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GENDER AND OVARIAN HORMONE EFFECTS ON THE RELATIVE CONTRIBUTION OF CHROMATICITY TO BRIGHTNESS

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by

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To Becky

Thank you for providing a peaceful place for me to rest my mind.

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ABSTRACT

The chromatic contribution to brightness perception was compared in males and females. Direct brightness matching (DBM) and heterochromatic flicker photometry (HFP) were used to measure relative luminous efficiency, and DBM/HFP ratios were predicted to be higher for females than males on repeated measures and for each "primary" color. No gender differences were predicted in DBM or HFP measures. Within-females effects of estradiol (E2) and progesterone (PG) levels, contraceptive use, and menstrual cycle phase were also investigated. It was expected that E2 would directly predict DBM/HFP ratios and that PG would antagonize that relationship. Based on that prediction, DBM/HFP ratios would be at a maximum during the ovulatory phase, intermediate during the menstrual phase and minimum during the luteal phase. No effects were predicted for DBM or HFP measures.

DBM/HFP ratios were significantly higher for female subjects than male subjects. Contraceptive use had no effect on overall DBM/HFP ratios. There were limited effects of hormone levels, menstrual cycle phase and contraceptive use on DBM/HFP ratios and on DBM and HFP measures analyzed separately. However, hormone effects on DBM/HFP ratios and DBM measures at 650 nm agree with previous findings involving long-wavelength sensitive (L-) cone mechanisms.

The present findings supporting a female advantage in chromatic contribution to brightness are robust. The significant results are discussed in the context of previous findings suggesting organizational and short-term effects of ovarian steroid hormones.

INTRODUCTION

PROPERTIES OF LIGHT

Humans are capable of detecting electromagnetic radiation from 380 to 700 nanometers (nm). This visible radiation, or light, has two basic properties—luminance and chromaticity. Luminance, or lightness, is transformed by the visual system and is perceived as brightness. Chromaticity likewise undergoes a transformation and is perceived as color, which has two properties—hue and saturation. Hue defines where a light stimulus falls on the visual spectrum and can be thought of as the name we give a color (blue, red, orange, etc.) Saturation is a more difficult concept and has been defined as "the attribute of a visual sensation which permits a judgment to be made of the degree to which a chromatic stimulus differs from an achromatic stimulus regardless of their brightness" (Wyszecki and Stiles, 1982). A simpler approach is to say that highly saturated stimuli contain more color and pale colors contain more white (Pokorny et al., 1991). Hue then tells us the color of an object, and saturation tells us how colorful the object is.

ANATOMY AND FUNCTIONAL MODELS OF THE VISUAL SYSTEM

Parallel visual pathways

There are numerous processes involved in transforming visual sensations into the perception of brightness and color. In humans and other primates, physiological and behavioral evidence indicate two anatomically and functionally distinct pathways originating in the magnocellular (M) and parvocellular (P) retinal ganglion cells

(Leventhal et al., 1981; Perry et al., 1984). Object location, movement, low contrast sensitivity and global analysis of visual scenes are processed more efficiently through the M-cell pathway, whereas object and pattern recognition as well as color (in particular, red-green opponency) are processed more efficiently through the P-pathway (Schiller and Malpeli, 1978; Livingstone and Hubel, 1987; De Yoe and Van Essen, 1988; Brannan and Bodis-Wollner, 1991; Plainis and Murray, 2005). The M and P retinal ganglion cells project to the dorsal portion of the lateral geniculate nucleus (dLGN) of the thalamus, and the dLGN projects to cortical areas involved in visual processing, chiefly to primary visual cortex (V1). These M and P pathways can be followed deep into visual processing areas of posterior parietal (PP) and inferior temporal (IT) cortex and are thought by some to form anatomically distinct dorsal (M) and ventral (P) processing streams (Mishkin and Ungerleider, 1982). A third functional system, the koniocellular (K) pathway, involves a neurochemically distinct population of neurons in the interlaminar zones of the dLGN (Casagrande, 1994). Though these K cells are more variable in size and function, they have the lowest spatial acuity and intermediate temporal resolution when compared to M and P cells. A subset of K cells relay low-acuity visual information to V1, while others innervate cortical areas outside of visual cortex likely to augment visual behaviors in the absence of normal V1 function (Hendry and Yoshioka, 1994).

There is general agreement concerning the existence and clinical relevance of these parallel pathways (reviewed by Bassi and Lehmkuhle, 1990). The persistent debate is whether individual differences in parallel pathways lead to measurable differences in visual processing. Since my research intimately involves the debated mechanisms, a

summary follows of major findings in retinal anatomy, physiology and models of visual sensation and perception.

Retinal anatomy and physiology

There are three major types of neurons in the retina—photoreceptors, bipolar cells and ganglion cells. The light gathering elements (photoreceptors) in the vertebrate retina are long, tubular neural cells whose names describe the shape of their free ends—rods and cones. It is generally accepted that there is one type of rod and three types of cones in normal human retina (Dowling, 1987). Cone types are categorized based on their peak sensitivity to long (L-cone), medium (M-cone) or short (S-cone) wavelengths (Cohen, 1992). Rods are primarily responsible for visual detection in dim light, while cones are more adapted for the sensation of form and color in higher light levels. While there are individual differences, the number of rods in the human retina is estimated at 120 million, and cones number 6.5 million (Snell and Lemp, 1989). The density of photoreceptors varies in different parts of the retina. The foveal region of the retina—important for fine central vision tasks such as driving and reading—contains no rods, while the density of rods is about 30,000/mm^2 in the far peripheral retina. The opposite is true for cones, which are much denser in the fovea and decrease in number in the periphery. The fovea, however, contains almost no S-cones. This probably indicates the secondary role of S-cones in fine spatial vision (Cohen, 1992).

In his review of retinal contributions to color pathways, Lee (2004) provided the following view of retinal organization. Bipolar cells connect photoreceptors with

ganglion cells, the axons of which form the nerve layer carrying initial visual transformations to the dLGN. Rod bipolar cells synapse with several rods and up to four ganglion cells called parasol cells. Diffuse bipolar cells connect several L and M cone cells to parasol cells, which project to the magnocellular layers of dLGN. Parasol ganglion cells have large cell bodies that are tuned for low spatial and high temporal frequency. Midget bipolar cells (which can be inhibitory or excitatory) connect individual L or M cones to individual midget ganglion cells in the ganglion layer. This arrangement is thought to be responsible for fine spatial resolution and red-green (L-M) color comparisons of the P pathway. A final type of bipolar cell, the bistratified bipolar cell, connects S cones to bistratified ganglion cells. Bistratified cells also receive an inhibitory input from L and M cones (through diffuse bipolar cells) and result in the ability of the visual system to make +S-(M+L) or blue-yellow comparisons. A subset of these bistratified cells is thought to form the basis for the koniocellular neural pathway (reviewed by Lee, 2004).

In addition to serial connections, there is a parallel arrangement in the primate retina facilitated by horizontal and amacrine cells. As predicted from their name, horizontal cells project laterally in the retina and connect distant photoreceptors to one another and with bipolar cells. In mammals, there are two horizontal cell types (H1 and H2). In primates, H1 cells avoid S cones, while H2 cells make substantial connections to S cones and to L and M cones. Horizontal cells integrate the visual signal by responding to neurotransmitter released from rods and cones, sending an inhibitory signal to bipolar cells. Horizontal cells are also thought to be involved in cone-specific or local gain-

control in the outer retina (Lee, 2004). Amacrine cells synapse with ganglion cells and bipolar cells as well as with one another. They are stimulated by bipolar cells and provide excitatory input to ganglion cells.

Even at the retinal level of visual transformation, one can see the genesis of two basic aspects of visual sensation—color and brightness. These are not completely independent functional systems, as the M- and P-pathways interact (Milner and Goodale, 1995). That is, the M-pathway is not entirely achromatic and the P-pathway is not the sole processing system for chromatic information. After all, the M-pathway does contain information from wavelength coded sensors and contributes to color even at relatively low light levels (Lee, 1999). In addition, the interactions of rods with all cone types via lateral connections involve the P-pathway in visual transformations at all perceivable intensity and contrast levels (Lee, 1999; Buck et al., 2000). However, the P-pathway, tuned primarily for high spatial frequency and color information, provides limited information about the overall form of objects (Livingstone and Hubel, 1987). In fact, it is nearly impossible to discriminate between forms in natural scenes without activation of contrast mechanisms of the M-pathway. Therefore, careful stimulus and experimental design can be used to largely isolate magnocellular, parvocellular and even koniocellular function.

Models of color sensation and perception

Although the concept that white light is composed of separate colors was discovered as early as 100 AD, Sir Isaac Newton is usually credited with being the first to

analyze the color spectrum of light in terms of vision (Gouras, 1991). That is, he recognized that objects would take on the color of light they reflected the most and concluded that light was capable of stimulating the retina to produce colors (Newton, 1671). Although his theories on color mixing have endured, even evolving into the current science of colorimetry, Newton was not able to understand that the retina—not the light itself— is the limiting aspect in color mixing.

