 5 to 11 of the menstrual cycle) and mid-luteal (1 to 7 days prior to the onset of menses) phases of the menstrual cycle. Results indicated that PMDD was associated with a clear increase in baseline startle magnitude in the luteal phase compared to the follicular phase, while healthy female controls did not show cyclic changes in this measure of physiologic arousal. The direction and degree to which picture viewing modulated the startle magnitude did not vary by group or menstrual cycle phase. The results of the ASR studies suggest that menstrual cycle phase exerts a modulatory effect on physiologic reactivity in women with PMDD, but not healthy women. Specifically, women with PMDD show more physiological reactivity during the late-luteal phase. Taken together, the results of the PPI and ASR studies indicate that lower or changing levels of reproductive steroids may interact with other neurotransmitter systems such as the GABAergic system in a way that interferes with the normal inhibitory actions of certain physiological and perceptual systems.

The Visual Perceptual System in Brief

As previously noted, the production and secretion of melatonin are mediated largely by postganglionic retinal nerve fibers, which themselves are largely responsible for the transmission of information regarding the daily pattern of light and darkness. Thus, mechanisms within the visual system are critical in regulating the availability of melatonin and the sleep/wake cycle. Given that sleep problems accompany mood disruptions in many psychological disorders including PMDD, it is plausible that individuals with these disorders also have functional distinctions associated with the visual system, both with the more vegetative, nonperceptual pathways (i.e., retinotectal and retinohypothalamic pathways) and those associated with higher-end visual perception (i.e., retinogeniculostriate pathways).

With regard to the latter, in human and non-human primates, it was previously espoused that two populations of retinal ganglion cells transmit signals to V1 by way of a relay through two sets of layers in the Lateral Geniculate Nucleus (LGN). Midget and parasol ganglion cells send separate projections to parvocellular (PC) and magnocellular (MC) layers of the LGN, respectively which in turn, innervate striate cortex (VI) (Hendry & Reid, 2000). Electrophysiological studies suggest that the MC streams communicate information relevant to higher-ordered movement and depth perception, while the PC stream communicates information relevant to higher-ordered shape, colour, and form perception (Livingstone & Hubel, 1988). More recently, a third visual stream with projections interleaved between PC and MC layers in the LGN has been identified (Hendry & Reid, 2000).

Research on this newly elucidated third visual stream, or koniocellular (KC) stream has begun to garner attention since its discovery (Hendry & Reid, 2000). The KC stream appears to be involved primarily with colour opponency as it specifically relates to “blue-yellow” colour

discrimination, although it may also provide feeds to other types of retinal circuitry that underlie a contribution to the cortical areas involved with motion discrimination (Calkins, 2001). Colour vision in humans can be attributed to the activation of three types of cone photoreceptors which are maximally sensitive to short, medium, and long wavelengths. Upon activation, signals from each of these cone types are relayed to retinal ganglion cells (RGCs) via cone-specific bipolar cells (Reid & Shapley, 1992). Signals for the KC system originate mainly from short-wavelength-sensitive (S) cones, which have been shown to respond optimally to wavelengths ranging from 419 to 433 nm (see review by Calkins, 2001). Unlike the long-wavelength-sensitive (L) and middle-wavelength-sensitive (M) cones, the opponent S-cones are less common and constitute only 5 to 10 percent of the total cone population (Curcio & Hendrickson, 1991). Retrograde tracing and intracellular recording techniques have revealed that the S-ON/(M+L)-OFF signal input into the LGN originates from the small bistratified retinal ganglion cells (Dacey & Lee, 1994; Martin, White, Goodchild, Wilder, & Sefton, 1997), bypassing layers IV of V1 that receive input from PC "red-green" chromatic opponent signals. These bistratified RGCs lie within and between the parvocellular and magnocellular layers, and have been shown in the macaque to project directly to the colour selective blobs in V1 (Martin et al., 1997). Dacey and Lee state: "the distinctive morphology of the small bistratified cells suggests a specific neural circuitry that may give rise to "blue-yellow" colour opponency" (Dacey & Lee, 1994, p. 733). Through these cells, S-cones are responsible for the S-ON component of the S-ON/(M+L)-OFF portion of colour opponency. The (M+L)-OFF process occurs when the signals from L and M-cones converge at the ganglion cell level and offset the input from the S-cones (Dacey & Lee, 1994).

Given that the KC stream is responsible for “blue-yellow” opponency, and may be associated with a distinct neural circuit responsive to short wavelengths, advances in colour vision and theories tied to the neurological processes that underlie them have been greatly extended by the invention of short-wavelength automated perimetry (SWAP). SWAP, or "blue-on-yellow" perimetry, is a visual field test designed to assess early visual field loss in several conditions including glaucoma, retinitis pigmentosa, and diabetes. As implied by the name, SWAP makes use of a short-wavelength target (i.e., 440 nm) to assess visual field loss. The use of a bright 100 cd/m^2 "yellow" background saturates the rods as well as the L and M cones. Therefore, this test primarily activates only the S-cones and their associated small bistratified ganglion cells (Sample, 2000). In terms of psychophysical research, SWAP provides a valuable measure of S-cone operations, and consequently the “up front” KC stream health and functionality.

The tracking of visual stimuli is achieved via the concurrent coordination of the saccadic (SAC) and smooth pursuit eye movement (SPEM) systems. Srihasam, Bullock, and Grossberg (2008) report that the SAC eye movement system generates rapid, temporally spaced open loop processing signals that cancel the difference between an initial angle of gaze and the angle necessary to foveate a target, while the SPEM system generates continuous, moderate-velocity, closed-loop signals that prolong foveation of mobile goal stimuli by trying to match gaze velocity to stimulus velocity. For rapidly moving stimuli, prolonged foveation requires reengagement of the SAC system to generate ‘‘catch-up’’ saccades. Both systems must coordinate their actions such that the same target stimulus is selected and maximum visibility is achieved (Srihasam et al. 2008). An elegant summary of the neurophysiology and neuroanatomy of the SPEM system is provided by Lencer and Trillenberg (2008). During smooth pursuit eye

tracking, the retinal image of the stimulus target is encoded and projected from the LGN to neurons in V1 that respond to motion signals from moving objects. The receptive fields of neurons in V1, however, are small, and interpretation of target information requires the recruitment of neurons from both the middle temporal (MT) and medial superior temporal areas in MT (V5). While MT neurons have been found to respond to speed, acceleration, and direction of moving stimuli, neurons in MST are optimally active when an individual is performing smooth pursuit tasks (see review by Lencer and Trillenberg, 2008). Area V5 is therefore recognized as a key area responsible for control in smooth pursuit tasks. From V5, signals are fed forward to the frontal eye field (FEF) from which the occulomotor command for smooth pursuit tasks is generated. Thus, posterior brain regions such as V5 are responsible for stimulus detection and response to motion, while frontal areas, through control of ocular muscles, are responsible for the maintenance of pursuit, as well as smooth pursuit initiation and prediction. Pursuit-related neural activity is reliably observed in the rostral regions of superior colliculus (rSC) which contain cells that respond to both SPEM and SAC eye movements (Krauzlis, Basso, & Wurtz, 2000). The cortical MT area sends strong excitatory projections to via rSC (Collins, Lyon, & Kaas, 2005), to the pontine nuclei, which in turn provide information to the cerebellum. Two cerebellar regions, the paraflocculus/flocculus and posterior vermis, involved in the coordination of pursuit and vestibular-ocular reflex and precise adjustments of the eye movements such as adaptation of pursuit information respectively then direct outputs to the motor neurons of the extra-ocular muscles in the brainstem (Ilg & Their, 2008). Thus, procedures such as eye tracking are of relevance in that they suggest specific neural networks that are potentially affected by hormones such as estrogen.

Estrogen and S-cone Functionality

In the last few years, evidence has accumulated indicating that estrogen may play a role in modulating the koniocellular visual stream. Using a desaturated D-15 test, a test that assesses fine colour discrimination for congenital and acquired colour vision defects, Gorin, Day, Constantino, Fisher, Redmond, Wickerham et al. (1998) reported that women who had used tamoxifen, an orally active selective estrogen receptor modulator (SERM), for many years tended to have poorer colour discrimination capabilities than women who had never used tamoxifen.

Additional studies have examined the effects of estrogen on the KC stream by using groups consisting of women with varying degrees of circulating estradiol. Eisner et al. (2004a) designed a study to evaluate the hypothesis that hormonal change can affect retinally-based lower level light-adaptation processes. Foveal visual sensitivities were measured across three consecutive menstrual cycles of four women not using hormonally acting medication and across three consecutive menstrual cycles of three women using the triphasic oral contraceptive, Ortho Tricyclen®. Ortho Tricyclen® is comprised of two synthetic hormonal agents, one of which varies in dose from week one to week three. Women generally take one per day over a 28-day cycle. In one 28-day package, tablet numbers 1 to 21 each contain 0.035 mg ethinyl estradiol, while tablet numbers 1 to 7 each contain 0.180 mg of norgestimate, tablets 8 to14 each contain 0.215 mg norgestimate, and tablets 15 to 21 each contain 0.250 mg norgestimate. Tablets taken from days 22 to 28 contain inert compounds. One woman, diagnosed with premenstrual syndrome (PMS), was a subject in both groups. Sensitivities were measured for a series of test wavelengths. During the testing procedure, both 2.0 and 4.0 log td background illuminances were employed. To assess threshold versus illuminance effects, the background illuminances

ranged from 1.2 log td through 4.4 log td in 0.4 log unit steps. Test wavelengths of 440, 460, 490, 510, 540, 580 and 640 nm were presented with 2.0 and 4.0 log td, 580-nm backgrounds. All test stimuli were presented at 1.5 Hz using a 50% square-wave duty cycle. Thresholds were detected using a method of limits in which the test illuminance was incremented in 0.06 log unit steps until women responded by pushing a button indicating that they detected the stimulus. After each threshold setting, test illuminances were decreased by 0.6 - 1.0 log units pseudorandomly. Of the six individuals tested, one had clear evidence of visual-adaptation changes occurring in phase with the menstrual cycle. Prior to using the oral contraceptive, this individual (the PMS subject) experienced S-cone sensitivity changes of up to 1.4 log unit on the 4.0 log td background (580 nm). Her S-cone sensitivities tended to be highest near ovulation and lowest premenstrually, indicating that higher levels of estrogen may promote enhanced discrimination with respect to the S-cone system and that the magnitude of these changes may be affected by the use of triphasic hormonal contraceptives.

Additional evidence for the role of estradiol in altering visual stream functionality comes from studies on women taking tamoxifen. As previously described, tamoxifen is a selective estrogen receptor modulator (SERM) that is used as adjuvant therapy for early stage breast cancer. It acts by competitively binding to estrogen receptors on tumors and other tissue targets; thereby preventing stimulation at the nuclear level and the subsequent proliferation of steroid-induced protein production/synthesis and second messenger systems (O'Malley, 2005).

The first study, conducted by Eisner and Incognito (2006), employed two groups consisting of women using tamoxifen as adjuvant therapy following successful treatment of early stage breast cancer (n=54; 30 reporting use for less than two years, and 24 reporting use for more than two years), and healthy amenorrheic peri-or post menopausal control subjects not using any

hormonally acting medications (n=32). A three-alternative forced-choice paradigm was used for assessing the perceived colour appearance of a 440 nm foveal test stimuli superimposed on a larger 3.6 log td, 580 nm background. In contrast to the control subjects, who indicated that the test stimulus appeared "white" 41.9% of the time, 87.5% of long-term tamoxifen users indicated that the test-stimulus appeared "white". These results indicate that simple colour perception processes may be altered in women with low levels of estrogen receptor binding.

Similarly, Yucel, Akar, Dora, Akar, Taskin, and Ozer (2005) examined the effects of the menstrual cycle on standard achromatic and "blue on yellow" field analysis of women with migraine. Using standard achromatic automated perimetry (SAP) and SWAP, the authors examined both eyes of 73 normally menstruating women (31 subjects with migraine and 42 healthy control subjects). Participants underwent a complete SAP and SWAP analysis in both the follicular and luteal phases of two consecutive menstrual cycles. In both groups, serum estradiol levels were significantly lower and serum progesterone levels were significantly higher during the luteal phase (one to two days prior to the onset of menses) than during the follicular phase (days 12-13 of menstrual cycle) as measured by basal blood samples drawn from the antecubital vein. For the non-migraine control group the mean sensitivity values with SWAP were significantly lower in the luteal than the follicular phase. A similar decrease was observed for the migraine group. The fact that estradiol levels were lower in the luteal phase provides additional evidence that low levels of estradiol are associated with decreased short-wavelength sensitivity. It also provides evidence for hormonal influences on retinal sensitivity given that visual field tests generally ascertain low-end functionality. This study differs from the present study, however, as the average age of participants was older (all aged between 30 and 39) and those with a history of severe PMS or PMDD were screened out. As well, women with migraine may

possess visual field anomalies not seen in control women or women without a history of migraine headaches. Thus, these findings make it somewhat difficult to infer hypotheses for the present study.

It was previously assumed that retinal photoreceptors were the only structures that were capable of interpreting and transmitting signals related to wavelengths within the visual spectrum. This has been challenged based on the discovery of intrinsically photosensitive retinal ganglion cells (ipRGCs), a class of retinal ganglion cells that are capable of being activated by certain wavelengths without traditional photoreceptor input (e.g., Panda, Provencio, Tu, Pires, Rollag, Castrucci et al., 2003). These ipRGCs are not only believed to be the prime candidate for visual modulation of circadian rhythms based on neurophysiological, electrophysiological, and genetic studies, but they may also be critical to the production of melatonin (Rea, Figueiro, & Bulloch, 2005). Recent studies suggest that the classical photoreceptors may moderate output from ipRGCs (Brainard & Hanifen, 2005; Dacey, et al., 2005; Panda et al., 2003). Originally, this moderation was thought to occur at the retinal level or at various points of neural convergence along the visual streams. Among the classical photoreceptors, the S-cones are of particular interest as they share similar peak sensitivities (i.e., 440 nm) as ipRGCs. Moreover, cellular recordings have shown that when the S-cone bipolar is stimulated with short wavelength radiation, it contributes to the excitatory depolarizing response of the ipRGC through a bipolar RGC synapse in the proximal ON layer of the IPL.

The moderating input of ipRGCs on known retinal photoreceptors (rods and cones) was shown by Panda and colleagues (2003). In this study, groups of genetically engineered mice were observed to respond disparately to artificial circadian conditions. Because there is evidence that suggests melanopsin-deficient mice retain most non-visual photic responses, the authors

hypothesized that either there is another class of photoreceptor present or, there are contributions from the classical photoreceptors to non-visual responses. To test this hypothesis, mice deficient in melanopsin *and* classical photoreceptors were engineered. These mice were then compared to wild type mice, mice lacking melanopsin only, and mice lacking classical photoreceptors only. The assessment of circadian entrainment was achieved by subjecting all groups of mice to a 24 hour light-dark cycle (8 hours light and 16 hours dark) while monitoring wheel running activity. Wild-type mice, melanopsin-deficient mice, as well as mice lacking classical photoreceptors all entrained normally and consolidated their wheel running activity to the dark period of the prescribed cycle. Mice lacking both melanopsin and classical photoreceptors, however, failed to do so, and continued to exhibit free-running rhythms. Increasing the light photoperiod and light intensity also failed to produce entrainment in this group of mice. Panda et al. (2003) concluded that there must be at least partial functional redundancy between classical rods and/or cones, and the melanopsin containing ipRGCs, as mice lacking one of the two types of receptors were still able to transduce photic information to critical brain areas important for the measured rhythmic behaviors. The substantial overlap that exists between symptoms of SAD and PMDD, and the differential results observed in studies assessing biorhythmic profiles of women with PMDD (such as those examining melatonin rhythms) suggest that women with PMDD may possess visual perceptual deficits that could be assessed by further investigation of their visual pathways, specifically with respect to the S-cone (KC) pathway.

Gender Differences in the Visual System

Although there are few studies examining gender differences on tasks specific to the visual functioning, there is some evidence that suggests morphological differences between males and females. Nuñez, Sodhi and Juraska (2002) reported that ovarian hormones introduced

after day 20 of the rat life cycle lead to an irreversible and critical loss of V1 cortical neurons, indicating an organizational effect of reproductive steroids during the peripubertal period in the binocular areas Oc1m and Oc1B. In an earlier study, Nuñez, Jurgens, and Juraska (2000) observed that neonatal androgens had an inhibitory effect on developmental cell death in rat primary visual cortex. Sexual dimorphism has also been observed in the retina. Salyer, Lund, Fleming, Lephart, and Hovarth (2001) showed than control male rats and female rats treated with testosterone had significantly larger retinal thicknesses than control female rats. The observation that estrogens are capable of exerting morphological changes throughout various levels of the visual system is interesting as it suggests a broader influence of reproductive steroids on the visual system and potential differences in perception as the result of hormonally mediated events such as the menstrual cycle, especially in those with PMS symptoms who may be suprasensitive to such changes.

The Present Study

The anomalous circadian responses observed with respect to melatonin release and secretion, and the differences in sleep parameters and sleep architecture sometimes reported in women with PMDD suggest that these women may suffer from a deficit in circadian entrainment. This hypothesis is further substantiated by the fact that patients with PMDD appear to respond to light therapy (Lam et al., 1999). The recent discovery of the short-wavelength sensitivity of the KC stream and the influence it may exert on ipRGCs suggests that these systems are worthy of examination in the PMDD population. Moreover, the performance of individuals with PMDD on tasks such as visual texture discrimination, which involves learning and plasticity contingent upon the acquisition of REM sleep, could serve to contribute to or

clarify mixed findings with respect to data obtained solely from the examination of sleep architecture in this group of women.

The present study sought to elucidate additional behavioral evidence implicating the potential neurological mechanisms through which hormonal levels (e.g. estradiol) and/or sensitivity may moderate visual perceptual functioning. We seek to test the theory that reproductive steroids such as estradiol exert functional or modulatory effects on the visual system using two groups of women. Given that women with PMS symptoms are hypothesized to be hormonally sensitive, we would expect that the visual systems of such women may respond differently to changes in hormonal levels that those of control women. Thus, in addition to examining the effects of hormonal changes on the visual system using menstrual cycle phase, we use a group of women with PMS symptoms to investigate whether any changes manifest differentially in a population that is hypothesized to be especially sensitive to such hormones. This was accomplished through the use of psychophysical tasks designed to examine both higher cortical perceptual and lower retinal functioning at two intervals across the menstrual cycle. At points in both the LF and LL phases, women with and without PMS symptoms were invited to complete: (1) measures of chromatic contrast sensitivity (CCS) based on "blue-yellow" tritan-matched and "red-green" deutan-matched, near-isoluminant spatial patterns of varying spatial frequencies, (2) an assessment of SWAP, (3) measures of texture discrimination involving spatial tasks designed to assess steroidal influences on V1 plasticity, and (4) a smooth pursuit eye-tracking procedure. The eye-tracking procedure was used as a tool to explore the potential link to REM disturbance in women with PMS symptoms, as well as to examine any deficiencies in retinal, extrastriate, and retinotectal smooth pursuit oculomotor operations. In addition to

completing the above tasks, measures of estradiol were obtained for the purposes of examining the influence of estradiol, and objectively determining menstrual cycle parameters.

During the CCS procedure, near isoluminant, bichromatic "blue-to-yellow" or "red-to-green" (peak to trough) sinusoidal gabor gratings were presented to assess cortical KC and PC stream functionality, respectively. Three different gabor orientations were also used to better isolate cortically-based chromatic orientation selectivity. We predicted that all groups would show increased chromatic contrast sensitivity in the LF phase as opposed to the LL phase (phase effect) (hypothesis 1), with women with PMS symptoms potentially exhibiting higher (overall) sensitivities than control women (group effect) (hypothesis 2). This would be in keeping with the findings of Wesner and Tan (2006) and Wesner and Pavlou (2008), whose results demonstrated heightened KC and PC contrast sensitivity in those with MDD and SAD. With respect to SWAP measurements, we hypothesized that both women with PMS symptoms and normal control women would exhibit lower short-wavelength sensitivity in the LL phase of the menstrual cycle when compared to the LF phase (hypothesis 3). In keeping with this hypothesis, however, it was further posited that women with PMS symptoms might exhibit differential sensitivities compared to control women in both phases (hypothesis 4), owing to a potentially heightened response to increases and decreases in levels of serum estradiol. Specifically, we hypothesized that that women with PMS symptoms would demonstrate higher S-cone sensitivity than controls during the peri-ovulatory, LF phase, and a lower short-wavelength sensitivity during the LL phase, when levels of estradiol decrease dramatically.

Given that learning acquired during an initial session of texture discrimination demonstrates resistance to decay (Karni & Sagi, 1993), testing sessions after training occurred not on subsequent days, but rather on days matched to the participant's appropriate menstrual

cycle phase (e.g., LL and LF). Should REM sleep differ amongst those with PMDD, as was found by Parry et al. (1989), we predicted, similar to the observations of Stickgold et al. (2000; 2005) and Stickgold and Walker (2006), that individuals with PMS symptoms might express deficiencies in overnight, texture-dependent learning tasks (group effect; hypothesis 5), thereby providing further support for a potential melatonin, and/or ovarian, steroid-based aberrant sleep cycle. Whether hippocampal modulatory differences, visual stream abnormalities, or combinations thereof mediate this was examined through the different hierarchical processing stages tied uniquely to the tasks described above.

Finally, with respect to the eye-tracking procedure and in accord with the findings of Kask et al. (2008) and Epperson et al. (2007) we hypothesized that compared to controls, women with PMS symptoms would exhibit a deficit in eye-tracking performance that may be more pronounced during the LL phase of the menstrual cycle (group x phase effect; hypothesis 6). Although findings between women with and without PMS symptoms are mixed with respect to sleep architecture, the observation that women exhibit altered sleep parameters such as longer REM latency and less time in REM during the LL phase suggests that normal, phasic, hormonal changes may affect the neural circuits responsible for circadian functioning. The observation that women with PMS symptoms have been shown to exhibit lower PPI and psychomotor slowing on tests of neuropsychological functioning, as well as disruptions in rapid, lateral eye-movement (REM sleep) warrants investigation into oculomotor functioning and steroidal influences on underlying relevant smooth pursuit retinal and extrastriate pathways.

Method

Participants

One-hundred-and-eighty women (mean age = 21.16 years, SD = 5.23 years) from Lakehead University and the local community were recruited to participate in a study investigating *Women's Health and Visual Functioning* following approval by Lakehead University's research Ethics Board (REB). The majority of these women were students recruited from introductory and upper level psychology courses. Those in introductory psychology courses received one bonus point of course credit for participation in the screening phase of the study. Women meeting the criteria necessary to participate in the laboratory phase of the study received up to two additional bonus points (one per lab session).

Based on information provided by the screening questionnaire, 34 women (mean age = 22.37 years, SD == 6.42 years) ranging in age from 18 to 44 years were recruited for the laboratory phase of the study. These women were representative of two groups: (1) a group of 16 women who reported the experience of adverse physiological and psychological symptoms approximately one week prior to the onset of menses (*PMS group*) and (2) a control group of 18 women who reported a comparatively low level of such symptoms. Women were initially selected into the above groups based on their scores from the Menstrual Distress Questionnaire (MDQ) (Moos, 1968) and a questionnaire developed by the authors assessing DSM-IV criteria for PMDD, with women scoring highest selected into the experimental PMS group. Specific criteria for group classification are provided below. Excluded from participation were: (1) women meeting criteria for a current major depressive episode as assessed by the Hamilton Rating Scale for Depression (HRSD) (details below), (2) women who reported the use of psychotropic medication(s) at the time of screening, (3) women with irregular menstrual cycles,

(4) women who were currently taking hormonal contraceptives or had taken them within the past six months, (5) pregnant women, (6) women who tested as "red-green" or "blue-yellow" colour blind (assessed in laboratory session one), and (7) women with ophthalmological disorders (e.g., glaucoma, retinitis pigmentosa, optic neuritis, etc..). Women who endorsed a history of anxiety or depression, but who had not sought pharmacological or psychological treatment within the last six months were eligible for inclusion. All efforts were made to select women with regular (predictable) menstrual cycles (e.g. women for whom the onset of menses could be reliably predicted within one to two days) based on their responses to such a question on the questionnaire. All women had normal or corrected-to-normal near visual acuity.

Measures

Screening Questionnaire (SQ).

The screening questionnaire (SQ) (see Appendix A) included several different sections, but was primarily used to select the two groups of women for the study: (1) women reporting high levels of PMS symptoms, and (2) a control group of women reporting low levels or an absence of PMS symptoms; none of whom reported the use of hormonal contraceptives. Other sections of the SQ provided information regarding psychiatric diagnoses, demographics, reproductive history, contraceptive history, medical and health history, morning/evening preference, and caffeine consumption. Many of the questions were adapted from questionnaires developed for two previous projects on hormonal effects (Oinonen & Mazmanian, 2007; Oinonen, 2009), as reliability and validity statistics were available. The demographic section included questions relating to age and education. Medical and health history questions provided information regarding height, weight, known medical and hormonal conditions, medication use, and family history of hormonal medical conditions (e.g., thyroid disorders, diabetes, breast cancer) and fertility problems. Additionally, participants were asked if

they had ever been diagnosed with a psychiatric illness; or treated with psychotherapy, pharmaceutical agents, or a combination of the two. Reproductive history was assessed with questions regarding age of menarche, parity, length of menstrual cycle, length of menses, premenstrual symptoms, time of last menstrual period, and cycle regularity. The contraception section contained questions about contraceptive use (e.g., type), and duration of use, as well as specific questions concerning the experience of certain OC side effects, including positive and negative mood change.

Symptoms of PMS were assessed using the Menstrual Distress Questionnaire (MDQ) (Moos, 1968) and a 33-item measure of PMS severity created for this study: The Lakehead Inventory of Premenstrual Symptoms (LIPS). In order to ensure that the two groups were not confounded by additional current diagnoses, the Hamilton Rating Scale for Depression (Hamilton, 1960) was included to screen for Depression. Scores from the Beck Anxiety Inventory (BAI) (Beck, 1990) were also examined as a post-hoc check to ensure groups did not differ in terms of anxiety, but were not used initially to screen women into groups. Participants meeting criteria for depression at the time of screening were excluded from experimental procedures. Finally, the Seasonal Assessment Pattern Questionnaire (SAPQ) (Rosenthal et al., 1984) was included to collect information on seasonal patterns of mood disturbance. Items from this questionnaire will be correlated with items on both measures of PMS for use in subsequent investigations.

Menstrual Distress Questionnaire (MDQ). The MDQ (Moos, 1968) consists of 47 items, each of which is rated on a five-point scale from 0 (*no experience of the symptom*) to 4 (*present/ severe*) with respect to either current experiences (MDQ-T) or experiences during three phases (menstrual, intermenstrual, premenstrual) of the most recent cycle (MDQ-C). The MDQ

consists of eight scales derived from empirically distinct, although correlated, sets of symptoms. Based upon principal components analysis with orthogonal rotation it has been established that these clusters consist of: pain, concentration, water retention, behavior change, negative affect, autonomic reactions, arousal and control. A more recent examination of the factor structure of the MDQ revealed that the original eight factors identified by Moos (1968) effectively represent the structure of menstrual cycle symptoms (Ross, Coleman, & Stojanovska, 2003). Furthermore, they reported that these factors appear to be replicable across different samples and across different methods of data collection. For the purposes of the present study, women were administered the MDQ-C, and asked to rate each item on a five-point Likert scale.

Lakehead Inventory of Premenstrual Symptoms (LIPS). This 33-item scale developed by the authors was designed to thoroughly assess the extent to which participants meet the APA's preliminary DSM-IV criteria for PMDD (APA, 2000). For each of the eleven criteria listed in the DSM-IV, participants were asked three questions assessing: (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. All questions were rated using seven-point likert scales 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. All questions asked women to estimate whether the described symptoms have occurred in more menstrual cycles than not over the past 12 months. Possible scores on this measure range from 0 to 198, with higher scores indicating an increased severity of symptoms and a greater degree of impairment.

Hamilton Rating Scale for Depression (HRSD). The HRSD (Hamilton, 1960) is used widely to assess current symptoms of depression in both clinical and research settings (Dozois,

Dobson, & Henny 2004). The core scale consists of 21 items, scored from 0 *(not at all)* to 4 (*marked or severely*) with items 18 to 21 assessing patterns of diurnal variation, depersonalization, obsessions/compulsions, and paranoia. For this study, the 28-item HRSD was employed. This form includes an additional seven items reflecting atypical and vegetative symptoms. In accordance with Wesner and Tan (2006), who used the HRSD to screen for participants with Seasonal Depression, efforts were made to exclude women scoring 20 or higher. Given the previously described overlap between symptoms of PMS and Seasonal Depression, scores on this measure allowed for the examination of depressive symptoms and the evaluation of potential confounds between depressive and PMS symptoms.

Beck Anxiety Inventory (BAI). The BAI measures the severity of self-reported anxiety (Beck & Steer, 1990). It consists of 21 descriptive anxiety symptoms that are rated on the following four-point scale: 0 (*not at all*), 1 (*mildly, it did not bother me much*), 2 (*moderately, it was very unpleasant, but I could stand it*), and 3 (*severely; I could barely stand it*). Analysis has revealed that the BAI correlates highly with other measures of anxiety, including the Hamilton Anxiety Scale Revised (r = .51) and the anxiety subscale of the Cognition Check List (CCL-A) (r = .51) (Beck, Epstein, Brown, & Steer, 1988). The BAI was constructed to measure symptoms of anxiety that are minimally shared with those of depression (Beck & Steer, 1990). With their diagnostically mixed sample of 160 outpatients, Beck et al. (1988) reported that the BAI had a high internal consistency (Cronbach coefficient alpha = .92). This same study reported a test retest reliability of .75. The maximum score on this measure is 63. Scores on this measure were collected to use as a check to ensure the groups did not differ in terms of anxiety, as well as for use in future investigations.

Seasonal Assessment Pattern Questionnaire (SAPQ). For the Seasonal Pattern Assessment Questionnaire (Rosenthal et al., 1984), participants are asked about seasonal variations in mood, weight, appetite, sleep length, social activity, concentration, and energy. This assessment has been shown to be high in internal consistency and test-retest reliability (Magnusson, Friis, & Opjordsmoen, 1997), and has an estimated identifying efficiency of 57% (Raheja, King, & Thompson, 1996). It is the most commonly used measure in the identification of SAD (Sullivan & Payne, 2007). The SAPQ is scored by adding up the participant's ratings on items assessing seasonal change in terms of alternations in the depressive symptoms and the degree to which they feel that the changes affect their lives. A total rating score of 12 or higher, out of a possible score of 24, is generally indicative of a set of symptoms consistent with SAD (Rosenthal et al., 1984; Sullivan & Payne, 2007). Scores on this measure will be correlated with scores on the two measures assessing PMS symptoms in later studies.

Conscientiousness Scale from The Revised NEO Personality Inventory (NEO-PI-R). The NEO-PI-R (1994) is a 240-item questionnaire designed to assess the traits of Neuroticism, Extraversion, Openness, Agreeableness, and Conscientiousness. Questions are rated on a five-point likert scale ranging from *strongly disagree* (0) to *strongly agree* (4). Each of the five scales is further divided into six facets. Internal consistency (reliability) coefficients are reported to range from .86 to .95 for domain scales, and from .62 to .82 for facet scales (Costa & McCrae, 1994). The NEO-PI conceptualizes conscientiousness as subsuming both proactive and inhibitive aspects, with proactive aspects dealing with behaviors relating to success at work (e.g., the need for achievement and commitment to work), and inhibitive aspects relating to self-control and cautiousness (Costa, McRae, & Dye, 1991). These aspects are encompassed by the six facets of dutifulness, achievement striving, competence, order, self-discipline, and deliberation. According

to the authors, dutifulness reflects the propensity to honor and uphold commitments to social justice and social obligations, often found in work contexts; achievement striving reflects the propensity to be hard working and driven; competence refers to one's sense of being capable, effective, and sensible; order refers to one's propensity to be well organized, neat, and clean; self-discipline is the ability to carry tasks through to completion despite boredom and other potential distractions; and deliberation reflects impulse control, patience, and maturity (Costa & McCrae, 1994). This measure was included to potentially control for differences in perceived obligation, stamina, and/or responsibility between women with and without PMS symptoms, as those scoring higher on such traits may exhibit a tendency to persist at difficult tasks despite experiencing feelings of discomfort (e.g., during the lab session occurring in the LL phase).

Horne-Ostberg Morningness-Eveningness Questionnaire (MEQ). Developed by Horne and Östberg in 1976, the MEQ is a 19-item measure designed to determine whether people function more effectively in the morning or evening hours. Questions assess preferences pertaining to habitual rising and bed times, preferred times of physical and mental performance, and subjective alertness after rising and before going to bed. It yields scores on a single scale of “morningness" vs. “eveningness" ranging from 16 to 86. Higher scores suggest greater “morningness". Lower scores indicate greater “eveningness" (i.e., a preference to sleep later, and for evening activities). The MEQ classifies participants as follows: 70-86 Definitely Morning Type, 59-69 Moderately Morning Type, 42-58 Neither Type, 31-41 Moderately Evening Type, and16-30 Definitely Evening Type. This questionnaire was used to collect data for future studies.

Laboratory Measures and Materials

Laboratory Questionnaire. Prior to completing laboratory procedures, participants were asked to complete a brief laboratory questionnaire (see Appendix B) designed to assess their current affective state and level of cycle-related discomfort. Women were also asked to indicate whether they had taken any pain medication or consumed any caffeine. They were further asked to indicate the amount of sleep they acquired the previous evening.

Positive and Negative Affect Scale (PANAS). The PANAS (Watson, Clark, & Tellegen, 1988) was included in the laboratory questionnaire. The PANAS consists of two scales: one for positive affect (PA), and one for negative affect (NA). High positive affect reflects a state of high energy, full concentration, and pleasurable engagement; whereas low PA is characterized by sadness and lethargy. In contrast, NA is a general dimension of subjective distress and unpleasant mood states, including anger, contempt, and disgust. Low NA indicates a sense of calm and security. The PA PANAS items include *attentive, interested, alert, excited, enthusiastic, inspired, proud, determined, strong,* and *active;* while the PANAS scale for NA includes the items *distressed*, *upset, guilty, scared, hostile, irritable, ashamed, nervous, jittery,* and *afraid*. For the current study, participants were asked to indicate how they felt at the moment they were completing the surveys. They were asked to rate each adjective on a five-point response scale ranging from 1 *(very slightly or not at all)* to 5 (*extremely).* These ratings will be used for exploratory analyses in subsequent studies.

Menstrual Distress Questionnaire (MDQ). In order to prospectively assess menstrual cycle related pain and/or discomfort, three scales from the MDQ were included in the Laboratory Questionnaire. These included the pain (six questions), water retention (four questions), and negative affect (8 questions) scales. Scores on these scales were collected to be used as potential

covariates in analyses comparing performance across the menstrual cycle or between groups. The scales were also used as a prospective confirmation of PMS symptomatology for the two groups of women.

Visual screening. A Freiberg visual acuity task (FrACT; Bach, 1996) was used to measure participants' near visual acuity for a 75 cm distant stimulus. Landolt 'C' stimuli were presented on a 27 cm CRT monitor and participants were required to select the proper orientation of the C's gap—a gap whose degrees visual angle systematically varied from a 5° subthreshold to a 1° suprathreshold size. The orientation responses were made using a number pad on a standard keyboard. A total of eight possible orientations were presented with the test adapting to the responses of the participants. Participants were positioned with a chin rest to ensure constant viewing distance and stimulus visual angles.

Color vision was also assessed using the Ishihara pseudoisochromatic plate test, 24-plate edition (Ishihara, 1993). Participants showing deviations along the protan, deutan or tritan lines (e.g., colour blindness) were excluded from the study. Ishihara plates consist of a series of colour-defined numbers embedded within different coloured dots. The plates were always presented under a standard D65 "daylight" lamp. The plates are designed so that grouping of dots by colour causes a number to emerge from the background that can be recognized correctly by people with normal colour vision, but in the absence of normal colour signals, all of the dots appear falsely of the same colour (pseudoisochromatic). Therefore, colour-deficient observers either fail to see the number altogether or make mistakes in recognizing it correctly. Using the above screening procedures, all women participating in the laboratory sessions reported normal or corrected-to-normal near acuity and no deficits in colour vision.

Spatial chromatic contrast sensitivity (CCS) measurements. CCS was measured using near isoluminant, heterochromatic "red-to-green" and "blue-to-yellow" peak-to-trough gabor gratings with center-spatial frequencies at 0.5, 1.5 and 4.0 cycles per degree (c/deg). These bichromatic gabors were presented within a 1000 msec temporal Gaussian window. Gabors are spatially discrete patterns composed of sinusoidal gratings that are convolved with a spatial gaussian blur. In this study, three different near isoluminant gabors were used, each consisting of either a vertical, horizontal, or 45° oriented sinusoidal pattern. All gabors were presented on a gamma corrected Viewsonic® G225 21-inch monitor for one second either to the left or right of a center-positioned 0.38° white crosshair (18.00 cd/m^2). Near isoluminance was used to minimize the influence of luminance stream operations in the determination of threshold. The chromaticities were derived from a hue scaling study conducted by DeValois, DeValois, Switkes, & Mahon, (1997). The "red-to-green" (R/G) gabor was used to assess PC processing ability. The *relative* initial contrast of the gabor was set to 1.0 for the maximum allowable "red" peak and "green" trough while still maintaining near isoluminance. In CIE (1931) coordinates, the "red" peak was x = .3828 and y = 2846 and the "green" trough was x = .2639 and y = .3772. For the "blue-to-yellow" (B/Y) gabor, the *relative* initial contrast was set to 1.0 as well for the maximum allowable "blue" peak and "yellow" trough. This near-isoluminant gabor was designed to probe the KC cortical stream, with the initial "blue" set to x = .2739 and y = .2263, and the initial "yellow" set to x = .4280 and y = .4976 in CIE coordinates. An illustration showing these endpoints within the 1931 CIE space is shown in Figure 2. Our spectroradiometric measurements confirmed near isoluminance of the stimuli, with luminance values corresponding to 35.3 cd/m^2 ("red"), 35.1 cd/m^2 ("green"), 35.6 cd/m^2 ("blue") and 35.4 cd/m^2 ("yellow"). Luminance for the background screen was 36.8 cd/m^2 for the "red-green" condition and 33.9

cd/m^2 for the "blue-yellow" condition. Examples of both sets of chromatic R/G and B/Y gabors at the above frequencies are illustrated in Figure 3.

The stimuli were presented binocularly with the participant seated 75 cm from the display. Testing began after a seven-minute dark adapt followed by a three minute light adapt to the background. Chromatic contrast thresholds were assessed using a spatial, two-alternative forced choice (2AFC), 2-interleaved staircase procedure. Chromatic contrast was defined along either the deutan ("red-to-green") or tritan ("blue-to-yellow") confusion lines in CIE space; that is, modulation of the relative chromatic contrast for the R/G or B/Y gabors was configured along these two dichromatic confusion axes, respectively. Relative chromatic contrast modulations were made starting from the near isoluminant endpoints towards the neutral background. This was done by incrementing or decrementing chromatic contrast in 0.1 logarithmic (gamma corrected) steps in which the iterations were based on a *relative* Michelson periodic contrast of 1.0 (or 100%) set to the maximum peak and trough RGB endpoint values for "red-to-green" and "blue-to-yellow" modulations. The use of *relative* contrast iteration ensured proper near-isoluminant modulation along the CIE deutan and tritan confusion lines. It is important to point out again that the thresholds obtained, and therefore the CCS, are *not* absolute but relative measures within each chromatic condition. Three correct responses were necessary for an increase and one incorrect response for a decrease in contrast. Each staircase terminated after four practice and six experimental trial reversals. The arithmetic and geometric means of both staircase experimental reversals defined the chromatic contrast threshold (both values were used in the analyses) for each spatial frequency condition while the inverse of the arithmetic threshold

defined CCS.[1] Using either the left or right mouse buttons, participants were asked to choose which side of a "white" (CIE 1931 chromaticity of x = .3050, y = 0.3275, and luminance value of 18 cd/m^2) crosshair the gabor appeared on (left or right side).

Short-wavelength automated perimetry (SWAP). Following the screening procedures and completion of the laboratory questionnaire, SWAP measurements were collected for all participants. SWAP, or "blue-on-yellow" perimetry, is a visual field test designed to assess early visual field loss and is used to assess S-cone sensitivity. SWAP makes use of a short-wavelength target (i.e., 440 nm) superimposed on a 580-nm, 100 cd/m^2 "yellow" background, the latter of which saturates the L and M cones and rods, thus ensuring that the 440-nm test spots activate only the S-cones and their associated connected KC streams (Sample, 2000). In terms of psychophysical research, SWAP provides a valuable measure of early retinal S-cone operations. KC stream functionality can also be inferred using the SWAP coupled with higher-ordered psychophysical measures such as B/Y orientation-specific contrast sensitivity measurements (see above).

SWAP measurements were made with an AP200BY Automated Perimeter (Opto-Global, Adelaide, South Australia) using the participant's dominant eye (the non-dominant eye was patched). Right or left eye dominance was determined by having participants indicate which eye they favored during the performance of a monocular task. Following the determination of eye-dominance, participants were positioned on a chin-rest situated in front of a stimulus bowl, extending a 100° monocular visual angle. Following a five-minute dark adaptation, participants were required to focus on a 640-nm "red" fixation point for a period of 3 minutes to ensure proper retinal light adaptation and ocular position. Following light adaptation, participants were

[1] NOTE: Statistical and plot analyses revealed no difference between the arithmetic- or geometric-derived CCS. We therefore only report the arithmetic means in this paper.

passed a joystick and asked to depress the top button to indicate when a target stimulus was detected. The target stimulus consisted of a Goldmann Size V 440-nm "blue" circular point (1.72º) that was randomly presented along 162 points across the visual field. Results from this experiment provided a map of the participant's sensitivity to the target stimuli across the visual field. Throughout testing, the automated perimeter tracked the women's pupil position using an infrared eye-tracking camera ensuring proper foveal fixation and target imaging at designated retinal positions. Participant responses were not recorded if the eye position moved, or was out of alignment.

Texture discrimination task (TDT). Spatial learning properties were assessed across groups and over menstrual cycle phases using a texture discrimination task adapted from Karni and Sagi (1991). During the TDT, participants were presented with computer-generated textures, consisting of a foreground target of three 566-nm "green" elements (textons) differing only in orientation from a background of horizontal elements. The grouping of the three-element textons was presented randomly as either three vertically stacked textons or three horizontally positioned, side-by-side textons. This texton arrangement was always presented in the same quadrant per participant. That is, every participant, during training and testing, was always presented with the texton groupings in Quadrant I, II, III or IV (position was based on standard Cartesian coordinate system). This was done to maintain retinotopic consistency for each participant. To guarantee proper central fixation, a 'T' or 'L' was presented in the middle of the monitor at the same time as the texture element. All participants were asked to first identify the target letter (fixation) followed by the orientation of the target texton group (horizontal or vertical). Following presentation of this target display and a brief inter-stimulus blank, a patterned mask was presented. The inter-stimulus blank, known as the stimulus-to-mask onset

asynchrony (SOA), varied in duration ranging (in order) from 360 to 20 ms. Specific SOA iterations were dependent on the lab session and trial block.

The 22.0 cd/m^2, ”green” line segments in the initial presentation were 23 arc minutes in length spaced 0.1° or 6 arc minutes apart. The line segments were ‘jittered’ 2.5° to 5° eccentric from central fixation on a black background. Jittering was done within a 19 x 19 element lattice that was 13.5° wide x 13° high. The spatial averaged luminance of the target display with all the elements was 38 cd/m^2.

Following each trial, a 100 ms mask consisting of randomly rotated, ‘green- coloured’ ‘V’ characters ‘jittered’ on a black background presented in the same 19 x 19 element lattice as the test session. All characters within the mask were 22.5 cd/m^2 and spaced 15 arc-minutes apart. The overall space-averaged luminance of the mask display was 30.0 cd/m^2 with a peak wavelength of 566 nm. The top panel of Figure 4 illustrates the presentation of a single trial in which the central fixation target is a rotated letter ‘T’ and the texture element is found simultaneously in the top left-hand panel (Quadrant II). The lower panel of Figure 4 shows the mask pattern presented for all trials at a given SOA.

All trials began with the 250 ms presentation of a small fixation cross subtending 15.5 arc-minutes. A custom response pad with four buttons was used to record participants’ responses. The buttons were arranged in a diagonal pattern to eliminate handedness confounds and reduce response position bias (e.g., Simon, 1969). One pair of buttons was designated for each of the possible fixation targets (T or L); the other pair was used to indicate the texton arrangements (horizontal or vertical). Women were notified via an audible beep if an incorrect response was indicated for the fixation letter, but no feedback was given for an incorrect identification of texton arrangement. This was necessary to ensure proper central fixation of the

participants throughout the trials, thereby guaranteeing consistent retinal quadrant positioning of the target textons. Again, as mentioned above, measuring potential plasticity changes demands this kind of spatial consistency in the retinotopic maps of V1. Performance was measured as the mean percent correct response for different SOAs. Reaction times (in msec) were also measured. All stimuli were presented on a 21" Image Systems Corporation monitor, model M21L-0332 (subtending 20 degrees visual angle) that used a monochrome p46-phosphor tube with a 290 ns response time. This ensured accurate timing as well as the elimination of ghosting artifacts. Participants were positioned with a chinrest so that their entrance pupils were 110 cm from the high-speed (200.4 Hz refresh rate) monitor.

Participants completed 16 to 20 blocks of trials (stimulus presentations) with 50 trials in each block, constituting a presentation of approximately 1000 stimuli per session. The first lab session conducted (either during the LF or LL phase) was designated as a training session, and consisted of six SOAs decreasing progressively from 360 to 40 ms. The second lab session (testing session) was conducted during the other menstrual cycle phase (either LF or LL depending on the first phase) and consisted of seven SOAs decreasing progressively from 300 to 20 ms.

Smooth pursuit eye tracking task. The eye tracking procedure was adapted from that of Smyrnis, Evdokimidis, Mantas, Kattoulas, Stefanis, Constantinidis et al. (2007) using a Cambridge Systems, infrared eyetracker [Cambridge Research Systems (CRS), Rochester, UK]. A 22-inch Mitsubishi Diamond Pro 2070 monitor powered by a CRS ViSaGe stimulus generator with a 14-bit resolution per colour channel and 200 Hz frame rate displayed the sinusoidal movement of the stimulus. The monitor was driven by a Dell Precision Workstation with a Pentium 4 processor running at 3.6 GHz. Calibration and gamma-correction of the software and

monitor was conducted using a Minolta colorimeter and calibration software provided with the ViSaGe system.

Each participant began the experiment by performing a calibration procedure with their dominant eye in which system-specific software calculated the correlation between measured Purkinje glints in the camera image to specific eye positions. During the calibration procedure, women were asked to foveate upon a "white" crosshair (0.5° x 0.5°) while the computer measured Purkinje glints. Different crosshairs were presented at a series of different screen positions. Following completion of the measurement at one position, the crosshair was removed and immediately replaced by another crosshair at a different area on the screen. Twenty such measurements were made, forming a 4 x 5 lattice covering the entire viewing area of the monitor, allowing for interpolation of any recorded eye position to a particular screen region. The visual angle extended from left to right and top to bottom extended 26° and 15° visual angle respectively.

During the experimental procedure, participants were instructed to follow a horizontally moving circular, 89.2-cd/m^2, "yellow" target (dominant wavelength of 569 nm) superimposed upon a "gray" background (mean luminance of 23.1 cd/m^2). The angular subtense for the full horizontal excursion was13.3° from fixation center, with a horizontal motion conforming to a sinusoidal (right to left) pattern of acceleration and deceleration. The velocity of the target varied between 0 deg/sec at maximal excursion and 10.43 deg/sec to 83.29 deg/sec, depending upon the frequency of the trial. Target frequencies were presented in the same ascending order for all participants: 0.25 Hz, 0.5 Hz, 1.0 Hz, 1.25 Hz and 2.0 Hz. For each frequency condition, participants pursued the target for five complete 360° cycles, forming a total of 25 tracked cycles for the entire experiment. Trials began with a five second presentation of a fixation crosshair at

the center of the monitor. Two seconds following the removal of the fixation crosshair, the target was centrally presented and immediately began to move. Target movement began at the center of the screen towards the extreme left, 13.3° angular subtense position, which we identify as a 90° excursion phase, and then towards the extreme right, -13.3° angular subtense position, which we identify as a 270° excursion phase. Central position is therefore identified as 0° phase at start, 180° phase halfway through the first half of a complete cycle, and 360° phase at the end of the complete cycle. Following completion of the five cycles, the target ceased movement and was removed from the screen revealing, once again, only the grey background. Participants were cued as to the initiation of a subsequent trial. To avoid excessive local memory demands on the Dell computer hard drive, infrared Purkinje-image positions were acquired as x,y ASCI files and downloaded to a large memory Apple storage facility (RAID® system) linked to a network server. Given the convergent evidence for depressive and reproductive cyclic influences on oculomotor physiology, we were particularly interested in onset and offset latency, and the amplitude and smooth pursuit tracking accuracy for the different cycle phases and frequencies.

Salivary Estradiol Collection. The collection of saliva for estradiol measurement was accomplished via the use of a passive drool technique. Upon initial contact with participants in the orientation session, instructions (as per Salimetrics® protocol) were given asking all women to avoid the following prior to attending their laboratory appointment: (1) brushing teeth within one hour, (2) using salivary stimulants such as chewing gum, lemon drops, granulated sugar, or drink crystals, (3) consuming a major meal within one hour, (4) consuming alcohol for twelve hours, or (5) consuming acidic or high sugar foods within 20 minutes. One 2 ml sample of saliva was collected within each lab session and the sample was collected across three different times within the 3-hour session. Women were offered distilled water following the collection of the

first two salivary samples allowing for an adequate amount of time to pass prior to collection of the final sample at the end of the laboratory session. During collection, participants were given a straw approximately five cm in length and one Cryovial®. Prior to their testing session, participants were asked to rinse their mouths out with distilled water. In order to collect the samples, participants were instructed to imagine eating something palatable and allow saliva to pool in their mouths. With heads tilted forward, participants salivated down the straw allowing for the collection of saliva in the cryovial. In order to ensure accurate hormonal measurements and to attempt to control for the pulsatile release of steroids, this procedure was repeated three times during each laboratory session until approximately 2 ml of sample was collected in the same tube (see Salimetrics® Specimen Collection protocol in Appendix C). That is, participants were asked to contribute saliva to one test tube at three times during each session, resulting in one test tube of two mL of saliva that was collected over a three hour period. Samples were provided at the beginning and end of each session as well as after the first experimental procedure (SWAP) corresponding to approximately one hour into the session. This procedure was used in order to obtain a saliva sample that contained a level of estradiol that was representative of the levels present during the actual testing session and at that particular cycle phase. Such a procedure controlled for the fluctuations in estradiol levels over time due to the pulsatile release of gonadal steroid hormones (Hampson & Young, 2008). Samples were refrigerated between collection intervals. All women completed the laboratory sessions between three and three-and-a-half-hours. Following collection, samples were frozen at -21º C until the time of analysis. Samples were analyzed via the use of enzyme linked immunosorbent assay (ELISA), to detect optical density (Groschl, 2008). Following the collection of all samples,

analyses were undertaken at the Lakehead University Centre for Biological Timing and Cognition Bioassay Laboratory.

Ovulation Test Strips. To corroborate self-report information, and position within the menstrual cycle, the determination of ovulation was accomplished via the use of generic ovulation test strips purchased from a bulk medical supply source. (http://www.early-pregnancy-tests.com/) (e.g. Claydon, Younis, & Hainsworth, 2006; Johnson, Shaw, Parkinson, Ellis, Buchanan, Zinaman et al., 2011). These strips are sensitive to the pre-ovulatory LH surge and change colour when the presence of the hormone is detected in urine. The strips are sensitive to 25 mIU/mL of LH, as per kit instructions. This LH test was used to ensure that the menstrual cycle in question was indeed an ovulatory cycle and that women were invited into participate in lab sessions targeting the LF phase at the appropriate time (i.e., just after the LH surge). Depending on the reliability of their cycle, women were asked to test for a spike in LH between days -20 to -13 of their menstrual cycles (based on a method of reverse-count). Participants were asked to test between the hours of 10:00 h and 20:00h, when the presence of LH can be detected in urine (see test strip instructions in Appendix D). In order to achieve a result, women were required to direct the test strip into a collected sample of urine for seven to ten seconds. Collector cups facilitating the positioning of test-strips were provided to all women during the laboratory orientation. Results were indicated by the appearance of coloured bands in the control (C), and/or test (T) regions of the strip. The appearance of one colour band in the control C region or a lighter appearance of the T band is indicative of a negative result for the presence of LH. The appearance of a T band equal to, or darker than the C band indicates the presence of LH, and the likelihood of ovulation occurring within 24 to 48 hours. The absence of a control band is indicative of an invalid test, which can occur if an insufficient volume of specimen is added to

the test strip. A trained female researcher provided instructions to all participants on the use of the test strips as well as their interpretation during their orientation session. All women were provided with five collector cups, five ovulation test strips, and a set of printed instructions following the orientation session.

Procedure

Screening Phase. Following recruitment, all participants were directed to a website where they read a brief overview (letter) of the study and what would be required of them in all phases. After reading this letter (see Appendix E) and completing Consent Form A (see Appendix F), women were immediately guided to the online screening questionnaire. Following the completion of the screening questionnaire, all women were provided with Debriefing Form A (see Appendix G) that included the contact information of all researchers involved in the study. Subsequently, participants meeting criteria for the study (described above) were contacted by telephone or email and invited to participate in the laboratory phase of the experiment. Prior to scheduling the initial sessions, participants were asked to attend an orientation session in which they were asked to provide information regarding their menstrual cycle, including the date of their last menstrual period and the usual duration of their menstrual cycle. Criteria for establishing the presence or absence of PMS symptoms are defined below.

Laboratory Orientation. After responding to an initial request to participate in the study, all women were invited to the laboratory for an explanation of experimental tasks and scheduling of sessions. During this session, current menstrual cycle day was determined using calendars and self-report information. Women were told that one laboratory session was to be undertaken in the LF phase, just after the LH surge, when estradiol concentrations were rising or high, and a second session was to be undertaken later in the LL phase prior to the beginning of a

subsequent cycle. All women were provided with five ovulation test-strips and instructed on their use and interpretation of results. The primary researcher or a trained assistant always explained the difference between positive and negative test results. Printed copies of test instructions with photos and step-by-step directions for interpreting LH test results were provided to all women to take home. While in the lab, they also watched a demonstration video illustrating proper use and interpretation of test results. Women were asked to begin using the LH strips beginning on day -17 or -18 of their cycle and to continue using the strips until a positive result was achieved. If necessary, additional strips were provided at their request.

While in the orientation session, women were preliminarily scheduled for a laboratory session corresponding to their current position within the menstrual cycle. Those who were uncertain of their cycle-day while in the lab were not scheduled immediately and instead asked to make note of the first day of their subsequent cycle and contact the primary researcher upon reaching it. If women, who were preliminarily scheduled, obtained a positive test result using the ovulation test strips prior to the scheduled session, they were instructed to contact the researchers immediately to reschedule their session to within 24 hours of the achievement of the positive result. The LL phase was confirmed retrospectively by asking all women to contact the researchers upon the start of their next menstrual period. Counting backwards from this day provided information which served to corroborate both participant self-reports of cycle regularity, as well as the approximate day of the cycle on which participants were initially tested. This information was also used to book second laboratory sessions if necessary. At the end of the initial laboratory session, a subsequent laboratory session was scheduled corresponding to participants' current position in the menstrual cycle (as described above, these appointment times

may have been altered slightly following contact with participants indicating day one of a subsequent cycle).

For each group of women (PMS and control groups), descriptive data including mean cycle day at the time of laboratory testing in both menstrual phases, range of days tested during each phase, and phase at time of session one, is found in Table 1. Information provided in Table 1 is based on the method of reverse counting in which the LF and LL cycle phases are measured in relation to day one of the menstrual cycle, thereby controlling for cycle length (Jöchle, 1973). Thus, for the purposes of reporting, day counts were established by counting backwards from day one of menses. This method has been found to be more reliable than forward counting, as research has shown greater variability in follicular phase duration than that of the luteal phase. In other words, the number of days in the luteal phase is more consistent than the number of days in the follicular phase, and variability in the follicular phase therefore accounts for most of the variation in total cycle length (e.g., Pillsworth, Haselton, & Buss, 2004). Using the day counts, two independent samples *t*-tests were employed to assess differences between groups at the time of testing in each phase. These tests revealed no differences between control women and women with PMS symptoms at the time of testing in either the LF, $t(32) = -0.30$, $p = .77$, or LL, $t(32) = -0.15$, $p = .89$, phases of the menstrual cycle. As well, no differences were indicated between control ($M = 157.41$, $SD = 7.83$) women and women with PMS symptoms ($M = 157.56$, $SD = 8.37$) in terms of conscientiousness scores, $t(31) = -0.05$, $p = .96$. Finally, no differences were observed between women in control ($M = 6.28$, $SD = 10.55$) and PMS ($M = 7.19$ $SD = 9.13$) groups in terms of general anxiety, $t(32) = -0.27$, $p = .79$.

Laboratory Sessions. Prior to beginning testing, and upon completion of the laboratory questionnaire, participants were asked to complete Consent Form B (see Appendix H).

Table 1

Mean Cycle Day at Time of Laboratory Testing in the Late Follicular (LF) and Late Luteal (LL) Phases of the Menstrual Cycle for Women in Control (N = 18) and PMS (N = 16) Groups, Range of Testing Days in Each Phase, and Number of Participants Tested in Session One for Each Phase

	Group		
Menstrual Cycle Parameter	Control (*N* = 18)	PMS (*N* = 16)	Total (*N* = 34)
Mean Cycle Day at LF Testing	-14.67	-14.44	-14.56
Range of LF Testing Days	-11 to -20	-10 to -19	-10 to -20
Mean Cycle Day at LL Testing	-4.22	-4.13	-4.18
Range of LL Testing Days	-1 to -8	-1 to -7	-1 to -8
Number of Participants in LF Phase at Session One	13	9	22
Number of Participants in LL Phase at Session One	5	7	12
Number (Percentage) of Women testing Positive for LH Surge[a]	13 (72)	11 (69)	25 (74)

Note. Cycle day counts are based on a method of reverse count to the Luteinizing Hormone (LH) surge

[a]All women testing positive for the LH surge were tested within 24 hours following the result. Women not reporting a positive result (*N*=9) were included in analyses given that their reverse count testing day was within the appropriate phase.

Following the completion of Consent Form B, all participants underwent the visual screening protocol (i.e., the FrACT and the Ishihara pseudoisochromatic plate test). They then provided the first of three portions of the salivary estradiol sample. Each laboratory session involved the assessment of S-cone and L+M cone functionality via the use of both SWAP and CCS procedures. Additionally, participants were administered the TDT, and asked to participate in the eye-tracking procedure. While in the lab, the order of tasks administered to all women following the SWAP procedure was counterbalanced. The remainder of the salivary sample was collected at two intervals during the lab session: once following the SWAP procedure and again after completing all of the experiments. Samples were refrigerated during collection intervals and frozen at -21°C following the final collection and completion of the lab session. Upon completion of the study, all women were provided with Debriefing Form B (see Appendix I). In order to attempt to control for time of day effects, efforts were made to schedule each participant's two lab sessions at roughly the same time of day. With the exception of two participants whose lab sessions were scheduled five and six hours apart, the remainder of participants' sessions were scheduled within three hours of each other. Analysis of the time of day data revealed that the average time of testing for laboratory sessions was in the early afternoon. Independent samples *t*-tests revealed no differences between the time of testing in either the LF or LL phase for control women $t(31) = -0.10$, $p = .99$ (LF: $M = 14{:}07$, $SD = 2{:}03$, LL: $M = 14{:}07$, $SD = 2{:}11$), women with PMS symptoms, $t(30) = -0.84$, $p = .41$ (LF: $M = 13{:}32$, $SD = 2{:}32$, LL: $M = 14{:}27$, $SD = 3{:}36$), or the overall sample of women, $t(63) = -0.70$, $p = .49$ (LF: $M = 13{:}50$, $SD = 2{:}17$, LL: $M = 14{:}17$, $SD = 2{:}56$). Thus, time of day is not a potential confound for the interpretation of group differences in either phase or between groups.

Salivary Estradiol Assay. Saliva samples were analyzed using a standardized Salimetrics™ protocol (for a detailed overview of this protocol, see Appendix C). Plate arrangements were determined a priori and 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 standard (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, 4 pg/mL, 2pg/mL, and 1pg/mL for tubes 1 through 6 respectively. Adhering to the plate layout determined *a priori*, 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. Concentrations of controls and unknowns were determined using SoftMax Pro® software through interpolation with a 4-parameter sigmoid minus curve fit.

Data Analysis

Final group assignments.

As noted above, women were initially screened using retrospective reports of PMS symptoms in the LL phase of the menstrual cycle as assessed by all 8 scales on the MDQ. However, final group assignments for the PMS and control groups were determined using: (1) retrospective menstrual cycle data provided by six MDQ scales found within the screening questionnaire (pain, concentration, behaviour change, autonomic reactions, water retention, and negative affect), (2) prospective data provided by three MDQ scales (pain, water retention, and negative affect) within laboratory questionnaires completed during experimental sessions corresponding to both menstrual cycle phases, and (3) scores on the LIPS questionnaire, a measure of pre-menstrual symptoms created by the authors.

Five criteria were used to categorize women into either PMS or control groups. Women were assigned values of either *0* or *1* (corresponding to a control or PMS assignment) respectively for each of the five criteria listed below, resulting in a possible summed score ranging from 0 to 5. After assessing information provided by each woman for each criterion, women with scores of three or more were assigned to the PMS group, while those with scores less than three were assigned to the control group. The five criteria used are as follows: (1) retrospectively reported mean premenstrual phase MDQ scores for the entire sample of women who completed the screening questionnaire (N= 188) (i.e., the mean of the sum of scores for the PMS symptoms four days prior to the onset of menses for the entire sample). Women with a

score above the mean were coded as *1* while those with a score below were coded as *0*; (2) The second criterion used was the retrospectively reported change in MDQ score for each woman between the late luteal phase of the menstrual cycle and the remainder of the menstrual cycle. Percent change was used to examine the increase in scores for all women who participated in the study. Those whose scores increased (or changed in a positive direction) from the non-late luteal to the late luteal phase were coded as *1*, while those whose scores remained the same or decreased were coded as *0*; (3) Prospective data on PMS symptoms provided during the sessions by the laboratory questionnaires were used to create a late luteal phase MDQ score based on the sum of the three scales (i.e., pain, water retention, and negative affect). The mean score for all participants was then calculated. Using this information, women with scores higher than the mean were coded as *1*, while those women with scores equal to or lower than the mean were coded as *0;* (4) Percent change in prospectively measured PMS symptoms between the LF and LL phase laboratory sessions was used as criterion four. The aggregate score based on the three MDQ scales for both sessions was examined. Women with an increase in symptoms between the late follicular phase and the late luteal phase were coded as *1* while those whose score decreased or remained the same were coded as *0*; (5) Finally, the mean score on the LIPS for the entire group of women who completed the screening questionnaire (*N*=188) was used as a fifth criterion, with women scoring above the mean coded as *1,* and women with scores falling at or below coded as *0*. Using these five criteria, 18 women were grouped as controls and 16 women were grouped as having PMS symptoms. A final total score consisting of the sum of *z*-scores for criteria 1 to 5 (PMS 5) was also created, given evidence that aggregate scores are most reliable (Ossenkopp & Mazmanian, 1985). Means and standard deviations for control and PMS groups for all classification parameters are found in Table 2. In order to examine group differences on

Table 2

Means and Standard Deviations of Classification Parameters for Women in Control (N= 18) and PMS Groups (N=16). Parameters Included: 1) Retrospective Menstrual Distress Questionnaire (MDQ) Scores (6 Scales) Four Days Prior to the Onset of Menses, 2) Percent Change in Retrospective MDQ Score Between the Remainder of Cycle and Four Days Prior to the Onset of Menses, 3) Prospective MDQ Ratings (3 Scales) Obtained During the Laboratory Session Conducted in the Late Luteal Phase of the Menstrual Cycle, 4) Percent Change in Prospective MDQ Scores from Laboratory Sessions in the Late Follicular (LF) and Late Luteal (LL) Phases of the Menstrual Cycle, and 5) Scores on the Lakehead Inventory of Premenstrual Symptoms (LIPS).

	Group	
Classification Measure	Control *M* (SD) (*N*=18)	PMS *M* (SD) (*N*=16)
1. Retrospective MDQ Scores Four Days Prior to the Onset of Menses***	41.94 (12.53)	67.47 (25.38)
2. Percent Change in Retrospective MDQ Scores Between the Remainder of Cycle and Four Days Prior to the Onset of Menses**	0.74 (11.06)	18.04 (19.36)
3. Prospective MDQ Scores During the Late Luteal Phase Laboratory Session**	7.17 (5.71)	16.53 (10.58)
4. Percent Change in MDQ Scores Between Laboratory Sessions	-29.47 (84.41)	13.12 (59.98)
5. Scores on the Lakehead Inventory of Premenstrual Symptoms (LIPS)**	11.83 (5.59)	22.53 (9.65)
PMS 5 Score	-4.47 (20.05)	12.92 (18.67)

NOTE: The PMS 5 score is based on the summation of the z-scores of the five numbered classification variables

^{t} $p < .10$, $*p \leq .05$, $**p \leq .01$. $***p \leq .001$

the five classification parameters, a MANOVA was calculated in which the five classification variables were entered as dependent variables, and group (control or PMS) was entered as the between subjects variable. For this test, Box's test of equality of variance was returned within acceptable limits ($p > .05$). The MANOVA revealed a significant overall effect indicating group differences in classification scores between control and PMS women, $F(5, 28) = 51.69$, $p = .011$, $\eta^2 = .40$, observed power = .87. Follow-up ANOVAs revealed that women with PMS symptoms exhibited significantly: (1) higher scores on retrospective reports of PMS symptoms four days prior to the onset of menses, $F(1, 32) = 13.17$, $p = .001$, $\eta^2 = .29$, observed power = .94, (2) a higher amount of change on retrospective scores between the LF and LL phases of the menstrual cycle, $F(1, 32) = 11.54$, $p = .002$, $\eta^2 = .27$, observed power = .91, (3) higher scores on prospective ratings of symptoms of premenstrual syndrome in the laboratory session conducted in the LL phase, $F(1, 32) = 9.54$, $p = .004$, $\eta^2 = .23$, observed power = .85, and (4) higher scores on the LIPS, $F(1, 32) = 14.35$, $p = .001$, $\eta^2 = .84$, observed power = 1.00. No group difference was found for the change in prospective symptom ratings between the two laboratory sessions, $F(1, 32) = 2.19$, $p = .15$, $\eta^2 = .06$, observed power = .30, although the direction of the means was consistent with the PMS group having more symptoms in the LL phase. Finally, significant between group differences were observed on the PMS 5 variable, $t(32) = -2.32$, $p = .027$.

Data reduction and organization. The testing of women in two groups at two distinct phases of the menstrual cycle created a nested model in which the data produced was organized at more than one level. For all experiments, menstrual cycle phase constituted a within subjects independent variable (IV) and group constituted a between subjects IV. Each experimental procedure contributed a unique set of dependent variables (DVs) represented by task-

performance. This design allowed for the examination of group effects, phase effects and group x phase effects.

Chromatic contrast sensitivity (CCS). Chromatic "red-green" (R/G) CCS and "blue-yellow" (B/Y) CCS functions were examined across three spatial frequencies (0.5, 1.5, and 4 cpd) presented at three orientations (0°, 45°, and 90°) in each menstrual cycle phase for each participant.

In order to establish the unique properties of each visual stream, as well as to examine initial and overall effects of group and phase for CCS, a four factor repeated-measures ANOVA was conducted in which colour (2-levels), menstrual cycle phase (2-levels), frequency (3-levels), and orientation (3-levels) were entered as within subjects factors (IVs) and group was used as the between subjects factor (IV). Following this analysis, two additional three-factor repeated measures ANOVAs were conducted to examine effects unique to each visual stream. As with the overall analysis, phase, frequency and orientation constituted the within subjects IVs and group represented the between subjects IV.

Mean CCS values corresponding to unadjusted data were plotted to compare between-subjects groups. Curves were fit according to a double exponential template (Movshon & Kiorpes, 1988), with error bars expressing the standard error of the means. Of note, significant effects involving only spatial frequency variables were omitted from the Results section for brevity and because the observed effects conformed to expected, nonlinear, frequency-dependent low or band-pass CCS functions that are unrelated to the questions posed in the current study. For example, significant main effects of spatial frequency on contrast sensitivity across the range of 0.5 to 4.0 cpd were expected, and found, for all groups of participants. Of course, significant interactions of spatial frequency with any of the between-subjects group and/or within menstrual

cycle phase variables were reported in the Results sections, as were significant main effects for group and cycle phase.

The influence of estradiol on CCS was examined using a series of hierarchical regressions in which concentrations of estradiol in each of the LF and LL phases were entered at step one, and percent change in estradiol between phases was entered at step two. These analyses were performed for the pooled sample of women and women in each of the control and PMS groups for all three conditions of orientation (0°, 45°, and 90°), and for both R/G and B/Y presentations.

Short-wavelength automated perimetry (SWAP). S-cone sensitivity was assessed °initially within three radial zones of retinal eccentricity: central, paracentral, and peripheral. In order to further elucidate more localized areas of sensitivity, these regions were subdivided further into five hemiretinal zones: central, superior nasal, inferior nasal, inferior temporal, and superior temporal. As with the CCS measurements, initial measurements of S-cone sensitivity were assessed using two repeated measures ANOVAs. The first set of analyses divided the retina into three zones according to eccentricity, with the central zone referring to 1°- 6° of visual angle, the paracentral zone referring to 10°- 22° visual angle, and the peripheral zone referring to 30°- 50° visual angle. The second set of analyses divided the retinal area into five hemiretinal locations involving a central section (1°- 6°) and four outer quadrants (10°- 50°) corresponding to the superior nasal, inferior nasal, inferior temporal, and superior temporal sections. For both analyses, menstrual cycle phase (LF or LL) was entered as a within subjects IV, and group (control or PMS) was used as a between subjects IV. Subsequent to these analyses, a series of forced entry linear regressions was used to investigate the influence of estradiol levels on S-cone sensitivity in six areas of retinal eccentricity. For these analyses, concentrations of estradiol in

each phase were used as predictors, while the total sensitivity scores for each region in each phase were used as DVs. Finally, the relationship between S-cone sensitivity and changes in estradiol across the cycle was examined for the pooled sample of women and for each group using a series of bivariate correlations.

Texture discrimination task (TDT). With respect to the TDT task, all women were trained in their first laboratory session irrespective of whether they were in their LF or LL menstrual cycle phase. Although optimal conditions would have allowed for complete counterbalancing of training and testing sessions such that women were trained and tested in each phase (creating a total of four sessions), practical constraints such as timing, scheduling, and participant availability as well as task specific confounds such as the retinotopic nature of the TDT paradigm, and practice effects prohibited this. Thus, in order to examine the influence of group and cycle phase at the time of training and testing, performance at each level of SOA was examined using a two-level repeated measures ANOVA. For this ANOVA, group (control or PMS) and training-testing order (LL-LF or LF-LL) were used as between subjects IVs and relative training-testing change scores for the five levels of SOA were used as DVs. Finally, as with the CCS and SWAP analyses, five multiple regressions (one for each level of SOA) were performed to examine the influence of session order and estradiol levels in each phase on the proportion of improvement in scores between training and testing for each SOA. For these regressions, the five proportion improvement scores corresponding to each level of SOA, were used as DVs and concentrations of estradiol in the LF phase, concentration of estradiol in the LL phase, and session order were employed as the three predictors. These regressions were performed separately for each group of women as well as for the pooled sample of participants.

Smooth pursuit eye tracking. During the eye-tracking procedure, all women tracked with their dominant eye for 10 complete cycles for each of 5 temporal frequencies: 0.25 c/sec or hertz (Hz), 0.5 Hz, 1.0 Hz, 1.25 Hz, and 2.0 Hz. Following completion of all trials, the 10 cycles were reduced to an average sinusoidal function for each participant with the assumption that the aggregate score would best capture women's average eye tracking accuracy (e.g., Ossenkopp & Mazmanian, 1985). Sinusoidal gaze patterns were created during the procedure as participants followed the "yellow" circular stimulus from center to left and back through center to right on the computer monitor. The left and right positions made up the sinusoidal pattern with extreme left and right position excursions defining the peaks and troughs. Excursions of the stimulus and the registered eye movements were made in degrees visual angle: left being positive and right being negative. The extreme left and right positions minus the center position defined the amplitudes of the sinusoidal smooth pursuit pattern (i.e., ±13.3°). In order to facilitate data analysis, the final averaged sinusoidal function for each woman was deconstructed into five positional phases at 0°, 90°, 180°, 270°, and 360° allowing for examination of gaze at several points. Again, 0°, 180° and 360° defined the center position from start to completion of an entire cycle. Ninety and two-hundred-and-seventy degrees defined left (peak) and right (trough) positions, respectively. Following the averaging of 10 complete cycles, the five phase eye positions were entered into a three-factor mixed ANOVA using menstrual cycle phase, frequency, and position as within subjects IVs and group as a between subjects IV. As with all other experiments, the two levels of analysis for menstrual cycle phase were represented by the LF and LL positions. Frequency and position were each defined using five levels as indicated above.

Results

Data Screening

Prior to conducting all analyses, the dependent variables (DVs) were examined as a function of group for the presence of univariate and multivariate outliers (e.g., Tabachnick & Fidell, 2007), as well as for skewness and kurtosis. Examination of Mahalonobis distances revealed no multivariate outliers; however, a number of univariate outliers were noted (see below).

With respect to the salivary estradiol measurements, three univariate outliers were detected. Two of these were for the same participant in the PMS group in each of the LF (25.85 ρg/mL) and LL (20.61 ρg/mL) phases of the menstrual cycle, with the third outlier found for a women in the PMS group in only the LL (10.85 ρg/mL) phase. Consultation with the literature (e.g. Hampson, 2008; Rosoen & Lopez, 2009) revealed that although the shift in concentration was appropriate between phases (e.g., estradiol concentration higher in late follicular than late luteal phase), the participant with two outliers had concentrations of estradiol higher than what is typically observed in women of the same age. Similarly, the third outlier was slightly outside of the range of reported estradiol levels during the LL phase of the menstrual cycle. Comparison with the estradiol values of pregnant women suggests that it is highly unlikely that the high estradiol levels were due to pregnancy. It is more likely that the higher values are due to contamination of the sample. Thus, these three estradiol values were not used in any analyses, yet the remainder of the participants' data was retained.

Using the criteria of standard error (SE) skewness/skewness and examination of histograms with normal curves (e.g. Tabachnick & Fidell, 2007), the relative CCS data was revealed to be significantly skewed. This was not surprising, given the inherent nonlinear nature

of human CCS. Logarithmic transformation of this data improved the shape of the distributions, however, a proportion of univariate outliers were still noted. Of the 1224 CCS values recorded across 36 unique spatial frequency, orientation, and chromatic conditions measured in each menstrual cycle phase, 55 (4.5%) cases were identified as extreme values based upon interquartile range distances, *z*-scores, and box plots. Given the potential influence of these extreme scores in data analysis, the transformed values were winsored at a *z*-score of +/- 3.29 (or $p = .001$) of the mean value for the respective condition. Thus, they retained their rank order and some distance from the remainder of values, but the possible effect of their separation was reduced. These points were distributed across 9 women in the control group and 16 women in the PMS group. Given the heterogeneity of conditions in the CCS study and the fact that none of these women demonstrated a greater than one log unit separation between their data and that of the rest of the sample for any given condition, it was unlikely that the adjustment of their scores would lead to artificial effects of phase. Thus, the decision was made to make an adjustment in favour of correcting the skewness of the distribution.

Similar screening and adjustment procedures were employed with SWAP data, resulting in modification of 12 (2.5%) extreme cases among 476 total observations. Following these adjustments, the distributions of contrast sensitivity and S-cone sensitivity values within groups and across conditions fell within acceptable limits of normality. Examination of the changed data points indicated that for each of the control and PMS groups one participant accounted for 10 of 12 (4 for the control women and 6 for the women in the PMS group) of these changes and that the data was split evenly between phases. Thus, given that these women tended to operate in the extreme range of sensitivities across both phases, it is less likely that a phase effect would be artificially detected.

For the TDT task, participant data was screened using two criteria. First, only women who completed both training and testing sessions were included. Second, results were included in analyses only if women were able to discriminate the T/L fixation in the majority of conditions in both testing and training conditions. Discrimination of fixation was defined as the ability to correctly identify 60% or more of the initial letter presentations. This was especially relevant at the beginning of the task at higher SOA levels. As women progressed through the procedure and the SOAs decreased accordingly, some women were also unable to reach the 60% fixation discrimination. Those, however, whose data fit a profile indicating an ability to discriminate at higher SOA levels, thereby implying an understanding of the task, but who could not reach 60% discrimination at lower, more difficult SOAs were nonetheless included. For the training data, 14 fixation averages below 60% between SOA 80 and SOA 40 were included, while for the testing data 8 fixation averages below 60% corresponding to either SOA 60 or SOA 40 were included. These two criteria resulted in the removal of seven women from the final analysis. Finally, as most women were performing at or below chance at SOA 20 in the testing phase, this condition was removed from the final analysis.

With respect to the eye-tracking procedure, each woman completed 10 cycles at each of five target oscillating frequencies (in order): 0.25, 0.5, 1.0, 1.25, and 2.0 Hz. All eye movement data were fitted with sinusoidal wave functions corresponding to the sinusoidal movement of the stimulus. For each cycle, eye position was measured continuously at a temporal resolution of 20 msec. Precision of timing sequence and target position was confirmed with high-speed video synchronized with oscilloscopic tracings of a voltage signal marker generated by the VISage program every 20 msec. Smooth pursuit eye movements (averaged across 10 cycles) were plotted with the target movement in degrees visual angle as a function of time (in msec).

Saccades and missed tracks (e.g., blinking) were filtered using a cutoff amplitude of ±20° visual angle. Women with values exceeding these filter constraints at all tracked positions were assigned a value of zero degrees for that point within the function. This was done for all cycle values prior to the 10-cycle averaging. Those women who showed extreme eye positions and exceeded the filter parameter more than 6 times across the 10 tracked cycles were not included in the final grand means used across clinical groups. This procedure resulted in six women being omitted from the control group and three women being omitted from the PMS group.

Salivary Estradiol Assay

Results of the salivary estradiol assay were initially examined using a 2 x 2 ANOVA with estradiol levels in each menstrual phase (LF and LL) used as within subjects variables, and group (control or PMS) used as the between subjects factor. The means and standard deviations for the estradiol levels are reported in Table 3 as a function of group and phase. For the ANOVA, Box's test of equality of covariance was returned within normal limits. The ANOVA did not indicate an effect of group, $F(1, 30) = 0.008$, $p = .93$, $\eta^2 = .00$, observed power = .05. However it did reveal an overall effect of phase $F(1, 30) = 6.12$, $p = .019$, $\eta^2 = .17$, observed power = .67, with higher estradiol in the LF than LL phase; as well as a trend for a group x phase effect, $F(1, 30) = 3.34$, $p = .078$, $\eta^2 = .10$, observed power = .42.