Almost a century later, Palmer reasoned that the three primary pigments used in art and textiles had three matching receptors in the retina (Gouras, 1991). Palmer's theories were groundbreaking. He understood that color deficiency would result from missing receptor types. He also recognized that receptors with peak sensitivity to a particular pigment would respond to other pigments if intense enough. Having been exposed to Palmer's work, Thomas Young proposed in his second Bakerian lecture that the human visual system contained three nerve fiber types, each producing a red, green or violet sensation.

> Now, as it is almost impossible to conceive each sensitive point of the retina to contain an infinite number of particles, each capable of vibrating in perfect unison with every possible undulation, it becomes necessary to suppose the number limited, for instance, to the three principal colours, red, yellow, and blue, of which the undulations are related in magnitude nearly as the numbers 8, 7, and 6; and that each of the particles is capable of being put in motion less or more forcibley by undulations differing less or more from a perfect unison; for instance the undulations of green light being nearly in the ratio of 6 ½ will affect equally the particles in unison with yellow and blue, and produce the same effect as a light composed of these two species: and each sensitive filament of the nerve may consist of three portions, one for each principal colour. – Young (1802: pp. 20-21)

While advancing his wave theory of light as vibrations in luminous elastic ether, Young observed, "The sensation of different Colours depends on the different frequency of Vibrations, excited by Light in the Retina." He also suggested that the retina might be sensitive to only three principal colors and that all color appearance, including blacks and whites, might be attributable to varying degrees of excitation of these three receptors.

The psychophysical studies of Helmholtz (1852) also suggested three receptor types that respond at different rates depending on the wavelength of incoming light rays. The Young-Helmholtz theory, which argued that the visual system is able to derive all color perception (including overall lightness) from three types of photoreceptors, has been repeatedly supported by modern studies. For example, Marks et al. (1964) as well as Sperling and Harwerth (1971) used microspectrophotometric techniques to verify the existence of three photoreceptor types with peak sensitivities to low, medium and long wavelength light. So, at the level of photoreceptors, a strong relationship emerges between the wavelength of light and the neural signals.

Regardless of how prescient the Young-Helmholtz trichromatic theory was in predicting three wavelength-dependent photoreceptors, it could not completely describe color perception. Ewald Hering, the father of the opponent process theory, made some very interesting observations that could not be accounted for by the trichromatic theory (Hurvich and Jameson, 1964). For example, he noted that there are certain pairs of complementary colors one never sees together at the same place and at the same time. For example, while humans can perceive yellowish-greens or bluish-reds, it is impossible to perceive reddish-greens or yellowish-blues. Hering hypothesized an opponent process

that caused red and green, as well as blue and yellow, to cancel each other. Hering also observed that there was a distinct pattern to the color of perceived afterimages. That is, if one looks at a certain color for about a minute and then switches the gaze to a homogeneous white field, a patch of complementary (or opponent) color will appear.

Another problem for the trichromatic theory is in the nature of the human subjective response of yellow hues. Trichomatic theory predicts three primary colors—blue, green and red—with none appearing to be a combination of the other two. This agrees with our subjective experience. After all, blue is not a combination of green and red and so on. Trichromatic theory also predicts that non-primary colors are combinations of primary colors. Cyan is the combination of blue and green, and purple is the combination of blue and red. These also agree with our subjective experience. However, yellow—physically resulting from the combination of green and red—does not make sense in the same way.

Hering hypothesized that trichromatic signals from the cones fed into subsequent neural or post-retinal stages and allowed for two major opponent classes of processing. The first process—the substrate of color sensation—was the spectrally opponent process involving red vs. green and blue vs. yellow. He also hypothesized a second spectrally non-opponent (black vs. white) process resulting in brightness sensation. This opponent process model lay relatively dormant for many years until two vision scientists at Eastman Kodak, Leo Hurvich and Dorothea Jameson, conceived and engineered the hue cancellation method to psychophysically evaluate opponent processing in color vision (Hurvich and Jameson, 1957).

It was predicted from opponent process theory that red and green should not mix to form reddish-green. Rather, red and green cancel each other, and the addition forms yellow. Blue and yellow also do not mix. They cancel each other, and the addition forms white. Hurvich and Jameson then reasoned that if one started with a bluish-red color, it should be possible to add unique green (green containing minimal influence of other hues) to cancel out the redness, leaving only blue. Unique green was also used to cancel out the redness in orange, leaving only yellow. For yellow-green stimuli, unique blue was used to cancel out the yellow, leaving only green. The amount of the cancellation color used was taken as the strength of the cancelled hue. These data were then converted to produce red-green and blue-yellow opponent process curves as shown in Figure 1 (adapted from Hurvich and Jameson, 1957).

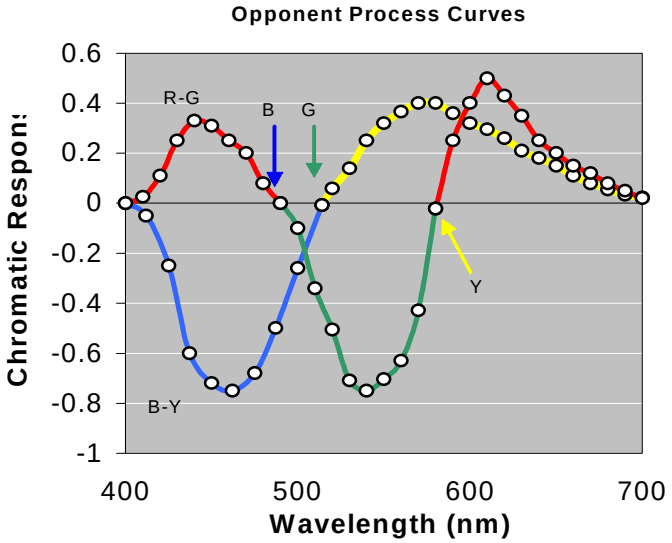


Figure 1 – Opponent process curves for a single observer (adapted from Hurvich and Jameson, 1957). The arrows indicate the wavelength positions of unique blue, green and yellow for this observer. The sign (plus or minus) of the chromatic response is arbitrary and represents the opposite nature of the opponent processes.

It may seem at first that trichromatic and color-opponent theories are incompatible. However, modern *dual process* (or zone) theories view the models together with the three cone classes combined in an antagonistic fashion at the post-receptor level (Boynton, 1979). The dual process theory, which is currently the accepted model of color processing, also proposes a non-color opponent class of neurons (DeValois et al., 1966). The spectral sensitivity function of these neurons is broad with a peak around 555 nm, which has led some to conclude that their activity is due to the combined activation of M- and L-cone mechanisms. Some authors even credit these non-opponent neurons with the shape of photopic spectral sensitivity functions (Cavanagh et al., 1987). Analogous to this suggestion is the idea that S-cone mechanisms are "color only" (Cavanagh et al., 1987), although this is a minority opinion. Individual differences in post-receptoral retinal or ganglion cell organization may also result in M+L (yellow) perception differences. Therefore, it is easy to imagine that individual differences in the number of cone photoreceptors would alter achromatic and chromatic processes across all wavelengths.

Previous studies have suggested that individual differences in color perception may result from gender, and several of these studies are discussed in the following section.

GENDER DIFFERENCES

General gender differences

Physical gender differences exist early in fetal development and are the result of a series of prenatal hormone processes that may also produce gender differences in neural development (Levine and Mullins, 1966). There are also known sex differences in cortical (Luders et al., 2004) and subcortical (Shors et al., 2001) structures which may contribute to gender-specific cognitive or memory function. In addition, there are gender differences in the incidence of certain illnesses (Kudielka and Kirschbaum, 2005). Women, for example, suffer more often from autoimmune disorders while men are more likely to contract infectious diseases or develop cardiovascular disease earlier in life. Functional differences may also result from different experiences and expectations (Slonim et al., 1975; Hamilton, 1995). There is also some experimental evidence of structural and functional differences in visual processing between the sexes (reviewed by Alexander, 2003).

Gender differences in ocular anatomy and visual processing

Although the results of previous gender studies of visual processing are heterogeneous, they suggest sexual dimorphism in visual structure and function that may extend to color processing. Some reviewers have concluded that women differ from men in ocular features such as tear production, lens clarity, and corneal shape and thickness (Midelfart, 1996). Zadnick et al. (2003) found that girls had more powerful corneas and lenses but shallower anterior chambers than boys. Others have found, however, no

gender differences in the anatomy of the eye (reviewed by Jones, 1990). In squirrel monkeys, Jacobs (1983b) described sex differences in spectral responses of LGN cells. Male monkeys had a significantly smaller proportion (14%) of spectrally opponent cells than females (21%).