In order to further examine these findings, paired samples *t*-tests were used to examine phase effects within the two groups. Differing results were found for the PMS and control groups. For the PMS group, a paired samples *t*-test revealed no differences between estradiol concentrations in the LF and the LL phases, $t(14) = 1.00$, $p = .33$. For the control group, however, a paired samples *t*-test revealed significantly higher estradiol concentrations in the LF than the LL phase of the menstrual cycle, $t(17) = 3.48$, $p = .003$. Thus, these analyses revealed that, unlike the

Table 3

Means and Standard Deviations of Estradiol Levels as Determined by SALIMETRICS® Salivary Immunoassay in the Late Follicular (LF) and Late Luteal (LL) Phases of the Menstrual Cycle in a Group of Women Reporting Symptoms of Premenstrual Syndrome (PMS) (N= 14) and a Group of Control Women (N= 18)

	Estradiol Level (ρg/mL)		
Group	Late Follicular Phase (*M, SD*)	Late Luteal Phase (*M, SD*)	Overall Mean (*M, SD*)
Women Reporting PMS Symptoms	4.91 (1.64)	4.74 (1.77)	4.83 (0.35)
Control Women	5.43 (1.77)	4.31 (1.24)	4.87 (0.31)
Grand Mean	5.20 (1.71)	4.50 (1.27)	4.85 (0.24)

control women, women with PMS symptoms did not show phasic cycling of estradiol across the menstrual cycle (see Figure 5). Overall, it appears that women are exhibiting the expected tendency to show higher estradiol levels in the LF phase and lower levels in the LL phase; thereby providing additional confirmation that women were tested at the appropriate times in their menstrual cycles. Further analysis of the same data, however, shows that the control group may be accounting for this phase effect as they, and not the group of women with PMS symptoms, show an overall effect for a change in estradiol between cycle phases. As noted earlier, this effect cannot be explained by differences in the testing days, as there were no significant differences in testing days between the groups.

Chromatic Contrast Sensitivity (CCS)

Initial effects for relative CCS were explored using a four-factor repeated measures ANOVA. In this analysis, the within subjects factors consisted of colour (2 levels), phase (2 levels), frequency (3 levels), and orientation (3 levels). For colour, the two levels of analysis were R/G and B/Y contrast presentations; for phase the two levels of analysis were LL and LF; levels of analysis for spatial frequency were represented by 0.5, 1.5, and 4 cycles/degree (cpd); and finally the three levels of orientation were defined as 0°, 45°, and 90°, where 0° was representative of a horizontal orientation. Group (control and PMS) was entered as the between subjects factor. For both R/G and B/Y conditions, means and standard deviations for CS are reported for frequency, orientation, and phase in Tables 4, 5, and 6. This analysis confirmed an overall effect of colour, indicating higher overall relative CCS for RG stimuli ($M = 2.07$, $SD = .032$) compared to BY stimuli ($M = 1.81$, $SD = .020$), $F(1, 32) = 105.99$, $p < .001$, $\eta^2 = .77$, power = 1.0, indicating higher overall sensitivity for these stimuli. There was also a significant effect of phase, indicating higher relative CCS in the LL ($M = 1.96$, $SD = .029$) compared to the LF phase

Table 4

Means and Standard Deviations for "Red-Green" Log Contrast Sensitivity Measurements for Three Spatial Frequencies at 0°, 45°, and 90° Spatial Orientations in the Late Follicular (LF) and Late Luteal (LL) Phases of the Menstrual Cycle for Control (N=18) and PMS Groups (N=16)

Control Group

Frequency	Late Follicular Phase (*M, SD*)			Late Luteal Phase (*M, SD*)			Grand Mean (*SE*)
	0°	45°	90°	0°	45°	90°	
0.5 Hz	2.36 (0.19)	2.33 (0.21)	2.34 (0.22)	2.32 (0.36)	2.37 (0.30)	2.39 (0.24)	2.35 (0.05)
1.5 Hz	2.21 (0.29)	1.97 (0.19)	2.11 (0.26)	2.31 (0.40)	2.11 (0.26)	2.24 (0.35)	2.16 (0.05)
4.0 Hz	1.80 (0.29)	1.72 (0.27)	1.74 (0.22)	1.88 (0.25)	1.70 (0.35)	1.89 (0.25)	1.79 (0.05)
Marginal Mean (*SE*)	2.12 (0.05)	2.01 (0.05)	2.07 (0.05)	2.17 (0.06)	2.06 (0.06)	2.17 (0.06)	2.10 (0.04)

PMS Group

Frequency	Late Follicular Phase (*M, SD*)			Late Luteal Phase (*M, SD*)			Grand Mean (*SD*)
	0°	45°	90°	0°	45°	90°	
0.5 Hz	2.19 (0.25)	2.12 (0.29)	2.26 (0.24)	2.26 (0.30)	2.26 (0.27)	2.18 (0.21)	2.21 (0.05)
1.5 Hz	2.13 (0.25)	2.06 (0.29)	2.07 (0.35)	2.14 (0.40)	2.07 (0.33)	2.14 (0.26)	2.10 (0.05)
4.0 Hz	1.76 (0.25)	1.68 (0.24)	1.80 (0.20)	1.86 (0.28)	1.62 (0.21)	1.91 (0.23)	1.77 (0.05)
Marginal Mean (*SE*)	2.03 (0.05)	1.95 (0.05)	2.05 (.053)	2.09 (0.06)	1.98 (0.06)	2.08 (0.06)	2.03 (0.05)

Table 5

Means and Standard Deviations for "Blue-Yellow" Log Contrast Sensitivity Measurements for Three Spatial Frequencies at 0°, 45°, and 90° Spatial Orientations in the Late Follicular (LF) and Late Luteal (LL) Phases of the Menstrual Cycle for Control (N=18) and PMS Groups (N=16)

Control Group

Frequency	Late Follicular Phase (*M, SD*)			Late Luteal Phase (*M, SD*)			Grand Mean (*SE*)
	0°	45°	90°	0°	45°	90°	
0.5 Hz	2.03 (0.15)	2.11 (0.15)	2.03 (0.20)	2.09 (0.19)	2.22 (0.23)	2.14 (0.18)	2.10 (0.03)
1.5 Hz	1.93 (0.16)	1.99 (0.12)	2.01 (0.23)	1.97 (0.23)	1.97 (0.17)	2.03 (0.24)	1.98 (0.03)
4.0 Hz	1.43 (0.18)	1.36 (0.11)	1.49 (0.16)	1.42 (0.18)	1.41 (0.11)	1.49 (0.21)	1.43 (0.03)
Marginal Mean (*SE*)	1.79 (0.03)	1.82 (0.04)	1.84 (0.03)	1.83 (0.04)	1.86 (0.04)	1.89 (0.04)	1.84 (0.03)

PMS Group

Frequency	Late Follicular Phase (*M, SD*)			Late Luteal Phase (*M, SD*)			Grand Mean (*SE*)
	0°	45°	90°	0°	45°	90°	
0.5 Hz	1.96 (0.22)	2.04 (0.15)	2.06 (0.17)	2.02 (0.25)	2.06 (0.16)	2.03 (0.23)	2.02 (0.04)
1.5 Hz	1.94 (0.16)	1.92 (0.12)	1.93 (0.15)	1.96 (0.18)	1.91 (0.22)	1.96 (0.26)	1.94 (0.03)
4.0 Hz	1.40 (0.17)	1.30 (0.12)	1.40 (0.15)	1.48 (0.20)	1.36 (0.20)	1.43 (0.18)	1.40 (0.03)
Marginal Mean (*SE*)	1.77 (0.03)	1.75 (0.02)	1.80 (0.03)	1.82 (0.04)	1.78 (0.04)	1.81 (0.04)	1.79 (0.03)

Table 6

Means and Standard Errors for "Red-Green" and "Blue-Yellow" Log Contrast Sensitivity Measurements Collapsed Across Frequency and Orientation for Control Women (N=18) and Women with PMS Symptoms (N=16) in the Late Follicular (LF) and Late Luteal (LL) Phases of the Menstrual Cycle

"Red-Green" Chromatic Contrast Sensitivity		
Group	Late Follicular Phase (*M*, *SE*)	Late Luteal Phase (*M*, *SE*)
Control	2.07 (.044)	2.14 (.053)
PMS	2.01 (.046)	2.05 (.056)
Grand Mean	2.04 (.032)	2.09 (.039)

"Blue-Yellow" Chromatic Contrast Sensitivity		
Group	Late Follicular Phase (*M*, *SE*)	Late Luteal Phase (*M*, *SE*)
Control	1.82 (.023)	1.86 (.035)
PMS	1.77 (.025)	1.80 (.037)
Grand Mean	1.80 (.017)	1.83 (.026)

(M = 1.92, SD = .021) of the menstrual cycle, $F(1, 32) = 5.83$, $p < .02$, $\eta^2 = .15$, power = .65. No main effect was indicated for group. Results of the ANOVA reflecting trends and main effects are reported in Table 7. The overall effect of colour served to confirm the operation of discrete system-specific operations with respect to the PC and KC visual streams.

As such, two mixed ANOVAs were conducted for each of the relative R/G and B/Y chromatic contrast conditions. Consequently this enabled us to examine any effects unique to the associated visual streams.

R/G chromatic contrast sensitivity. For both PMS and control groups, log sensitivity was plotted as a function of log spatial frequency for each of 0°, 45°, and 90° orientations (see Figure 6). For this condition, a three-level mixed ANOVA was conducted using menstrual cycle phase (LF and LL), frequency (0.5 cpd, 1.5 cpd, and 4.0 cpd) and orientation (0°, 45°, and 90°) as within subjects IVs and group (PMS or control) as the between subjects variable. A summary of effects is presented in Table 7.

For this analysis, Mauchley's test of sphericity was returned within acceptable limits ($p > .05$). As such, no corrections were employed in the reporting of the following findings. All efforts were made to counter-balance women according to menstrual cycle phase at time of session one and time of session two, however, to determine the potential influence of a phase x session effect, a confirmatory ANCOVA using cycle phase at time of training as a covariate was conducted to rule out possible practice effects. This analysis revealed no effect of session order on performance, $F(1, 31) = 0.29$, $p = .59$.

For the R/G stimulus condition, there was no evidence of a group effect, indicating that PMS and control groups did not show overall differences in R/G CCS. There was a significant main effect of orientation on CCS, $p < .001$, indicating that relative sensitivity for the 45° gabor

Table 7

ANOVA Table Showing Overall and System Specific Parvocellular and Koniocellular Effects in a Contrast Sensitivity Experiment with Three Frequency and Three Orientation Conditions

Overall Effects					
Dependent Variable	*F*	*df*	Error *df*	*p*	η^2
Group	1.64	1	32	.21	.05
Color***	105.99	1	32	< .001	.77
Phase*	5.83	1	32	.02	.15
Frequency***	748.27	2	64	< .001	.96
Orientation***	14.92	2	64	< .001	.30
Phase x Group	0.29	1	32	.59	.01
Color x Frequency***	32.22	2	64	< .001	.41
Color x Orientation***	10.19	2	64	< .001	.24
Frequency x Orientation***	8.12	4	128	< .001	.21
"Red-Green" (Parvocellular) Effects					
Dependent Variable	*F*	*df*	Error *df*	*p*	η^2
Group	1.19	1	32	.28	.04
Phase[t]	3.77	1	32	.061	.11
Frequency***	252.56	2	64	< .001	.89
Orientation***	16.48	2	64	< .001	.34
Group x Frequency*	4.03	2	64	.022	.11
Phase x Group	0.26	1	32	.610	.01
Frequency x Orientation**	3.86	4	128	.005	.11
Phase x Frequency x Orientation[t]	2.43	4	128	.051	.07
Frequency x Orientation x Group[t]	2.22	4	128	.070	.065
Phase x Frequency x Group*	1.26	2	64	.29	.04
"Blue-Yellow" (Koniocellular) Effects					
Dependent Variable	*F*	*df*	Error *df*	*p*	η^2
Group	1.73	1	32	.20	.05
Phase[t]	3.47	1	32	.07	.10
Frequency***	564.32	2	64	< .001	.95
Orientation*	3.36	2	64	< .04	.10
Group x Frequency	0.025	2	64	.70	.01
Phase x Group	0.09	1	32	.76	.003
Frequency x Orientation***	6.21	4	128	< .001	.16
Phase x Frequency x Orientation[t]	2.43	4	128	.59	.67
Frequency x Orientation x Group[t]	2.22	4	128	.80	.53
Phase x Frequency x Group*	3.46	2	64	.04	.10

[t] $p < .10$, *$p \leq .05$, **$p \leq .01$. ***$p \leq .001$

presentations was lowest of the three orientation conditions, and significantly lower than that for the 90° gabor, $p < .001$, $\eta^2 = .45$, observed power = 1.00. Sensitivity for 0° gabors did not differ from that of 90° gabors $F(1, 32) = .43$, $p = .52$, $\eta^2 = .013$, observed power = .098. Finally, a trend was revealed for the effect of phase, $p = .061$, indicating increased CCS in the LL phase as compared to the LF phase of the menstrual cycle. No group x phase effect was observed. For F values, and effect sizes, for all reported R/G CCS analyses, refer to the middle panel of Table 7.

With respect to two-way interactions between variables, a significant group x frequency effect was noted, $p = .022$, indicating that CCS measures at different frequencies differed for the PMS and control groups. To further examine this effect, contrasts were performed comparing each level of frequency across PMS and control groups. These revealed a significant difference between group performance at 0.5 cpd and 4.0 cpd, $F(1, 32) = 5.60$, $p = .024$, $\eta^2 = .15$, observed power = 0.63. Figure 7 illustrates the group x frequency interaction indicating that there are larger performance differences between control and PMS women at the lower 0.5 cpd frequency condition as opposed to the higher 4.0 cpd condition (which conforms to a typical, chromatic low-pass function as highlighted in the Data Analysis section of the Methods above). Follow-up analyses revealed that at the 0.5 cpd frequency condition, control women performed significantly better than women in the PMS group, $F(1, 33) = 4.50$, $p = .042$. Thus, the group difference was strongest at the lowest frequency condition.

Two three-way trends were reported for the chromatic R/G stimuli. Of particular note was a trend indicating a phase x frequency x orientation interaction, $p = .051$. With respect to this trend, contrasts illustrated that at the 45° the orientation, women exhibited higher CCS in the LL phase at 0.5 cpd and higher CCS in the LF phase at 4.0 cpd. In contrast, at 90° presentations, women exhibited greater CCS in the LF phase at 0.5 cpd and higher CCS at 4.0 cpd in the LL

phase of the menstrual cycle. $F(1, 32) = 12.24$, $p = .001$, $\eta^2 = .28$, observed power = 0.92. This effect is partially illustrated in the middle and right-hand panels of Figure 8. Thus, with respect to R/G stimuli, this interaction shows that CCS is differentially influenced by menstrual cycle phase when comparing oblique (45°) and vertical (90°) gabors at high frequency (4.0 cpd) presentations. Specifically, it shows that higher CCS performance at 4.0 cpd is found in the LF phase when gabors are rotated obliquely and the LL phase when gabors are presented vertically.

Finally, a trend was noted for a frequency x orientation x group effect, $p = .07$. Again, contrasts were used to deconstruct the finding. These comparisons revealed that for the 45° orientation condition, women in the control group exhibited lower CCS than women with PMS symptoms at 1.5 cpd, and higher CCS than women with PMS symptoms at 4.0 cpd. The reverse was observed with respect to the 90° orientation condition, in which women in the control group exhibited greater R/G chromatic CS than women with PMS symptoms with stimuli presented at 1.5 cpd, and lower CCS than women with PMS symptoms at 4 cpd presentations, $F(1, 32) = 4.84$, $p = .035$, $\eta^2 = .13$, observed power = 0.57 . Figure 9 shows that at both 45° and 90° orientations, however, CCS was noted to converge at 4 cpd presentations, revealing few differences between performances in groups at higher spatial frequencies.

B/Y chromatic contrast sensitivity. For both PMS and control groups, log sensitivity as a function of log spatial frequency was plotted for each of 0°, 45°, and 90° orientations (see Figure 10). As with the R/G stimuli, a three level mixed ANOVA was conducted using menstrual cycle phase, frequency, and orientation as within subjects factors and group as the between subjects factor. Levels of analysis were as above for the R/G CCS analyses. All trends and main effects are summarized in the bottom panel of Table 7.

Again, for all reported effects and trends, Mauchley's test of sphericity was returned within acceptable limits ($p > .05$) and as such, no corrections were employed in the reporting of the following findings.

As with the R/G contrast condition, there was no evidence of a significant group effect ($p = .20$), indicating that PMS and control groups did not show overall differences in B/Y CCS. A significant main effect of orientation on CCS for B/Y gabors was observed, $p = .041$. Contrasts revealed that sensitivity for 45° stimuli was significantly less than that for 90° stimuli, $F(1, 32) = 4.60$, $p = .04$, $\eta^2 = .13$, observed power = .55. A strong trend was also indicated for greater sensitivity for 90° stimuli over 0° stimuli, $F(1, 32) = 4.06$, $p = .052$, $\eta^2 = .11$, observed power = .50.

In addition to the main effect of orientation, a trend was revealed for the effect of menstrual cycle phase, $p = .072$, indicating increased CCS in the LL phase as compared to the LF phase of the menstrual cycle. Examination of Table 7 shows the highest sensitivity for women in the LL phase of the menstrual cycle at all orientation conditions. This effect can be seen in Figure 8.

With respect to two-way interactions, a significant frequency x orientation effect was found for the B/Y gabors, $p < .001$. Contrasts revealed that for 45° and 90° stimuli, sensitivity was significantly greater for 0.5 cpd than in for 4.0 cpd presentations, $F(1, 32) = 16.98$, $p < .001$, $\eta^2 = .35$, observed power = .98, and that for 0.5 cpd presentations, sensitivity was higher for 45° compared to 0° and 90° orientations. At 4.0 cpd, however, sensitivity for both orientations converges.

Finally, an effect of phase x frequency x group was observed, $p = .037$, in which differences in performance by phase and by group were most pronounced when comparing the

lower, 0.5 cpd presentations to the higher, 4.0 cpd frequency presentations, $F(1, 32) = 1.01$, $p = .016$, $\eta^2 = .17$, observed power = .70, with women in both groups showing higher CCS performance at 0.5 cpd presentations compared to 4.0 cpd presentations. Figure 11 best illustrates the three way interaction and shows that, CCS at all frequencies is highest for women in the LL phase of the menstrual cycle, and that this effect is most noticeable at the lower 0.5 and 1.5 cpd frequency presentations. Statistical contrasts, revealed that for both groups CCS for the highest 4.0 cpd frequency presentations was significantly less than that of the 0.5 cpd presentations $F(1, 32) = 703.95$, $p < .001$, $\eta^2 = .69$, observed power = 1.0, and 1.5 cpd presentations, $F(1, 32) = 90.29$, $p < .001$, $\eta^2 = .97$, observed power = 1.0 (see Figure 11). Paired samples *t*-tests revealed that only women in the control group demonstrated a significant difference in performance at a frequency of 0.5 cpd between the LF ($M = 6.16$, $SD = .40$) and LL phases ($M = 6.44$, $SD = .50$), $t(17) = 3.40$, $p = .003$. No additional significant differences were observed between phases for any group at any other frequency conditions.

Chromatic contrast sensitivity and estradiol. The influence of estradiol on R/G and B/Y CCS (collapsed across phases) at all three conditions of orientation for the pooled sample of participants, as well as for each of the control and PMS groups was explored using a hierarchical model of regression using concentrations of estradiol in each phase at step one and percent change in estradiol between phases {([LF]-[LL]/[LL]) *100} at step two. Results for the R/G analyses are reported in Table 8. These analyses revealed that for the R/G gabors, none of the overall models significantly predicted CCS. However, percent change in estradiol significantly added to prediction over and above estradiol levels in each phase for all three orientations. Furthermore, at all three orientations all three variables were significant unique predictors of CCS at step 2 (see Table 9; all $p < .05$). The model for the 45° condition also showed an overall

Table 8

Table Showing statistics for a Hierarchical Model of Regression Using Concentrations of Estradiol in both the Late Follicular (LF) and Late Luteal (LL) Phases at Step One, and Percent Change Estradiol Between the LF and LL Phases at Step Two in the Prediction of R/G Chromatic Contrast Sensitivity Across Phases at Three Conditions of Orientation for a Pooled Sample of Women (N= 32), a Control Group of Women (N= 18) and a Group of Women Reporting PMS Symptoms (N= 14)

Pooled Sample (*N*=32)

Orientation	Step	*df*	Error *df*	*R*	Adjusted R^2	*R* Square Change	*F*	*F* change
0°	1	2	29	.17	-.04	.03	0.45	
	2*	1	28	.40	.07	.13	1.79	4.36
45°	1	2	29	.10	-.06	.01	0.16	
	2**	1	28	.49	.16	.23	2.93	8.39
90°	1	2	29	.13	-.05	.02	0.26	
	2*	1	28	.42	.09	.16	1.97	5.32

Control Group (*N*=18)

Orientation	Model	*df*	Error *df*	*R*	Adjusted R^2	*R* Square Change	*F*	*F* change
0°	1	2	15	.08	-.13	.01	0.053	
	2*	1	14	.52	.11	.26	1.72	5.01
45°	1	2	15	.15	-.11	.02	0.18	
	2**	1	14	.68	.35	.44	4.04	11.50
90°	1	2	15	.02	-.13	.00	0.002	
	2*	1	14	.58	.20	.34	2.38	7.13

PMS Group (*N*=14)

Orientation	Model	*df*	Error *df*	*R*	Adjusted R^2	*R* Square Change	*F*	*F* change
0°	1	2	11	.30	-.08	.09	0.53	
	2	1	10	.31	-.18	.01	0.35	.07
45°	1	2	11	.11	-.17	.01	0.06	
	2	1	10	.25	-.22	.05	0.22	.54
90°	1	2	11	.21	-.13	.04	0.25	
	2	1	10	.25	-.22	.02	0.22	.18

NOTE: Significance is indicated for steps within the model not the overall model

$^{t}p < .10$, $*p \leq .05$, $**p \leq .01$. $***p \leq .001$

Table 9

Coefficients for a Hierarchical Model of Regression Using Concentrations of Estradiol in both the LF and LL Phases at Step One and Percent Change Estradiol Between the Late Follicular (LF) and Late Luteal (LL) Phases at Step Two in the Prediction of R/G Chromatic Contrast Sensitivity Collapsed Across Phases at Three Conditions of Orientation.

		Pooled Sample (*N*= 32)		
0°	*B*	SE *B*	β	*p*
[Estradiol LF]	1.29	0.57	1.66	.03
[Estradiol LL]	-1.60	0.71	-1.53	.03
% change [E]	-0.05	0.02	-1.45	.05
45°				
[Estradiol LF]	1.27	0.48	1.87	.01
[Estradiol LL]	-1.65	0.59	-1.82	.009
% change [E]	-0.06	0.02	-1.91	.007
90°				
[Estradiol LF]	-1.18	0.51	1.70	.03
[Estradiol LL]	-1.52	0.63	-1.64	.02
% change [E]	-0.05	0.02	-1.59	.03
		Control Group (*N*= 18)		
0°				
[Estradiol LF]	1.55	0.74	2.13	.06
[Estradiol LL]	-1.83	0.92	-1.75	.07
% change [E]	-0.07	0.03	-1.81	.04
45°				
[Estradiol LF]	1.57	0.52	2.65	.009
[Estradiol LL]	-2.00	0.64	-2.37	.007
% change [E]	-0.07	0.02	-2.35	.004
90°				
[Estradiol LF]	1.57	0.62	2.45	.02
[Estradiol LL]	-1.92	0.77	-2.09	.03
% change [E]	-0.07	0.03	-2.06	.02
		PMS Group (*N*= 14)		
0°				
[Estradiol LF]	0.49	1.12	0.60	.64
[Estradiol LL]	-0.67	1.40	-0.65	.67
% change [E]	-0.01	0.05	-0.37	.80
45°				
[Estradiol LF]	0.69	1.09	0.87	.54
[Estradiol LL]	-0.96	1.37	-0.97	.50
% change [E]	-0.03	.05	-1.06	.48
90°				
[Estradiol LF]	0.49	1.05	0.64	.65
[Estradiol LL]	-0.77	1.33	-0.81	.57
% change [E]	-0.02	0.05	-0.62	.68

strong trend at step 2 ($p = .051$) indicating that the three estradiol variables showed a trend to predict general CCS ability in women. The same pattern of results was observed for control women, except that the model for the 45° condition was significant at step 2 ($p = .029$), indicating that the three estradiol variables together predicted general CCS ability in women across the cycle. For the PMS women, none of the models showed any associations between the estradiol variables and CCS for R/G stimuli at any of the orientations. Thus, the strongest effects of estradiol on R/G CCS were found for control women with the 45°condition.

Examination of beta coefficients (see Table 9) reveals that the relationship between CCS performance and percent change in estradiol across all orientation conditions is negative. Thus, higher CCS is associated with smaller changes in estradiol across the cycle from the LL to LF phase.

Unlike the R/G CCS where estradiol best predicted CCS in the 45° orientation condition for control women, estradiol best predicted B/Y CCS in the 0° orientation condition for all women. As indicated in Tables 10 and 11, when all women were examined together, the three estradiol variables significantly predicted CCS at step 2, and all three estradiol variables were unique predictors for 0° and 45°. When the control women were examined, similar weaker effects were found only for 0°. Regressions using the PMS women indicated the same strong effects were found only for 0°. Thus, the strongest effects of estradiol on B/Y CCS were found for the PMS and control women with the 0° condition. As with the R/G stimuli, coefficients for the B/Y regression analyses (see Table 11) indicated that higher CCS was associated with a smaller shift in estradiol across the cycle from the LL to the LF phase.

Table 10

Table Showing Statistics for a Hierarchical Model of Regression Using Concentrations of Estradiol in both the Late Follicular (LF) and Late Luteal (LL) Phases at Step One and Percent Change Estradiol Between the LF and LL Phases at Step Two in the Prediction of B/Y Chromatic Contrast Sensitivity Across Phases at Three Conditions of Orientation for a Pooled Sample of Women (N= 32), a Control Group of Women (N= 18) and a Group of Women Reporting PMS Symptoms (N= 14)

Pooled Sample (*N*=32)

Orientation	Step	*df*	Error *df*	*R*	Adjusted R^2	*R* Square Change	*F*	*F* Change
0°	1	2	29	.37	.07	.13	2.24	
	2**	1	28	.61	.31	.24	5.53	10.61
45°	1	2	29	.33	.05	.11	1.75	
	2*	1	28	.54	.22	.19	3.89	7.40
90°	1	2	29	.12	-.06	.01	0.20	
	2*	1	28	.41	.08	.16	1.92	5.29

Control Group (*N*=18)

Orientation	Step	*df*	Error *df*	*R*	Adjusted R^2	*R* Square Change	*F*	*F* Change
0°	1	2	15	.24	-.07	.06	0.48	
	2*	1	14	.55	.16	.25	2.07	5.01
45°	1	2	15	.29	-.04	.09	0.70	
	2	1	14	.44	.02	.11	1.14	1.93
90°	1	2	15	.29	-.04	.09	0.71	
	2	1	14	.43	.01	.10	1.06	1.70

PMS Group (*N*=14)

Orientation	Step	*df*	Error *df*	*R*	Adjusted R^2	*R* Square Change	*F*	*F* Change
0°	1	2	11	.58	.21	.33	2.76	
	2*	1	10	.78	.48	.27	5.03	6.72
45°	1	2	11	.29	-.08	.09	0.51	
	2	1	10	.56	.11	.23	1.52	3.31
90°	1	2	11	.28	-.09	.08	0.47	
	2	1	10	.47	-.02	.14	0.92	1.75

NOTE: Significance is indicated for steps within the model not the overall model
$^{t}p < .10$, $*p \leq .05$, $**p \leq .01$. $***p \leq .00$

Table 11

Coefficients for a Hierarchical Model of Regression Using Concentrations of Estradiol in both the Late Follicular (LF) and Late Luteal (LL) Phases at Step One and Percent Change Estradiol Between the LF and LL Phases at Step Two in the Prediction of B/Y Chromatic Contrast Sensitivity Collapsed Across Phases at Three Conditions of Orientation.

	Pooled Sample (*N*= 32)			
0°	*B*	SE *B*	β	*p*
[Estradiol LF]	0.98	0.29	2.18	.002
[Estradiol LL]	-1.36	0.36	-2.26	.001
% change [E]	-0.40	0.01	-1.95	.003
45°				
[Estradiol LF]	0.79	0.27	1.97	.007
[Estradiol LL]	-1.07	0.33	-2.00	.003
% change [E]	-0.03	0.01	-1.73	.01
90°				
[Estradiol LF]	0.84	0.36	1.72	.03
[Estradiol LL]	-0.95	0.44	-1.46	.04
% change [E]	-0.04	0.02	-1.59	.03
	Control Group (*N*= 18)			
0°				
[Estradiol LF]	0.84	0.41	2.02	.06
[Estradiol LL]	-1.17	0.51	-1.98	.04
% change [E]	-0.04	0.02	-1.76	.04
45°				
[Estradiol LF]	0.49	0.37	1.40	.21
[Estradiol LL]	-0.74	0.46	-1.49	.13
% change [E]	-0.02	0.02	-1.18	.19
90°				
[Estradiol LF]	0.45	0.47	1.03	.36
[Estradiol LL]	-0.48	0.58	-0.78	.42
% change [E]	-0.03	0.02	-1.12	.21
	PMS Group (*N*= 14)			
0°				
[Estradiol LF]	1.37	0.45	2.69	.01
[Estradiol LL]	-1.84	0.58	-2.90	.01
% change [E]	-0.05	0.02	-2.44	.03
45°				
[Estradiol LF]	0.97	0.49	2.34	.08
[Estradiol LL]	-1.24	0.62	-2.40	.07
% change [E]	-.04	0.02	-2.25	.10
90°				
[Estradiol LF]	1.01	0.66	1.91	.16
[Estradiol LL]	-1.11	0.84	-1.68	.22
% change [E]	-0.04	0.03	-1.74	.22

S-cone Sensitivity: Short-Wavelength Automated Perimetry (SWAP)

Retinal location: Eccentricity. In order to determine the effect of group and menstrual cycle phase on S-cone sensitivity, two mixed ANOVAs were performed. In the first analysis, phase and retinal eccentricity were used as within-subjects IVs. As with the above CCS analyses, menstrual cycle phase was defined using two levels represented by both the LF and LL phases. Retinal eccentricity consisted of three levels corresponding to three different radial regions of the retina (central, paracentral, and peripheral zones). Again, group (PMS or control) was used as a between subjects factor. Means and standard deviations for S-cone sensitivity for all areas of retinal eccentricity for control and PMS groups in both LF and LL menstrual cycle phases are shown in Table 12.

For this analysis, Mauchley's test of sphericity was returned as significant ($p < .05$) and as such, the Greenhouse Geisser correction is used for the reporting of the following findings. Not surprisingly, results revealed a significant effect of retinal zone, $p < .001$, indicating that S-cone sensitivity varied as a function of retinal eccentricity. For this analysis, no significant effects of phase or group and or interactions with either phase or group were observed. All effects are summarized in Table 13.

Contrasts revealed significant differences in S-cone sensitivity between central and peripheral regions, $F(1, 32) = 1159.39$, $p < .001$, $\eta^2 = .97$, observed power = 1.0, and paracentral and peripheral regions, $F(1, 32) = 1104.47$, $p < .001$, $\eta^2 = .97$, observed power = 1.0, with women showing higher S-cone sensitivities in central and paracentral regions compared to peripheral regions. In addition to the main effect of retinal zone, a trend was noted for the effect of phase x retinal zone, $p = .10$. No other trends or significant main effects or interactions were noted for phase.

Table 12

Means and Standard Deviations (in decibel power ratios, dB) for S-cone Sensitivity as Assessed using Short-Wavelength Automated Perimetry (SWAP) for Three Zones of Retinal Eccentricity in the Late Follicular and Late Luteal Phases of the Menstrual Cycle for Control (N=18) and PMS Groups (N=16)

Control Group			
Retinal Zone	Late Follicular Phase (*M, SD*)	Late Luteal Phase (*M, SD*)	Grand Mean (*M, SD*)
Central	28.41 (1.01)	29.04 (0.85)	28.73 (0.29)
Paracentral	25.38 (1.85)	25.92 (1.50)	25.65 (0.47)
Peripheral	17.38 (2.05)	17.36 (2.64)	17.37 (.64)
Marginal Mean	23.72 (0.48)	24.11 (0.46)	23.92 (0.44)
PMS Group			
Retinal Zone	Late Follicular Phase (*M, SD*)	Late Luteal Phase (*M, SD*)	Grand Mean (*M, SD*)
Central	29.32 (1.74)	29.70 (1.81)	29.51 (0.31)
Paracentral	26.89 (2.54)	27.36 (2.67)	27.12 (0.49)
Peripheral	18.28 (3.86)	17.97 (3.19)	18.12 (0.67)
Marginal Mean	24.83 (0.51)	25.00 (0.49)	24.92 (0.46)

Table 13

ANOVA Table Showing Effects of Retinal Location (Eccentric and Hemiretinal) on a Performance of SWAP

			Eccentric Regions		
Dependent Variable	*F*	*df*	Error *df*	*p*	η^2
Group	2.05	1	32	.16	.06
Phase	1.11	1	32	.30	.03
Phase x Group	.148	1	32	.70	.01
Retinal Zone***	967.51	2	64	< .001	.97
Group x Zone	1.14	2	64	.33	.03
Phase x Zone[t]	2.63	2	64	.10	.08
Group x Phase x Zone	0.18	2	64	.86	.003
			Hemiretinal Sections		
Dependent Variable	*F*	*df*	Error *df*	*p*	η^2
Group	2.07	1	32	.16	.06
Phase	2.47	1	32	.13	.072
Phase x Group	.066	1	32	.80	.002
Hemiretinal Section***	360.64	4	29	< .001	.98
Group x Hemiretinal Section	0.49	4	29	.74	.063
Phase x Hemiretinal Section	1.78	4	29	.16	.20
Group x Hemiretinal Section x Phase	0.27	4	29	.90	.036

[t] $p < .10$, *$p \leq .05$, **$p \leq .01$. ***$p \leq .001$

In order to further investigate the trend for a phase x retinal zone effect, three paired samples *t*-tests were performed for each of the central, paracentral, and peripheral zones using SWAP measurements repeated in the LF and LL phases as the IV pairs. These analyses revealed that women exhibited significantly higher SWAP sensitivity in the LL phase (M = 29.35, SD = 1.40) compared to the LF phase (M = 28.84, SD = 1.45) in the central zone, $t(33) = 2.29$, p = .029. A similar trend for higher overall sensitivity in the LL phase (M = 26.60, SD = 2.22) over the LF phase (M = 26.09, SD = 2.30) was additionally observed for the paracentral zone, $t(33)$ = 1.69, p = .10. No differences were revealed between the LF (M = 17.80, SD = 3.02) and LL (M = 17.65, SD = 2.88) phases for peripheral regions $t(33) = -.37$, p = .72. A histogram showing S-cone sensitivity in the three retinal zones for both menstrual cycle phases is shown in Figure 12.

Retinal location: Hemiretinal Superior and Inferior Zones. A second mixed ANOVA was conducted on S-cone sensitivity, with phase (LF and LL) entered as the first within-subjects factor, and hemiretinal zones as a second, five-level, within-subjects variable. The five levels for hemiretinal zones included: central, superior nasal, inferior nasal, superior temporal, and inferior temporal. Group was used as the between-subjects variable. Means and standard deviations of S-cone sensitivity for all hemiretinal sections for PMS and control groups in both menstrual cycle phases are found in Table 14.

For this analysis, no main effects for phase or group were observed indicating that women did not differ in S-cone sensitivity as a function of hormonal sensitivity or phase. A significant effect of hemiretinal section was observed, $F(4, 128) = 425.53$, $p < .001$, $\eta^2 = .93$, observed power = 1.0, with the highest S-cone sensitivity observed in the central region (refer to Table 13 for the ANOVA results). Examination of means and standard deviations in Table 14 revealed that S-cone sensitivity was highest, irrespective of menstrual cycle phase or group, in

Table 14

Means and Standard Deviations for S-cone sensitivity (dB) as assessed using Short-Wavelength Automated Perimetry (SWAP) for Superior and Inferior Hemiretinal Sections in the Late Follicular (LF) and Late Luteal (LL) Phases of the Menstrual Cycle for Control (N=18) and PMS Groups (N=16).

Control Group

Retinal Zone	Late Follicular Phase (*M, SD*)	Late Luteal Phase (*M, SD*)	Grand Mean (*M, SD*)
Central	28.41 (1.01)	29.04 (0.85)	28.73 (0.29)
Superior Nasal	19.81 (2.17)	20.27 (2.05)	20.04 (0.56)
Inferior Nasal	24.07 (2.31)	24.45 (2.21)	24.26 (0.52)
Superior Temporal	18.60 (2.26)	19.46 (1.74)	19.03 (0.60)
Inferior Temporal	21.64 (2.28)	21.94 (2.22)	21.79 (0.53)
Marginal Mean	22.50 (0.49)	23.03 (0.51)	22.77 (0.46)

PMS Group

Retinal Zone	Late Follicular Phase (*M, SD*)	Late Luteal Phase (*M, SD*)	Grand Mean (*M, SD*)
Central	29.32 (1.74)	29.70 (1.81)	29.51 (0.31)
Superior Nasal	21.00 (2.91)	21.25 (3.34)	21.13 (0.60)
Inferior Nasal	25.60 (2.74)	25.54 (2.76)	25.55 (0.55)
Superior Temporal	19.45 (3.33)	20.55 (3.66)	20.00 (0.64)
Inferior Temporal	22.38 (2.40)	22.56 (3.08)	22.47 (0.56)
Marginal Mean	23.54 (0.52)	23.92 (0.54)	23.73 (0.49)

the central retinal location. Follow up *t*-tests indicated that S-cone sensitivity in the central region ($M = 58.19$, $SD = 2.54$) was significantly higher than in the superior nasal region ($M = 41.10$, $SD = 4.84$), $t(33) = 29.89$, $p < .001$; the inferior nasal region ($M = 49.73$, $SD = 4.57$), $t(33) = 15.99$, $p < .001$; the superior temporal region ($M = 38.97$, $SD = 5.10$), $t(33) = 32.07$, $p < .001$; and the inferior temporal region ($M = 44.22$, $SD = 4.48$), $t(33) = 27.80$, $p < .001$. In addition, women showed higher S-cone sensitivities in both inferior nasal and inferior temporal retinal locations as compared to parallel areas in the superior portion of the retina. Combined scores for all women comparing the superior and inferior retinal regions revealed significantly higher sensitivity in inferior ($M = 93.95$, $SD = 8.68$) compared to superior retinal locations ($M = 80.07$, $SD = 9.53$), $t(33) = -17.03$, $p < .001$.

S-Cone Sensitivity and Estradiol. In order to examine the effect of estradiol levels in the LF and LL phases on S-cone sensitivity, a series of multiple regressions was performed using the sensitivity of the retinal zones as DVs, and concentrations of estradiol in the LF and LL phases as predictors. Given that theoretical evidence links estrogen levels to S-cone sensitivity, but does not suggest a strong rationale for estradiol levels in either the LF or LL predicting differential outcomes, a forced entry method was employed in which both predictors were entered into the model simultaneously. As well, given the large number of conditions in this experiment, a decision was made to collapse retinal zones within each phase in order to prevent conducting a large number of analyses and inflating error rates. Collapsing of retinal zones was accomplished by creating new variables in which S-cone sensitivities were combined according to phase. The new variables used as DVs in the six regression analyses were as follows: (1) retinal eccentricity LL, (2) retinal eccentricity LF, (3) inferior retina LL, (4) inferior retinal LF, (5) superior retina LL and (6) superior retina LF. The first two regression variables (1 & 2) were

created by adding the S-cone sensitivity values in the central, paracentral and peripheral retinal regions together for each of the LL and LF phases, respectively, while the last four regression variables (3-6) were created by combining the superior nasal and superior temporal S-cone sensitivities together, and the inferior nasal and inferior temporal S-cones sensitivities together for each of the LL and LF phases.