Human sex differences in visual function and associated behaviors also have been reported (reviewed by Alexander, 2003). In free drawings, boys tend to describe more motion and include more mechanical descriptions of imagined environmental scenes while girls tend to use more color (Iijima et al., 2001). In a study designed to determine the effects of gender and age on dynamic visual acuity, Ishigaki and Miyao (1994) found that boys tended to detect a small gap at faster drifting rates than girls. Kramer et al. (1996) found that, in describing large shapes made up of smaller geometrical elements, boys use more global descriptions (relying on the overall shape). Girls, however, are more detailed (or local) in their descriptions, referring to the smaller shapes to describe the figures. Of course, these differences may result from early association by boys and girls to "gender expected" behaviors. For example, Hamilton (1995) found that the most significant predictor of visual-spatial performance was not gender but self-perceived masculinity. Still, the overall body of evidence suggests possible innate gender differences in parallel visual processes. That is, males may rely more on magnocellular processing, while females may rely more on parvocellular processing.

As part of a study designed to compare the contrast response of the dominant eye to stimuli processed more strongly by magnocellular and parvocellular pathways, Foutch

and Bassi (2005) found a significant interaction ($p = 0.025$) of stimulus type (magno vs. parvo) with gender. The *magno* stimuli were large (4° by 6°), low spatial frequency (1 cycle per degree), quickly drifting (30 cycles/sec) rectangular blue/yellow gratings while *parvo* stimuli were small (2°), high spatial frequency (20 cycles per degree), stationary circular red/green gratings. There was also a simple effect of gender on contrast thresholds for the stimuli more strongly processed by the parvocellular pathway. Females were more sensitive than males ($p = 0.05$) to the high spatial frequency, red-green stimulus, but there was no simple effect of gender on contrast thresholds for the magno stimuli (Figure 2). There were also gender-related trends in reaction times (reported separately by Foutch and Fletcher, 2006). Female subjects were slower than male subjects in reacting to magno stimuli but faster than males in reacting to parvo stimuli.

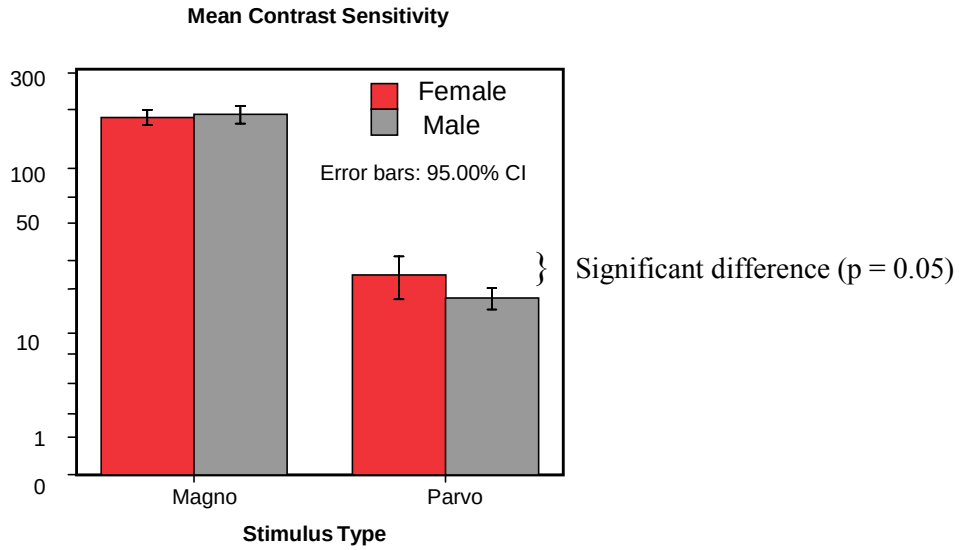


Figure 2 – Contrast sensitivity results from Foutch and Bassi (2005).

The contrast and reaction time findings may have resulted from differences in either spectral sensitivity or in sensitivity to the spatial and temporal characteristics of the targets. In a study designed to test visual acuity as a function of hue (blue, green, yellow and red), Kelton et al. (1978) found an interaction effect of gender with hue on visual acuities. Therefore, it is possible that differences in spatial frequency processing account for gender differences in contrast thresholds of chromatic grating stimuli.

The results are mixed in studies that carefully consider the influence of gender on spatial frequency processing. Oen et al. (1994) investigated the effects of race, gender and occupation on spatial contrast thresholds and found that females had lower contrast sensitivity than males at all spatial frequencies, suggesting a male advantage in magnocellular processing. However, the results of a subsequent study specifically designed to investigate gender differences in spatial frequency indicated no gender differences in contrast thresholds between 20 males and 20 females (Solberg and Brown, 2002). Zaroff et al. (2003) also found no significant gender differences in stereoacuity thresholds. The equivocal nature of reported gender differences in spatial processing suggests that gender differences in parvocellular processing may result from color processes.

Gender differences in color vision

The majority of investigations into gender differences in color vision deal with preferences and naming. In a study of 1279 university students, Walton et al. (1933) found that men preferred orange over yellow, whereas the opposite was found for

women. St. George (1938) and Eysenck (1941) verified these results in separate college populations. In a modern study involving naming of color chips, women identified significantly more colors than men yet had no significant difference in vocabulary (Greene and Gynther, 1995).

Several studies also have investigated directly the perceptual aspects—lightness, hue and saturation—of color transformations. In an attempt to measure saturation sensitivity, Nichols (1885) found that males could detect smaller amounts of red, yellow, and green powder added to white powder while females could detect smaller amounts of blue powder. His methods were flawed by modern standards as the colors were broadband and could have been influenced by neighboring hues. A modern study used color caps from the standard Farnsworth D-15 and L'Anthony desaturated (DS-15) tests to investigate the possible effects of gender on lightness and saturation (Bimler et al., 2004). Each observer was shown a series of three small color disks, and their task was to eliminate the disk that did not belong. Males eliminated color disks based on different lightness whereas females eliminated disks based on hue—particularly red-green—differences. That is, males placed more perceptual weight on lightness and less on color discrimination, providing more evidence for gender differences in magnocellular vs. parvocellular processing. Volbrecht et al. (1997) found that the wavelength positions of a uniquely green hue were distributed differently for males than females. Distributions for males were normal and non-skewed whereas female distributions were positively skewed, non-normal and bimodal. Kuehni (2001) reported the same result for unique

green and similar results for the distributions of unique yellow and red. These studies suggest a pattern of variability *within females* not observed in males.

In order to reduce the influence of spatial frequency on the results of Foutch and Bassi, Foutch and Peck (2005) compared contrast thresholds between genders for 2° circular patches of uniform color—blue, cyan, green, yellow, orange, red and magenta—that alternated *temporally* with a broadband (white) stimulus. An alternation rate of 5 cycles/sec was used to minimize the contribution of achromatic channels, and repeated measures analysis of variance (ANOVA) revealed that females had significantly higher thresholds for all seven stimuli ($F = 10.75$, $p < 0.001$). Analyzing the data for planned (green and red) comparisons revealed significant gender differences for green ($F = 10.90$, $p < 0.001$) and red ($F = 6.24$, $p = 0.014$). Even applying the conservative Bonferroni correction, contrast thresholds were significantly higher for females for unplanned comparisons of cyan, yellow, and orange.

The higher thresholds for females did not represent lower spectral sensitivities. The algorithm to determine thresholds added white to the color in the first patch and kept the color constant in the second until the observer could no longer discriminate between the two when temporally alternated at 5 Hz. Thus, the more sensitive an observer is to a given color, the more white that will be required to disrupt that sensation. Therefore, a higher threshold on this task indicates more contribution of color to the overall sensation. It could be argued that these results were overly influenced by the presence of white in the stimuli. In that case, the results would represent differences in saturation, rather than chromatic, sensitivity. However, Graham and Hsia (1969) concluded that saturation

sensitivity is equivalent to the difference between chromatic and achromatic sensitivity. Guth and Lodge (1973), in their description of a vector color model, also suggest that the ratio of chromaticity to overall sensitivity is predicted by saturation.

Foutch and Peck (2005) also found significantly higher variability in females for the primary hues of blue, green, yellow, and red. This is consistent with Volbrecht et al. (1997) and Kuehni (2001) in suggesting a *within female* effect on variability not present in males. There was no effect of gender on the variability for all three non-primary hues (cyan, orange, and magenta).

Gender differences have also been reported in perception of color after-images (Hoynga et al., 1979). When subjects were shown brief colored or white flashes and asked to report the appearance and changes in afterimages, males perceived more movement in after-images, and there was an interaction effect of stimulus color with gender on after-image duration. A similar study revealed shorter overall afterimage durations for females, but longer durations in response to long-wavelength flash stimuli (McGuiness and Lewis, 1976). Females reported afterimages in response to a red stimulus twice as often as males, but less often for all other colors.

While all of these studies suggest differences in color processing, there is debate over what mechanisms account for this gender dimorphism. Some proposed mechanisms are discussed in the following section.