A summary of all regression analyses is found in Table 15. For the eccentric regions, levels of estradiol in both menstrual cycle phases predicted S-cone sensitivity in the LF phase, p = .02, adjusted R^2= .18. A trend was found for the same combination of variables in the prediction of S-cone sensitivity in the LL phase, p = .09, adjusted R^2 = .10. For the inferior and superior hemiretinal zones, estradiol levels at the LF and LL positions in the menstrual cycle predicted S-cone sensitivity in both regions when women were tested in the LF phase only. Thus, for testing sessions occurring in the LF phase, estradiol concentrations in the LF and LL phases significantly predicted S-cone sensitivity in the inferior hemiretinal section, p = .007, adjusted R^2 = .24 , and the superior hemiretinal section, p = .05, adjusted R^2 = .13.

These analyses revealed that S-cone sensitivity is best predicted in the LF phase by a combination of estradiol levels in the LF and LL phases. Interestingly, the results also indicated that, for all analyses, the β weights for the predictors were in opposite directions, with those for estradiol levels in the LF phase having negative weights and those for estradiol levels in the LL phase having positive weights (see Table 16). This would suggest that higher levels of estradiol in the LL phase are related to higher S-cone sensitivity in the late follicular phase of a subsequent cycle, while high levels of estradiol in the late follicular phase predict poorer S-cone sensitivity at that time in the cycle. The same combination of predictors did not significantly predict S-cone sensitivity in the LL phase. Thus, higher estradiol in the LL phase and lower

Table 15

Table Showing Statistics for a Forced Entry Model of Regression Using Mean Concentrations of Estradiol in Each of the Late Follicular (LF) and Late Luteal (LL) Phases of the Menstrual Cycle as Predictors of S-cone Sensitivity in Central, Paracentral, and Peripheral Locations in both the LF and LL Phases

Dependent Variable	df	Error df	*R*	Adjusted R^2	*R* Square	*F*	*p*
Eccentric LL	2	29	.40	.10	.16	2.69	.09[t]
Inferior LL	2	29	.26	.001	.07	1.01	.38
Superior LL	2	29	.34	.06	.12	1.94	.16
Eccentric LF*	2	29	.48	.18	.23	4.35	.02
Inferior LF**	2	29	.54	.24	.29	5.89	.007
Superior LF*	2	29	.43	.13	.19	3.31	.05

[t] $p < .10$, $*p \leq .05$, $**p \leq .01$. $***p \leq .001$

NOTE: Dependent Variables represent S-cone sensitivity for the respective retinal location

Table 16

Coefficients (from Analyses in Table 15) for Mean Concentrations of Estradiol in Each of the Late-Luteal (LL) and Late-Follicular (LF) Phases for a Forced Entry Model of Regression in the Prediction of S-Cone Sensitivity in Eccentric, Inferior, and Superior Regions for Each Menstrual Cycle Phase in an Overall Sample of Participants (N=32)

Retinal Location and Menstrual Cycle Phase	*B*	SE*B*	β	*p*
Eccentric Regions LL				
Mean [Estradiol] LF*	-1.58	0.72	-.44	.035
Mean [Estradiol] LL	1.67	0.96	.35	.092
Inferior Regions LL				
Mean [Estradiol] LF	-.89	0.62	-.30	.17
Mean [Estradiol] LL	.57	0.83	.14	.50
Superior Regions LL				
Mean [Estradiol] LF[t]	-1.12	0.64	-.36	.09
Mean [Estradiol] LL	1.43	0.86	.34	.11
Eccentric Regions LF				
Mean [Estradiol] LF*	-1.91	0.71	-.51	.012
Mean [Estradiol] LL*	2.31	0.95	.46	.022
Inferior Regions LF				
Mean [Estradiol] LF**	-1.55	0.50	-.56	.005
Mean [Estradiol] LL**	1.94	0.67	.53	.007
Superior Regions LF				
Mean [Estradiol] LF*	-1.37	0.58	-.46	.026
Mean [Estradiol] LL*	1.66	0.78	.41	.043

[t] $p < .10$, *$p \leq .05$, **$p \leq .01$. ***$p \leq .001$

estradiol in the LF phase were associated with higher S-cone sensitivity in the LF phase, with similar directional, though non-significant trends observed for S-cone sensitivity in the LL phase. These results seem to suggest that women with the least cyclicity in estradiol levels may perform best on the SWAP (i.e., those with a lower estradiol periovulatory surge and a higher late luteal phase estradiol level). This was explored further (see below) by examining change in estradiol between the two phases.

Correlations between changes in estradiol concentrations between the LF and LL menstrual cycle phases (LF-LL) and S-cone sensitivity measurements in the eccentric and hemiretinal sections from each testing session were examined (see Table 17). These correlations were performed for the pooled sample of women, as well as for women in each of the control and PMS groups. The results of these correlations indicated an inverse relationship between amount of change in estradiol between menstrual cycle phases and SWAP sensitivity in several eccentric and hemiretinal zones. Specifically, a greater amount of change in estradiol levels between the LF and LL phases (e.g. across the cycle) was significantly associated with either higher or lower S-cone sensitivities in most retinal locations in both phases. Results indicate that women who experience a higher level of the expected decrease in estradiol levels between the LF and LL phases demonstrate poorer SWAP performance (e.g. lower S-cone sensitivity) in both phases. Conversely women who show increases in estradiol from the LF to the LL phase demonstrate better SWAP performance (e.g. higher S-cone sensitivity). Thus, decreases in estradiol across the cycle were associated with low S-cone sensitivity, while increases in estradiol were associated with heightened S-cone sensitivity. These relationships were present for both PMS and control women, but the results were most pronounced for the pooled sample of women ($N = 32$). Examination of the scatterplot of the relationship between the change in

Table 17

Correlations Examining the Relationship Between Change in Estradiol Concentrations between the Late-Follicular (LF) and Late-Luteal (LL) Phases (LF-LL) and S-Cone Sensitivity Measurements in Eccentric and Hemiretinal Sections in Each Cycle Phase for Women with PMS Symptoms (Column A), Control Women (Column B), and the Combined Sample of all Women (Column C)

Correlations Between Change in Estradiol ([LF]-[LL]) and Change in SWAP Scores

Retinal Location	Women with PMS symptoms (*N*=14)	Control Group (*N*=18)	Total Sample (*N*=32)
Central LL	-.55*	-.41	-.52**
Paracentral LL	- .48	- .06	-.39*
Peripheral LL	-.31	- .20	-.27
Central LF	-.32	-.52	-.44*
Paracentral LF	-.30	- .39	-.40*
Peripheral LF	.49	- .45	-.48*
Superior Nasal LL	-.33	- .04	-.25
Inferior Nasal LL	-.33	.31	-.10
Superior Temporal LL	-.39	-.32	-.38*
Inferior Temporal LL	-.42	-.25	-.37
Superior Nasal LF	-.45	- .22	-.40*
Inferior Nasal LF	-.44	-.33	-.44*
Superior Temporal LF	-.35	- .47*	-.41*
Inferior Temporal LF	-.55**	- .55*	-.56**

[t] $p < .10$, *$p \leq .05$, **$p \leq .01$. ***$p \leq .001$

estradiol concentrations between the LF and LL phases and S-cone sensitivity in the central location for a testing session occurring in the LL phase in Figure 13 indicates that that is indeed the case. That is, women with lower estradiol during the LL than the LF phase (the expected pattern) did more poorly on the SWAP while those with higher estradiol during the LL than the LF phase performed relatively better.

Texture Discrimination Task (TDT)

Means and standard deviations of trials responded to correctly in training and testing for the overall sample of women, women in the control group, and women with PMS symptoms are found in Table 18. Figure 14 shows the percentage of trials responded to correctly (i.e., %C) as a function of SOA for all women. Figure 15 shows the percentage of trials responded to correctly (i.e., %C) as a function of SOA for control women and women with PMS symptoms. The figures illustrate that women's scores improved between day of training and day of testing as indicated by the leftwards shift in the psychometric function (i.e., %C improves with decreasing levels of SOA). This finding conforms to expected patterns of learning for TDT experiments (Karni & Sagi, 1991; 2006).

Given that scores of 50% correct at any level of SOA parallels chance performance in this 2AFC design, the requisite threshold for assuming learning is assumed to be 75% correct or higher. The mean %C scores of women at training and testing in this study, however, seldom met the criterion necessary to achieve threshold (see Table 18). These truncated data did not allow us to make adequate Weibull-function curve fits needed to interpolate fitting parameters such as sensitivity (i.e., 1/threshold) or slope (i.e., performance efficiency). Despite the failure of our data to meet sufficient lapse rates of 95% or greater correct, overall gain changes characteristic of psychometric shifts along the SOA axis indicating improvement (learning) from training to

Table 18

Mean Percent of Trials Identified Correctly for Six Levels of Stimulus Onset Asynchrony (SOA) in the Training and Testing Sessions of a Texture Discrimination Task (TDT) for: (1) a Pooled Sample of Women from Control and PMS Groups (Top Panel), (2) a Group of Control Women (Middle Panel) and (3) a group of Women with PMS Symptoms (Bottom Panel).

Total Sample (*N*= 27)

Training SOA	Percent of Trials Correct at Training (*M, SD*)	Testing SOA	Percent of Trials Correct at Testing (*M, SD*)
360	70.88 (16.21)	300	78.59 (21.03)
160	69.17 (19.02)	200	77.25 (20.29)
100	54.76 (11.90)	140	75.69 (19.16)
80	52.93 (13.61))	100	68.21 (16.35)
60	51.71 (6.22)	60	54.95 (9.67)
40	50.03 (5.41)	40	52.14 (5.14)

Control Women (*N*= 14)

Training SOA	Percent of Trials Correct at Training (*M, SD*)	Testing SOA	Percent of Trials Correct at Testing (*M, SD*)
360	72.84 (16.29)	300	76.37 (22.35)
160	73.02 (17.13)	200	77.64 (21.37)
100	53.50 (11.53)	140	74.79 (20.12)
80	53.61 (17.33)	100	69.03 (15.43)
60	50.77 (6.52)	60	53.34 (6.40)
40	50.80 (4.60)	40	52.89 (4.82)

Women with PMS Symptoms (*N*= 14)

Training SOA	Percent of Trials Correct at Training (*M, SD*)	Testing SOA	Percent of Trials Correct at Testing (*M, SD*)
360	68.76 (16.50)	300	80.98 (20.13)
160	65.02 (20.74)	200	76.83 (19.93)
100	56.12 (12.62)	140	76.67 (18.84)
80	52.19 (8.66)	100	67.32 (17.86)
60	52.65 (6.01)	60	56.68 (12.32)
40	49.26 (6.21)	40	51.33 (5.53)

testing were nonetheless observed. Thus, in order to control for individual idiosyncratic performance differences with the task, as well as to make comparisons, relative change scores were calculated for each SOA. These scores were calculated using the differential proportion of correct responses obtained by women for each SOA level: 330, 150, 100, 60, and 40 msec with the following formula:

eq.1 (%C during Test Session - %C during Train Session) /
(%C during Test + %C during Train)

Prior to performing any analyses examining group and phase effects, an independent samples *t*-test was conducted to determine any between group differences in the number of days between the TDT training and testing sessions. This test revealed no differences ($p = .19$) between the control ($M = 13.07$, $SD = 4.68$) and PMS groups ($M = 18.62$, $SD = 13.89$) in terms of days between TDT sessions. Given the absence of differences between groups in terms of days between training and testing, the influence of 1) group and 2) order of training and testing on performance at each level of SOA was examined using a two level repeated measures ANOVA using group (control or PMS) and training order (LL-LF or LF-LL) as between subjects IVs and relative change scores for the five levels of SOA as DVs (see Table 19). This analysis did not reveal a significant effect of group, $p = .27$, however, a trend was noted for the effect of menstrual cycle phase at the time of testing and training (phase order), $p = .06$. This trend indicated a higher proportion of correct responses for women who trained in the LL phase and tested in the LF phase, as opposed to those who performed the experiment in the reverse order. A second trend was also noted for an SOA x group effect, $p = .07$. A summary of the main effects and interaction results can be found in Table 20.

The relative proportion of improvement between SOAs at training and testing days are shown according to menstrual cycle phase for the pooled sample of women in Table 21. To

Table 19

Means and Standard Deviations for the Relative Proportion of Improvement Between Training and Testing Sessions for Five Levels of Stimulus Onset Asynchrony (SOA) for a Group of Control Women (N= 14) and a Group of Women with PMS symptoms (N= 13) Participating in a Texture Discrimination Task (TDT)

SOA Level	Proportion Improvement		
	Control (*M, SD*)	PMS (*M, SD*)	Total
330	.012 (.09)	.076 (.13)	.043 (.11)
150	.007 (.08)	.088 (.12)	.046 (.11)
100	.122 (.12)	.082 (.14)	.103 (.13)
60	.029 (.08)	.029 (.10)	.029 (.09)
40	.017 (.07)	.021 (.06)	.019 (.06)

Table 20

ANOVA Table Showing the Effects of Group and Cycle Phase at Time of Training and Testing on Proportion Improvement at Five levels of SOA in a Task of Texture Discrimination

Effect	*F*	*df*	Error *df*	*p*	η^2
Group	1.27	1	22	.27	.06
Phase Order[t]	3.86	1	22	.06	.15
Group x Phase Order	2.23	1	22	.15	.09
SOA**	4.26	4	88	.003	.16
SOA x Group[t]	2.29	4	88	.07	.09
SOA x Phase Order	0.70	4	88	.59	.03
SOA x Group x Phase Order	1.73	4	88	.15	.07

[t] $p < .10$, *$p \leq .05$, **$p \leq .01$. ***$p \leq .001$

Table 21

Descriptive Data (Mean Proportion Improvement from Training to Testing) and ANOVA Results for the Texture Discrimination Task (TDT): Performance as a Function of Menstrual Cycle Phase at Training and Testing for Proportion of Correct Responses at Five Levels of Stimulus Onset Asynchrony (SOA) for a Pooled Sample of Women (N= 26)

Dependent Variable	Session Order	*M* (*SD*)	*F*	*df*	*Error df*	*p*	η^2
SOA 330*	LF-LL	.02 (.09)	4.86	1	23	.038	.17
	LL-LF	.11 (.12)					
SOA 150[t]	LF-LL	.03 (.10)	3.47	1	23	.075	.13
	LL-LF	.11 (.09)					
SOA 100	LF-LL	.10 (.15)	0.42	1	23	.53	.02
	LL-LF	.13 (.88)					
SOA 60	LF-LL	.02 (.09)	0.74	1	23	.40	.03
	LL-LF	.05 (.08)					
SOA 40	LF-LL	.01 (.06)	1.61	1	23	.22	.07
	LL-LF	.04 (.07)					

Note: Women Trained in the Late-Follicular Phase and Tested in the Late-Luteal Phase (*N*=18) are Indicated by LF-LL while Women Trained in Late-Luteal Phase and Tested in Late-Follicular Phase (*N*=9) are Indicated by LL-LF.
[t] $p < .10$, *$p \leq .05$, **$p \leq .01$. ***$p \leq .001$

further examine the trend for order of menstrual cycle phase at time of training and testing, a follow-up univariate ANOVA was conducted in which relative proportion change in performance for the five levels of SOA were used as DVs, and phase order at time of training and testing was used as the between subjects IV. See Table 21 for a summary of all trends and main effects. For this test, homogeneity of variance calculations were returned within acceptable limits ($p > .05$), and as such, no corrections were employed in the interpretation of results. These post-hoc analyses indicated a significant difference in proportion change scores for SOA 330, $p = .038$, and a trend for those at SOA 150, $p = .075$. In both cases, women who trained in the LL phase and tested in the LF phase showed a higher proportion of correct answers between sessions than woman who trained and tested in the reverse order (see Figure 16).

In order to investigate the second trend of an SOA x group effect, a follow up ANOVA was conducted using the five levels of proportion change scores for each SOA as DVs and group as the between subjects factor. Means and standard deviations for proportion improvement for women in the control group, women with PMS symptoms, and across groups are found in Table 19. The ANOVA revealed a significant between group effect at SOA 150, $F(1, 26) = 4.20$, $p = .05$, indicating that women with PMS symptoms had a higher proportion of improvement at the 150 ms SOA than control women. Although no other trends or significant findings were revealed for the other SOAs, examination of Figure 17 shows the group x SOA interaction and indicates that the PMS women also demonstrated a higher, though non-significant proportion improvement at SOA 330.

As with the SWAP analyses, five multiple regressions were performed using the proportion improvement scores for each of the five SOAs as the DV in each analysis with measured estradiol concentrations in the LF and LL phases and session order as the three

predictors. Session order was coded as *1* for women training in the LF phase and testing in the LL phase and *2* for the reverse. The five regressions were performed for the pooled sample of women, and then repeated for each of the PMS and control groups (see the overall regression findings in Table 22). For the pooled sample of women, the regression analyses revealed that the combination of IVs successfully predicted the proportion of correct responses for SOA 330, $p = .049$, adjusted $R^2 = .21$, and SOA 150, $p = .013$, adjusted $R^2 = .31$. While the same series of regressions performed on the control group yielded no significant effects, the combination of IVs predicted the proportion of performance improvement at three SOAs for women with symptoms of PMS: (a) SOA 330, $p = .008$, adjusted $R^2 = .71$, (b) SOA 150, $p = .032$, adjusted $R^2 = .56$, and (c) SOA 40, $p = .002$, adjusted $R^2 = .82$. These results suggest that the performance on the TDT task in women with PMS symptoms may be influenced by session order and estradiol concentrations in each session to a much greater degree than control women. Furthermore, for the noted SOAs, estradiol levels and session order explained 56% to 82% of the variance in performance improvement between sessions for the PMS group. Examination of beta weights and the significance of the individual predictors in each regression analysis (see Tables 23 to 25) revealed that for all SOAs with a significant main effect, among the three predictors, order of testing session was usually the only unique predictor (although the exception was with SOA 40 in the PMS group), and there was a positive relationship between session order and performance. Specifically, improvement in texture discrimination performance was predicted best by a session order characterized by training in the LL phase and testing in the LF phase. This finding that the menstrual cycle phase of testing and training affected improvement in texture discrimination performance was strongest for the PMS group at the extreme SOAs (40, 150, and 330). This finding indicates a possible link between menstrual cycle phase and texture learning in women

Table 22

Summary Table Denoting Effects for a Forced Entry Model of Regression using Concentrations of Estradiol at Training and Testing, and Session Order as Predictors of Improvement in a Texture Discrimination Task (TDT) with Five Levels of Stimulus to Onset Asynchrony

SOA	df	Error df	*R*	Adjusted R^2	*R* Square Change	*F*	*p*
Pooled Sample (*N* = 24)							
330*	3	21	.55	.21	.31	3.10	.049
150*	3	21	.63	.31	.39	4.53	.013
100	3	21	.31	-.03	.10	0.76	.53
60	3	20	.63	-.04	.10	0.70	.56
40	3	20	.37	.01	.14	1.08	.38
Control Group (*N* = 13)							
330	3	10	.24	-.23	.06	0.20	.11
150	3	10	.66	.27	.44	2.62	.89
100	3	10	.34	-.15	.12	0.44	.73
60	3	9	.44	-.07	.20	0.74	.55
40	3	9	.37	-.15	.13	0.47	.71
Women with PMS Symptoms (*N* = 11)							
330**	3	7	.89	.71	.80	9.32	.008
150*	3	7	.83	.56	.70	5.32	.032
100	3	7	.58	.04	.33	1.16	.39
60	3	7	.34	-.26	.12	0.31	.82
40**	3	7	.93	.82	.87	15.79	.002

[t] $p < .10$, *$p \leq .05$, **$p \leq .01$. ***$p \leq .001$

Table 23

Coefficients for a Forced Entry Model of Regression for Prediction of Improvement in a TDT Paradigm with Five SOAs using Concentrations of Estradiol at Training and Testing, and Session Order as Predictors in a Pooled Sample of Women ($N = 24$)

SOA	*B*	SE*B*	β	*p*
SOA 330				
[Estradiol] at Training	.020	.014	.33	.16
[Estradiol] at Testing	-.021	.019	-.27	.26
Session Order**	.16	.052	.65	.007
SOA 150				
[Estradiol] at Training	-.005	.011	.093	.67
[Estradiol] at Testing	-.017	.014	-.26	.25
Session Order**	.12	.041	.61	.007
SOA 100				
[Estradiol] at Training	-.003	.018	-.047	.86
[Estradiol] at Testing	-.016	.024	-.18	.52
Session Order	.08	.069	.29	.26
SOA 60				
[Estradiol] at Training	-.003	.012	-.069	.80
[Estradiol] at Testing	-.002	.016	-.030	.91
Session Order	.052	.045	.29	.26
SOA 40				
[Estradiol] at Training	.006	.010	.15	.56
[Estradiol] at Testing	-.016	.012	-.36	.20
Session Order	.057	.035	.40	.12

[t] $p < .10$, *$p \leq .05$, **$p \leq .01$. ***$p \leq .001$

Table 24

Coefficients for a Forced Entry Model of Regression for Prediction of Improvement in a TDT Paradigm with Five SOAs using Concentrations of Estradiol at Training and Testing, and Session Order as Predictors in a Sample of Control Women ($N = 13$)

SOA	*B*	SE*B*	β	*p*
SOA 330				
[Estradiol] at Training	.011	.020	.23	.60
[Estradiol] at Testing	-.017	.027	-.28	.55
Session Order	.060	.079	.33	.47
SOA 150				
[Estradiol] at Training	-.008	.014	-.18	.60
[Estradiol] at Testing	-.027	.019	-.49	.19
Session Order	.087	.056	.52	.15
SOA 100				
[Estradiol] at Training	-.025	.026	-.38	.37
[Estradiol] at Testing	.004	.035	.05	.91
Session Order	-.047	.10	.19	.66
SOA 60				
[Estradiol] at Training	-.010	.017	-.24	.57
[Estradiol] at Testing	.016	.022	.31	.50
Session Order	.029	.066	.18	.67
SOA 40				
[Estradiol] at Training	-.012	.017	-.29	.51
[Estradiol] at Testing	-.004	.022	-.08	.86
Session Order	-.030	.063	-.20	.65

[t] $p < .10$, $*p \leq .05$, $**p \leq .01$. $***p \leq .001$

Table 25

Coefficients for a Forced Entry Model of Regression for Prediction of Improvement in a TDT Paradigm with Five SOAs using Concentrations of Estradiol at Training and Testing, and Session Order as Predictors in a Sample of Women with PMS symptoms ($N = 13$)

SOA	*B*	SE*B*	β	*p*
SOA 330				
[Estradiol] at Training[t]	.030	.015	.42	.09
[Estradiol] at Testing	-.022	.020	-.23	.31
Session Order***	.27	.053	.95	.001
SOA 150				
[Estradiol] at Training	.004	.014	.07	.80
[Estradiol] at Testing	-.011	.019	-.15	.58
Session Order**	.19	.048	.86	.006
SOA 100				
[Estradiol] at Training	-.001	.028	-.016	.97
[Estradiol] at Testing	-.005	.038	-.047	.91
Session Order	.17	.099	.58	.13
SOA 60				
[Estradiol] at Training	.003	.022	.07	.88
[Estradiol] at Testing	-.021	.030	-.31	.50
Session Order	.06	.076	.27	.50
SOA 40				
[Estradiol] at Training*	.017	.006	.51	.02
[Estradiol] at Testing*	-.018	.008	-.40	.05
Session Order***	.134	.020	1.00	<.001

[t] $p < .10$, *$p \leq .05$, **$p \leq .01$. ***$p \leq .001$

with PMS symptoms, but not women without such symptoms, and that the effect is present at particular SOAs (i.e., 40, 150, and 330). Examination of the results from SOA 40 suggests that PMS women with higher estradiol at testing and lower estradiol at training, and those in the LL-LF group, showed the greatest improvement in texture discrimination.

To further examine the effect of cycle phase on learning in this paradigm an additional series of hierarchical regressions was performed in which percent change in estradiol was entered at step one and session order was entered at step two, These analyses were performed for the pooled sample of women as well as for each group, A summary of all regression analyses is reported in Table 26. Coefficients are reported in Table 27. For the pooled sample of women, this series of analyses revealed that percent change in estradiol did not significantly predict improvement, but session order was a significant predictor of improvement on the TDT beyond that of percent change in estradiol at both SOA 330, $p = .005$, and SOA 150, $p = .012$. For the control group neither percent change in estradiol or session order was found to predict improvement on the TDT at any level of SOA. For the PMS group, however, session order was found to significantly predict performance above and beyond percent change in estradiol for SOA 330, $p < .001$, SOA 150, $p = .004$, and SOA 40, $p < .001$. Thus, the effect of session order seems to hold only for the PMS group and not the control group. In addition, percent change in estradiol was also a significant unique predictor of TDT improvement at SOA 330 and SOA 40; and both session order and change in estradiol explained 80 and 85 percent of the variance in TDT improvement, respectively. As with the first set of regressions, beta coefficients for significant models revealed that the relationship between proportion improvement and session order was positive, indicating that training in the LL phase and testing in the LF phase was associated with a higher proportion of improvement on the TDT.

Table 26

Summary Table Denoting Effects for a Hierarchical Regression using Percent Change Estradiol Between Training and Testing at Step 1, and Session Order at Step 2 as Predictors of Improvement in a Texture Discrimination Task (TDT) with Five Levels of Stimulus to Onset Asynchrony

SOA	Step	df	Error df	*R*	Adjusted R^2	*R* Square Change	*F*	*F* change
				Pooled Sample (*N*= 25)				
330	1	1	23	.04	-.04	.002	0.041	
	2**	1	22	.56	.25	.31	4.94	9.82
150	1	1	23	.24	.02	.06	1.42	
	2*	1	22	.55	.24	.24	4.68	7.55
100	1	1	23	.01	-.04	.00	0.05	
	2	1	22	.26	-.02	.07	0.82	1.64
60	1	1	22	.10	-.04	.10	0.21	
	2	1	21	.30	.001	.08	1.02	1.81
40	1	1	22	.12	-.03	.02	0.34	
	2	1	21	.36	.04	.11	1.51	2.66
				Control Group (*N*= 14)				
330	1	1	12	.02	-.08	.00	0.004	
	2	1	11	.19	-.14	.04	0.21	0.42
150	1	1	12	.23	-.03	.05	0.67	
	2	1	11	.36	-.03	.07	0.80	0.94
100	1	1	12	.18	-.05	.03	0.39	
	2	1	11	.28	-.09	.05	0.48	0.58
60	1	1	11	.47	.15	.22	3.07	
	2	1	10	.48	.08	.01	1.51	0.18
40	1	1	11	.03	-.09	.001	0.07	
	2	1	10	.19	-.16	.04	0.19	0.36
				Women with PMS Symptoms (*N*= 11)				
330	1	1	9	.15	-.09	.02	0.20	
	2***	1	8	.90	.77	.80	17.95	34.97
150	1	1	9	.15	-.09	.02	0.21	
	2**	1	8	.83	.60	.66	8.58	16.59
100	1	1	9	.03	-.11	.001	0.08	
	2[t]	1	8	.60	.20	.36	2.24	4.46
60	1	1	9	.17	-.08	.03	0.27	
	2	1	8	.31	-.13	.06	0.41	0.56
40	1	1	9	.25	-.04	.06	0.59	
	2***	1	8	.95	.89	.85	40.74	76.00

[t] $p < .10$, *$p \leq .05$, **$p \leq .01$. ***$p \leq .001$

Table 27

Coefficients for a Hierarchical Model of Regression using Percent Change Estradiol Between Training and Testing at Step 1, and Session Order at Step 2 as Predictors of Improvement in a Texture Discrimination Task (TDT) with Five Levels of Stimulus to Onset Asynchrony

		Pooled Sample (*N*= 25)		
SOA	*B*	SE *B*	β	*p*
330				
% change [E]	-0.001	.001	-0.31	.13
Session Order	0.15	0.05	0.62	.01
150				
% change [E]	< 0.01	< 0.01	0.01	.97
Session Order	0.11	0.04	0.54	.01
100				
% change [E]	< 0.01	0.001	-0.11	.63
Session Order	0.08	0.06	0.29	.21
60				
% change [E]	< 0.01	< 0.01	-0.03	.90
Session Order	0.06	0.04	0.31	.19
40				
% change [E]	> 0.01	> 0.01	-0.28	.23
Session Order	0.05	0.03	0.37	.12
		Control Group (*N*= 14)		
330				
% change [E]	< 0.01	0.01	-0.18	.65
Session Order	0.05	0.07	0.25	.53
150				
% change [E]	< 0.01	0.001	-0.003	1.00
Session Order	0.06	0.06	0.36	.35
100				
% change [E]	0.001	0.001	0.37	.36
Session Order	-0.07	0.09	-0.29	.46
60				
% change [E]	0.001	0.001	0.37	.33
Session Order	0.02	0.06	0.15	.68
40				
% change [E]	< 0.01	0.001	0.13	.76
Session Order	-0.04	0.06	-0.24	.56
		Women with PMS Symptoms (*N*= 11)		
330				
% change [E]	-0.001	< 0.01	-.037	.05
Session Order	0.26	0.05	0.92	< 0.01
150				
% change [E]	< 0.01	< 0.01	-0.05	.81
Session Order	0.18	0.04	0.84	.004
100				
% change [E]	-0.001	0.001	-0.18	.55
Session Order	0.18	0.09	0.62	.07

60				
% change [E]	< 0.01	0.001	-0.23	.52
Session Order	0.05	0.07	0.26	.47
40				
% change [E]	-0.001	< 0.01	-0.48	.002
Session Order	0.13	0.02	0.95	< 0.01

Eyetracking

The final averaged and fitted eye movement plots for all temporal frequencies (0.25 to 2.0 Hz) can be seen in Figure 18. The first column shows traces made during the LL menstrual cycle phase and the second column shows traces made during the LF phase. Data are the result of signal averaging as outlined in the Data Analysis section of the Procedure. This averaging procedure resulted in six women being omitted from the control group and three women being omitted from the PMS group due to extensive eye blinks or missed tracking positions. Continuous lines are sinusoidal functions fitted to the data. Characteristic lag shifts are evident in the higher frequency target movements (details in Figure 18 caption). The means and standard deviations for the latency between eye-gaze and stimulus position as a function of frequency and menstrual cycle phase are reported in Tables 28 (control group) and 29 (PMS group). Means and standard deviations for latency between eye-gaze and stimulus position collapsed across sinusoidal wave position for the five frequencies for both groups in both menstrual cycle phases are found in Table 30.

As with the CCS and SWAP procedures, the effect of group and menstrual cycle phase on smooth pursuit eye-movement was tested using a three factor mixed ANOVA. Again, menstrual cycle phase consisted of two levels (LF and LL phases) and constituted the first within subjects IV. The second within subjects factor and was defined by the five oscillating target frequencies: 0.25 Hz, 0.5 Hz. 1.0 Hz, 1.25 Hz, and 2.0 Hz., while the final factor entered in the analysis was sinusoidal phase position, which was defined by five levels corresponding to 0°, 90°, 180°, 270° and 360° along the function. These positions represent tracking a target position start (center), extreme left-of-center, through the center, extreme right-of-center, and return-to-end at center, respectively. As with all other analyses, group was used as a between subjects

Table 28

Means and Standard Deviations for Latencies Between Eye-gaze and Stimulus Position (°) at Five Positions along an Averaged Sinusoidal Function in an Eye-Tracking Procedure. Late Follicular (LF) and Late Luteal (LL) Phase Latencies for a Control Group of Women (N=12) are shown Across the Five Oscillating Target Frequencies.

	Control Group									
Frequency (Hz)	Late Follicular Phase (*M, SD*)					Late Luteal Phase (*M, SD*)				
	0°	90°	180°	270°	360°	0°	90°	180°	270°	360°
0.25	-0.48 (2.91)	-12.79 (1.34)	1.47 (2.52)	13.07 (.69)	-0.50 (2.94)	-.59 (2.08)	-12.79 (0.83)	0.43 (2.10)	12.82 (0.81)	-0.53 (2.20)
0.50	0.26 (2.71)	-12.42 (1.38)	1.17 (3.68)	13.04 (.82)	0.12 (3.23)	.30 (2.81)	-12.36 (1.23)	-.52 (3.25)	12.56 (0.89)	.017 (3.15)
1.0	4.44 (5.00)	-8.17 (1.69)	-2.01 (5.48)	9.46 (1.71)	4.68 (5.49)	4.58 (3.02)	-8.88 (1.60)	- 1.68 (5.30)	10.00 (2.15)	5.17 (3.15)
1.25	5.42 (3.41)	-7.05 (2.26)	-5.23 (3.62)	8.32 (2.03)	5.99 (3.78)	5.34 (3.52)	-7.33 (1.30)	-6.03 (3.03)	7.63 (1.86)	6.03 (3.50)
2.0	5.07 (3.06)	1.20 (4.62)	-3.38 (3.17)	0.96 (2.64)	5.06 (3.02)	5.08 (2.08)	-.43 (3.66)	-4.10 (2.42)	1.46 (4.09)	5.44 (2.10)
Marginal Mean	2.94 (0.63)	-7.85 (0.38)	-1.60 (0.57)	8.97 (0.35)	3.07 (0.67)	2.89 (0.58)	-8.36 (0.52)	-2.29 (0.60)	8.89 (0.46)	3.23 (0.62)

Table 29

Means and Standard Deviations for Latencies Between Eye-gaze and Stimulus Position (°) at Five Positions along an Averaged Sinusoidal Function in an Eye-Tracking Procedure. Late Follicular (LF) and Late Luteal (LL) Phase Latencies for a Group of Women Reporting PMS Symptoms (N=13) are shown Across the Five Oscillating Target Frequencies

	PMS Group									
Frequency (Hz)	Late Follicular Phase (*M, SD*)					Late Luteal Phase (*M, SD*)				
	0°	90°	180°	270°	360°	0°	90°	180°	270°	360°
0.25	-0.78 (2.48)	-13.21 (1.17)	0.34 (1.84)	12.97 (.72)	-0.62 (2.85)	0.24 (2.41)	-12.94 (.98)	0.54 (2.02)	13.79 (1.32)	.38 (2.37)
0.50	0.27 (3.39)	-10.74 (5.66)	0.65 (3.19)	12.35 (1.01)	0.37 (3.62)	2.09 (4.29)	-10.25 (4.49)	-0.98 (3.92)	11.99 (3.55)	2.14 (4.89)
1.0	4.88 (1.98)	-8.41 (1.54)	-2.30 (3.97)	9.48 (1.86)	5.23 (1.89)	4.22 (4.67)	-7.17 (2.16)	-.84 (3.42)	10.65 (2.20)	4.65 (4.98)
1.25	5.23 (3.64)	-6.43 (1.79)	-5.41 (2.75)	7.49 (2.22)	5.96 (3.95)	5.38 (4.49)	- 6.25 (2.75)	-3.93 (4.44)	8.10 (3.16)	5.80 (4.56)
2.0	5.12 (2.32)	0.89 (2.86)	-3.64 (3.34)	.012 (2.95)	5.09 (2.24)	5.10 (2.84)	0.21 (3.71)	-2.64 (3.75)	1.74 (3.43)	5.47 (2.77)
Marginal Mean	2.95 (0.61)	-7.58 (0.36)	-2.07 (0.54)	8.46 (0.34)	3.21 (0.64)	3.41 (0.56)	-7.28 (0.50)	-1.57 (0.57)	9.26 (0.44)	3.69 (0.59)

Table 30

Means and Standard Deviations for Latencies Between Eye-gaze and Stimulus Position Collapsed Across Sinusoidal Position (°) for the Five Oscillating Target Frequencies in the Late Follicular (LF) and Late Luteal (LL) Phases of the Menstrual Cycle for Both Women with PMS symptoms (N= 13) and Control (N= 12) Groups.

	Control		PMS		
Frequency	Late Follicular	Late Luteal	Late Follicular	Late Luteal	Marginal Mean
0.25	0.16 (.30)	-0.13 (.25)	-0.26 (.29)	0.40 (.24)	0.04 (.16)
0.50	0.43 (.51)	0.04 (.35)	0.58 (.49)	1.00 (.34)	.51 (.22)
1.0	1.68 (.56)	1.84 (.53)	1.78 (.54)	2.30 (.51)	1.90 (.33)
1.25	1.49 (.45)	1.13 (.45)	1.37 (.43)	1.82 (.43)	1.45 (.25)
2.0	1.78 (.65)	1.49 (.50)	1.50 (.62)	1.97 (.48)	1.69 (.36)
Grand Mean	1.11 (.36)	0.87 (.29)	0.99 (.35)	1.50 (.28)	

variable. For this analysis no significant main effects or interactions were observed for either menstrual cycle phase or group (see Table 31). As expected, significant main effects were observed for frequency, $p < .001$, and position, $p < .001$. These effects indicated that women's eye position differed as a function of stimulus position as well as the speed at which the stimulus was traveling at each of the five frequencies. This was not surprising given that target velocity increased with each presentation of a trial.

In addition to the anticipated effects described above, a trend was noted for a menstrual cycle phase x group interaction, $p = .057$. This trend reflected the fact that in the LL phase of the menstrual cycle, women with PMS symptoms demonstrated a greater excursion distance with a bias toward eye movements from the left to right. In order to further elucidate this trend, women's scores were consolidated across all five averaged gaze positions for each frequency for both menstrual cycle phases. A series of independent samples *t*-tests revealed group differences in excursion distance (i.e., amplitude) in the LL phase of PMS women compared to controls with the slower oscillating target frequencies of 0.25 Hz, $t(31) = -2.43$, $p = .021$ (control mean = -1.66, $SD = 4.29$, PMS mean = 2.22, $SD = 4.87$) and 0.50 Hz, $t(34) = -2.63$, $p = .013$, (control mean = -0.75, $SD = 5.38$, PMS mean = 4.39, $SD = 6.37$). No group differences in frequency were noted in the LF phase of the menstrual cycle. In addition, no significant within group differences were noted between menstrual cycle phases. Thus, the eye-tracking experiment revealed a trend for differences between groups in the LL phase of the menstrual cycle, in which women with PMS symptoms demonstrated higher left-to-right excursions at lower-end frequencies (see Figure 19).

Table 31

ANOVA Table Showing the Effects Between Smooth Pursuit Stimulus Frequency (0.25 Hz, 0.5 Hz, 1.0 Hz. 1.25 Hz, and 2.0 Hz), Sinusoidal Target Position (0°, 90°, 180°, 270° and 360°), Menstrual Cycle Phase, and Group in an Eye tracking Experiment

	Eccentric Regions				
Dependent Variable	*F*	*df*	Error *df*	*p*	η^2
Group	.38	1	23	.54	.02
Phase	.54	1	23	.47	.02
Phase x Group[t]	4.06	1	23	.06	.15
Frequency***	.72	4	20	< .001	.72
Frequency x Group	.70	4	20	.60	.12
Position***	431.76	4	20	< .001	.99
Position x Group	.30	4	20	.88	.06
Phase x Frequency	.19	4	20	.94	.04
Phase x Frequency x Group	.11	4	20	.98	.02
Phase x Position	.74	4	20	.57	.13
Phase x Position x Group	.39	4	20	.81	.07
Frequency x Position***	97.20	16	8	< .001	1.00
Frequency x Position x Group	1.42	16	8	.32	.74
Phase x Frequency x Position	.51	16	8	.88	.51
Phase x Frequency x Position x Group	.72	16	8	.73	.59

[t] $p < .10$, *$p \leq .05$, **$p \leq .01$. ***$p \leq .001$

Discussion

Summary of Findings

Psychophysical techniques have proven to be an informative and noninvasive method of exploring visual functioning in neurodegenerative disorders such as Parkinson's disease (Archibald, Clarke, Mosimann, & Burn, 2009), Multiple Sclerosis (Caruana, Davies, Weatherby, Williams, Haq, Foster et al., 2000) and psychiatric disorders such as depression (Wesner & Tan, 2006; Zomet, Amiaz, Grunhaus, & Polat, 2008). Initial psychophysical investigation of the influence of reproductive hormones on visual stream functionality has additionally suggested anomalies in KC pathways related to hormone exposure (e.g., Eisner & Incognito, 2006). Given the perceptual differences noted in such clinical presentations, the substantial overlap between the physiological and emotional symptoms of PMS and seasonal depression, and the absence of literature examining the effects of reproductive hormones on neurological mechanisms responsible for learning and memory, further investigation of cognitive, cortico-perceptual, and brainstem operations was warranted. The purpose of the present study therefore was to investigate the effect of menstrual cycle phase and hormonal sensitivity on a series of psychophysical tasks designed to best isolate visual pathway hierarchical functioning in women with and without PMS symptoms.