POSSIBLE MECHANISMS FOR GENDER DIFFERENCES

Gender differences in neural transmission

When the retina is stimulated by light, amacrine cells release the neurotransmitter dopamine, modulating the neural processes leading to sensation (Feigenspan et al., 2000). Several studies have suggested that dopamine depletion that accompanies Parkinson's disease leads to defects in contrast sensitivity (Masson et al., 1993), spatial processing (Diederich et al., 2002) and color discrimination (Büttner et al., 1994; Haug et al. 1995; Müller et al., 1997; Sartucci et al., 2003). Other studies correlate dopamine function with gender and provide evidence that gender differences can result from *retinal* (originating in dopaminergic retinal neurons), *thalamic* (involving dLGN), or *central* (involving dopaminergic neurons in V1 and/or higher cortical areas) processes (Cowan et al., 2000; Yilmaz et al., 1998).

In a blood-oxygen-level-dependent functional magnetic resonance imaging (BOLD fMRI) study involving twenty (10 males, 10 females) age-matched subjects, Cowan et al. (2000) found significantly different gender responses to blue light in primary visual cortex. Females had a 0.14% BOLD signal increase to both red and blue light, but males had only a 0.01% increase in BOLD signal to red light. This difference was not significant at the $p < 0.05$ level. Males did, however, have a 0.52% BOLD signal increase to blue light, which was significantly greater than the increase to blue light for females ($p < 0.05$). The direction of the latter finding appears to contradict suggestions of a female physiological advantage in chromatic processing. Levin et al. (1998) also found that males had higher BOLD fMRI signals than females in response to simple light

stimulation. However, BOLD signals are complex. While they are sensitive to blood oxygenation that accompanies increased activity, they depend on blood flow and tissue volume as well. Therefore, they are better indicators of relative changes to baseline function.

Cowan suggested that the entire difference could be due to sex-related retinal variations. After all, dopamine receptors are present on all known retinal cell types (Nguyen-Legros et al., 1999). However, dopamine receptors are also present in the thalamus (Mrzijak et al., 1996) and visual cortex (Berger et al., 1991), so central nervous system (CNS) alterations could occur as well. It is not known how dopamine effects on the retina, thalamus, or CNS pathways contributed to the observed differences, as dopamine was not directly manipulated by Cowan nor were patients rigorously screened for conditions affecting dopamine function.

Sex hormones and cyclical fluctuations

Steroid sex hormone levels differ between males and females and are known to affect neurotransmitter function, including that of dopamine (Smith et al., 1987; Smith, 1989). Estrogen is a steroid hormone that has significant effects on both male and female reproductive systems. Estrogens also regulate tissue functions outside of the reproductive system. Estrogen receptor (ER) proteins mediate estrogen effects, and there are two subtypes of ERs, ER_{α} and ER_{β}. Ogueta et al. (1999) observed at least one type, ER_{α}, in both male and female ocular structures, including the iris, lacrimal gland, choroid and retina. However, ER_{α} proteins were found only in the retinas of *premenopausal* women,

not in men or in postmenopausal women. These findings suggest the possibility of direct estrogenic influences on visual processing in pre-menopausal women.

It is also possible that estrogens act through an intermediate mechanism. Estrogens influence dopamine release by augmenting glutamate and inhibiting gamma amino butyric acid (GABA) syntheses (Smith, 1989). It is generally accepted that GABA-mediated cortical inhibition is important in determining visual responses (Zemon et al., 1986), and there is research attributing differences in visual processing abilities to sex hormones. Diamond et al. (1972) found increased visual sensitivity to briefly presented broadband (white) lights during peak estrogen levels in women experiencing "normal cycles", but not in age-matched males or women taking contraceptives that partially suppress the normal fluctuations in estradiol. Yilmaz et al. (1998) found evidence for a facilitating effect of estrogen on visual neural transmission. This study revealed significant decreases in latency and increases in amplitude of pattern reverse visual evoked potentials when estradiol levels peaked during the ovulatory phase of the menstrual cycle. Ward et al. (1978) found that detection sensitivity to white stimuli on black backgrounds depended on the menstrual cycle phase of the woman. Although the results were not well correlated with presumed estradiol levels, sensitivity was maximal during the menstrual phase, intermediate during the luteal phase, and minimal during the pre-ovulation phase. A review by Parlee (1983) highlighted evidence for cyclical effects on visual processing. In a later review of this research, Guttridge (1994) suggests there is an increased cortical capacity for visual information processing in women during peak estradiol levels of the menstrual cycle. She concluded in her review that gender

differences in visual processing may be due to fluctuations in sex hormone levels during the menstrual cycle.

Only a few studies have investigated the effects of the menstrual cycle on color vision. Finkelstein (1887) found restricted color visual fields during the menstrual (low estradiol) phase of the menstrual cycle. Finkelstein also reported an increase in the wavelength position of unique green (toward yellow) during the menstrual phase. Lorenzetti (1926) also found constricted red and green color fields with decreased yellow and green sensitivity during the menstrual phase. While Guttridge (1996) could not demonstrate repeatable changes in standard achromatic visual fields across the menstrual cycle, other modern studies have linked the menstrual cycle to chromatic visual field changes. The results of Akar et al. (2005) and Yucel et al. (2005) both agreed with those of Guttridge (1996) that standard achromatic automated perimetry (SAP) did not depend on the phase of the cycle. However, both studies suggested decreased sensitivity to short-wavelength stimuli during the relatively low estradiol luteal phase ($p < 0.05$ for both studies). In a study of cyclical effects on isolated S-, M- and L-cone mechanisms, Eisner et al. (2004) found cyclical effects on S-cone mechanisms. There were, however, only limited cyclical effects on M- and L-cone mechanisms.

In addition to estrogen, progesterone is implicated in visual processing. Progesterone is thought to antagonize estrogens by increasing CNS sensitivity to GABA and decreasing the CNS response to glutamate (Smith, 1987). Although the results have limited relevance to my current study, Phillips and Sherwin (1992) suggested that increased progesterone levels during the luteal phase limit visual memory. More related

to my study are the results of Dunn and Ross (1985) who found improvements in contrast sensitivity during the luteal phase when progesterone/estradiol ratios are at their maximum. Since the magnocellular pathway is sensitive to low contrast, this result possibly indicates a progesterone-related improvement in magnocellular function. Kim and Tokura (1998) found that preferences for hue classes ("warm" vs. "cool") depended on an interaction of ambient temperature and phase of menstrual cycle. During the luteal phase (when progesterone is highest), warmer colors (reds) were preferred over cooler colors (blues) at warmer temperatures (28° C vs. 23° C).

Finally, Kramer et al. (1996) implicate androgens (male sex hormones) in their demonstrated preponderance of "global bias" in boys compared to girls. In describing large shapes made up of smaller geometrical elements, boys used more global descriptions (relying on the overall shape) whereas girls were more detailed (or local), referring to the smaller shapes to describe the figures. However, I am unaware of any studies linking androgen levels to gender differences in color vision.

Manifest heterozygosity of defective carriers

Normal males have two sex chromosomes—one X-chromosome inherited from their mother and one Y-chromosome inherited from their father. As a result, males are hemizygous (only one chromosome) for the expression of sex-linked color vision deficiencies and thus have either normal or abnormal color vision (Deeb, 2004). Normal females differ from males in that they generally inherit two X chromosomes, one from

each parent. Females can then be homozygous normal (XX) or heterozygous carriers (XX'), where X and X' denote normal and abnormal genes, respectively, for color vision. Color vision deficiencies are recessive, sex-linked traits that generally involve amino acid substitutions or mutations on M- and L-cone gene opsins (Deeb, 2004). Therefore, XY males are normal, and X'Y males express abnormal color vision. With the exception of rare conditions involving multiple copies of X chromosomes in males, there is no intermediate state. Females do have an intermediate state—manifest heterozygous carriers of color deficiencies.

Approximately fifteen percent of human females are such carriers of sex-linked color deficiencies (Jordan and Mollon, 1993). In theory, these individuals should have normal color vision since the normal color gene on one of their X chromosomes is dominant. However, heterozygous carriers often exhibit mild color vision deficiencies on clinical testing (Jordon and Mollon, 1993). Lyon (1963) hypothesized that heterozygous carriers are actually hemizygous in some cells due to X-inactivation during early development of the female embryo. That is, one gene (normal or abnormal) is active at any given point during embryonic development of certain tissues. In the eye, this results in a mosaic of normal and abnormal retina. While some studies have suggested that heterozygous carriers have essentially normal trichromatic vision (Miyahara et al., 1998), others have found reduced luminous efficiency to long wavelength (Schmidt, 1955; Harris and Cole, 2005) or medium wavelength light (Crone, 1959) as well as deficient red-green discrimination (Krill and Schneiderman, 1964; Lang and Good, 2001; Hood et al., 2006).

Neitz and Jacobs (1986) suggested that the significant individual variation in color matches was due to polymorphism of L-cones in heterozygous carriers. In his review of the question of gender and color, Mollon (1986) concluded there was evidence that the mutation provides carriers with a color vision advantage. Since the substrate for color vision is the presence of multiple cone classes, a fourth cone class should improve color discrimination. A study of this possible advantage revealed significantly greater delineation of spectral bands (i.e. more colors were seen) in heterozygous female carriers than in male or normal female trichromats (Jameson et al., 2001). It is possible, then, that the results of previous studies indicating gender differences were influenced by undetected heterozygous carriers. Because of this possibility, I used careful family history and examination of red-green discrimination to identify and exclude carriers from participation in the current study.