Given the natural fluctuation of hormones across the menstrual cycle, it was expected that estradiol levels would be higher during the LF than during the LL phase of the menstrual cycle. Results confirmed this effect and also indicated a trend for a phase x group interaction. Exploration of this trend revealed that women in the control group demonstrated expected fluctuations in estradiol levels between laboratory sessions, showing significantly higher concentrations of the hormone during the LF phase of the menstrual cycle. Women in the PMS

group, however, did not show the expected change in estradiol levels across the menstrual cycle despite being scheduled using the same procedures as women in the control group. Thus, the typical shift in estradiol levels between the LF and LL phases was attenuated in women with PMS symptoms.

With respect to CCS, there was evidence of an overall phase effect ($p = .02$) whereby analyses revealed higher overall CCS in the luteal versus the follicular phase across both colour systems. In addition, discrete system specific effects for "red-green" and "blue-yellow" stimulus conditions were found ($p = .001$). For the R/G condition, a trend for phase indicated higher overall sensitivity for stimuli presented in the LL as opposed to the LF phase of the menstrual cycle ($p = .06$). A group x frequency effect ($p = .02$) revealed that differences in performance between groups were most notable in the lower frequency (e.g. 0.5 cpd) conditions and that in these conditions, women in the control group demonstrated greater CCS than women with PMS symptoms. A phase x frequency x orientation interaction ($p = .05$) also revealed that, at the 45 degree orientation LL phase CCS was greater than LF CCS at low frequencies but not high frequencies, while at the 90 degree orientation, LL CCS was better than LF at high frequencies but not low frequencies. A group x orientation x frequency trend ($p = .07$) indicated that, at 45 degrees, PMS women outperformed control women at 1.5 cpd but not 4.0 cpd, while at 90 degrees, PMS women outperformed control women at 4.0 cpd but not 1.5 cpd. Furthermore, regressions revealed that estradiol levels were the best predictors of R/G CCS for the control women in the 45° orientation condition.

As with R/G stimuli, results for B/Y stimuli showed a trend for the effect of phase ($p = .07$), with higher sensitivity values observed in the LL as opposed to the LF phase of the menstrual cycle. A group x phase x frequency interaction ($p = .04$) revealed that the control

group showed a phase effect (LL > LF) at 0.5 cpd ($p = .016$), but the PMS group did not. Lowest CCS values were observed across all groups and all orientation conditions at 4.0 cpd with the convergence of values especially notable in the 45° and 90° orientation conditions. In addition, between session changes in estradiol predicted CCS at all orientations for all women (pooled sample), and estradiol levels and changes in estradiol best predicted B/Y CCS in the 0° orientation condition for all women.

Although no group differences were observed with respect to S-cone sensitivity in either eccentric or hemi-retinal sections, a trend for a phase x zone effect ($p = .10$) was observed for the eccentric regions. This trend showed heightened S-cone sensitivity in the LL phase of the menstrual cycle in the central region of the retina. Regression analyses using total S-cone sensitivity in six retinal locations as dependent variables and estradiol levels in the LL and LF phases of the menstrual cycle as independent variables revealed that SWAP performance is predicted in the LF phase by a combination of estradiol concentrations in the LL and LF phases, and that each of these variables serves as a unique predictor. Surprisingly, this analysis indicated that the weights of the predictors were in opposition to one another suggesting that high levels of estradiol in the LL phase and low levels of estradiol in the LF phase best predict S-cone sensitivity in the LF phase of the menstrual cycle. No combination of variables was found to predict S-cone sensitivity in the LL phase of the menstrual cycle. Correlational analyses and examination of scatterplots indicated that women whose estradiol levels increased from the LF to the LL phase generally had the highest SWAP performance at both phases.

For the TDT experiment, women's scores demonstrated an expected leftward shift from training to testing, indicating consolidation of the task, however, no significant between group differences were observed with respect to the percentage of trials responded to correctly for any

level of SOA in training or testing. A trend for a group x SOA interaction ($p = .07$) revealed better TDT performance in PMS than control women at a SOA of 150 with a similar trend at another low SOA. To evaluate women's performance between training and testing more thoroughly, the proportion of improvement between sessions was also examined. As well, the influence of menstrual cycle phase and level of estradiol at training and testing was taken into consideration. Results indicated that the order of menstrual cycle phase at the points of training and testing exerted a significant effect on performance such that women training in the LL phase and testing in the LF phase exhibited a higher proportion of correct responses than women who trained and tested in the reverse order. The effect of session order exerted a more pronounced influence on texture learning than concentrations of estradiol in either phase and than percent change in estradiol. This effect was found for the PMS group only. Session order was found to be a unique predictor of improvement at SOAs of 330, 150, and 40 in PMS women. At the SOA of 40, estradiol levels at both training and testing were also unique predictors. Finally, percentage change in estradiol between training and testing was a unique predictor of improvement for PMS women at SOAs of 330 and 40.

The final eye-tracking experiment examined whether women differed in their ability to track stimuli moving at five different frequencies as a function of menstrual cycle phase or group. Notwithstanding a main effect of frequency, no significant effects were observed for differences in SPEM between women in the control and PMS group or between menstrual cycle phases. However, a group x phase trend ($p = .06$) revealed differences in eye movement excursion (amplitude) with women with PMS symptoms showing a bias for higher excursion distances from left to right during the LL phase.

Findings for Salivary Estradiol Analyses

The finding that estradiol levels fluctuated differentially between women in the control and PMS groups was unexpected given that differences of this kind in women with PMS or PMDD have not been previously reported. The hypothesis that luteal phase-specific differences in gonadal steroids accompany mood and behavioral symptoms has been inconsistently reported in previous studies. Rubinow and Schmidt (2006) did not observe any group differences in the levels or patterns of secretion of several different reproductive hormones, including: progesterone, estradiol, follicle-stimulating hormone, luteinizing hormone, testosterone-estradiol-binding globulin, dehydroepiandrosterone sulfate, dihydrotestosterone, prolactin, and cortisol. Other researchers such as Seippel and Backström (1998) have found associations between high LL phase concentrations of estradiol and the severity of PMS symptoms. Thus, our findings, while compelling, require replication and should be interpreted with caution.

In the present study, control women and women with PMS symptoms did not differ significantly in their levels of estradiol within phases. That is, in both the LL and LF phases, the groups' estradiol levels were comparable ($p > .05$), and despite an overall effect for the full sample of women to exhibit a shift in the expected direction between menstrual cycle phases (higher estradiol levels in the LF phase than in the LL phase), only control women demonstrated a phase shift change that was significant. Thus, the data obtained from women in the present study indicates that those reporting a history of few, if any, premenstrual symptoms have a tendency to exhibit a greater drop in estradiol levels between the late follicular and late luteal phases when compared with women reporting a high level of PMS symptoms. Certainly, the relative stability of estradiol levels between the LF and LL phases in our PMS group could be of importance in understanding any evidence of dysregulated visual-based perceptual and non-

perceptual mechanisms of the systems in PMS women that often appear to be responsive to the dynamic changes of estradiol levels in non-PMS women. More research is necessary, however, as it will be important to replicate the estradiol assay results in another sample before the findings can be tied to the experimental findings with confidence.

As described above, findings in this area are inconsistent. Previous studies have shown no evidence of differences in estradiol levels between women with and without PMS symptoms (Schmidt et al., 1998) while others have found differences between amounts of free and bound estradiol and overall levels of estradiol in the LL phase. In a sample of 129 women who provided eight serum and urine samples across the cycle, Thys-Jacobs, McMahon, and Bilezikian (2007) found significantly lower free (e.g. unbound) estradiol, percent free estradiol, and significantly higher serum hormone binding globulin in the LL phase of PMDD women compared to asymptomatic controls. No differences were found between groups in overall levels of estradiol within phases. This is in contrast to the findings of Wang, Seippel, Purdy, and Backström, (1996) who reported higher serum estradiol and lower serum progesterone during the luteal phase in women with PMS symptoms. Although neither of these studies commented specifically on the shift in hormone levels between phases, Wang et al. (1996) provided raw data which was similar to the present study in that control women, and not women with PMS symptoms, exhibited a characteristic and expected shift in levels of estradiol between the LF and LL phases. This supports a hypothesis of dysregulated cyclicity in women with PMS women and validates our findings in the study herein. The interpretation and generalization of findings in studies comparing steroid concentrations in women with and without PMS symptoms is difficult as often different methods are used to operationalize PMS symptoms, and at times, the meanings of subjective ratings are unclear (Smith, Schmidt, & Rubinow, 2003). This is complicated further

by the absence of an agreed upon method of classification for women with symptoms of PMS or PMDD and difficulty tracking the parameters of the menstrual cycle. For example, it is not clear whether PMS definitions should require an increase in symptoms between the rest of the cycle and the luteal phase, how much of a shift is required, the extent to which high symptoms in the luteal phase is important to the overall definition of pms, and whether having a high level of luteal phase symptoms is more or less important than the having a large increase in symptoms during the luteal phase.

When considering the degree of change between phases, these data are unique in that they suggest that women with PMS symptoms demonstrate smaller fluctuations in their estradiol levels between the LF and LL phases than women without PMS symptoms. This suggests that larger shifts in estradiol levels may act as a protective factor against the aversive constellation of physiological and affective symptoms that occur in some women with regularity across the menstrual cycle and which characterize PMS and PMDD.

Evidence suggests that PMS symptoms are likely the result of several complex factors whose interactions and interplay, may culminate in its phenotypic expression. Among these include the other neuroendocine systems, neurotransmitter systems, genetics (e.g. epistasis), and environmental characteristics. Recent evidence has implicated four single nucleotide polymorphisms in intron four of the ESR1 gene as conferring genotypic differences between women with and without PMDD (Miller, Vo, Huo, Roca, Schmidt, & Rubinow, 2010). This finding alone does not guarantee the presence of mood dysfunction, but it does implicate such factors as heredity, and potentially epigenetics (as symptoms were associated with SNPs and not more substantial alterations of DNA) in the expression of dysphoric symptoms associated with the menstrual cycle. Although overly simplistic on its own, the contribution of shifts in estradiol

(or lack of a shift) between menstrual cycle phases as a potential etiological factor of PMS in concert with the additional factors described above warrants further investigation.

Although extremely intriguing, these results should be interpreted with caution as salivary sampling occurred on only one occasion for each phase for each woman participating in the study. Thus, these data suggest a pattern of hormonal fluctuation characteristic of one menstrual cycle only, and may not hold up if pooled with data collected across several consecutive menstrual cycles. However, it is also worth noting that our method of saliva sampling was superior to the majority of methods published so far. That is, each salivary sample we collected contained saliva that was provided on three occasions over a period of approximately three hours. This differs from the protocols of some previous studies that have permitted participants to collect their own salivary estradiol samples at home (e.g. Chatterton, Mateo, Hou, Rademakeer, Acharya, Jordan, et al., 2005), sampled at only one interval (Rosen and Lopez, 2009) or employed the use of aids such as cotton salivettes to assist with the production of saliva (Budde, Voelcker-Rehage, Pietrassyk-Kendziorra, Machado, Ribeiro, & Arafat, 2009), which is not typically recommended (Shirtcliff, Granger, Schwartz, & Curran, 2001). The fact that our samples were collected in a laboratory under controlled conditions and across a three hour interval to control for pulsatile steroid release may mean that our salivary sampling method better captured actual estradiol levels at each cycle phase, and may constitute a strength of this study.

Findings for Chromatic Contrast Sensitivity (CCS)

Cortical PC and KC functionality was assessed in the CCS experiment. Hypothesis 1 held that higher CCS would be observed in the LF as opposed to the LL phase, while hypothesis 2 examined whether PMS symptoms would be associated with potential enhancements in CCS

owing to the supposition that these symptoms may be the result of a heightened sensitivity to normally fluctuating levels of ovarian steroids. The constellation of symptoms that define PMS and PMDD are primarily captured by two clusters represented by disturbances in somatic and affective functioning, and the broad spectrum of these symptoms suggests several potential avenues of investigation into the effects of estradiol on central nervous system functioning. Given the substantial overlap between symptoms of PMS and those of other affective disorders such as seasonal depression, and the recently identified deficits associated with visual system functionality in those with seasonal depression (Wesner & Tan, 2006), menopausal women (Eisner & Incognito, 2006), and patients using the anti-cancer agent tamoxifin (Eisner et al., 2004b), an examination of the influence of estradiol on these systems was warranted.

The finding of a significant overall phase effect and trends for a phase effect indicating higher CCS in the LL phase of the menstrual cycle in both R/G and B/Y CCS conditions supports a putative effect of reproductive steroids on V1 and associated ventral streams, however, this finding was not in keeping with the expected hypothesis (hypothesis 1) of enhanced performance in women with PMS symptoms or women in the LF phase of the menstrual cycle when levels of estradiol are highest. The present findings suggest that lower estradiol levels (LL phase) and low hormone sensitivity (control group) are associated with high cortical-based CCS. Interestingly, although not significant, with respect to the R/G stimuli, women in the PMS group demonstrated poorer performance than women in the control group at corresponding menstrual cycle phases for all orientation conditions and for the 0.5 cpd frequency condition. A similar, though non-significant observation of superior performance in CCS for control women in the LL phase was also noted with B/Y stimuli. These observations suggest that

further investigation may be warranted to better elucidate the effect of hormonal sensitivity on CCS.

As well, results demonstrated that the differentiation of groups and, in many cases, menstrual cycle phase is possible using only one of the three frequency conditions. Separation of performance on measures of CCS was evident using 0.5 cpd gabor presentations. Convergence of CCS measurements between groups and phases, however was noted with the higher frequency 4.0 cpd gabors. This may simply be due to the fact that the discernment of chromatic contrast changes are significantly reduced at higher frequencies (i.e., the low pass nature of chromatic channels), and because of this overall reduction in sensitivity, less salient CCS separations across experimentally manipulated variables are to be expected. The convergence of group and phase CCS data at the higher frequency was most pronounced with respect to the R/G gabors and less so with the B/Y condition. This suggests that somehow, factors such as receptive field orientation and reproductive hormones may play a larger role in PC operations than in KC operations. We base this conclusion on two assumptions: (1) R/G and B/Y gabors typically elicit early cortical operations (e.g., Elliott, Roth, Highsmith, Werner, & Webster, 2010; Wachtler, Sejnowski, & Albright, 2003), and 2) our near-isoluminant gabors were specifically modulated along deutan and tritan confusion lines which we infer optimally elicit PC and KC responses, respectively.

The higher overall CCS and greater performance separation between menstrual cycle phases with R/G stimuli suggests a stronger influence of the effects of estradiol on PC systems, and that this occurs perhaps further downstream in primary visual cortex (V1). Color information from L and M cones is supplied to layer 4Cβ of V1 (Chatterjee & Callaway, 2003) via the outer layers of the LGN forming the PC pathway which has also been shown to provide detailed form

information via small centre-surround receptive fields (Sumner, Anderson, Sylvester, Haynes, & Rees, 2008). Researchers believe that the PC pathway was shared by ancestral primates (O'Keefe et al., 1998) prior to the evolutionary divergence of L and M cone pigments in modern primates. This supposition has led investigators to hypothesize that colour information communicated along the PC pathway could be exploiting a pre-existing older mechanism used to convey form perception (Mollon, 1989). It is thus possible that information destined for form perception and L and M chromatic information share a common population of cells in V1 (Sumner et al., 1998). In contrast, chromatic information from S cones is supplied to V1 via layers 3B and 4A (Chatterjee & Callaway, 2003) through afferent projections of the KC system and is therefore segregated from parvocellular input at layer 4Cβ. However, recent evidence from fMRI investigations has demonstrated effects in V1 for S-cone defined orientation selectivity as well (Sumner et al., 2008). This suggests that a proportion of S-cone cell projections throughout primary visual cortex have joint responsiveness to both color and early form features such as orientation that are not anatomically distinguishable . Thus, the KC stream may be unique in that chromatic and orientation selective information share common processing areas in V1. Such a convergent pathway could therefore explain the less prominent estradiol modulation effects we noted in the B/Y conditions.

The trend for the effect of phase was strongest for the R/G as opposed to the B/Y chromatic condition indicating a more robust change in performance between phases for the R/G stimuli. The fact that chromatic information (R/G and B/Y) is processed through distinct streams could account for the apparent lack of change in CCS with changes in phase and/or orientation when the B/Y gabors were chromatically modulated along the tritan line (e.g. the KC pathway).

These results also indicate that parvocellular as opposed to koniocelluar pathways may be more responsive to changes in reproductive steroids such as estradiol.

That overall sensitivity values were highest for R/G stimuli was not surprising given the inherent properties of the parvocellular system and the apparatus used to perform these measurements (e.g., CRT characteristics). Noteworthy, however, were the trends for the effect of menstrual cycle phase for both chromatic conditions. In this experiment, we observed trends for phase effects in both chromatic conditions indicating heightened CCS in the LL phase of the menstrual cycle and better performance when estradiol levels are low. In addition, and although not significant, control women nearly always outperformed women with PMS symptoms in corresponding phases of the menstrual cycle suggesting better performance in women who are not hormonally sensitive. These results suggest downstream effects of estradiol on cells of V1 layers 3B and 4A. Although phase differences were also noted with the implied KC systems, the inter-phase relative differences in CCS were less apparent than those observed for the parvocellular system and may indicate a greater resistance to the influence of estradiol in layers unique to these projections (i.e. layer 4Cβ).

A hypothesis of a potentially differential response of cortical cells to menstrual cycle phase position in the present study indicates that low levels of estradiol in the LL phase are associated with a higher performance, while higher levels of estradiol in the LF phase are associated with a reduced ability to discriminate chromatic stimuli and thereby lower performance. This effect is especially pronounced in women without premenstrual symptoms and in PC-inferred systems. With respect to inferred KC operations, a similar but somewhat attenuated result was observed in both groups, with the highest CCS observed in control women during the LL phase of their cycle. However, this finding was accompanied by less separation

between phases in controls and near complete overlap between phases for women with PMS symptoms. This suggests that KC feeds to areas of V1 and associated extrastriate regions may be less influenced by the phasic shift of estrogens over the course of the menstrual cycle, especially for women that experience premenstrual symptoms. This was somewhat of a surprising finding given that previous investigations of SWAP have shown differential S-cone response as a result of variations in estradiol concentrations (e.g. across the menstrual cycle) or modifications in estradiol binding (e.g. with tamoxifen). It is possible that estradiol-enhanced S-cone responses may in fact be compensating for an attenuated post-receptoral differencing signal characteristic of B/Y opponency. Or alternatively, the KC pathway may be tempering the estradiol-enhanced S-cone responses to avoid bias in the opponency channels. Either way, it is the cortical opponency channels that are called upon to enable individuals to discern contrasts and compensatory mechanisms are often used to avoid higher-end signal saturation (e.g., neural gain systems). Distinctions in steroidal influences on retinal versus cortical functions therefore, should not be that surprising.

At a very general level, the findings that distinguish inferred PC from KC processing abilities supports a hypothesis of disrupted or potentially altered functioning in women with PMS symptoms in not only the receptor-based periphery, but in regions of the central nervous system that have not been previously examined. This phenomenon is exemplified by the finding that women with PMS symptoms exhibited the same relative pattern of results as control women, but to a lesser degree (non-significant difference). Given the collapsed CCS performance between LF and LL phases in these women, disrupted receptor functionality may be especially notable with respect to areas of V1 specific to KC streams. For both R/G and B/Y gabor presentations, a greater separation in CCS between menstrual phases was observed in control

women. These women also demonstrated a significant shift in estradiol levels between phases as compared to women with PMS symptoms who did not. The corresponding changes in CCS and estradiol levels between menstrual cycle phases in control women provides some initial evidence for the influence of estradiol levels on V1 cortical mechanisms, however, more research is required to fully establish this link.

Estradiol has been shown to modulate GABAergic systems in several brain regions. It has also been shown to reduce GABA levels in brain sections of female rats (Blurton-Jones & Tuszynski, 2006) and neuronal orientation selectivity has been shown in animal models to require corticocortical network cooperation that is dependent on GABAergic inhibition. For example, orientation specific cells have been shown to be reversibly influenced by the application of GABA antagonists such as bicuculline which reduce orientation selectivity (Wolf, Hicks, & Albus, 1986), or the direct application of GABA which promotes the sharper tuning of orientation selectivity (Li, Yang, Liang, Xia, Yang, & Zhou, 2008). Given the previous findings with respect to the influence of estradiol on GABAergic neurons, and the known effect of GABA on orientation selectivity, it is plausible that lower levels of estradiol characteristic of the LL phase of the menstrual cycle may promote a higher degree of GABAergic expression, which may account for the higher CCS performance observed in this phase. Conversely, higher levels of estradiol characteristic of the LF phase of the menstrual cycle may promote a greater degree of GABAergic inhibition, thus lowering the firing rate of orientation-specific cells, consequently reducing overall CCS levels. This could also account for some of the lower CCS levels found with the 45° orientated gabors (see Tables 5 and 7) in which detection is contingent on active oblique receptive fields – receptive fields that require greater GABAergic inhibitory activity to achieve selectivity than a 0 or 90° oriented receptive field (Edden, Muthukumaraswamy,

Freeman, & Singh, 2009). In other words, oblique receptive fields may actually be less susceptible to potential estradiol modulation thereby reducing behaviourally-defined sensitivity for all groups and phases, particularly at lower chromatically-relevant spatial frequencies. Once again, this finding was most evident for the inferred-PC stimuli. We argue that for the inferred-KC stimuli, it is more difficult to disentangle these subtle phase and orientation effects because of the overall CCS diminution with B/Y gabors.

The non-significant observation (as seen through visual inspection of Figures 6, 7, 9, 10 and 11) of a group effect indicating that women with PMS symptoms exhibited lower CCS in menstrual cycle phases parallel to those of controls, with less variable performance between menstrual cycle phases lends preliminary support to our hypothesis that estradiol may alter cortical PC and KC functions. Again, these effects may also have implications for GABAergic systems responsible for orientation discrimination. As described above, however, women with PMS symptoms also showed more stable salivary concentrations of estradiol during their LF and LL phases which could account for the restrained performance variation in that their levels of estradiol did not change enough to alter their performance comparably to controls.

For the R/G stimuli, supplementary regression analyses indicated that percent change in estradiol predicted CCS at all three orientation conditions across phases better than concentrations of estradiol in either phase alone. This finding was observed for the pooled sample of women as well as for women in the control group. Percent change in estradiol was best able to predict CCS in control women at the 45°orientation, explaining 35% of the variance in performance. For the B/Y stimuli, estradiol was best able to predict CCS in both PMS and control women at the 0° orientation, explaining 16% and 48% of the variance in CCS performance respectively. Given that the R/G stimuli make use of the PC pathway, which is

sensitive to orientation, it is plausible that oblique orientations may be maximally affected by changes estradiol and that this effect is not as robust in women with PMS symptoms. The differential CCS performance of women with PMS symptoms and control women suggests that the parvocellular system of those with PMS symptoms is less responsive to changes in estradiol possibly due to decreased receptoral sensitivity or a down-regulation in receptor number.

Findings for SWAP

The influence of S-cone sensitivity on upstream (retinal) KC functionality was assessed with SWAP. Hypothesis 3 held that both control women and women with PMS symptoms would demonstrate higher S-cone sensitivity in the LF as opposed to the LL phase, while Hypothesis 4 held that women with PMS symptoms might exhibit higher sensitivity relative to controls in both phases. The results of the SWAP experiment suggested no significant overall changes in S-cone sensitivity as a function of menstrual cycle phase or group. Thus, little support was found for Hypotheses 3 and 4. A trend for a menstrual cycle phase x zone effect, however, indicated significantly higher S-cone sensitivity in the central zone in the LL phase, and a similar but non-significant trend for higher S-cone sensitivity in the paracentral region in the LL phase, a time of lower estradiol. These findings are, however, in the opposite direction from the hypothesized effects.

Not, surprisingly, S-cone sensitivity was also found to differ as a function of retinal location. Specifically, results showed significantly higher sensitivities for women in the central and paracentral retinal regions compared to the periphery. Regression analyses indicated that high S-cone sensitivity in the LF phase is predicted by a combination of high levels of estradiol in the LL phase and low levels of estradiol in the LF phase. This finding is interesting as it, along with scatterplots, suggests that women whose estradiol levels increase from the LF to the LL

phase demonstrate higher S-cone sensitivity, as compared to those women whose estradiol levels show the expected decrease from the LF to the LL phase. In the present study, women with PMS symptoms demonstrated more stable levels of estrogen across the cycle (i.e., less of a decrease), and although a significant group effect was not observed, there were weak trends ($p = .16$). An examination of means and standard deviations (Table 12) shows that the PMS group exhibited higher S-cone sensitivity in nearly all conditions. Given the lack of significance of a group effect, however, this observation could be spurious and thus requires replication.

Previous research has implicated estrogen-altering pharmaceuticals such the ER antagonist tamoxifen, hormonal contraceptives, and reproductive events such as menopause in the modulation of S-cone pathways (Eisner et al., 2004b), suggesting the role of estradiol in its effect at the retinal level. However, very little research has previously examined the effect of PMS on S-cone sensitivity using SWAP.

To our knowledge, this is the first known study to examine the effects of changing levels of endogenous estrogen in a sample of healthy university aged women using SWAP. Although Eisner et al. (2004a) found higher S-cone sensitivity near ovulation and lower S-cone sensitivity premenstrually, S-cone sensitivity in that study was assessed using a 2-channel Maxwellian View visual testing apparatus and not SWAP. Moreover, their effect was observed in only one woman classified as having PMS symptoms and who began taking oral contraceptives at a point mid-way through the study. Thus, their results are difficult to generalize to a larger population of women and could be spurious or confounded by the use of hormonally-acting medications. Although the remaining six women in their study did not demonstrate S-cone mediated change across the cycle, three of the remaining six participants were taking oral contraceptives. Thus, comparisons with our participants are difficult. With respect to the findings of Eisner et al.

(2004b) in which SWAP was used to assess the S-cone sensitivity of women taking tamoxifen, the authors report that lower mean deviations in sensitivity were found in eccentric regions among those taking tamoxifen for a comparatively long period, however, the average duration for which participants in that study were using the drug was not reported. As well, those results are difficult to contrast with those of our study as the average age of women in that study was 55.2 years (range 42 to 69 years) and therefore several participants had likely transitioned from the fertile to the menopausal period. Despite inconsistencies between these studies, the findings of variable S-cone sensitivity in different populations of women (e.g., those with PMS symptoms, those taking tamoxifen, those taking oral contraceptives, and those in the menopausal period) suggests that estrogens likely play a role in S-cone mediated sensitivity, and more research is required to further elucidate these mechanisms, their effects across the cycle, their potential influence on post-receptoral circuits, and implications for these systemic changes over the lifespan.

The findings of the present experiment lend some support to the potential influence of estrogens at the level of the retina, and the concomitant alteration of S-cone functionality with naturally fluctuating levels of estradiol. In contrast to Eisner et al. (2004a), who found that a single participant with PMS symptoms showed higher S-cone sensitivity in the mid-follicular phase of her menstrual cycle, in this study, both groups of women demonstrated higher S-cone sensitivity in the LL phase of the menstrual cycle. To investigate this effect further, three paired samples *t*-tests were conducted using data from the entire sample of women to compare mean S-cone sensitivities between menstrual cycle phases in the three eccentric regions of the retina. These analyses revealed that S-cone sensitivity was significantly higher in the central region in the LL phase compared to the LF phase of the menstrual cycle. A similar directional trend was

also revealed for higher S-cone sensitivity in paracentral regions in the LL phase compared to the LF phase of the menstrual cycle. This may be due to the spatial heterogeneity of S-cone populations, although our paracentral region (10 ° to 22°) far exceeds the ±0.5° characteristic of prevailing S-cone numerosity (e.g., Williams, MacLeod, & Hayhoe, 1981). Certainly, it is plausible that S-cone spatial heterogeneity (i.e., receptor density highest foveally) can account for the few, if any sensitivity differences found in the peripheral zones.

The finding that in general (across groups) women demonstrated higher S-cone sensitivity in the LL phase of the menstrual cycle suggests that S-cone functionality may be contingent upon changing levels of estradiol, however, in a direction opposite of what was originally hypothesized. In addition, higher levels of estradiol in the LL phase of the menstrual cycle were predictive of higher S-cone sensitivity in the LF phase of the menstrual cycle, indicating that a priming effect may be occurring in which higher levels of estradiol in a phase of the menstrual cycle where estradiol levels are generally at their lowest (e.g. in the LL phase), may predispose, or sensitize women to better performance at points later in the menstrual cycle when estradiol is rising. Such hypotheses, however, require further investigation and replication of the current results.

Retinal Enhancements, Contrast Sensitivity, and PMS symptoms

The current study hypothesized that the experience of PMS symptoms (a putative indicator of higher hormonal sensitivity) would be associated with increased visual sensitivity manifested in measures of visual functioning owing to the actions of estrogens in the retina and further downstream in V1, and that the KC stream may be most affected by these enhancements. Previous findings have indicated that short-wavelength light is most effective in influencing neurometric and psychometric dysphoric symptoms (Glickman et al., 2005; Harrison & Wesner,

2010). Although no significant effects or trends were noted for women in either group to demonstrate higher S-cone sensitivity, examination of means show that sensitivity in central and paracentral regions was higher in women with PMS symptoms. Although our findings did not support an effect of group, the observation of higher, though non-significant, S-cone sensitivity for women with PMS symptoms across both cycle phases is interesting as it suggests the possibility of potential enhancements at the retinal level for women with menstrual cycle-related somatic and mood disturbances. Furthermore, these enhancements may occur in women with PMS in order to compensate for a lower sensitivity to estradiol in the parvocellular system. This would also explain why short-wavelength light might be more effective in influencing dysphoric symptoms in women with PMS.

There are several possibilities that may account for the discrepant performance of women with and without PMS symptoms across the two previous experiments. The higher performance of PMS women on measures of SWAP did not translate into enhanced B/Y CCS. Rather, in this experiment, the experience of PMS was associated with reduced B/Y CCS in parallel phases of the menstrual cycle and across most experimental conditions, suggesting the possibility of compensatory activity in post-retinal mechanisms. Alternatively, the neurological mechanisms underlying PMS may differentially impact the distinct S-on and S-off cell pathways of the KC stream, both of which are involved in the processing of B/Y CCS, whereas SWAP assessment relies more prominently on receptor functionality prior to post-receptoral increment/decrement processing and opponency (e.g., Racheva, & Vassilev, 2008; Vassilev, Zlatkova, Manahilov, Krumov, & Schaumberger, 2000). Finally, the relatively lower CCS observed in women with PMS symptoms across most spatial and chromatic conditions suggests that in hormonally sensitive women, the parvocelluar system may be less affected by changes in estradiol than in

controls. It may also suggest a stronger suppressive role for the actions of estradiol in layers of V1 for women with PMS symptoms as compared to control women; an action which may be contingent upon the interaction of estrogens with the GABAergic system, or reflective of compensation for a phase-dependent dysregulated receptor system.

Findings for Texture Discrimination

The potential influences of cycle phase and reproductive steroids on V1-specific plasticity were assessed via the use of the TDT. With respect to this experiment, it was hypothesized that a group effect would be observed whereby control women would demonstrate better performance (e.g. a higher proportion improvement between training and testing) than women with PMS symptoms (hypothesis 5). The results of the TDT experiment showed that group membership did not generally confer an advantage to performance at testing, however, training in the LL phase of the menstrual cycle and testing at the LF phase was associated with better performance than the opposite train-test order. Regression analyses confirmed that the effect of session order was significant for women with PMS symptoms and that for this group, session order and estradiol levels predicted improvement on the TDT for some SOAs while these variables were not significant predictors for control women. Given that women with PMS symptoms exhibited a non-significant change in estradiol levels between sessions, this finding suggests that more stable levels of estradiol across the cycle may be associated with enhancements in V1-specific task performance and/or plasticity. Improved performance on this task is indicated by an increasing efficiency with which observers are able to identify a target texton orientation from an array of horizontal line elements. Given that these textons are repeatedly presented to the same area of the retina, Karni and Sagi (1991) hypothesized that learning on this task involves areas of V1 in which involve both: 1) a high degree of

monocularity, and 2) orientation gradient sensitive specificity that likely occurs prior to processing at or before area 17 of V1. Thus, consistent levels of estradiol across the cycle from the LL to the LF phase may enhance mechanisms related to texture learning in or associated with areas of V1.

A combination of variables including concentrations of estradiol at training and testing and session order predicted TDT performance at SOA 330 and SOA 150 for the pooled sample of women and women with PMS symptoms. Given that the coefficients for session order were significant, task consolidation in PMS women may also be contingent upon changes in other reproductive hormones such as progesterone that were not examined in this study. Findings with respect to the effect of progesterone on learning and memory are mixed and based primarily upon laboratory and animal models. Frye and Walf (2010) report that progesterone administration improved performance on a number of memory dependent tasks including object placement, water maze, and cued and contextual fear conditioning in both wild-type mice and mice with an absence of functional progesterone receptors. Although such findings indicate progesterone-induced learning enhancement, a 2008 review of the effects of progesterone in the brain reported: no effect of progesterone on long-term potentiation in rat hippocampal CA1 slices; interference of progesterone with the estradiol mediated enhancement of synaptic transmission; progesterone induced impairment of performance on the Morris water maze; and a progesterone induced reduction of hippocampal pyramidal cell spine density which can be blocked by the progesterone antagonist RU-38486 (Diaz-Brinton, Thompson, Foy, Baudry, Wang, Finch et al. 2008). The evidence presented within this review suggests that progesterone is unlikely to account for increases in performance on tasks such as the TDT from the LL to the

LF phase, as training (e.g. consolidation) would occur when progesterone levels are high and more likely to affect learning negatively.

In contrast, several studies have demonstrated the effect of estradiol improving performance on tasks of learning and memory. For example, chronic estrogen treatment has been shown to enhance performance in radial arm maze navigation in ovariectomized rats (Luine, Richards, Wu, and Beck, 1998). In humans, transient levels of endogenous estrogens have been associated with changes in specific measures of spatial ability across the menstrual cycle (see review by Hampson, 1995) while exogenous estrogen administration has improved cognitive decline in post-menopausal women (Henderson, 2010). The observation of differential performance on tasks of spatial and/or cognitive ability in both animals and humans is complex, however, and it is widely held that these effects are both age and dose dependent. (Barha & Galea, 2010). Thus, different concentrations of estrogens are thought to exert variable effects depending upon such factors as the age of the subject (human or animal), their reproductive period (e.g. juvenile, pre, peri, or post-menopausal), cycle phase, and dose of hormone administered.

Improvements in task performance may be attributable to anatomical changes in hippocampal post-synaptic dendritic spines, which have been shown to increase proportionally with estradiol treatment and over the estrous cycle (Woolley, 1998). Both estrogen receptor subtypes α and ß (ER_α and ER_β) have been mapped at diffuse locations throughout the mammalian brain, including within the hippocampus, and although both share similar homology, the two receptor subtypes have been associated with differential functionality as demonstrated by studies in which ER_α knockout mice exhibit disrupted fertility but ER_β mice do not (Krege, Hodgin, Couse, Enmark, Warner, Mahler et al., 1998), suggesting that the ER_α may play a

greater role in reproductive functioning. Recent evidence has substantiated this hypothesis and suggests the action of the ER_β in the regulation of hippocampal synaptic plasticity and memory improvement. Liu, Day, Muniz, Bitran, Arias, Revilla-Sanchez et al. (2008) demonstrated that ER_β activation promoted long-term potentiation (LTP) in rat hippocampal slices. In addition, Liu et al. (2008) found elevated levels of phosphorylated CREB (pCREB) indicating the genomic influence of ER_β binding, Liu et al. (2008) also found elevated levels of a range of synaptic proteins including PSD-95, synaptophysin, and GluR1 in response to *in vivo* dosing with the ER_β agonist WAY-200070. All of these observed proteins have been implicated in mechanisms underlying synaptic plasticity. Thus, the present findings fit with previous evidence suggesting that estradiol plays a role in visuospatial learning and memory. With respect to the observed effect of women with PMS symptoms exhibiting a higher proportion improvement between sessions for the TDT, this renders a hypothesis of a non-significant shift in estradiol between phases as more plausible, and suggests future avenues for investigation that regard hormonal shifts and not individual cycle phases as predictors of task performance and expression of symptom severity.

Task consolidation was also observed to be robust between training in the LL phase and testing in the LF phase for the pooled sample of women, who as a group showed an overall increase in levels of estradiol between phases, suggesting that the dynamic changes (increases) in estrogen levels across the cycle may improve consolidation of the TDT task over time. Such effects may be produced through the actions of estrogen on neurotransmitter systems, which facilitate communication between brain areas outside of V1, thereby strengthening the consolidation process between training and testing, or perhaps through the actions of estrogens directly in retinotopic regions of V1. A possible hypothesis is that estradiol may aid in the

recruitment of "trained" neurons in V1, improving consolidation, and yielding a higher total neuronal response during testing, which translates into an overall improvement in performance.

In a more recent TDT study, Schwartz, Maquet, and Frith (2002) showed that repeated exposure to texture gradients provided direct evidence linking learning to enhanced neural activation in early retinotopically-specific regions of the human visual cortex. In this study, Schwartz et al. (2002) trained participants monocularly using a total of 1760 trails across 22 blocks. Following 24 hours, participants were tested in an fMRI session in which stimuli were presented binocularly with trials administered alternatively to trained and untrained eyes. Results showed an increased response in calcarine cortex to target texture stimuli presented to previously trained eyes, suggesting that plasticity in the adult brain can be induced during initial stages of visual cortical processing, where monocular inputs are still segregated. As the recruitment of additional brain areas outside of early visual cortex was not observed in task consolidation for trained eyes, their results support the effect of local neuronal plasticity in low-level V1 pathways. Interestingly, however, performance for the untrained eye was associated with a greater functional coupling between early visual cortex and distant areas in the parietal and frontal cortex, as well as the amygdala. The recruitment of these additional structures in the untrained eyes could be reflective of the invocation of brain areas involved in exposure to novel stimuli and/or tasks. With respect to TDTs these structures are likely involved in attentive processing, motivation, and the processing of visual cues necessary for task consolidation prior to testing (Schwartz et al. 2002). That additional brain areas outside of V1 were not activated in the untrained eye condition, provides further evidence for a localized effect of estrogens on texture learning and suggests early visual cortex as the primary area of plastic effects unique to TDT.

With respect to the TDT experiment, our findings suggest that naturally fluctuating levels of endogenous estrogens observed across the menstrual cycle may influence memory consolidation and subsequent performance in retinotopically-constrained tasks of spatial learning such that training in the LL phase confers an advantage to testing in the LF phase. That this effect was observed more prominently in the PMS group, suggests that this group is potentially more sensitive to phase order and changes in E; it also fits with the finding that for PMS women, both change in estradiol and session order significantly predicted TDT performance at SOA levels 330, 150, and SOA 40.