PHOTOMETRIC METHODS

Overall brightness, as well as the contributions from achromatic and chromatic channels, can be measured by photometric methods. These methods consist of visually matching the illuminance produced by a test light source against the illuminance produced by a reference source. Heterochromatic flicker photometry (HFP) is a technique that involves temporally alternating a monochromatic (narrowband) light with a reference broadband light at a relatively high (~20 Hz) rate. The intensity of the test or colored stimulus is adjusted so that the perception of flicker is minimized. After the observer has adjusted the intensity of the test (colored) stimulus to minimize flicker

perception, luminous efficiency is calculated. This is the ratio of the maximum testable spectral radiance to that required to minimize flicker at that stimulus wavelength. Dividing each luminous efficiency by the maximum measured efficiency yields the relative sensitivity function for an observer.

Since relative spectral sensitivity involves placing certain wavelengths in *equal classes* based on an assigned numerical value of light, all members of a class must have the same numerical value. In this way, a *class* can be represented by a single member of that class and likewise be replaced by any other equivalent member of that class (Ives, 1912). To be considered equivalent, the measurements must first be *additive*. That is, the mixture of two equiluminant colors must be of equal brightness and saturation to that of either of the original hues adjusted to twice its original value (Wagner and Boynton, 1972). A simpler approach is to say that if color C results from the mixture of two colors, A and B, the brightness of C should be equal to the sum of brightness A and brightness B. A second requirement of equivalence is that the measures are *transitive*. If A and B are colors and match in brightness to a reference white, then A and B should match in brightness. HFP measures are both additive and transitive, which is why results from flicker techniques played an important role in forming the standard luminous efficiency function, V(λ), frequently used in visual displays and calibrations (Lennie et al., 1993).

The flicker rate in HFP can be manipulated to isolate the influence of chromatic vs. achromatic pathways. Although rates vary for different wavelengths, one study suggested that the chromatic system is only fast enough to detect seven alternations per

second (Ikeda and Shimozono, 1978). Beyond that alternation rate, equally bright flickering stimuli will appear to be continuous. Achromatic processing is much faster, with critical (maximum) detectable flicker rates measured as high as 35-50 Hz, depending on the size and intensity of the test stimulus used (Hecht and Verrijp, 1933). HFP then isolates achromatic channels by staying below the critical flicker rate of the achromatic system (< 35 Hz) while avoiding influence of the chromatic channels (> 7 Hz). Generally accepted flicker rates for HFP are 18-25 Hz (Ikeda and Shimozono, 1978).

As noted above, slowly flickering systems involve a contribution of chromatic channels not detected by HFP techniques. The theory is that slow successive matches (heterochromatic brightness matching or HBM) result in additivity failures due to an additional color opponent system not involved in minimum flicker detection (Guth and Lodge, 1973). Differences in relative chromatic contributions along the spectrum also cause transitivity failures not found in HFP. The interference of the opponent process causing these failures can actually be used to quantify the additional contribution of the chromatic channels (Guth and Lodge, 1973). Either the difference (HBM-HFP) or the ratio (HBM/HFP) can be used as the "chromatic contribution" metric (Sagawa and Takahashi, 2001).

Provided an observer maintains the "same brightness" criterion, it is reasonable to assume that the chromatic channel influence on brightness would be maximized if the colored and reference stimuli are presented simultaneously. Direct brightness matching (DBM) is a photometric technique that involves simultaneous side-by-side (or up-and-

down) presentation of two stimuli in a bipartite field separated by a small gap. In DBM experiments, the observer adjusts the intensity of the colored field until it matches the reference field in perceived brightness. This technique seems to be more intuitive than slow successive matches. Although DBM measures are less repeatable (Meyer et al., 1978) and more subject to daily shifts in criteria (Yaguchi et al., 1993) than HFP measures, they yield greater sensitivities than HFP in the blue (short wavelength) and red (long wavelength) portions of the spectrum (Guth and Lodge, 1973). In the current study, I used the ratio of DBM to HFP as a measure of the contribution of chromaticity to brightness.

GOALS AND HYPOTHESES

In this study, I investigated the potential difference in chromatic processing between genders by comparing DBM/HFP ratios collected at five wavelengths—450 nm, 520 nm, 560 nm, 580 nm, and 650 nm (perceived as the hues blue, green, yellow-green, yellow and red). I predicted higher DBM/HFP ratios in females for the "primary" wavelengths: 450 nm, 520 nm, 580 nm, and 650 nm. No such prediction was made for the yellow-green (560 nm) stimulus, which was included in the study as a normalizing stimulus. I predicted there would be no gender effect on DBM measures in the present experiment. This prediction was based on the work of Crawford (1948), who found no gender differences in threshold spectral sensitivity. I also predicted there would be no gender difference in HFP measures on repeated measures or at any wavelength. This prediction was based on the results of Foutch and Peck (2006) and a post-hoc gender

analysis of Harrington et al. (2004), both suggesting that HFP measures do not depend on gender.

I also investigated the *within females* effects of hormone levels and menstrual phase on DBM/HFP ratios as well as HFP and DBM measures. I predicted a significant positive correlation of DBM/HFP with estradiol/progesterone ratios for all four "primary" wavelengths. I also predicted that DBM/HFP ratios would be at a maximum during the ovulatory phase, intermediate during the menstrual phase and minimum during the luteal phase. This prediction was based on the balance of previous studies suggesting an increase in chromatic sensitivity during the ovulatory phase and a decrease during the luteal phase. In my opinion, the balance of previous findings suggests very limited cyclical changes in HFP or absolute threshold measures. Therefore, I predicted there would be no significant relationships of HFP and DBM measures with either hormone levels or menstrual cycle phase.

METHODS

SUBJECTS

Subjects were recruited by convenience, and informed consent was obtained from 13 males and 21 females using separate consent forms. All volunteers were paid for participating. Subjects were eligible if they were between 18 and 45 years old, self-reported a complete eye examination within the last twelve months, had best-corrected visual acuity of 20/25 or better in each eye, and had normal color vision tested with psuedoisochromatic plates and Farnsworth D-15 color panels. Subjects were ineligible if they had a self-reported history of neurological or psychiatric disorders, used medications or nutritional supplements known to affect color vision, or had a family history of color defective vision. Female subjects were ineligible if they had abnormal color discrimination on Medmont C-100 or Nagel anomaloscope testing. In addition, female subjects were ineligible if they were menopausal or pregnant. Use of hormonal contraceptives was permissible. Tobacco use was allowed for all subjects if the number of pack years was below ten years, the amount previously shown to affect color sensitivity or discrimination (Erb et al., 1999; Bimler and Kirkland, 2004). The institutional review board of the University of Missouri –St. Louis approved the experimental protocol.

On selection, each female volunteer was asked if they were using oral contraceptives or intrauterine device (IUD). They were also asked if they were willing to disclose the date of the first day of menses for their current menstrual cycle. Two women were not willing to discuss details of their menstrual cycle and were placed in group I.

Sixteen women were willing to disclose the first day of menses and were placed in group II for data collection and analysis. Of the 16 subjects in group II, 7 subjects used either oral contraceptives or device that delivered exogenous estrogen and/or progesterone. Six subjects were taking combination oral contraceptives containing progestational and estrogenic compounds. Another subject was using a levonorgestrel intrauterine system. This system, also known as a progestin IUD, contains only progestin, a synthetic form of progesterone.

SCHEDULING

Men and group I women scheduled a single experimental session at their convenience. On selection, female subjects in group II were asked to estimate the date of day 1 (first day of menses) of their current menstrual cycle. The goal was to schedule experimental sessions to most closely coincide with postulated markers of minimum, intermediate and maximum estradiol/progesterone (E2/Pg) ratios. These markers are minimum E2/Pg = day 21, maximum E2/Pg = days 12-13, and intermediate = days 1-7 (see Figure 3). Experimental sessions were scheduled to most closely coincide with these markers. The dates were stored in a spreadsheet and recorded on a post-card. The post card was given to the subject as a reminder of scheduled experimental sessions. Each group II participant also recorded day one of the second (DO2S) and third (DO3S) menstrual cycles on her post-card, which was returned when all experimental sessions were completed. Some participants chose to email DO2S and/or DO3S dates rather than return the card.