Findings for Eye tracking

Finally, potential differences in cortico-collicularpontine circuitry responsible for eye movement were assessed using the eye tracking procedure. For this experiment, a group x phase effect was hypothesized in which compared to controls, women with PMS symptoms would exhibit a deficit in eye-tracking performance that was more pronounced in the LL phase (hypothesis 6). Previous studies have not examined the relationship between PMS symptoms and SPEM, however, given the previous research in which women with PMS symptoms were found to exhibit disrupted circadian functioning, REM sleep disturbances, and greater PPI, it was hypothesized that these disturbances may also involve eye movement pathways and manifest as anomalous SPEM eye tracking responses.

No significant findings indicating the influence of either menstrual cycle phase or group were observed for the eye-tracking experiment, however a phase x group trend was observed. This trend indicated that women in the PMS group exhibited higher excursion values than women in the control group in the LL phase of the menstrual cycle at lower tracking frequencies. Such group differences indicate more eccentric gaze patterns in tracking stimuli presented at

these speeds for the PMS women in the LL phase of their menstrual cycle. Thus, the findings of this experiment lend partial support to hypothesis 6. Our findings of increased eccentric gaze in the LL phase for women with PMS symptoms suggest that procedures that assess collicular and collicular-cerebellar networks may be worthy of examination in women who are hormonally sensitive or more concentration invariant, and may provide a non-invasive method of identifying additional clinical populations affected by atypical endocrine responses.

This finding is somewhat consistent with the literature suggesting anomalous sleep architecture in women with premenstrual symptoms, and implies a potential disruption in the neural circuits underlying eye movements. Knowledge of occulomotor circuitry during sleep is sparse, however, eye-movement characteristic of REM sleep are thought to be the result of desynchronized cortical activity and muscle atonia (Escudero & Marquez-Ruiz, 2008). As described in the Introduction, previous research has also found decreased PPI in the LL phase of the menstrual cycle in women with PMDD (Kask et al., 2008). The authors of that study suggested the progestin-induced alteration of the GABAergic system and/or disrupted responses of estrogen binding in brain areas such as the nucleus accumbens and amygdala as potential mechanisms underlying the observation of decreased PPI. Taken together, the results of CCS and eye-tracking experiments provide some evidence suggestive of potentially disrupted GABAergic functioning in women with PMS systems (e.g. lower CCS than controls, lack of responsiveness to estradiol in the parvocellular system, and higher excursion distance in the LL phase than controls). As with performance in the CCS experiment in which women with PMS symptoms demonstrated significantly lower sensitivities for stimuli across most conditions, the observation of higher values for gaze position at lower frequencies in the eye-tracking experiment could be attributable to a dysregulation in GABAergic systems attributable to a lack of response to

estradiol or progesterone binding thereby supporting a hypothesis of differential hormonal sensitivity in this population of women.

Limitations of the Present Study and Directions for Future Research

Limitations of the present study are associated with time constraints, sampling restrictions, and the use of relatively strict inclusion criteria. First, the research community lacks a universally accepted and reliable system with which the construct of PMS or PMDD can be validated. Given this difficulty, women were screened using a variety of measures and classified as experiencing or not experiencing PMS symptoms based upon a number of measures of PMS from a group of women largely in undergraduate university classes. Given these diagnostic issues and limited access to a population expressing full diagnostic criteria for PMDD, we elected to look at groups of women reporting high and low levels of PMS symptoms. In establishing the existence of PMS symptoms, other researchers have employed the use of diaries across two or more menstrual cycles. Given the already substantive commitment required of women in this study and the time constraints associated with the collection of data, the use of menstrual cycle journals was impractical. This logistical limitation, however, should not preclude the use of such journals when possible in future research investigating performance measures across the menstrual cycle. As well, there is little research regarding the epidemiology of PMS and PMDD across the lifespan, and sampling conducted using a cohort of women with greater variability in age may have yielded different results with respect to selecting participants into groups. This is because fertile women in their late twenties and thirties may exhibit inherently different effects associated with: (1) the physiology of the menstrual cycle, (2) the experience of PMS symptoms, and/or (3) the influence of reproductive steroid binding in the brain; and that such differences may have differentially affected the outcome of the four

laboratory experiments. Reproductive maturity is typically reached in the mid 20's from which point forward greater cycle-related hormone change is observed until perimenopausal and menopausal periods (Hampson & Young, 2008). While the use of women with low and high PMS symptoms, as opposed to women with and without PMDD, may represent a limitation of this study, it is expected that our findings would be even stronger if we had used a clinical group. Nevertheless, our use of five measures of PMS, including two prospective measures and one measure of functional impact, appears to be one of the most comprehensive methods of evaluating the presence of such symptoms in research on PMS to date.

Exhaustive efforts were made to ensure women participating in laboratory sessions were scheduled at appropriate intervals corresponding to points in both the LF or LL phases, and that those participating in the laboratory portion of the study experienced regular menstrual cycles. It is nonetheless possible, however, that a few women were not tested at the appropriate time. Sixty-seven percent of participants in the study reported positive test results using the luteinizing hormone detection test strips provided, and although this an acceptable rate of response, it implies that thirty-three percent of women did not present to laboratory sessions in the LF phase with the corroborating information afforded by a positive test, and their session was scheduled using self reported estimates of menstrual cycle parameters alone. However, it is important to note that we did get some collaborative evidence of proper phase scheduling with the control group using the estradiol bioassay. It is reasonable to assume, therefore that if significant erroneous scheduling were present, then larger standard errors in our estradiol concentration levels would have been obtained. Furthermore, if a few women were not truly in the LF phase at the time of testing this does not invalidate our findings. If anything, it suggests that our phase effects or phase interactions may be an underestimate of the true effect sizes.

With respect to the four experiments conducted in the laboratory, menstrual cycle phase during the LF and LL phase was especially relevant for the training and testing sessions of the TDT. Optimally, time would have permitted all women to be tested and trained in complementary menstrual cycle phases. In such a design, women, irrespective of group, would have been trained in both the LL and LF (with the stimulus presented in different quadrants to prevent further learning) phases and tested later in the opposite phase, for a total of four laboratory sessions. Such a schedule would have allowed for a more comprehensive examination of the influence of menstrual cycle phase and estradiol on learning and plasticity as it relates to this paradigm, however, as described previously, task-specific confounds such as retinotopic inconsistencies or practice effects prohibited this. As well the collection and analysis of progesterone may have been beneficial as a way to both confirm cycle phase as well as to examine putative effects of this hormone on task performance in terms of main effects or interactions with estradiol.

Given the nature of the TDT in which participants typically exhibit higher performance (e.g. approaching 100% correct) at higher levels of SOA, we sampled those SOA levels that better represent the shifting psychometric operating range of the testing session. This was done in accordance to previous TDT studies (Karni & Sagi, 1991; 1993). For statistical purposes, averaging was required for two SOA levels at the high end between 360 ms and 300 ms and 160 ms and 140 ms. These two averaged SOA levels produced the best performance (e.g. highest percent correct). The remaining SOA levels between training and testing sessions were matched, however, producing subliminal responses. Given the lower overall performance of our participants, the 20 ms SOA used during testing was omitted from analysis.

As well, the fact that a number of data sets had to be excluded from main analyses for the TDT would suggest a certain level of difficulty with acquisition of the task. Although the exact reason for this difficulty is unclear, future experiments may benefit from the presentation of all participants with a modified session of initial trial learning using slower SOAs to acquaint them with the task over a longer interval of time. Although all women were provided with an opportunity to practice trials prior to the initiation of the experiment, more trials at higher SOAs may have been necessary for some women to fully comprehend the task. As well, several of the TDT studies reported in the literature have employed graduate students or trained research assistants as participants in their experiments, and such participants may exhibit characteristics different from a sample of mostly undergraduate students. Such differences may not be limited to their more advanced level of training including a higher degree of familiarity with the nature of visuo-perceptual stimuli and more experience as participants in research studies in general, but also personal traits that accompany a high level of performance at the university level; such as a higher level of perseverance at difficult tasks or a lesser concern of asking questions if material is not fully understood.

Despite these limitations, however, this is the first known study examining the influence of PMS and menstrual cycle phase on retinal, cortical and brainstem pathways using more than single case study data, and provides relevant data on the underlying neurological mechanisms of PMS and the influence of normal, phasic, reproductive steroids on neurological functioning. Future research should be directed toward the continued evaluation of circadian parameters related to the menstrual cycle including conceptualizing the magnitude of the shift in hormones over time and not necessarily as unique indicators at circumscribed points within a cycle. Evidence suggests that reproductive steroids such as estradiol manifest themselves in

neurological functions beyond those of reproduction and that this may have implications for women who are predisposed to atypical concentrations of, or sensitivities to such hormones. It is plausible that hormonal effects on visual perception may contribute to the experience of somatic and emotional symptoms experienced by women with PMS. Replication of findings from the four visual tasks employed herein or the use of additional control groups such as women using hormonal contraceptives, peri or post menopausal women, and men would be of benefit in further clarifying the effects observed in this study and how they may relate or contribute to the experience of PMS symptoms and hormonal sensitivity.

Conclusions

This study provides evidence suggesting that hormones play a role in response patterns on psychophysical tasks assessing visuo-perceptual systems in women. First, we provide evidence of altered hormonal functioning and visuoperceptual response patterns in women with PMS symptoms. In addition to exhibiting non-significant phase shifts in estradiol between menstrual cycle phases, differential performance in women with PMS symptoms was evident based on tasks suggesting a lower orientation-based CCS at the post-retinal level and higher, though non-significant short-wavelength sensitivity at the retinal level. These observations suggest a potential compensatory mechanism occurring at the LGN or further downstream in cortical areas and implicate the role of reproductive steroids in moderating these responses. Also, with respect to eye-tracking, women with PMS symptoms demonstrated higher excursion values at lower frequencies; a finding which is in line with previous studies suggesting disrupted GABAergic functioning manifesting in decreased inhibition and, in this case, motoric control of SPEM.

Second, we provide evidence of changes in response patterns on psychophysical tasks assessing visuo-perceptual systems across the menstrual cycle and with changing estradiol levels. With respect to the TDT task, a higher proportion improvement in scores was observed for women who trained in the LL phase of the menstrual cycle and tested in the LF phase of the menstrual cycle. This increase in performance between sessions also corresponded to substantial increases in estradiol between sessions, and suggests the role of this hormone in the consolidation of memory for tasks requiring retinotopically consistent operations, not unlike the improved consolidation effects observed with hippocampus.

Although the exact mechanisms underlying these observations are unclear, the nature of these experiments suggest potential brain regions, behavioral outcomes, and phenotypes which can be targeted for the examination of the influence of reproductive steroids in the future.

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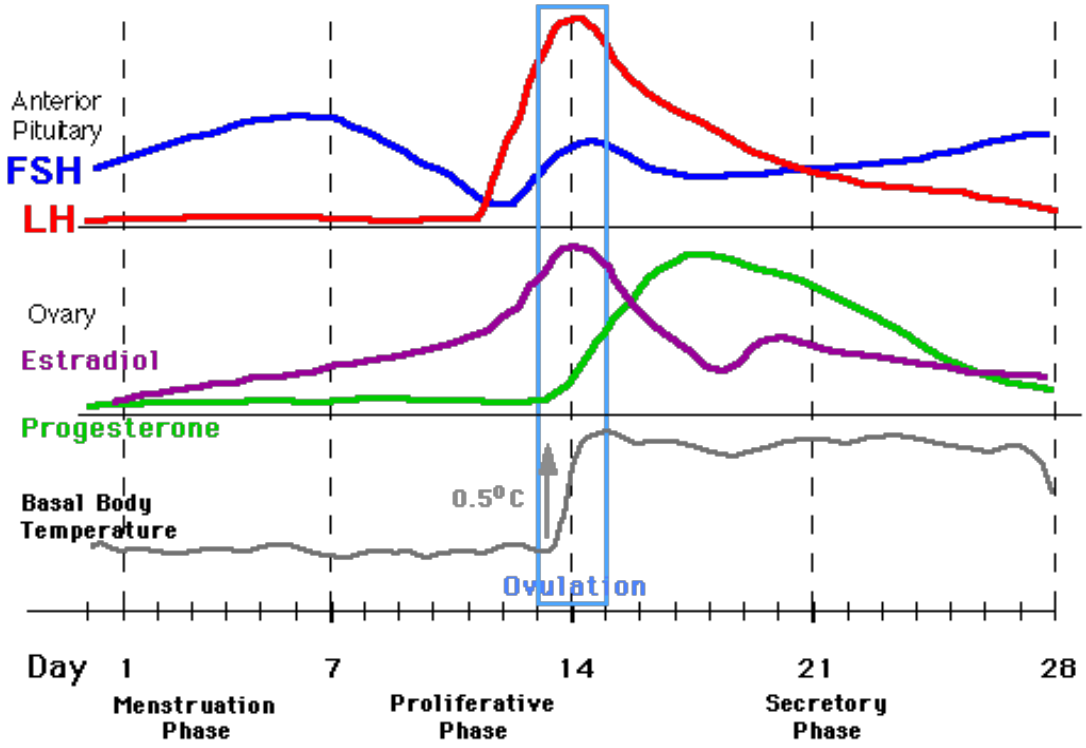


Figure 1. Overview of the adult female menstrual cycle. The habitual cycling of pituitary and ovarian hormones is shown in conjunction with corresponding menstrual cycle phases and approximate cycle days. Hormonal concentrations are in relative scales. Relative basal body temperature is also shown. Late Follicular (LF) and Late Luteal (LL) phases in this study coincided with -10 to -20 days and -1 to -8 days respectively (forward count equivalent to days 10 to 20 and days 22 to 28 respectively). Figure obtained from: http://embryology.med.unsw.edu.au/Science/ANAT2341lecture03.htm

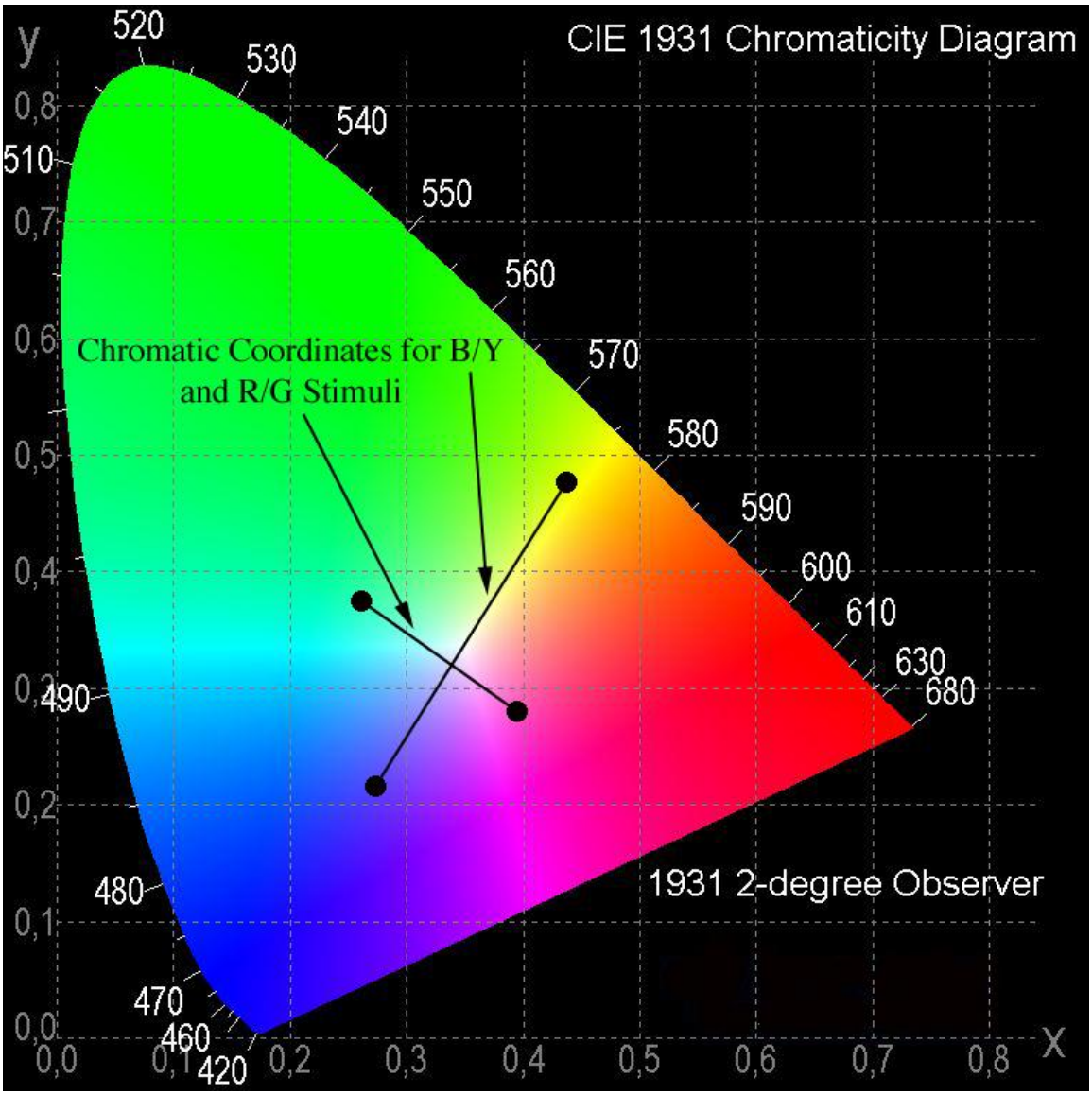


Figure 2. CIE (1931) Colour Space identifying the "Blue-Yellow" (B/Y) and "Red-Green" (R/G) "peak-to-trough" chromatic gabor modulations and endpoints. B/Y endpoints were [(.2739, .2263), (.4280, .4976) and R/G endpoints were [(.3828, .2846), (.2639, .3722)]. Background chromaticities were halfway between the two endpoints. Modulations from the endpoints through the background conformed to tritan and deutan confusion lines (Devalois & Devalois, 1990). Details in text.

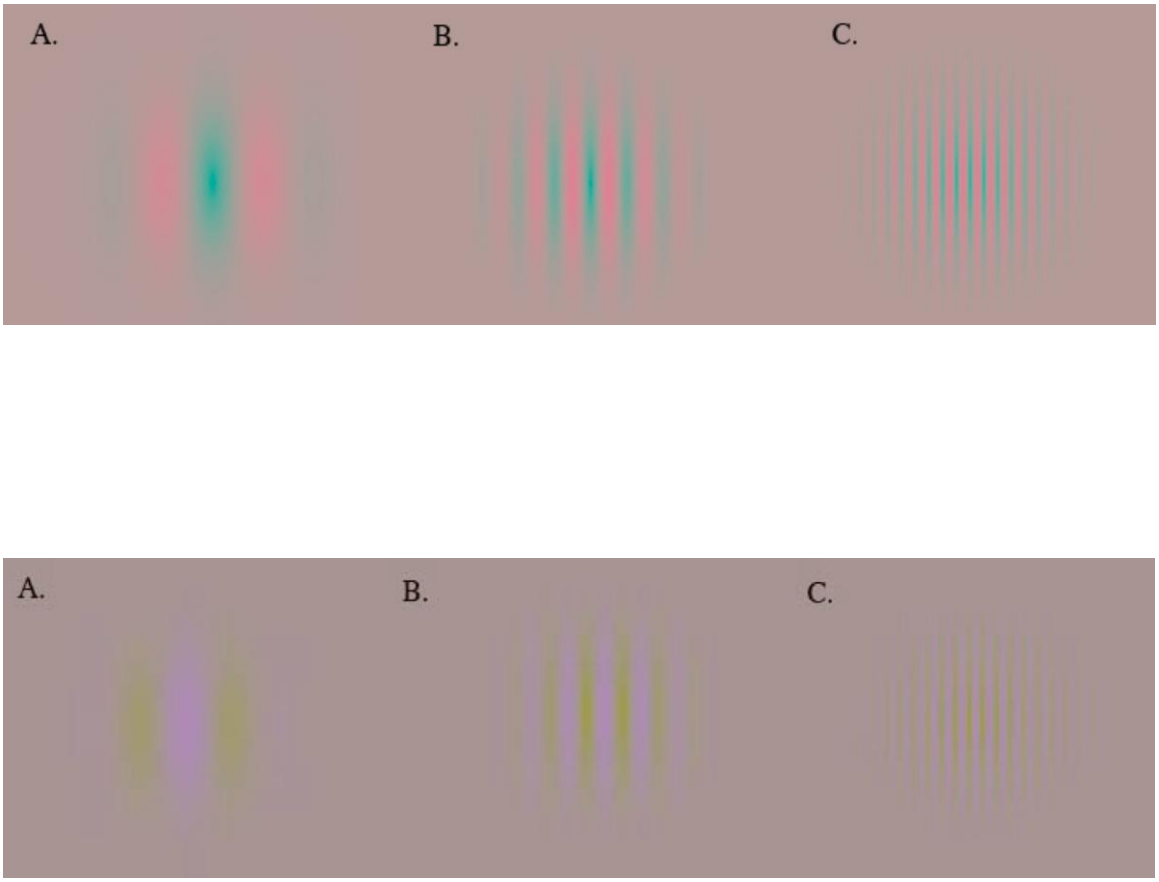


Figure 3. Representations of chromatic gabor stimuli. Top and bottom rows depict chromatic near isoluminant "red-green" and "blue-yellow" gabors, respectively. Anchor points for relative peak and trough contrasts are detailed in the Methods section. Panels A, B, and C illustrate the appearance of gabors viewed at a distance of 75 cm with center spatial frequencies of 0.5, 1.5 and 4.0 cpd, respectively.

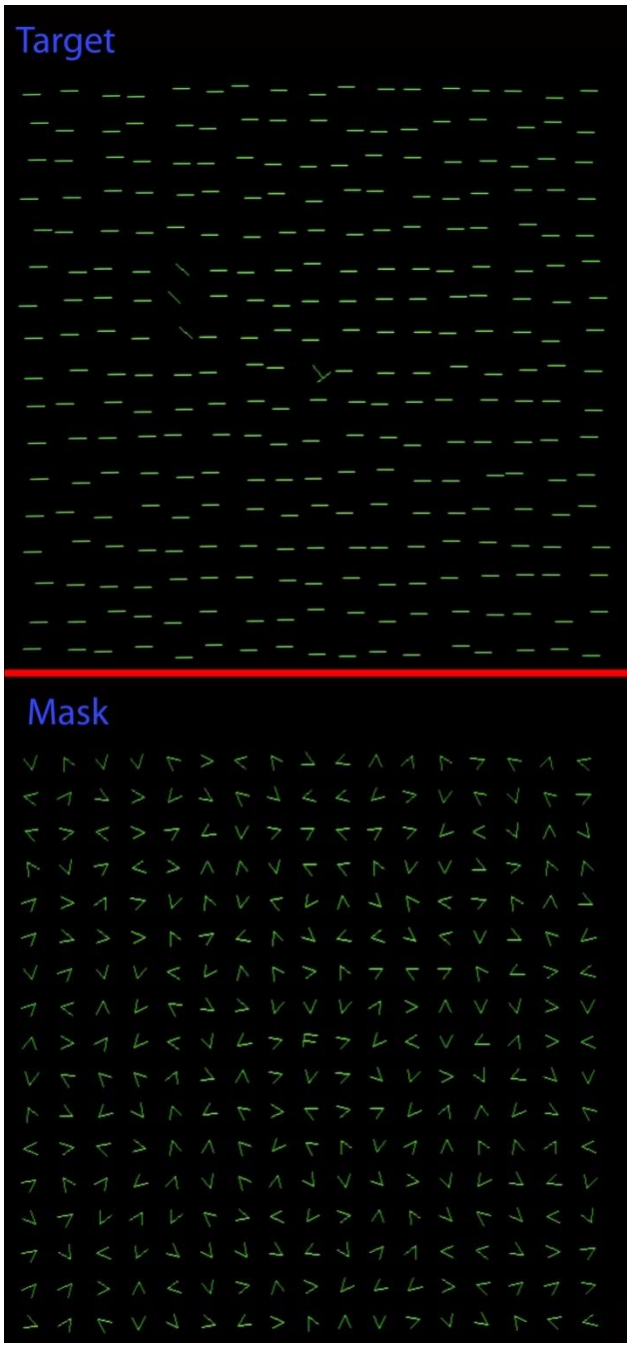


Figure 4. Illustration of the Texture Discrimination Task (TDT). Participants were presented with the above textures, consisting of a foreground target of three 566-nm "green", 23 arc-min long elements (textons) differing only in orientation from a background of horizontal, identical textons. The grouping of the three-element textons was presented randomly as either three vertically stacked textons or three horizontally positioned, side-by-side textons. The target textons were always presented to a participant in the same one of four quadrants for all training and testing trials (the example above shows the target in Quadrant I). To ensure central fixation a 'T' or 'L' was presented in the middle of the monitor concurrently with the textons. All participants were asked to first identify the fixation letter followed by the orientation of the target textons (i.e., horizontal or vertical) that was presented in the target presentation (top panel). Following the target display and a brief inter-stimulus blank, a patterned mask was presented (bottom panel). The inter-stimulus blank, known as stimulus onset asynchrony (SOA), decreased in duration from 360 to 20 ms depending on the lab session and trial block.

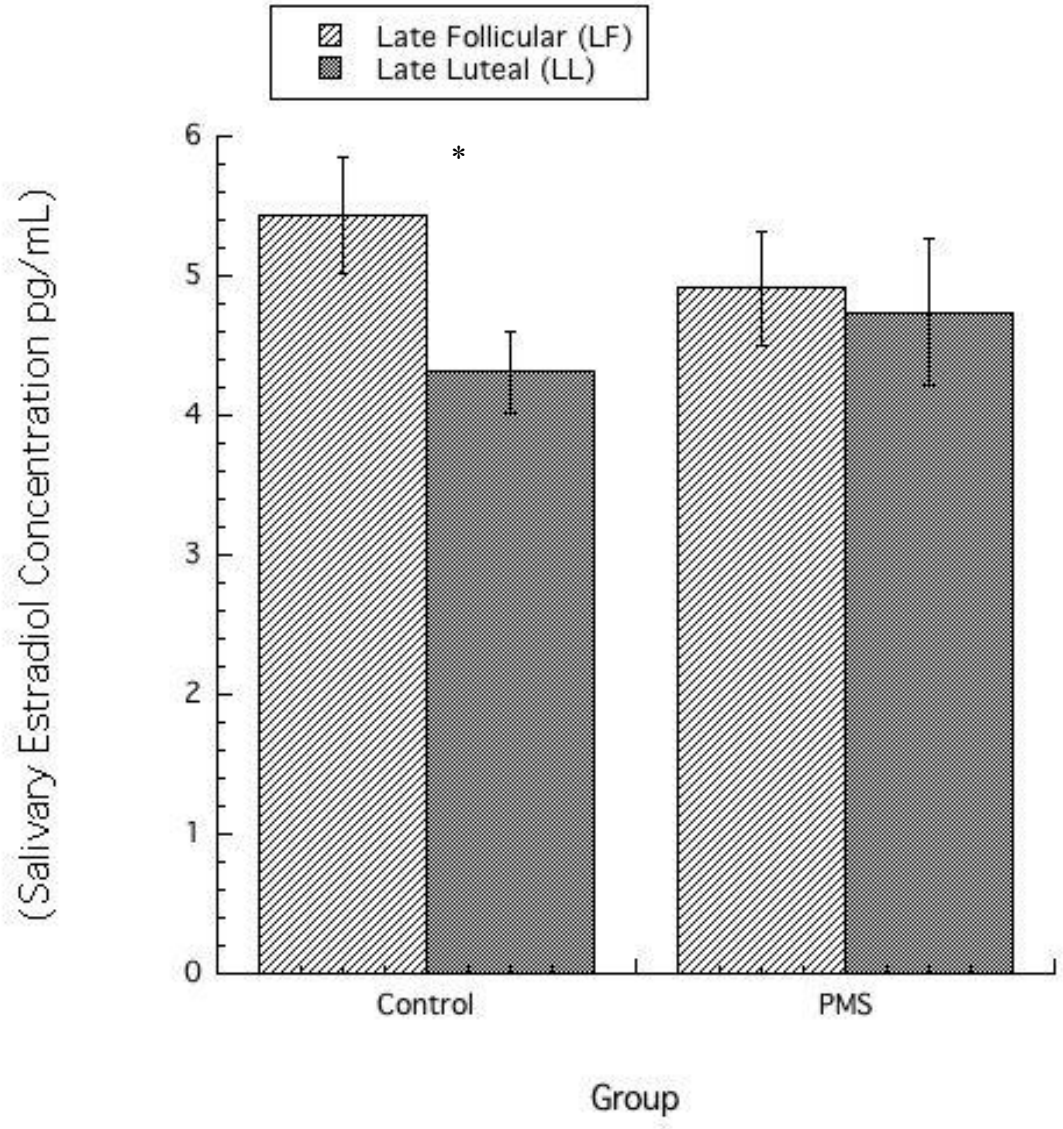


Figure 5. Salivary estradiol concentrations as a function menstrual cycle phase in a group of control women and a group of women with PMS symptoms. The figure illustrates an overall effect of phase $p = .019$, as well as a trend for a group x phase effect, $p = .078$. For the control group a paired samples *t*-test revealed significantly higher estradiol concentrations in the LF than the LL phase of the menstrual cycle, $t(17) = 3.48$, $p = .003$. For the PMS group, however, a paired samples *t*-test revealed no differences between estradiol concentrations in the LF and the LL phase, $t(14) = 1.00$, $p = .33$. Thus, with respect to the present study, these analyses revealed that, unlike control women, women with PMS symptoms did not undergo phasic cycling of estradiol across the menstrual cycle. Error bars equal ± 1SEM.

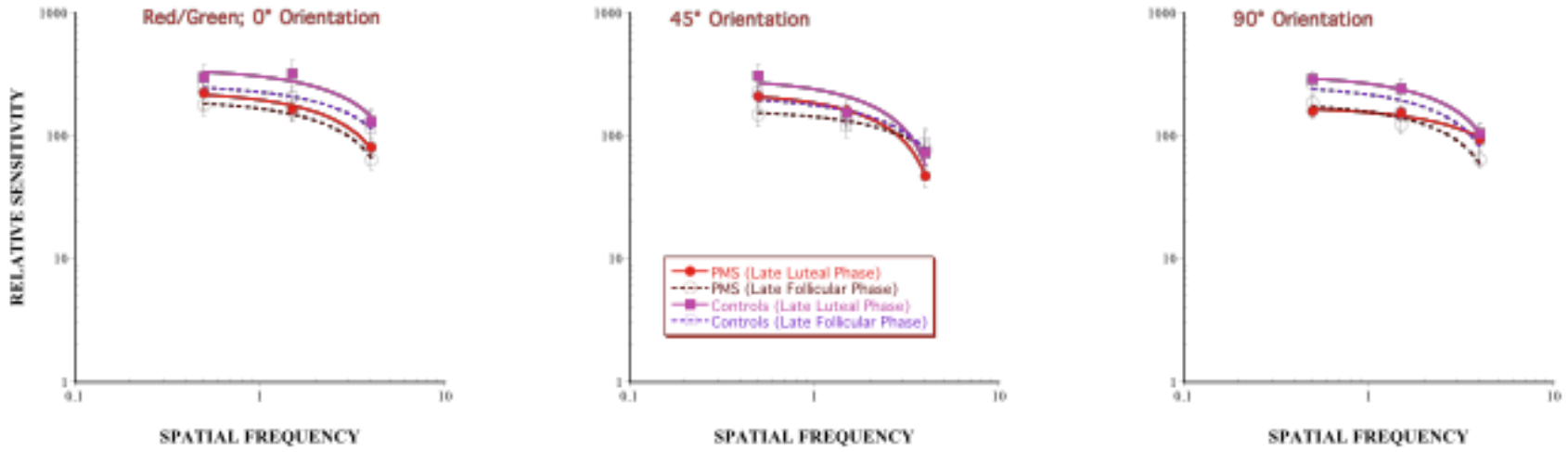


Figure 6. Data for the "red-green" chromatic contrast condition. Relative mean chromatic contrast sensitivity (CCS) as a function of spatial frequency plotted on log-log axes. Functions show typical low-pass chromatic contrast sensitivity with convergence towards the high, 4.0 cpd spatial frequency gabors. Control women (pinkish squares) and women with PMS symptoms (reddish circles) and are shown as filled or open symbols for the late luteal (LL) phase (continuous lines) or late follicular (LF) phase (dashed lines) data, respectively. The data for the different gabor orientations are shown across the three horizontal panels, with 0° representing horizontal, 45° diagonal and 90° vertical oriented center frequencies. The continuous (stippled) lines are fits from Movshon and Kiorpes' (1988) double exponential template. Error bars are ±1 SEM. Note that overall, the LL phase (solid symbols) shows a trend towards producing higher CCS than the LF phase (open symbols), $p = .061$. Performance at the three spatial frequencies is also observed to differ as function of group (group x frequency effect), with controls performing better than the pms group, $p = .02$. Additionally, trends for phase x frequency x orientation $p = .051$, and frequency x orientation x group, $p = .07$ were noted. Similar plots are shown in Figure 10 for complementary B/Y data. Details found in the correspomding section.

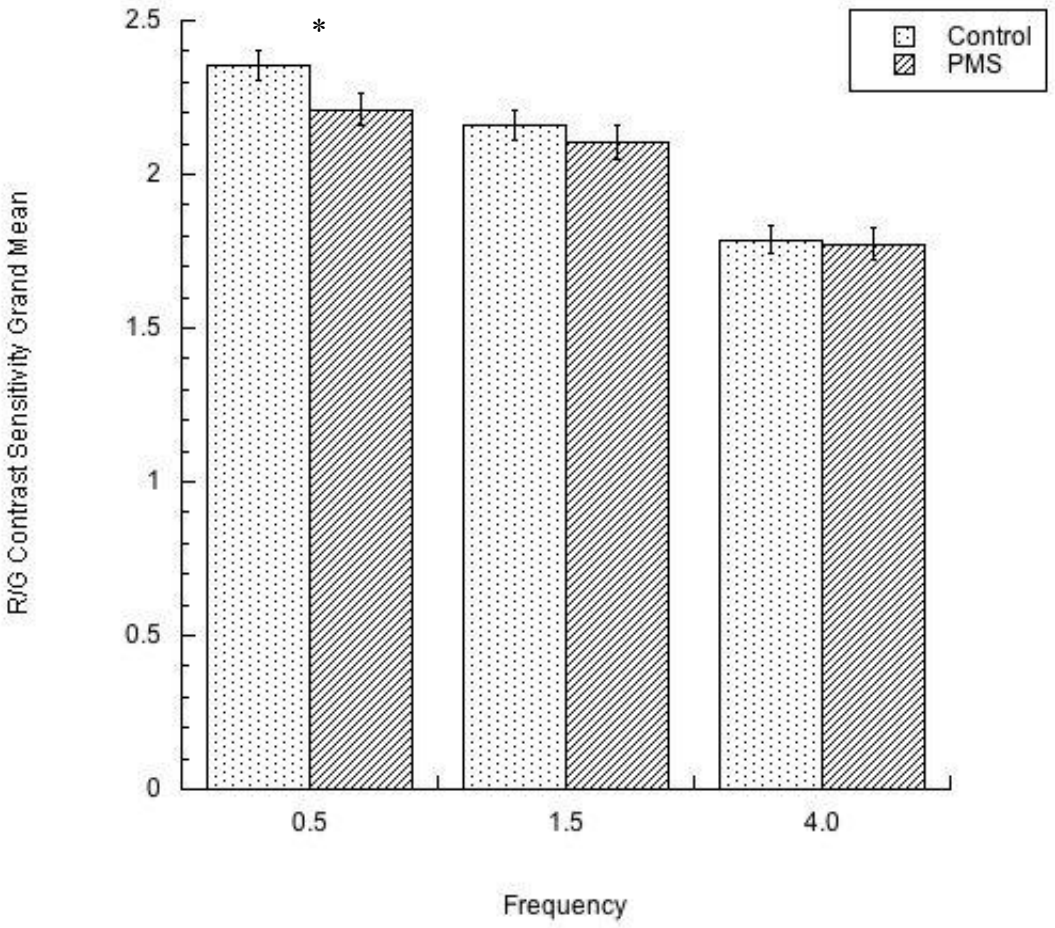


Figure 7. R/G CCS measurements for the three center spatial frequencies used in this study. The bar graph shows CCS for control women (*N*=18) and women with PMS symptoms (*N* = 16). For both groups, performance (sensitivity) at 4 cpd is lower that performance at 1.5 cpd. In addition, control women showed a tendency to perform better than women in the PMS group and significantly so at the lowest frequency, 0.5 cpd, $F(1, 33) = 4.50$, $p = .042$. Error bars equal ± 1SEM.

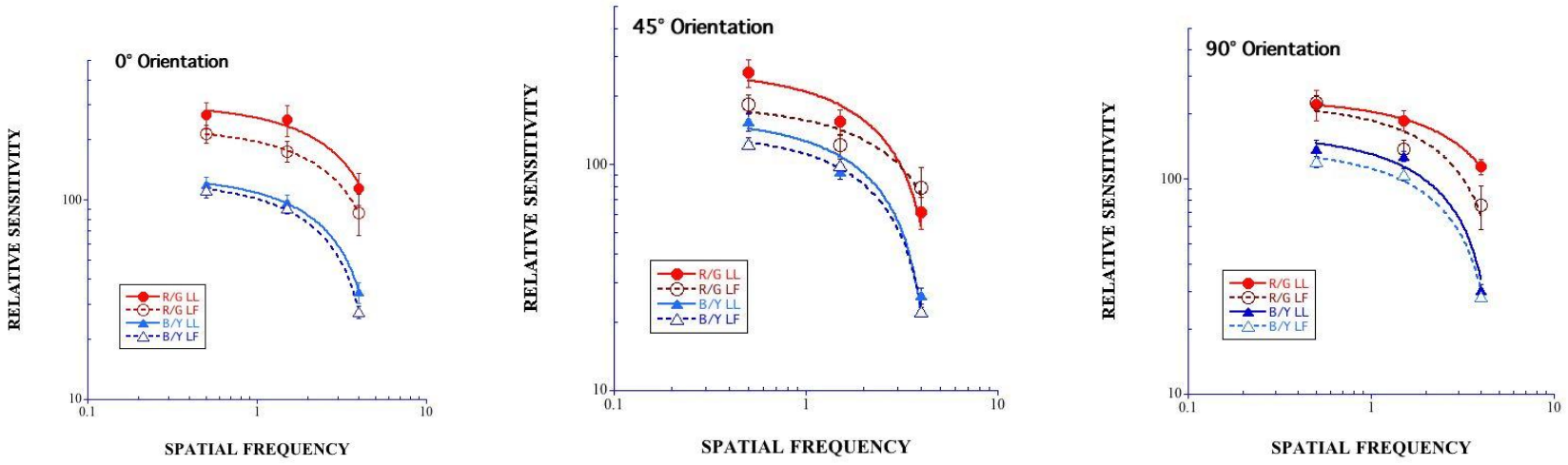


Figure 8. Chromatic Contrast Sensitivity (CCS) at three orientation conditions as a function of spatial frequency and menstrual cycle phase for "red-green" (R/G) and "blue-yellow" (B/Y) gabors. The data is collapsed across groups. Red lines depict data fits for R/G presentations in the late follicular (LF) (open symbol; dashed line) and late luteal (LL) (solid symbol; continuous line) phases respectively. Blue lines data fits for B/Y presentations in the LF (open symbol; dashed line) and LL (solid symbol; continuous line) phases respectively. For R/G data, the figure illustrates a trend for the effect of phase, $p = .061$, indicating increased CCS in the LL phase as compared to the LF phase. A second trend was revealed for a phase x frequency x orientation interaction, $p = .051$. With respect to this trend, contrasts illustrated that at the 45° orientation, women exhibited higher CCS in the LL phase at 0.5 cpd and higher CCS in the LF phase at 4.0 cpd. In contrast, at 90° presentations, women exhibited greater CCS in the LF phase at 0.5 cpd and higher CCS at 4.0 cpd in the LL phase of the menstrual cycle. $F(1, 32) = 12.24$, $p = .001$, $\eta^2 = .28$, observed power = 0.92. This effect is notable in the middle and right-hand panels. For the B-Y data, a trend was revealed for the effect of menstrual phase, $p = .072$, indicating increased CCS in the LL phase as compared to the LF phase of the menstrual cycle. Error bars equal ± 1SEM.