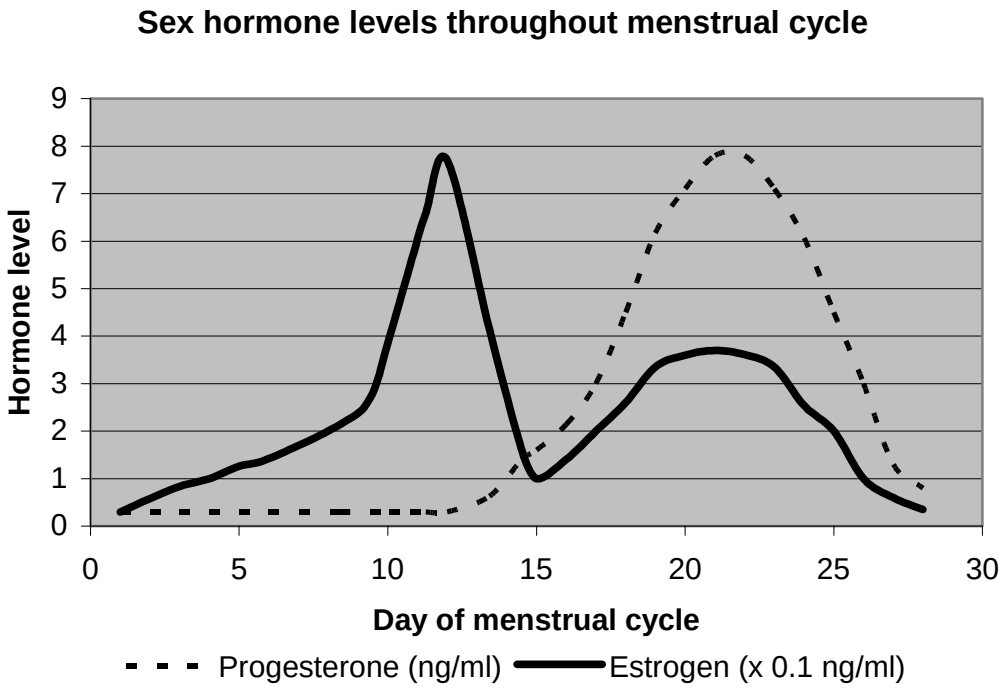


Figure 3 – Changes in steroid sex hormone plasma levels during a typical menstrual cycle. The solid line represents estradiol levels, and the dashed line represents progesterone levels. Days 1-7 correspond to the *menstrual phase* where both hormone levels are at a minimum, but the ratio of estradiol to progesterone is intermediate. *Ovulation* (release of ovum or egg from ovary) occurs around day 13 and is preceded by peak estradiol levels while progesterone levels remain low. The estradiol/progesterone ratio is maximized around days 11-12 just prior to ovulation. Progesterone levels then rise during the *luteal phase* until they peak around day 21 of the cycle. Estradiol levels decrease after ovulation then increase to a second peak also around day 21, where the estradiol/progesterone ratio is at a minimum compared to menstrual and ovulation phases. Note the different scales for estradiol and progesterone levels. (The figure is adapted and redrawn from Odell and Moyer, 1971.)

It is known that the length of the typical menstrual cycle from ovulation to menstrual phase is 14 days, but the length from start of menses to ovulation varies a great deal between women (Mozkowski et al., 1962). It has also been shown that peak

estrogen levels occur on average 34 hours prior to ovulation or 15.5 days prior to the start of the next cycle (Pauerstein et al., 1978). Mid-luteal phase was considered to occur at the midpoint between ovulation and the start of the next cycle. Therefore, I counted back 16 days (for peak E2/Pg near ovulation) and 7 days (for minimum E2/Pg at mid-luteal phase) from the expected start of the next cycle to determine ideal dates for data collection. Although all sessions were used for all subjects, DO2S and DO3S were used for discussion to determine how closely the experimental sessions tied to menstrual, ovulatory, and mid-luteal phases.

If an experimental session could not be scheduled or was not completed within three days of a predicted marker, that session was rescheduled during the second month. Several measurements were necessary during the second month, and the day within the cycle was verified by the first day of the third cycle (DO3S). If the ideal date of ovulatory or mid-luteal phase fell on Sunday, the session was scheduled for Monday. The same scheduling process was used for all female subjects in group II, but scheduling sessions for oral contraceptive users was more straightforward as the length of the menstrual cycle is fixed at 28 days. Two subjects could not complete three sessions within two cycles, and partial data (one and two sessions, respectively) were used in the analysis.

HORMONE ANALYSIS

On selection, female subjects in group II were provided three saliva collection kits (ZRT Laboratory, Beaverton, OR). Each was instructed to collect saliva at home on the

day of each experimental session. Subjects were instructed to collect saliva in a small vial prior to eating, drinking or brushing their teeth and then to bring the collected sample to the session. When a subject forgot to collect or bring the sample, she was instructed to collect and return the sample the following day. Although sex hormone levels have been proven relatively stable in saliva (Dabbs, 1991), subjects were asked to refrigerate the samples until returned since the vials contained no preservative. The day they were received, each sample was mailed to the laboratory for assay. Progesterone and progestin were measured with a direct competitive radioimmunoassay (RIA), while naturally occurring and synthetic estradiol were measured by double antibody RIA. All samples were analyzed within 30 days of collection as recommended by the laboratory. Results were received from the laboratory by email and recorded in a spreadsheet for analyses.

APPARATUS

A three-channel optical (open-view) system was used to produce a 2-degree, circular field for both experimental tasks. A uniform circular field was used for heterochromatic flicker photometry (HFP), while a side-by-side bipartite field was used for direct brightness measures (DBM).

Splitting the output light 99:1 from a 1000-watt Xenon arc lamp through an antireflective window formed two illumination channels. The first channel was a monochromatic test channel that consumed approximately 99% of the lamp output. The intensity of the test channel was adjusted with an iris aperture and a motorized, computer-

controlled set of dual counter-rotating variable neutral density filters. A motorized, computer-controlled narrow bandpass interference filter (NBIF) wheel produced each of

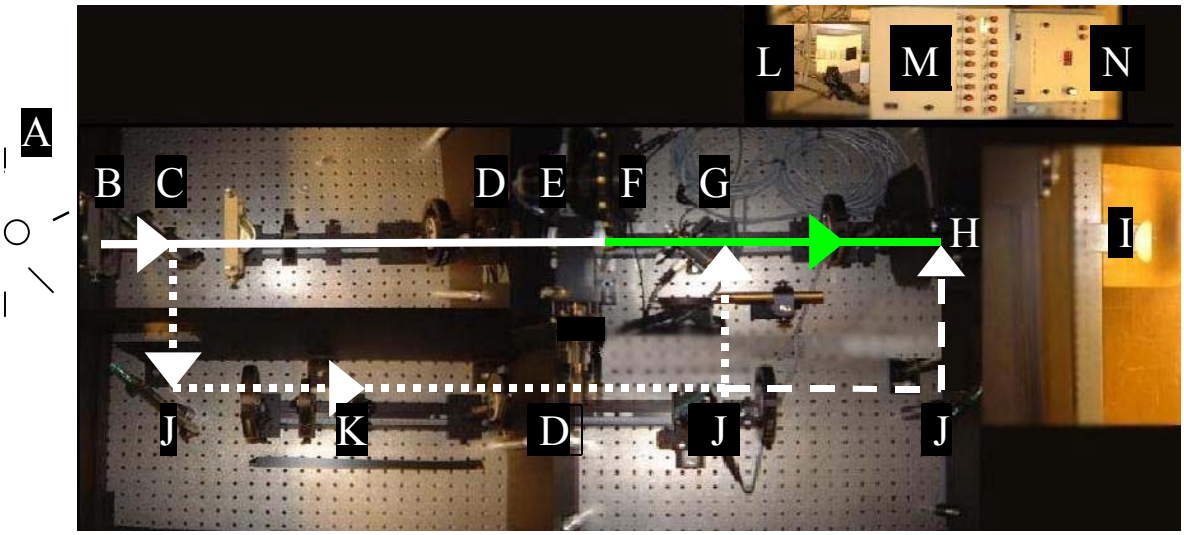


Figure 4 – Three channel optical system: The solid white and green lines represent Channel 1 before and after passing through narrowband interference filters, respectively. The dotted line represents Channel 2, which produces a broadband reference stimulus merged with Channel 1 for flicker photometry. The dashed line represents Channel 3, which is presented simultaneously with Channel 1 for direct brightness matching (DBM). Components include A) 1000 Watt Xenon arc lamp, B) heat absorbing lens, C) 99/1 beam splitter, D) variable iris apertures, E) dual counter-rotating variable neutral density filter system, F) narrow band pass interference wheel, G) mirrored optical chopper, H) diffusing optic cylinders for viewing stimuli, I) chin rest, J) front surface mirrors, K) variable neutral density filter, L) channel 2/3 selector, M) narrow band interference filter wheel controller, N) dual counter-rotating variable neutral density filter system controller. Several focusing/condensing lenses were placed throughout optical channels but are not labeled. A personal computer (PC) interfaced with M and N and was used for data acquisition and reduction.

the five test wavelengths. The second channel was a spectrally broad reference channel that consumed approximately 1% of the bulb output. The intensity of the reference channel was adjusted using a variable neutral density filter and an iris aperture. The reference channel was then split into two channels via a front surface mirror that could be translated in and out of the reference beam. With the mirror in place for the HFP task,

the test and reference beams were spatially merged yet temporally separated via a remote-controlled mirrored optical chopper rotating at 18 cycles/sec (Hz). The test and reference beams alternately illuminated a 0.75-inch magnesium chromate (acrylic) cylinder. Optically frosted ends of the cylinder acted to reduce variations in stimulus intensity and served as a diffuse circular viewing screen.