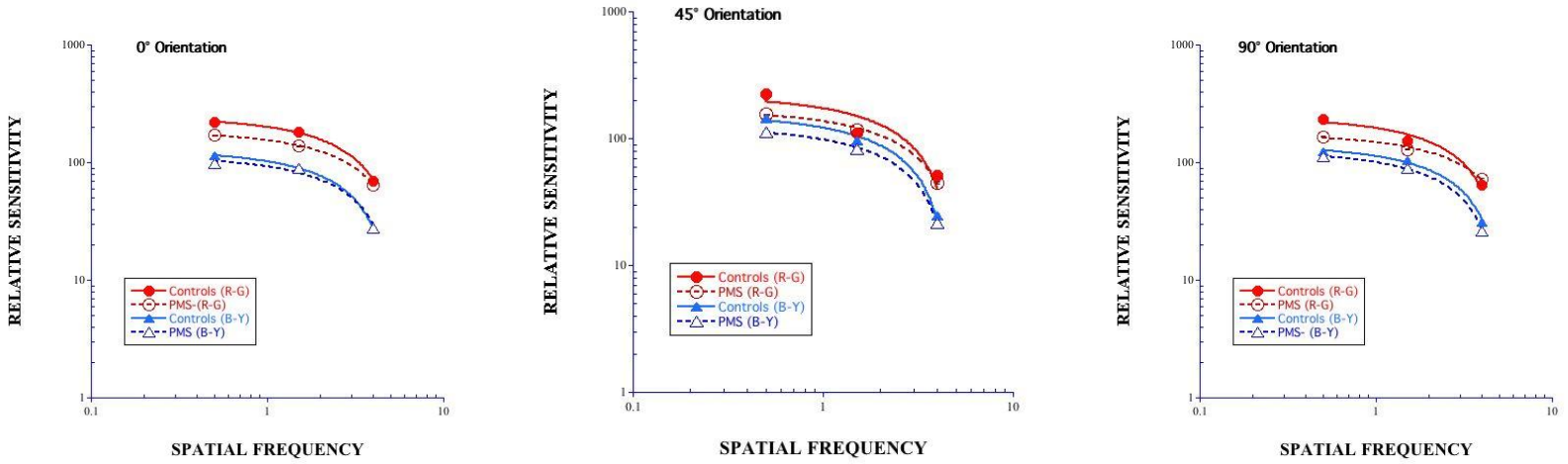


Figure 9. Chromatic Contrast Sensitivity (CCS) at three orientation conditions as a function of spatial frequency and group for "red-green" (R/G) and "blue-yellow" (B/Y) gabors. Red lines depict data fits for R/G presentations for the control group (solid symbol; continuous line) and PMS group respectively (open symbol; dashed line). Blue lines data fits for B/Y presentations for the control group (solid symbol; continuous line) and PMS group respectively (open symbol; dashed line). For R/G data, the figure illustrates a trend for a frequency x orientation x group effect, $p = .07$ in which, for the 45° condition, women in the control group exhibited lower CCS than women with PMS symptoms at 1.5 cpd, and higher CCS than women with PMS symptoms at 4.0 cpd. The reverse was observed with respect to the 90° orientation condition, in which women in the control group exhibited greater R/G chromatic CS than women with PMS symptoms with for stimuli presented at 1.5 cpd and lower CCS than women with PMS symptoms at 4 cpd presentations. Error bars equal ± 1SEM.

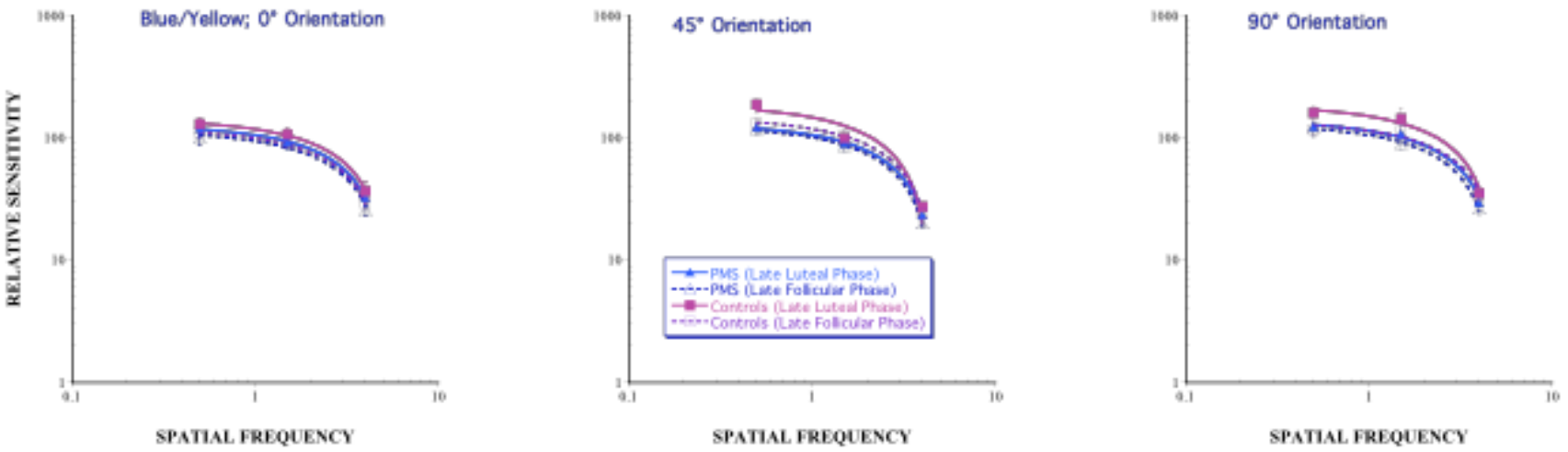


Figure 10. Data for the "blue-yellow" chromatic contrast condition. Relative mean CCS as a function of spatial frequency plotted on log-log axes. Functions show typical low-pass chromatic contrast sensitivity. Women with PMS symptoms (triangles) and control women (squares) are shown as filled or open symbols for the late luteal phase (solid lines) or late follicular phase (dashed lines) data, respectively. The data for the different gabor orientations are shown across the three horizontal panels, with 0° representing horizontal, 45° diagonal and 90°vertical oriented center frequencies. The continuous (stippled) lines are fits from Movshon and Kiorpes' (1988) double exponential template. Error bars are ±1 SEM. Overall, women in the LL phase (solid symbols) showed a trend to produce higher CCS than in the LF (open symbols), $p = .072$. A phase x frequency x group effect, $p = .022$, was also observed indicating better performance at higher compared to lower spatial frequencies, and for women in the control group to exhibit the highest overall CCS in the LL phase.

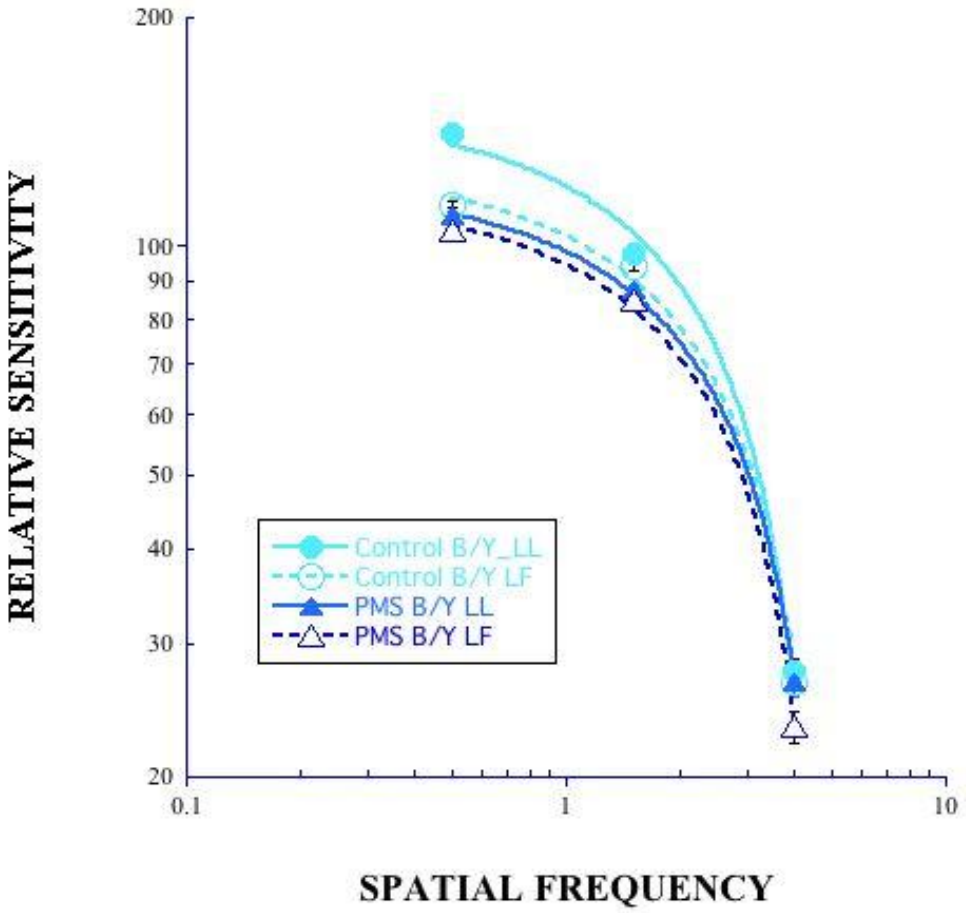


Figure 11. Figure illustrating a group by spatial frequency by phase effect (p =.037) for "blue-yellow" contrast sensitivity at the late-luteal (solid line) and the late-follicular (dashed line) phases of the menstrual cycle for women in control (light blue) and PMS (dark blue) groups. For this figure, means for phase and spatial frequency are collapsed across orientations. Higher performance for control women is noted across all three spatial frequencies in both phases, and especially in the LL phase. Only women in the control group demonstrated a significant difference in performance $t(17) = 3.40$, $p = .003$ between the LF ($M = 6.16$, $SD = .40$) and LL phases ($M = 6.44$, $SD = .50$), at a frequency of 0.5 cpd. Error bars equal ± 1SEM.

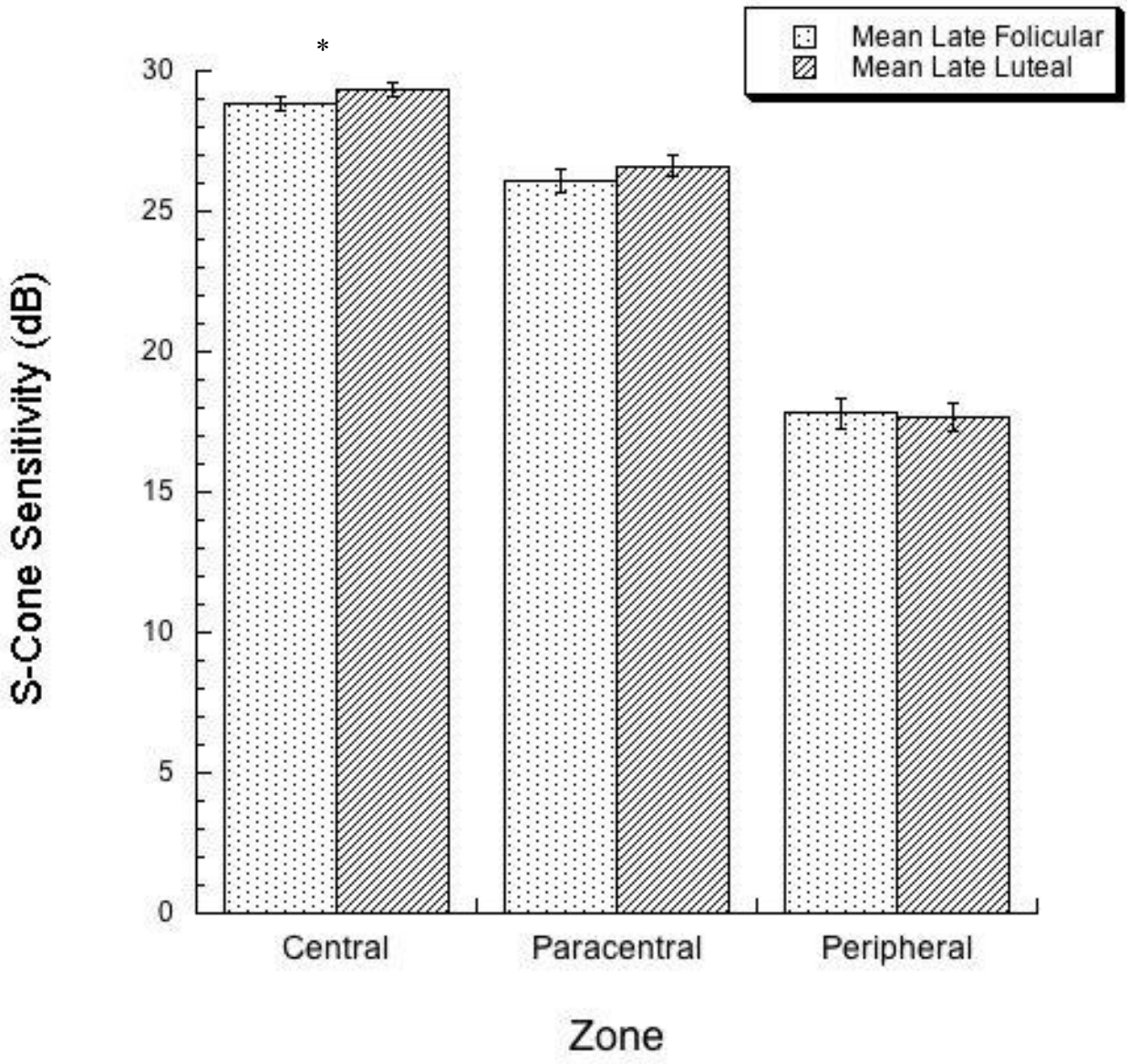


Figure 12. Figure illustrating a trend ($p = .10$) for a phase x retinal zone effect. Mean S-cone sensitivity as a function of menstrual cycle phase and retinal eccentricity (central, paracentral, and peripheral) for all women (N = 34; n = 18 control women and n = 16 women with PMS symptoms) as measured by Short-Wavelength Automated Perimetry (SWAP). Paired samples *t*-tests performed for each zone revealed that women exhibited significantly higher SWAP sensitivity in the LL phase compared to the LF phase in the central zone, $t(33) = 2.29$, $p = .029$. A similar trend for higher overall sensitivity in the LL phase over the LF phase was additionally observed for the paracentral zone, $t(33) = 1.69$, $p = .10$. No differences were revealed between phases for peripheral regions $t(33) = -.37$, $p = .72$. Error bars equal ± 1SEM.

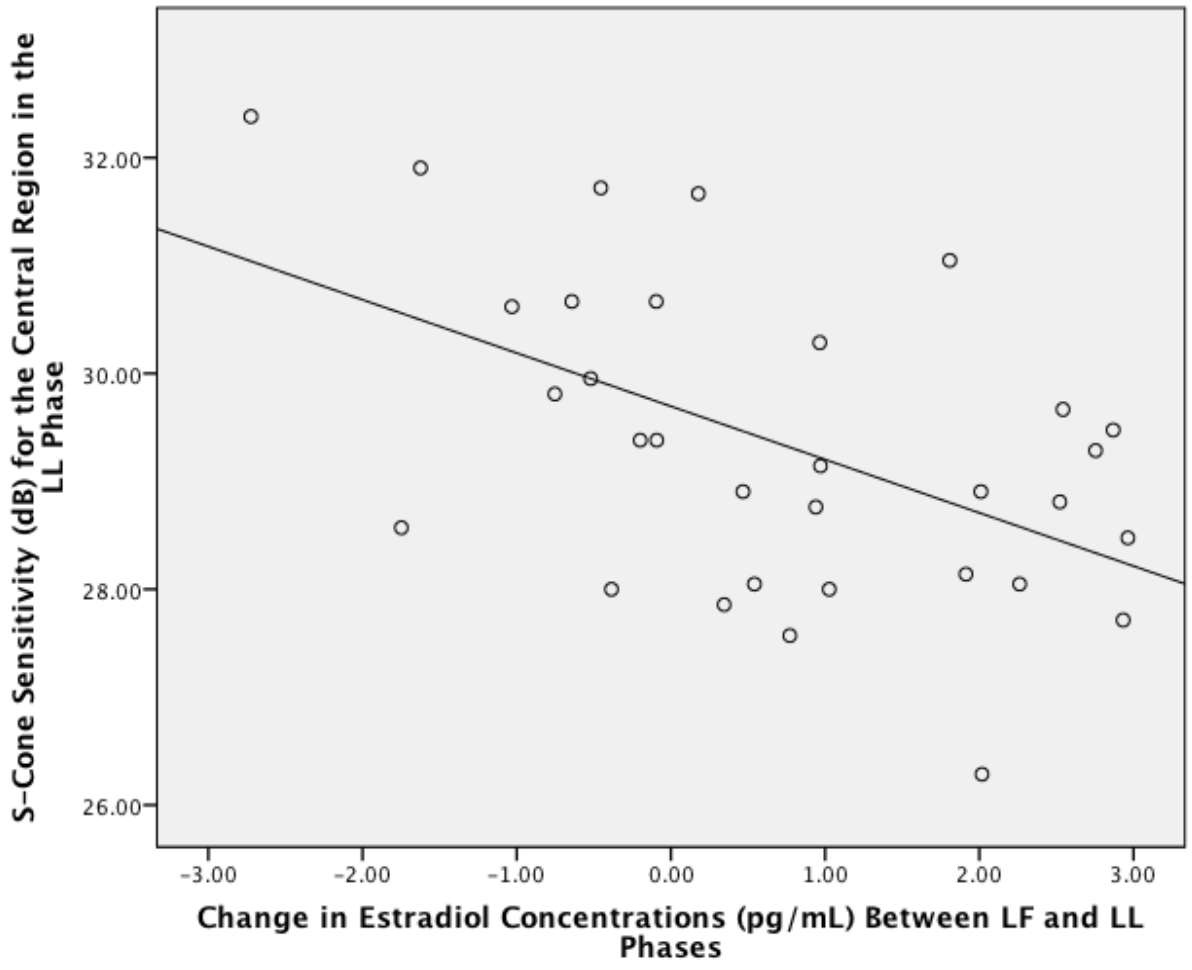


Figure 13. Scatterplot illustrating the relationship between the change in estradiol concentrations between the Late-Luteal (LL) and Late Follicular (LF) phases and S-cone sensitivity in the central retinal location for a testing session occurring in the LL phase. The scatterplot indicates that women showing the expected decreases in estradiol from the LF to the LL phase have the lowest S-cone sensitivity while those whose estradiol levels increase from the LF to the LL phase have the highest S-cone sensitivity.

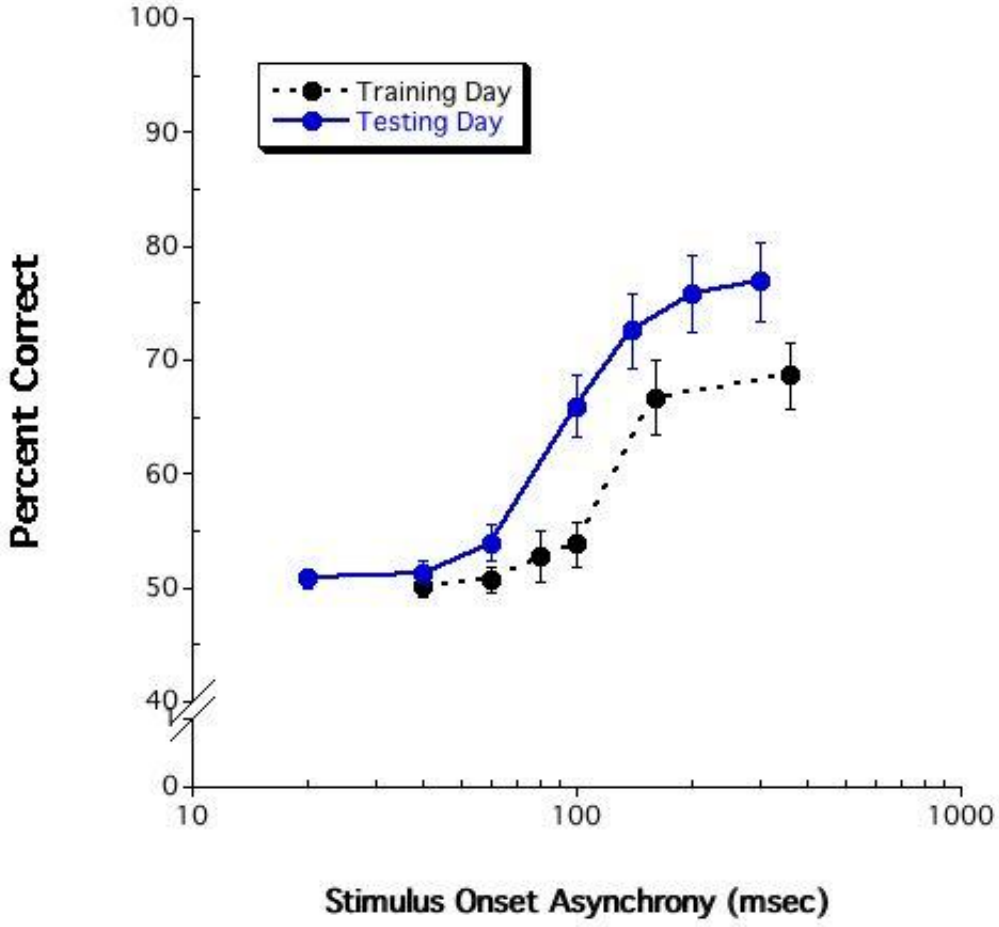
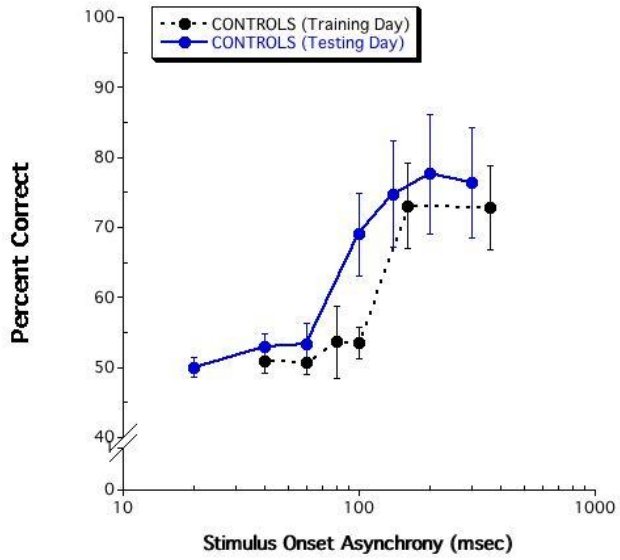


Figure 14. Mean percent of trials responded to correctly (%C) as a function of stimulus to onset asynchrony (SOA) in a texture discrimination task (TDT). Women's (*N*= 27) scores improved between day of training and day of testing as indicated by the leftwards shift in the psychometric function (i.e., %C improves with shorter SOA). Error bars equal ± 1SEM.



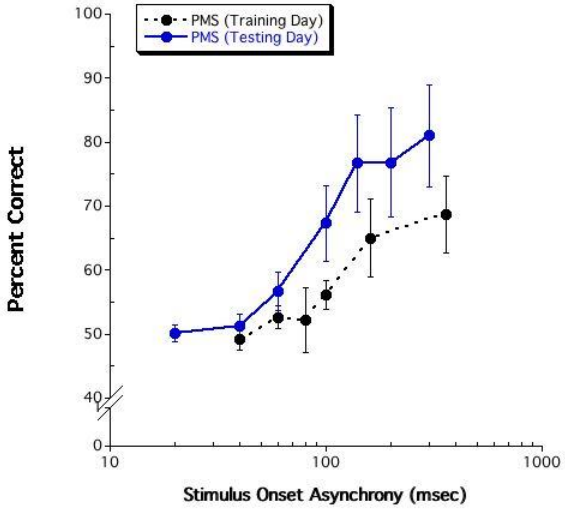


Figure 15. Mean percent of trials responded to correctly (%C) as a function of stimulus to onset asynchrony (SOA) in a texture discrimination task (TDT) for a control group (N=14) of women (top panel) and a group of women with PMS symptoms (N= 13) (bottom panel). With the data collapsed across menstrual cycle phase in these plots, both groups show the expected leftward shift of the curve on testing day indicating discrimination performance improvement. Error bars equal ± 1SEM.

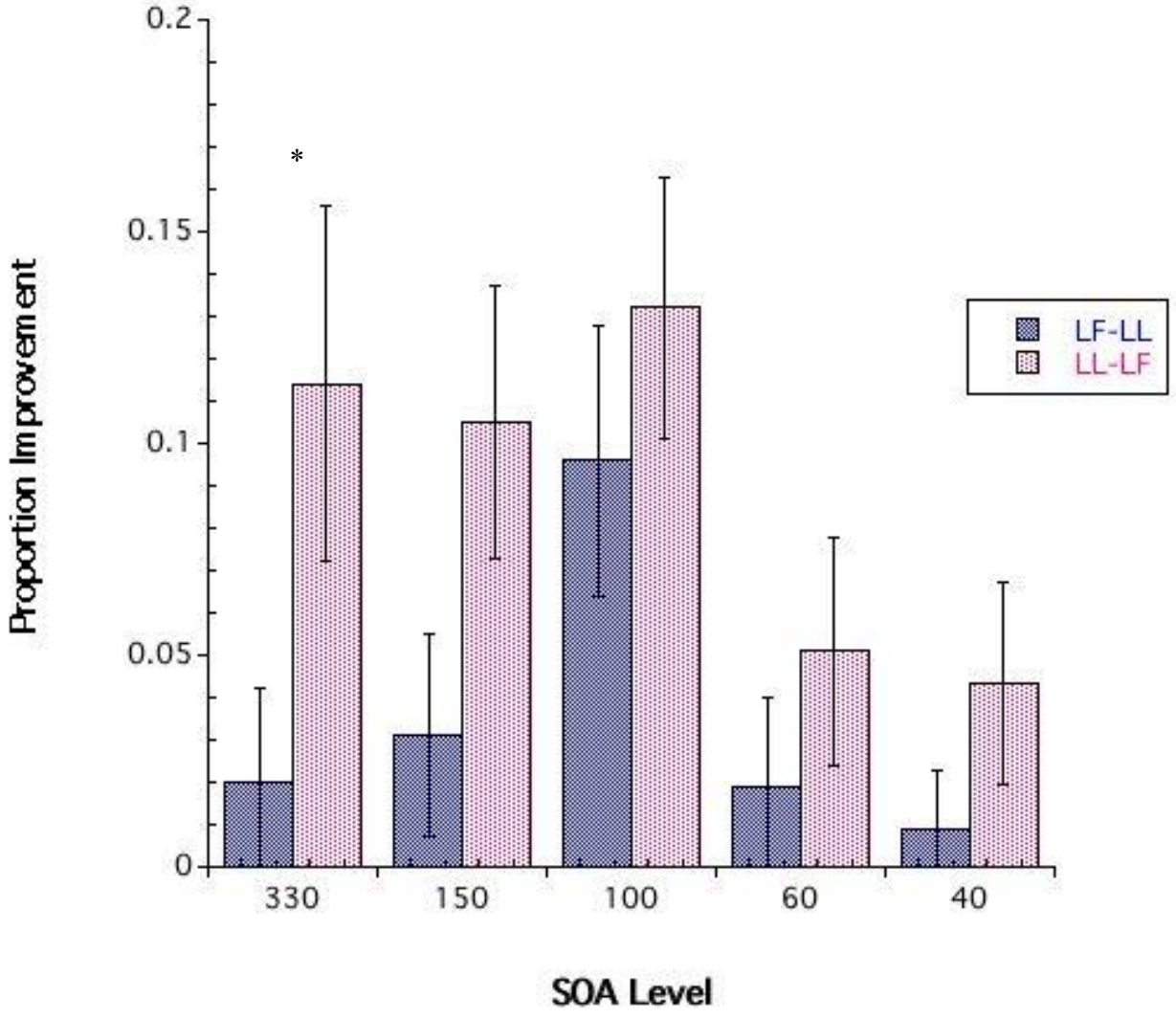


Figure 16. Mean proportion of improvement between training and testing sessions for a group of women training in the late follicular phase and testing in the late luteal phase of the menstrual cycle (LF-LL; $N = 18$) and a group of women training in the late luteal phase and testing in the late follicular phase of the menstrual cycle (LL-LF; $N = 9$) at five levels of averaged stimulus to onset asynchrony (SOA) in a texture discrimination task. The figure shows a trend ($p = .06$) indicating a higher proportion of correct responses for women who trained in the LL phase and tested in the LF phase, as opposed to those who performed the experiment in the reverse order. Post-hoc analyses indicated a significant difference in proportion change scores at SOA 330, $p = .038$, and a trend for those at SOA 150, $p = .075$. Error bars equal ± 1SEM.

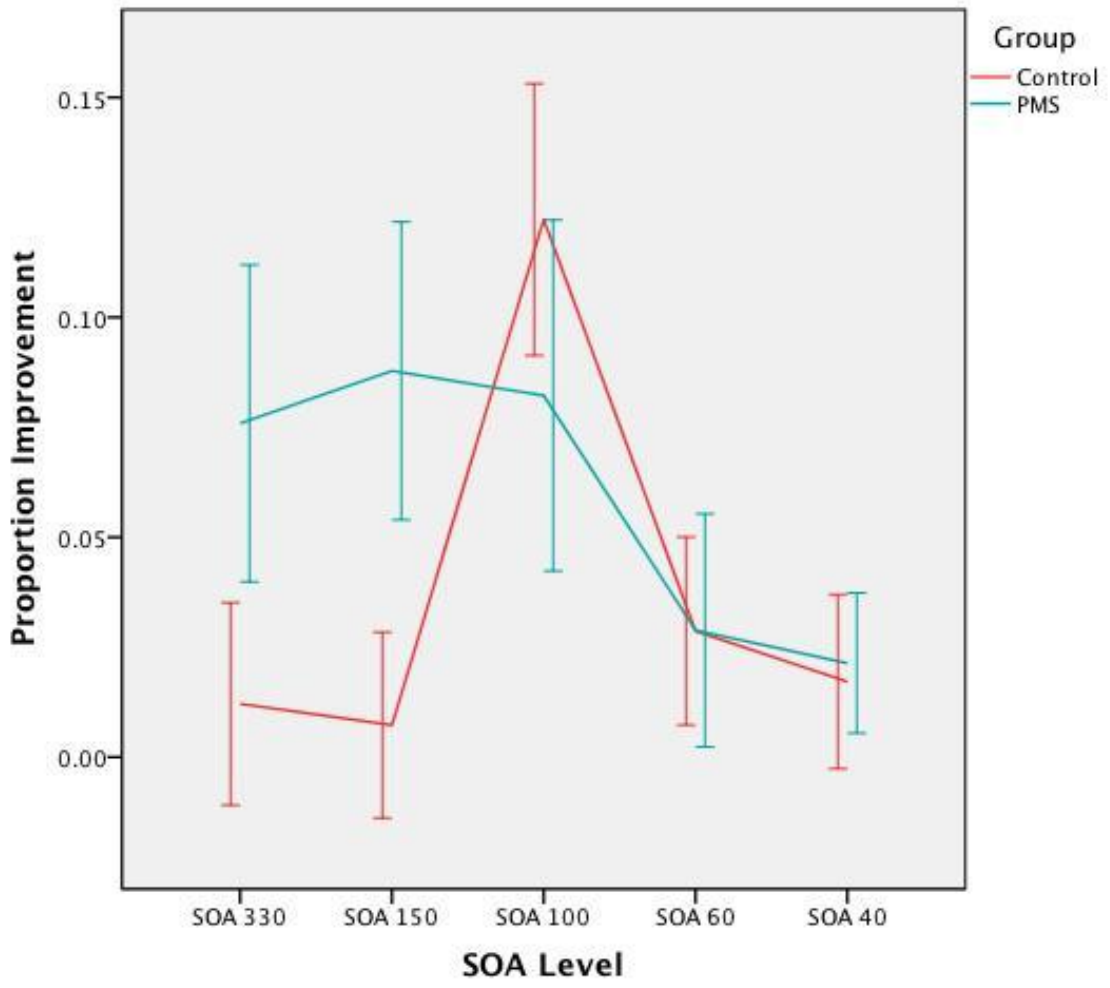


Figure 17. Mean proportion of improvement between training and testing sessions for a group of control women ($N = 14$) and a group of women with PMS symptoms ($N = 13$) at five levels of averaged stimulus to onset asynchrony (SOA) in a texture discrimination task. The trend for a group x SOA level effect is shown above ($p = .07$). Post-hoc analyses revealed a significant between group effect at SOA 150, $F(1, 26) = 4.20$, $p = .05$, indicating that women with PMS symptoms indicated a higher proportion of improvement at this SOA than control women. Error bars equal ± 1SEM.

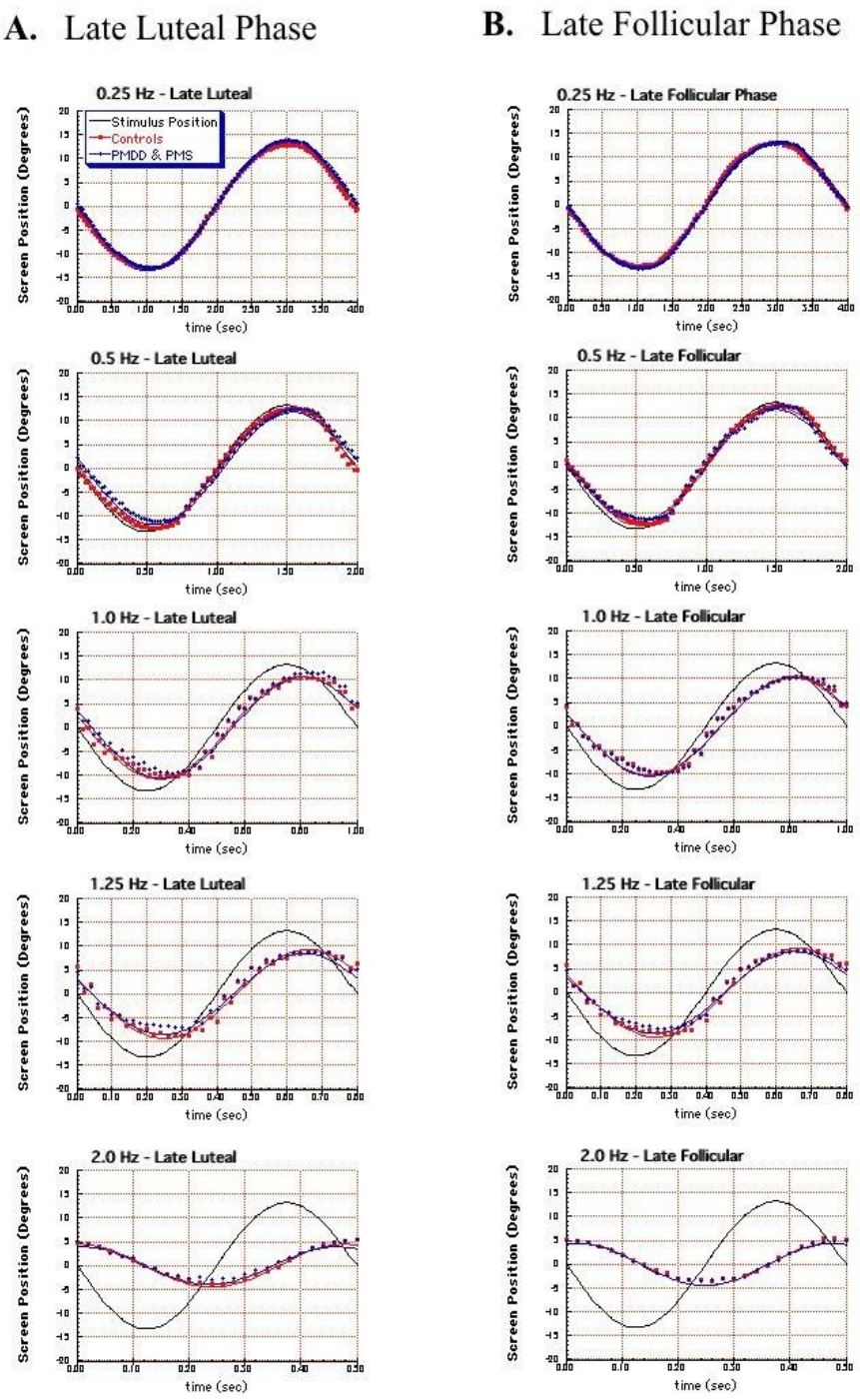


Figure 18. Spatial position of circular target and smooth pursuit eye position (in degrees visual angle) as a function of time. Final averaged smooth-pursuit positions (across 10 repeating cycles) are shown (from top to bottom) for all oscillating target frequencies: 0.25 Hz, 0.5 Hz. 1.0 Hz, 1.25 Hz, and 2.0 Hz. Data obtained during the LL and LF phases of the menstrual cycle are shown in the left and right columns, respectively. Control women responses are denoted with red symbols (N= 12) and women with PMS symptoms are denoted with blue symbols (N= 13). The continuous curves are fitted sinusoids to the data. For comparison purposes, the target position is indicated with a continuous black sinusoid. The quality of sinusoidal fits to the data confirm the validity of our averaging methods and saccadic/eyeblink filtering procedures. Note the phase lag and diminished eye movement amplitude with increased oscillatory target frequency. No significant main effects were found for group, however there was a significant increase in SPEM left-to-right amplitude for the PMS symptoms during LL phase and with the lower 0.25 and 0.5 Hz frequency oscillations.