The mirror was translated out of the reference beam to form a third channel used in the DBM experiments. A fixed front surface mirror reflected the reference beam onto the left viewing half of a bipartite viewing field, separated from the monochromatic test field by a 0.5 mm-thick sheet of aluminum. The fused and whole cylinders were part of a calibrated, mounted set that was remotely translated depending on task. A chin rest was used to position each subject 17 inches from the viewing end of the optic–which subtended 2.5° of visual angle. A detailed photograph of the experimental apparatus is shown in Figure 4.

CALIBRATION

Since relative sensitivity at each wavelength is the reciprocal of the radiance required to match the reference stimulus (DBM) or minimize flicker sensation (HFP), reliable measures of relative sensitivity depend on accurate measures of spectral radiance. Since time did not permit a direct measurement of each stimulus after each trial, stimulus radiance was calculated from filter wheel settings for each of the five wavelengths. The filter wheel controller provided the angle of rotation of the counter-rotating neutral density filters (from 50° to 330°). In order to obtain a radiance measure from these

settings, the output angle was calibrated to luminance using a Photo Research 650 (PR-650) spectroradiometer for every 10th degree shown on the output controller (i.e. 50°, 60°, 70°, … 310°, 320°, 330°).

Luminance was then modeled to filter settings according to the following equation: $L = k * e^{-\alpha(FS)}$, where L = luminance, FS = filter setting in degrees, and (k, α) are parameters of the exponential model. Each wavelength was modeled for three empirically derived ranges: Model I = ($80 \leq FS \leq 200$), Model II = ($210 \leq FS \leq 250$), and Model III = ($FS > 250$). Each model provided a nearly ideal correlation ($R^2 \approx 1.00$) for all five wavelengths (Figure 5). The only exception was with model I for the 450 nm stimulus. In this case, the filter settings only accounted for 97% of the variance in luminance values ($R^2 = 0.97$). Random variance in luminance from this lack of fit (3%) would be well below the expected effect size for gender differences. For this reason, the regression model was not further modified.

Each morning of data collection, the neutral density filter in channel 2 was used to adjust the reference luminance to 5.0 cd/m^2, measured using the spectroradiometer. Channel 1 luminance was also adjusted to 5.0 cd/m^2 with the NBIF wheel in the open (broadband) position. This level of luminance corresponded to a range of 100-200 retinal Trolands and ensured reliable, uniform cone contributions to both achromatic and chromatic systems (Lee, 1999) while staying in the recommended range for accurate HFP measures (de Vries, 1949). In addition, the maximum measurable luminance and lamp radiometric output for each stimulus was measured weekly and recorded in a spreadsheet used to calculate relative sensitivity data. This was important in calculating accurate

measures of spectral radiance required to match the reference stimuli, as repeated measures analysis of variance revealed differences over time in lamp radiometric output ($F = 27.4$, $p < 0.01$) and trends in maximum measurable luminance ($F = 5.42$, $p < 0.1$).

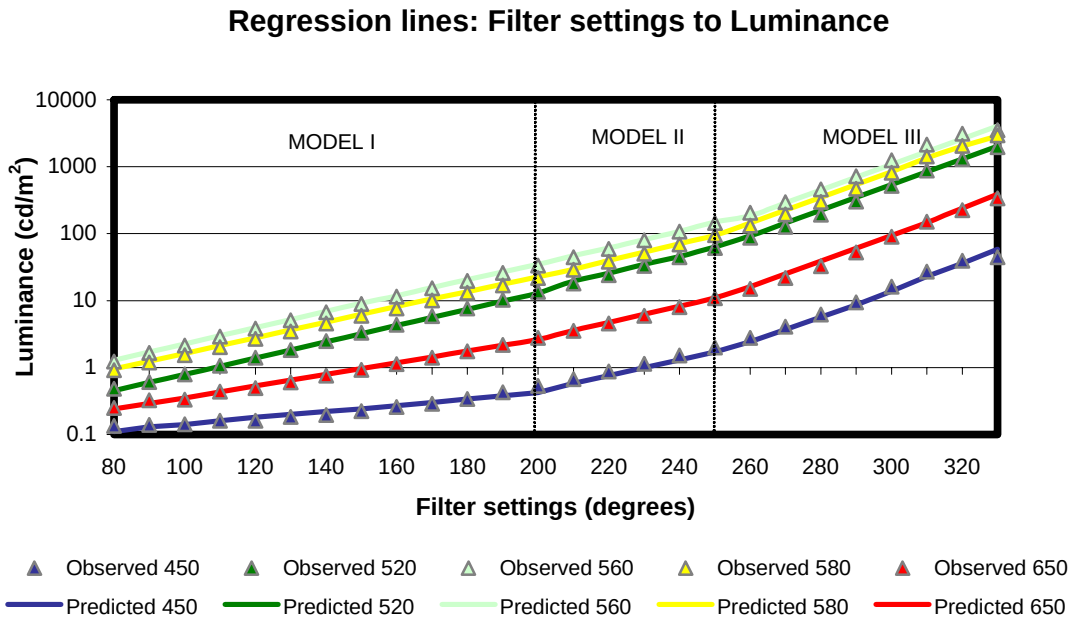


Figure 5 – Regression lines of filter settings to logarithmic values of luminance. For model I, $R^2 = 1.00$ for all wavelengths except for 450 nm ($R^2 = 0.97$). For models II and III, $R^2 = 1.00$ and 0.99, respectively, for all wavelengths.

PROCEDURE

Each subject completed a single practice session. During this session, a minimum of four trials were performed at each test wavelength—450, 520, 560, 580 and 650 nm—for both HFP and DBM tasks. The practice concluded when the range of trials at each wavelength fell within one standard deviation (SD) of the mean for both DBM and HFP

measures. This process was repeated until the subject was successful at making reliable sets of matches at all five test wavelengths for both methods.

Prior to each experimental session, a random number generator was used to determine the order of task and wavelength presentation. Each session began by adapting each subject to the background room luminance (< 0.4 cd/m^2) for at least five minutes. Subjects then practiced DBM and HFP matches while the Xenon bulb was allowed to warm up for at least thirty minutes. Before and after each set of trials, the subject adapted for thirty seconds to a broadband reference stimulus. This accomplished two things. It reduced selective bleaching from the previous narrowband stimulus and avoided the Purkinje shift in relative sensitivity to lower wavelengths due to low background and room luminance (Cornsweet, 1970). After adapting to the reference fields, subjects adjusted the intensity of the test stimulus until it matched the reference field (for brightness measures) or the flicker sensation was minimized (for flicker photometry measures). Pressing RECORD on the custom dual filter controller transferred the filter setting into a temporary spreadsheet (Excel, Microsoft Corporation, Redmond, WA). Custom software (Redd Software Inc., Glen Carbon, IL) then remotely decreased the filter wheel setting by a randomly determined value between 50 and 100 degrees (approximately 1-2 log units of intensity). Four trials were performed for each stimulus in succession and followed by another thirty-second adaptation period. This process was repeated for all five wavelengths for both tasks.

DATA REDUCTION AND ANALYSES

The filter settings were imported into an Excel spreadsheet and then converted into transmission values using the calibration regression equations. The transmission values from the filter settings on the four trials were averaged and divided by the maximum measurable luminance at the respective wavelength. The proportion of the maximum luminance at each wavelength provided a percent-transmission that was multiplied by the lamp radiometric output (in watts/cd-m^2) at each tested wavelength to yield the radiance required for match.

Measures of macular pigment optical density (MPOD) were used in adjusting the DBM and HFP measures for the 450nm stimulus. MPOD for each subject was measured with a commercial tabletop heterochromatic flicker photometer using a 1˚ circular 460 nm stimulus (QuantifEYE, Zeavision LLC, Chesterfield, MO). Since optical density (OD) is expressed by $\log_{10}(1/T)$, where T is transmittance, both HFP and DBM measures at 450 nm were multiplied by 10^{MPOD}. Due to scheduling difficulties, the instrument was unavailable for use at times convenient or even possible for some subjects. When this occurred, the HFP and DBM measures for the 450 nm stimuli were not analyzed. However, the DBM/HFP ratio measures were analyzed since 10^{MPOD} is present in both the numerator and the denominator.