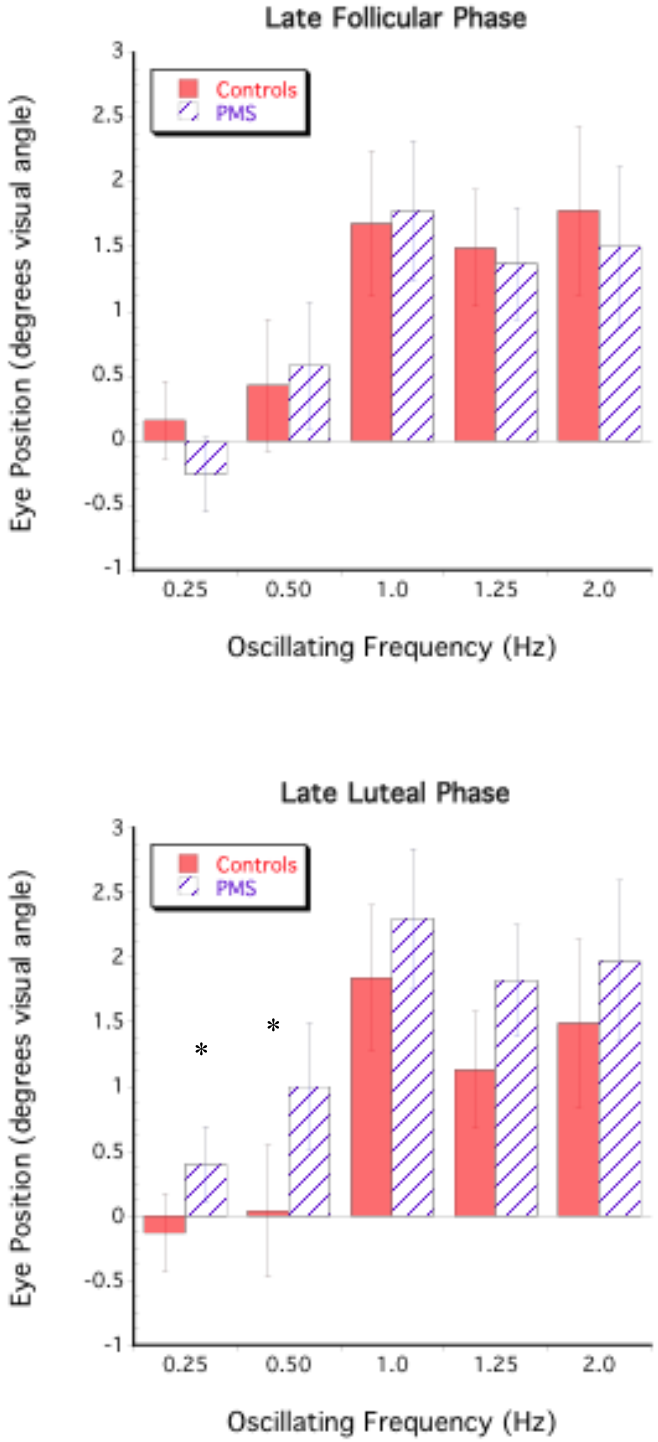


Figure 19. Eye position measured in degrees visual angle as a function of oscillating target frequency (in Hz). Control (red) and PMS (blue hatched) measures are averaged eye positions collapsed across five sampled target positions (0°, 90°, 180°, 270° and 360°; see text for details). Top and bottom panels show data from LF and LL phases, respectively. Note the greater excursions for PMS during LL phase at low 0.25 and 0.5 Hz oscillating frequencies. This distinction in movement amplitude is attributed to greater unilateral left-to-right movements. Error bars equal group standard deviations.

Appendix A

SCREENING QUESTIONNAIRE

1. Age: _____

2. Sex (Circle your answer): male female

3. Please check the box that best describes the highest level of education that you have completed:

[ ] some elementary	[ ] completed high school	[ ] some university
[ ] completed grade 8	[ ] some college	[ ] completed a university degree
[ ] some high school	[ ] completed college	[ ] some graduate studies
		[ ] completed a graduate degree

4. Today's date: _____
d/m/y

5. Height: _____ (feet & inches) or _____ (cm)

6. Weight: _____ (pounds) or _____ (kg)

7. Are you currently taking any medication? (circle one) YES NO

If YES, what medications are you taking? (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? (circle your answer)
YES NO MAYBE

10. Have you ever been diagnosed with or treated for bipolar disorder or manic depression? (circle your answer)
YES NO MAYBE

11. Have you ever been diagnosed or treated for an anxiety disorder? (circle your answer).
YES NO MAYBE

12. Do you think any of your relatives (i.e. parents, siblings, children, grandparents) have had any mental health problems (i.e. depression, anxiety, schizophrenia, alcoholism, eating disorders)? (circle your answer) 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 psychological 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 Disorder _____
[ ] Bipolar Disorder/Manic Depression _____
[ ] Eating Disorder _____
[ ] Alcoholism _____
[ ] Drug Abuse _____
[ ] Other: _____

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 experince 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.

0=no experience of symptom
1=present mild
2=present moderate
3=present strong
4=present severe

	Most recent menstrual period	Four days before	Remainder of cycle
1. Muscle Stiffness			
2. Weight Gain			
3. Dizziness, faintness			
4. Loneliness			
5. Headache			
6. Skin Blemish or Disorder			
7. Cold Sweats			
8. Anxiety			
9. Mood Swings			
10. Cramps			
11. Painful or tender breasts			
12. Nausea, vomiting			

13. Crying			
14. Backache			
15. Swelling (breasts/abdomen)			
16. Hot flashes			
17. Irritability			
18. Tension			
19. Fatigue			
20. Feeling guilty, sad, or blue			
21. General aches or pains			
22. Restlessness			
23. Insomnia			
24. Poor school or work performance			
25. Affectionate			
26. Feelings of suffocation			
27. Forgetfulness			
28. Take naps, stay in bed			
29. Orderliness			
30. Chest pains			
31. Confusion			
32. Poor judgment			
33. Stay at home			
34. Excitement			
35. Ringing in the ears			
36. Difficulty concentrating			
37. Avoid social activities			
38. Feelings of well-being			
39. Heart pounding			
40. Distractible			
41. Decreased efficiency			
42. Bursts of energy, activity			
43. Numbness, tingling			
44. Minor accidents			
45. Blind spots, fuzzy vision			
46. Poor motor coordination			
47. Increased appetite			

48. Was your last menstrual cycle in any way unusual? Yes No

If yes, please indicate the reason

_____

_____

_____

2. As best as you can, please indicate the frequency, severity, and level of impairment encountered for the following 11 symptoms during your menstrual cycle.

1a. In a12-month period, over how many cycles do you experience markedly depressed mood, feelings of hopelessness, or self-deprecating thoughts?
1- none 2- one to three 3. Four to six 4. Seven to Nine 5. Ten to Twelve

b. To what extent do these symptoms cause impairment in work, school, or occupational functioning:
1-not at all 2- a little 3- moderately 4- quite a bit 5- extremely

c. Please rate the degree of severity of these symptoms:
1- not at all severe 2- mild 3- moderate 4-severe 5-extremely severe/debilitating

2a. In a12-month period, over how many cycles do you experience marked anxiety, tension, tension, or feelings of being "keyed up," or "on edge?"
1- none 2- one to three 3. Four to six 4. Seven to Nine 5. Ten to Twelve

b. To what extent do these symptoms cause impairment in work, school, or occupational functioning:
1-not at all 2- a little 3- moderately 4- quite a bit 5- extremely

c. Please rate the degree of severity of these symptoms:
1- not at all severe 2- mild 3- moderate 4-severe 5-extremely severe/debilitating

3a. In a12-month period, over how many cycles do you experience marked affective lability (e.g. feeling suddenly sad or tearful, or increased sensitivity to rejection)?
1- none 2- one to three 3. Four to six 4. Seven to Nine 5. Ten to Twelve

b. To what extent do these symptoms cause impairment in work, school, or occupational functioning:
1-not at all 2- a little 3- moderately 4- quite a bit 5- extremely

c. Please rate the degree of severity of these symptoms:
1- not at all severe 2- mild 3- moderate 4-severe 5-extremely severe/debilitating

4a. In a12-month period, over how many cycles do you experience persistent and marked anger or irritability, or increased interpersonal conflicts?
1- none 2- one to three 3. Four to six 4. Seven to Nine 5. Ten to Twelve

b. To what extent do these symptoms cause impairment in work, school, or occupational functioning:
1-not at all 2- a little 3- moderately 4- quite a bit 5- extremely

c. Please rate the degree of severity of these symptoms:
1- not at all severe 2- mild 3- moderate 4-severe 5-extremely severe/debilitating

5a. In a12-month period, over how many cycles do you experience decreased interest in usual activities (e.g. work, school, friends, hobbies)?
1- none 2- one to three 3. Four to six 4. Seven to Nine 5. Ten to Twelve

b. To what extent do these symptoms cause impairment in work, school, or occupational functioning:
1-not at all 2- a little 3- moderately 4- quite a bit 5- extremely

c. Please rate the degree of severity of these symptoms:
1- not at all severe 2- mild 3- moderate 4-severe 5-extremely severe/debilitating

6.a. In a12-month period, over how many cycles do you experience a subjective sense of difficulty concentrating?
1- none 2- one to three 3. Four to six 4. Seven to Nine 5. Ten to Twelve

b. To what extent do these symptoms cause impairment in work, school, or occupational functioning:
1-not at all 2- a little 3- moderately 4- quite a bit 5- extremely

c. Please rate the degree of severity of these symptoms:
1- not at all severe 2- mild 3- moderate 4-severe 5-extremely severe/debilitating

7a. In a12-month period, over how many cycles do you experience lethargy, easy fatigability, or marked lack of energy?
1- none 2- one to three 3. Four to six 4. Seven to Nine 5. Ten to Twelve

b. To what extent do these symptoms cause impairment in work, school, or occupational functioning:
1-not at all 2- a little 3- moderately 4- quite a bit 5- extremely

c. Please rate the degree of severity of these symptoms:
1- not at all severe 2- mild 3- moderate 4-severe 5-extremely severe/debilitating

8a. In a12-month period, over how many cycles do you experience marked change in appetite, overeating, or specific food cravings?
1- none 2- one to three 3. Four to six 4. Seven to Nine 5. Ten to Twelve

b. To what extent do these symptoms cause impairment in work, school, or occupational functioning:
1-not at all 2- a little 3- moderately 4- quite a bit 5- extremely

c. Please rate the degree of severity of these symptoms:
1- not at all severe 2- mild 3- moderate 4-severe 5-extremely severe/debilitating

9a. In a12-month period, over how many cycles do you experie`nce sleeping too much or too little?
1- none 2- one to three 3. Four to six 4. Seven to Nine 5. Ten to Twelve

b. To what extent do these symptoms cause impairment in work, school, or occupational functioning:
1-not at all 2- a little 3- moderately 4- quite a bit 5- extremely

c. Please rate the degree of severity of these symptoms:
1- not at all severe 2- mild 3- moderate 4-severe 5-extremely severe/debilitating

10a. In a12-month period, over how many cycles do you experience a subjective sense of being overwhelmed or out of control?
1- none 2- one to three 3. Four to six 4. Seven to Nine 5. Ten to Twelve

b. To what extent do these symptoms cause impairment in work, school, or occupational functioning:
1-not at all 2- a little 3- moderately 4- quite a bit 5- extremely

c. Please rate the degree of severity of these symptoms:
1- not at all severe 2- mild 3- moderate 4-severe 5-extremely severe/debilitating

11a. In a12-month period, over how many cycles do you experience other physical symptoms, such as breast tenderness or swelling, headaches, joint or muscle pain, or sensations of "bloating" or weight gain?
1- none 2- one to three 3. Four to six 4. Seven to Nine 5. Ten to Twelve

b. To what extent do these symptoms cause impairment in work, school, or occupational functioning:
1-not at all 2- a little 3- moderately 4- quite a bit 5- extremely

c. Please rate the degree of severity of these symptoms:
1- not at all severe 2- mild 3- moderate 4-severe 5-extremely severe/debilitating

3 a. Are you currently taking oral contraceptives? YES NO

b. What is the average length of your menstrual cycle right now (i.e., How many days are there from the first day of one period to the first day of your next period – most people range between 25 and 35)? _____ days

c. Which statement best describes your menstrual cycle (Check the box with an "X" beside the appropriate response.)

[ ] 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.

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

e. 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
0	1	2	3	4

4a. Using the calendars below, please circle the first day of your last menstrual period. If you are not completely sure, please estimate the day that you believe you last menstrual period began. Also, please indicate with an X the day that you believe your next menstrual period will start.

January

Su	Mo	Tu	We	Th	Fr	Sa
				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

Su	Mo	Tu	We	Th	Fr	Sa
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

Su	Mo	Tu	We	Th	Fr	Sa
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				

April

Su	Mo	Tu	We	Th	Fr	Sa
			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		

b. How confident are you that the above circled day was the first day of your last period? (circle the best response).

0%		25%		50%		75%		100%
0	1	2	3	4	5	6	7	8

c. How confident are you that the above day with the X will be the first day of your next menstrual period (Circle the best response)

0%		25%		50%		75%		100%
0	1	2	3	4	5	6	7	8

d. Do you think that you have started to go through menopause: YES NO MAYBE

e. Are you currently pregnant? YES NO MAYBE

Section III: Emotions and Personality

1. Please read each of the following statements carefully and circle the one answer that best corresponds to your agreement or disagreement. Circle "sd" if the statement is definitely false or if you strongly disagree. Circle "d" if the statement is mostly false or if you disagree. Circle "a" if the statement is mostly true or if you agree. Circle "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.

SD = strongly disagree, D = disagree, N = neutral, A = agree, SA = strongly agree

1. I am known for my prudence and common sense.	sd	d	n	a	sa
2. I don't take civic duties like voting very seriously.	sd	d	n	a	sa
3. I keep myself informed and usually make intelligent decisions.	sd	d	n	a	sa
4. I often come into situations without being fully prepared.	sd	d	n	a	sa
5. I pride myself on my sound judgment.	sd	d	n	a	sa
6. I don't seem to be completely successful at anything.	sd	d	n	a	sa
7. I'm a very competent person.	sd	d	n	a	sa
8. I am efficient and effective at my work.	sd	d	n	a	sa
9. I would rather keep my options open than plan everything in advance.	sd	d	n	a	sa
10. I keep my belongings neat and clean.	sd	d	n	a	sa
11. I am not a very methodical person.	sd	d	n	a	sa
12. I like to keep everything it its place so I know just where it is.	sd	d	n	a	sa
13. I never seem to be able to get organized.	sd	d	n	a	sa
14. I tend to be somewhat fastidious or exacting.	sd	d	n	a	sa
15. I'm not compulsive about cleaning.	sd	d	n	a	sa
16. I spend a lot of time looking for things I've misplaced.	sd	d	n	a	sa
17. I try to perform all of the tasks that have been assigned to me conscientiously.	sd	d	n	a	sa
18. Sometimes I am not as dependable or reliable as I should be.	sd	d	n	a	sa
19. I pay my debts promptly and in full.	sd	d	n	a	sa
20. Sometimes I cheat when I play solitaire.	sd	d	n	a	sa
21. When I make a commitment, I can always be counted on to follow through.	sd	d	n	a	sa
22. I adhere strictly to my ethical principles.	sd	d	n	a	sa
23. I try to do jobs carefully so they won't have to be done again.	sd	d	n	a	sa
24. I'd really have to be sick before I'd miss a day of work.	sd	d	n	a	sa
25. I am easy going and lackadaisical.	sd	d	n	a	sa
26. I have a clear set of goals and work toward them in an orderly fashion.	sd	d	n	a	sa

27. When I start a self improvement project, I usually let it slide after a sew days.	sd	d	n	a	sa
28. I work hard to accomplish my goals.	sd	d	n	a	sa
29. I don't feel like I'm driven to get ahead.	sd	d	n	a	sa
30. I strive to achieve all I can.	sd	d	n	a	sa
31. I strive for excellence in everything I do.	sd	d	n	a	sa
32. I'm something of a "workaholic".	sd	d	n	a	sa
33. I'm pretty good about pacing myself so as to get things done on time.	sd	d	n	a	sa
34. I waste a lot of time before settling down to work.	sd	d	n	a	sa
35. I am a productive person who always gets the job done.	sd	d	n	a	sa
36. I have trouble making myself do what I should.	sd	d	n	a	sa
37. Once I start a project, I almost always finish it.	sd	d	n	a	sa
38. When a project gets too difficult, I am inclined to start a new one.	sd	d	n	a	sa
39. There are so many little jobs that need to be done that I sometimes just ignore them all.	sd	d	n	a	sa
40. I have a lot of self-discipline.	sd	d	n	a	sa
41. Over the years I have done some pretty stupid things.	sd	d	n	a	sa
42. I think things through before coming to a decision.	sd	d	n	a	sa
43. Occasionally I act first and then think later.	sd	d	n	a	sa
44. I always consider the consequences before I take action.	sd	d	n	a	sa
45. I often do things on the spur of the moment.	sd	d	n	a	sa
46. I rarely make hasty decisions.	sd	d	n	a	sa
47. I plan ahead carefully when I go on a trip.	sd	d	n	a	sa
48. I think twice before I answer a question.	sd	d	n	a	sa

2. Below is a list of common symptoms of anxiety. Please carefully read each item in the list. Indicate how much you have been bothered by each symptom DURING THE PAST 7 DAYS INCLUDING TODAY, by placing an X in the corresponding space in the column next to each symptom.

	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?

I have been feeling	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	0	1	2	3	4
2. less interested in doing things	0	1	2	3	4
3. less interested in sex	0	1	2	3	4
4. less interested in eating	0	1	2	3	4
5. that I have lost some weight	0	1	2	3	4
6. that I can't fall asleep at night	0	1	2	3	4
7. That my sleep is restless	0	1	2	3	4
8. that I wake up too early	0	1	2	3	4
9. heavy in my limbs or aches	0	1	2	3	4
in back, muscles, or head, more tired than usual	0	1	2	3	4
10. guilty or like a failure	0	1	2	3	4
11. wishing for death or suicidal	0	1	2	3	4
12. tense, irritable, or worried	0	1	2	3	4
13. sure I'm ill or have a disease	0	1	2	3	4
14. that my speech and thoughts are slow	0	1	2	3	4
15. fidgety, restless or antsy	0	1	2	3	4
16. that morning is worse than evening.	0	1	2	3	4
17. that evening is worse than morning	0	1	2	3	4
18. unreal or in a dream state	0	1	2	3	4
19. suspicious of people/paranoid	0	1	2	3	4
20. preoccupied/obsessed that I must check things a lot	0	1	2	3	4
21. physical symptoms when worried	0	1	2	3	4
22. like socializing less	0	1	2	3	4
23. that I have ganed weight	0	1	2	3	4
24. that I want to eat more than usual					
25. that I HAVE eaten more than usual	0	1	2	3	4
26. that I crave sweets and starches	0	1	2	3	4
27. that I sleep more than usual	0	1	2	3	4
28. that my mood slumps in the afternoons/evening	0	1	2	3	4

Section IV: Reproductive Questions:

1a. Are you currently taking oral contraceptives? (Circle your answer) YES NO

b. If you are NOT currently taking oral contraceptives, how many years and months has it been since you stopped taking your most recent oral contraceptive?_____years and_____months

c. Are you currently using any form of contraception? YES NO

d. If yes, please identify _____

e. If you have previously taken oral contraceptives, did you experience any negative side effects?
YES NO

If YES, please respond to question 1f.

f. If you are NOT currently taking oral contraceptives, but have in the past, please look at the following list of side effects that some people experience while taking oral contraceptives. Put a check beside any and all of the following PHYSICAL side effects that you have experienced while taking oral contraceptives. After indicating any side effects, please indicate what brand of oral contraceptive you were taking when you experienced the side effect and the severity of the symptom using the key below. Check all that apply.

1- not at all severe 2- mild 3- moderate 4-severe 5-extremely severe/debilitating

Effect	Brand	Severity	Effect	Brand	Severity
Nausea/vomiting			Headaches		
Breast size increase			Breast size decrease		
Decreased ability to orgasm			Increased ability to orgasm		
Weight gain			Weight loss		
Increased sex drive			Decreased sex drive/arousal		
Fewer menstrual cramps			More menstrual cramps		
Positive mood change			Negative mood change		
Tiredness/Fatigue			Dizziness/faintness		
High blood pressure			Painful or tender breasts		
Irregular heartbeat			Swelling of breasts or abdomen		
Clearer complexion			Complexion problems		
Complete loss of periods			Heavier periods		
Lighter periods			Concerned about hormones		
Breakthrough Bleeding			Fibrocystic breasts		

g. If you are NOT currently taking oral contraceptives, but have in the past, please look at the following list of side effects that some people experience while taking oral contraceptives. Put a check beside any and all of the following MOOD side effects that you have experienced while taking oral contraceptives. After

indicating any side effects, please indicate what brand of oral contraceptive you were taking when you experienced the side effect and the severity of the symptom using the key below. Check all that apply.

Effect	Brand	Severity	Effect	Brand	Severity
Slept more than usual			Less moody		
Slept less than usual			Lower self-esteem		
Depression			Cried more than usual		
More Pessimistic			More self critical		
More irritable			More moody		
Feelings of Inferiority			Less trust in partner		
Disrupted sleep			More sensitive to criticism		
More aggessive			Sadness		
More jealous			Less jealous		

f) Do you have a biological mother or sister who has experienced negative mood effects while taking oral contraceptives? (Circle answer): YES NO UNSURE

Section V: Caffeine Consumption

1a. Do you engage in the habitual consumption of caffeine? YES NO

b. How many servings of cafeine do you typically consume over the course of one day?
Coffee (please note Tim Horton's Large and Starbucks grande +venti sizes = 2 servings)_____
Tea _____ Canned Soda (355 ml)_____ Bottled Soda (710 ml)_____
Energy Drinks (Rock Star, Red Bull etc..)_____

c. Please indicate if you experience any of the following effects either during, or following the consumption of caffeine. Please also indicate the severity of the effect using the following scale:
1=not at all 2= a little 3= moderately 4= quite a bit 5= extremely

Affects mood positively ____
Affects mood negatively____
Irritability _____
Anger _____
Increase in alertness _____
Increase in Heart rate _____
Racing thoughts _____
Increased productivity _____
Increased Self Confidence ____
Increased Focus _____
Panic Attacks _____
Digestive problems_____
Heart burn _____
Ulcers _____
Increased urination _____
Increased creativity _____
Increased social interaction ____
Difficulty Concentrating _____
Migraines/Headaches _____
Fibrocystic Breasts _____

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

Time: ……

2. Considering only your own feelings, at what time would you go to bed if you were entirely free to plan your day?
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?

a. Not at all dependent [ ]
b. Slightly dependent [ ]
c. Fairly dependent [ ]
d. Very dependent [ ]

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

a. Not at all easy [ ]
b. Slightly easy [ ]
c. Fairly easy [ ]
d. Very easy [ ]

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

a. Not at all alert [ ]
b. Slightly alert [ ]
c. Fairly alert [ ]
d. Very alert [ ]

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

a. Not at all good [ ]
b. Slightly good [ ]
c. Fairly good [ ]
d. Very good [ ]

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

a. Very tired [ ]
b. Slightly tired [ ]
c. Fairly refreshed [ ]
d. Very refreshed [ ]

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

a. Seldom or never later [ ]
b. Less than one hour later [ ]
c. 1-2 hours later [ ]
d. 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?

a. Would be on good form [ ]
b. Would be on reasonable form [ ]
c. Would find it difficult [ ]
d. Would find it very difficult [ ]

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

a. 0800 – 1000 [ ]
b. 1100 – 1300 [ ]
c. 1500 – 1700 [ ]
d. 1900 – 2100 [ ]

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

a. Not at all tired [ ]
b. A little tired [ ]
c. Fairly tired [ ]
d. 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:

a. Wake up at the usual time and not go back to sleep [ ]
b. Wake up at the usual time and doze [ ]
c. Wake up at the usual time and go back to sleep [ ]
d. 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:

a. Not to go to bed until 0600h [ ]
b. Nap before 0400h and sleep after 0600h [ ]
c. Sleep before 0400h and nap after 0600h [ ]
d. 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:

a. 0800 – 1000 [ ]
b. 1100 – 1300 [ ]
c. 1500 – 1700 [ ]
d. 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:

a. Would be on good form [ ]
b. Would be on reasonable form [ ]
c. Would find it difficult [ ]
d. Would find it very difficult [ ]

17. Suppose that you can choose your own work hours, but had to work five hours in the day. Which five consecutive hours would you choose:

Hours: …….

18. At what time of day do you feel your best?
Time: ……..

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

a. Morning type [ ]
b. More morning than evening [ ]
c. More evening than morning [ ]
d. Evening type [ ]

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÷..

	jan	feb	mar	apr	may	jun	jul	aug	sept	oct	nov	dec
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 (1)	Slight Change (2)	Moderate Change (3)	Marked Change (4)	Extremely Marked Change (5)
a. Sleep length					
b. Social activity					
c. Mood (overall feeling of well being)					
d. Weight					
e. Appetite					
f. Energy level					

3a. If you experience changes with the seasons, do you feel that these are a problem for you? No Yes

b. If yes, is this problem: MILD MODERATE MARKED SEVERE DISABLING

4. 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

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

	Hours slept per day																		
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Over 18 hrs
Winter (Dec 21 -Mar 20)																				
Spring (Mar 21 -June 20)																				
Summer (June 21 -																				

Sept 20)																			
Fall (Sept 21- Dec 20)																			

6. Do you notice a change in food preference during the different seasons? No Yes

Appendix B

Laboratory Questionnaire

Date_____
Time_____
Participant number_____

Are you currently using oral contraceptives: YES NO

Phase: LL LF

1) Please indicate how you feel at this moment by circling the appropriate response; *(1) very slightly or not at all, (2) a little, (3) moderately, (4) quite a bit, (5) extremely.*

	Very Slightly or not at all (1)	A little (2)	Moderately (3)	Quite a bit (4)	Extremely (5)
	1	2	3	4	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 associated with menstruation. 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	4
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					
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 pain by circling the best answer.

1- not at all severe 2- mild 3- moderate 4-severe 5-extremely severe/debilitating

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

1- not at all severe 2- mild 3- moderate 4-severe 5-extremely severe/debilitating

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

1- not at all severe 2- mild 3- moderate 4-severe 5-extremely severe/debilitating

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

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

7a) Have you consumed ay 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? _____

Appendix C

SALIMETRICS®
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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

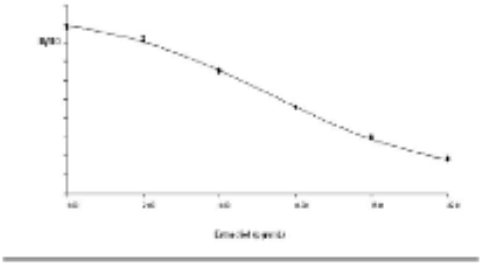
The Salimetrics' high and low salivary estradiol controls should be run with each assay. The control ranges established at Salimetrics are to be used as a guide. Each laboratory should establish its own range. Variations between laboratories may be caused by differences in techniques and instrumentation.

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
Cortisol	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
Triamcinolone	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:

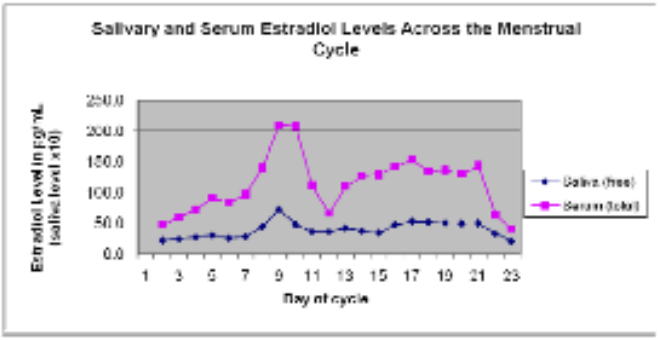
The correlation between saliva and serum estradiol in females was determined by assaying 11 matched samples. Samples were screened for pH and blood contamination. The magnitude of the saliva-serum correlation, $r(9) = 0.80$, $p = <0.001$, is consistent with the literature (4, 12, 18).

***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

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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 D

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Ovulation Test Strips

- **Free Shipping!**
- **Confidential Packaging**
- **Maximum Expiry Dates**
- ***Accurate, Easy-to-Use***

Instructions for LH Ovulation Tests

INTRODUCTION
For a Printable Version of Ovulation Test Instructions, please click here.

Luteinizing hormone (or LH) in elevated quantities causes ovulation (the release of the egg - or ovum - from the ovarian follicle). During the menstrual cycle only a small amount of LH is made, but in the middle of the cycle LH briefly and dramatically increases. This increase is called the "LH surge" and precedes ovulation. Conception is most likely to occur within thirty-six hours following the LH surge. The LH Ovulation Test is specifically designed to detect your LH surge - the time when you are likely to ovulate. If you receive a positive on an lh test, you are in your most fertile phase of your menstrual cycle.

SPECIMEN COLLECTION AND PREPARATION

COLLECTION
Collect urine once per day, at about the same time between 10:00 A.M. and 8:00 P.M. Do not use first morning urine as a sample. Collect urine in a clean, dry cup or container.

The sample may be stored at room temperature (15º-28ºC) for up to eight hours, or in the refrigerator for up to twenty-four hours. Do not freeze the urine sample. Let refrigerated samples reach room temperature before starting the test (this will take about 30 minutes). For best results, test the urine on the same day it is collected.

TEST PROCEDURE
STEP 1: WHEN TO START TESTING: Determine the length of the menstrual cycle. The length of the menstrual cycle is the number of days from the first day of menstrual bleeding to the day before bleeding begins on the next period.

Determine the usual length of the menstrual cycle over the last few months. Then, refer to the Ovulation Calendar Cycle Chart below to determine on which day of the menstrual cycle to begin testing. If your cycle is less than twenty-one days or greater than forty days, consult a physician.

Cycle Chart

Your Cycle Length	Day to Begin Testing
21 days	Day 5
22 days	Day 6
23 days	Day 7
24 days	Day 8
25 days	Day 9

26 days	Day 10
27 days	Day 11
28 days	Day 12
29 days	Day 13
30 days	Day 14
31 days	Day 15
32 days	Day 16
33 days	Day 17
34 days	Day 18
35 days	Day 19
36 days	Day 20
37 days	Day 21
38 days	Day 22
39 days	Day 23

STEP 2: TESTING PROCEDURE

1. To begin testing, open the sealed pouch by tearing along the notch. Remove the LH test from the pouch. Note: The ideal time to test is in the afternoon, not early morning, though testing may safely take please from 10am to early evening.

Error! Reference source not found.Dip end of strip into urine for 5 seconds. Do not exceed the max line.

2. Carefully place the LH test vertically into the urine cup for 5 seconds and lay the strip flat on a clean, dry, non-absorbent surface. IMPORTANT: Do not allow the urine level to exceed the line indicated by the arrows - MAX Line (Marker Line) - near the bottom of the test dipstick, otherwise the test will not perform correctly.

3. Wait for colored bands to appear. Depending on the concentration of LH in the test specimen, positive results may be observed in as little as 40 seconds. However, to confirm negative results, the complete reaction time of 10 minutes is required.

INTERPRETATION OF RESULTS
Within three to five minutes, two color bands will appear. For best results, interpret the test at **5 minutes**. Do not read the results after more than ten minutes.

To determine your result, compare the color intensity, i.e. shade of color, lightness or darkness of color, of the test band to the control band. In determining a positive or negative result, it is important to compare the color intensity, for this will indicate whether or not the LH surge (indicating ovulation) is in progress.

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1. Positive for the LH Surge
If the test band is of equal or greater intensity (equal or darker) than the control band, this is a positive result and a good indication that the LH surge is occurring.

2. Negative for the LH Surge
If the test band is of lesser intensity (lighter) than the control band or cannot be seen, this means the LH level of the sample is at or near its basal (normal) level and that the LH surge is not in progress.

3. Invalid Result
If no control band appears within five minutes, the test result is invalid and should be ignored. The control band will not appear if an insufficient volume of specimen is added into the test kit. Proper procedures may not have been followed in performing the test. Repeat with a new test kit.

To Optimize the Use of Ovulation Predictor Kits, please visit our online Ovulation Test FAQ and our extensive articles library.

HOW TO RECOGNIZE THE LH SURGE

After each test, you must decide if you are having an LH surge. If your test result is positive, you are probably having an LH surge. An LH surge can last from one to three days. Ovulation is most likely to occur sometime in the day and a half following the first day of the LH surge.

If your test result is negative, you are probably not having an LH surge. Remember that a pink-rose test band lighter than the control band shows that there is only a very low level of LH in your urine. Consult our online FAQ for more information about how to apply our test results.

WHEN TO STOP TESTING

Unless otherwise specified by a doctor, stop testing once the LH surge is detected. Six to ten days of testing may be needed to detect the LH surge, though additional testing may be required. Explanations for negative results include:

1. Use of first morning urine. First morning urine should not be used for LH .
2. The concentration of LH is too low to be accurately detect.
3. Testing is performed too early or too late in the menstrual cycle (please re-read cycle chart).
4. Testing is stopped before the surge occurs, and should have been continued for a few more days.

THE LH SURGE, OVULATION & PREGNANCY

A pregnancy begins with conception. A child is conceived when the male sperm successfully fertilizes the female egg. Successful fertilization is most likely during a 24 hour period one to three days following the LH surge. Since this ovulation "window" only opens once per month, and for only about 24 hours, being able to predict fertility it is very helpful when trying to become pregnant. Therefore, you should have intercourse during the one to three day period following the LH surge to increase chances of conception.

LIMITATIONS OF THE TEST

1) Directions must be followed carefully for accurate results.
2) Do not open the foil pouch until ready to conduct the test.
3) Do not use the results of this test as an aid for contraception.
4) Consult a doctor if irregular or unusually long cycles are experienced.
5) Do not use the test kit after the expiration date listed on the box.
6) A test device can only be used once. Discard the test after use.

QUESTIONS & ANSWERS

1) Should I restrict my diet before taking the test?
No, diet will not affect the test results.

2) Does alcohol, aspirin, or any other common drug affect the test?
No, but some hormonal medications can interfere with test results. If such medications are being taken or are suspected, seek professional advice from a physician to confirm the test results. Clomid can cause false positives if you begin testing too early in your cycles. Please use the above ovulation calendar guide to determine when to begin testing.

3) Should the test be used for contraception?
No, the test is not designed to prevent or help prevent conception and should not be used to do so.

4) Why is first morning urine not a good sample?
If first morning urine is used with the test, the first day of the LH surge may not be detected. The best time to collect the urine is between 10:00 A.M. and 8:00 P.M.. Always try to collect it at about the same time each day.

5) Today's control band is a different shade of red than yesterday's control band. Is this a concern?
No. Variations in the color of the control band will not affect the test result. Always compare the color of the test band to that of the control band of the same device on the day the test is performed. Do not compare bands from different devices.

6) Can test results be interpreted after five minutes?
No. Test results must be read at 5 minutes. Though a positive result should not change for several days, a negative result may change to a false positive within minutes after the end of the testing period, which would not be an accurate reading. It is always best to read the results at the 5 minute testing period and then discard the test to avoid confusion.

7) A pink background color and vertical streaking appeared in the result area during the testing period. Is this a concern?
No. Each urine sample will vary in its chemical makeup, as will the humidity of the air in testing chamber (room). Such variations in physical conditions can cause the vertical streaking and/or the pink-rose background color but will not affect the test results. As long as the control band appears within five minutes, the test is working properly.

STORAGE AND STABILITY
Store the test kit below 28ºC; do not freeze. Refer to the expiration dates of the individual components for stability information.

WARNINGS AND PRECAUTIONS
1. The test kit is for in vitro (external) diagnostic use only.
2. Do not use beyond the expiration date.

Appendix E

Introductory Statement/ Cover Letter

Dear Participant,

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

In recent years, the discovery of effects exerted by hormones outside of simple reproductive function, has began to influence our conceptualization of such things as learning, memory, and perception. Research has demonstrated that hormonal receptors are located in diffuse regions throughout the mammalian brain, thereby implicating these regions in a variety of ,cognitive operations never before considered. Among these regions are the retina, and the hippocampus, a structure involved in learning and memory. This study aims to psychophysical measures designed to assess functionality in these regions.

Following completion of the screening questionnaire, some participants will be selected to participate in two approximately one-and-half-hour laboratory sessions. Each laboratory session will involve the collection of a salivary sample, a brief questionnaire, and 4 visual tests. These tests are based on standard psychophysical procedures. In all visual tasks, participants will be presented with various images and asked to make judgments based on the appearance of the image. A sub-sample of participants will be asked to collect salivary samples at home and return them to the lab the following morning. All participants will be asked to sign a consent form, which will be reviewed with the researcher upon entry into the lab. Following completion of the laboratory sessions, all participants will be fully debriefed, and provided with a list of project references and resources.

All tasks involved in this study pose minimal risk to participants. If participants feel uncomfortable with any of the experimental tasks or procedures, or note any negative effects, they are free to withdraw their consent at any time. Participants will be supervised at all times. If at any time the researcher or participant feels that the experiments are causing any adverse effects, the session will be terminated. Early termination of the study will not have an impact on the offer of course credit.

All information collected during the course this project will be held in strict confidence. At no time will any identifying information be divulged to individuals outside of the research team. All information will be stored in a secure location at Lakehead University for a period of five years. Findings from the projects will be made available to participants upon completion of the project.

If you have any questions or concerns regarding the experiment, please do not hesitate to contact me directly. I can be reached by phone (807) 343-8418 or through e-mail (mrichar4@lakeheadu.ca). You may also contact 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,

Meghan Richards, M.A.
Doctoral Student, Clinical Psychology Program
Lakehead University

Appendix F

CONSENT FORM A

The purpose of the present study, conducted by Meghan Richards, Dr. Michael Wesner, and Dr. Kirsten Oinonen of the Department of Psychology at Lakehead University, is to examine visual functioning in women. If you are currently enrolled in an Introductory Psychology section, 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 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 morningness-eveningness.

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, the disclosure of any identifying information will be precluded in the dissemination of the results of this study. As per university requirements, all data will be stored for 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 on this form. 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. 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 prinicipal investigator, Meghan Richards (343-8418), or Dr. Micheal Wesner (343-8457), or Dr. Kirsten Oinonen (343-8096) directly.

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

Name (Please Print): _____ Phone Number: _____

Email address_____

Signature: _____ Date: _____

.

Appendix G

DEBRIEFING FORM A

Thank you for participating in the screening phase of our study. The study is being conducted by Ms. 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 and provide two salivary samples. A portion of participants will be asked to collect additional salivary samples at home. These participants will be eligible for an additional bonus point.

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.

Meghan Richards, M.A.
Department of Psychology
Lakehead University
955 Oliver Road
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(807) 343-8418

Dr. M. Wesner
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Dr. K. Oinonen
Department of Psychology
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955 Oliver Road
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(807) 343-8096

Appendix H

CONSENT FORM B

I agree to participate in the present study that is investigating visual functioning in women's health. 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 find out what factors are related to women's health. 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) and my body measurements (e.g. finger lengths). 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, Meghan Richards (mrichar4@lakeheadu.ca) or Dr. M. Wesner 343-8457 (mwesner@lakeheadu.ca) or Dr. K. Oinonen 343-8096, (koinonen@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: _____

_____ _____
Participant's Printed Name Signature

_____ _____
Date Experimenter Nam

Appendix I

DEBRIEFING FORM B

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 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 Premenstrual Dysphoric Disorder (PMDD), severe symptoms of PMS, as well as women who are prone to experiencing oral contraceptive side effects. We did not include this information in the participant letter or screening questionnaires because we felt that this information may have influenced the responses of some women in the study, and consequently prevented a thorough, accurate investigation into the topic at hand. We apologize, and hope you understand why it was necessary. In case you have any concerns regarding this procedure, we have attached the names of the principal investigators and provided you with a list of additional resources on the attached sheet.

Given that this study involves some aspects of which you were not fully informed at the start, 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 that 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 Meghan Richards, Dr. Michael Wesner, or Dr. Kirsten Oinonen, 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. Some participants may also be asked to collect measures of melatonin at home. These samples will assist us in examining additional parameters that could contribute to hormonal sensitivity, or have been previously found to differ in hormonally sensitive populations. 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:

Meghan Richards, M.A.
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Dr. K. Oinonen
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(807) 343-8096

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
- Family Services Thunder Bay: 626-1880
- Catholic Family Development Centre: 345-7323
- Emergency services are available at the Thunder Bay Health Sciences Centre
- Thunder Bay Crisis Response (24 hours): 346-8282.

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.

Lee, K. A., Shaver, J. F., Giblin, E. C., & Woods, N. F. (1990) Sleep patterns related to menstrual cycle phase and premenstrual affective symptoms, 13 (5), 403-409.

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

Parry, B. L., Mendelson, W. B., Duncan, W. C., Sack, D. A., Wehr, T. A. (1989) Longitudinal sleep EEG, temperature, and activity measurements across the menstrual cycle in patients with premenstrual depression and in age matched controls. *Psychiatry Research, 30,* 285-303.