All HFP, DBM and MPOD measures along with the date of the session were copied into a single Excel spreadsheet. The aggregate data were then copied into SPSS (SPSS for Windows, SPSS Inc., Chicago, IL) and R (R Programming Language, Free Software Foundation, Boston, MA) statistical packages for analysis. The HFP curves for

each subject were visually inspected for the presence of significantly reduced sensitivity to the 650 nm stimulus (Schmidt's sign), which is evidence of a manifest heterozygous protan defect. The contribution of chromaticity to brightness was calculated two ways, both involving the ratio of DBM to HFP measures. The non-standardized ratios were the ratios of the radiances required for match of HFP to DBM measures. The standardized ratios were the ratios of standardized DBM to standardized HFP measures. Factor analysis was used to determine if a single factor representing chromatic contribution to brightness across all wavelengths could account for a significant amount of the variance in each ratio. Modern item response theory (IRT) was also used to determine which ratio—standardized or non-standardized—was more suitable for use as a measure of chromatic contribution to brightness.

Based on the results of factor analysis and item response theory, non-standardized ratios were chosen to represent chromatic contribution to brightness. For the between-subjects design, DBM/HFP ratio measures from the first session for all subjects were analyzed by repeated-measures analysis of variance (ANOVA) with gender and contraceptive use as fixed factors. For the within-female analysis, only data from Group II female subjects were used for analyzing correlations of appropriate DBM/HFP ratios with estradiol, progesterone and estradiol/progesterone ratios. I further analyzed the effects of hormone by regressing DBM/HFP ratios onto hormone levels and contraceptive use. Lastly, I investigated the effects of menstrual phase and contraceptive use on DBM/HFP ratios by univariate ANOVA. All correlation, regression and variance analyses were repeated for HFP and DBM measures.

RESULTS

HORMONE ANALYSES

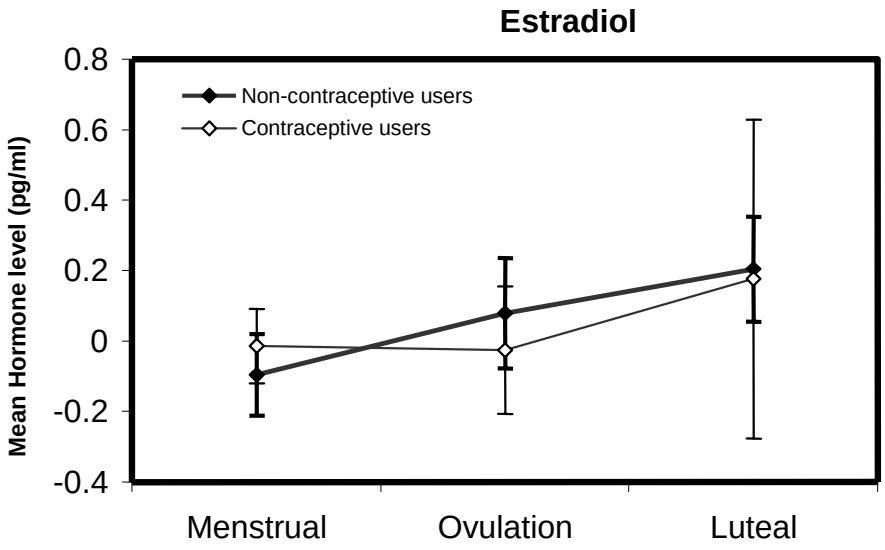
I obtained 45 saliva samples from 16 subjects, and they were assayed for progesterone and estradiol concentrations. Two samples from contraceptive users had estradiol concentrations below measurable levels (< 0.5 pg/ml), and eighteen progesterone concentrations were below measurable levels (< 15 pg/ml). Ten of the low progesterone measures were from subjects not using any contraceptive device, and the remaining eight were from contraceptive users. Another subject had an isolated high progesterone measure (2602 pg/ml) indicating a contaminated sample. This measure was treated as an outlier and not used in the analysis. Mean estradiol was 1.18 ± 0.64 pg/ml (range, 0.50 - 3.90 pg/ml). Because estradiol measures were positively skewed and kurtotic, estradiol measures were log-transformed for analysis. E2 will then refer to log-transformed estradiol measures. PG will refer to progesterone, which was distributed normally with a mean of 36.15 ± 21.24 pg/ml (range, 15-96 pg/ml).

For non-contraceptive users, the average length of the menstrual cycle was 28.9 ± 2.5 days (range, 25-32 days). The length of the menstrual cycle was normalized for each subject to 28 days, and the day of the cycle was re-calculated based on the normalized length. For example, subject 28 had a cycle length of 25 days, and her experimental sessions fell on days 6, 11, and 19 of her menstrual cycle. When the length of her cycle was normalized to 28 days, the days were re-scaled to days 7, 12, and 21.

Progesterone and estradiol measures for all subjects are plotted against the menstrual cycle phase in Figure 6. Estradiol measures for contraceptive users and non-

contraceptive users were coincident on profile analysis ($F = 1.69$, $p > 0.2$) but changed across menstrual phases ($F = 12.9$, $p < 0.01$). Mean E2 was at a minimum during menstrual phase (days 0-8) with a small peak during luteal phase (around day 20). However, E2 did not peak for non-contraceptive users as expected (Odell and Moyer, 1971) near ovulation (days 10-15). This was either due to subjects being non-ovulatory or saliva collection not coinciding with peak estradiol levels.

Only two subjects (one contraceptive user and one non-user) had usable progesterone measures for all three phases, therefore progesterone profiles could not be analyzed by repeated measures. Using a univariate ANOVA design with contraceptive use and phase as fixed factors, progesterone measures were significantly higher for non-contraceptive users than contraceptive users ($F = 5.23$, $p = 0.03$). There was no main effect of phase or interaction of phase with contraceptive use. Mean progesterone was at a minimum for non-contraceptive users during the menstrual and ovulatory phases (days 0-15) and peaked as expected during the luteal phase (Odell and Moyer, 1971). For contraceptive users, progesterone levels were at a minimum during menstrual phase but peaked during mid-cycle. Because of these results, contraceptive use was considered as a factor in all subsequent analyses of photometric measures.



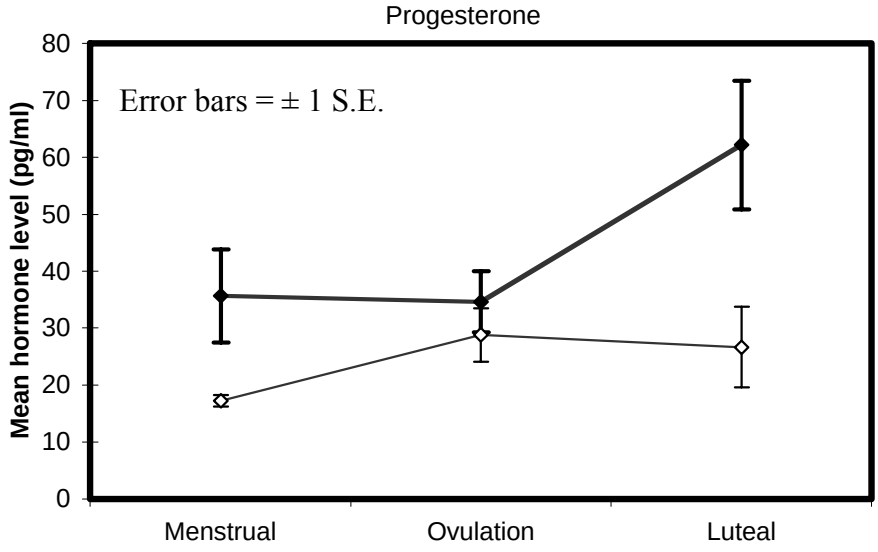


Figure 6 - Estradiol and progesterone profiles (in pg/ml) across the menstrual cycle phase. Estradiol profiles were coincident for contraceptive users and non-users ($F = 1.69$, $p > 0.2$) and changed across menstrual phase ($F = 12.9$, $p < 0.01$). There were not enough progesterone data to analyze the profiles across the menstrual phase. However, there was a significant difference in progesterone levels between contraceptive users and non-contraceptive users ($F = 5.23$, $p = 0.03$) using univariate ANOVA.

EXPERIMENTAL MEASURES

Thirteen male subjects and two female subjects in group I completed a single practice session. Sixteen female subjects in group II completed 45 experimental sessions. Sixteen, twelve and seventeen sessions were completed during menstrual, ovulatory and luteal phases, respectively. Because distributions of standardized heterochromatic flicker photometry (HFP) data were platykurtotic and negatively skewed, HFP measures were log-transformed for analysis. Since a decrease in luminous efficiency to red light (Schmidt's sign) can be evidence of a manifest heterozygous protan defect, HFP measures were compared to the standard luminous efficiency function,$V(\lambda)$, at 650 nm. HFP measures at 650 nm were within 0.1 log unit of $V(\lambda)$, indicating that Schmidt's sign was not observed. Standardized direct brightness matching (DBM) measures were normally distributed and were not transformed for analysis. Distributions for standardized and non-standardized DBM/HFP ratios were positively skewed and leptokurtotic and were log-transformed for analysis. For the rest of this paper, HFP will refer to log-transformed standardized HFP data, and DBM will refer to standardized DBM data.

Confirmatory factor analysis (CFA) was performed on DBM/HFP ratios using the data from the first session of all subjects, and two factors were extracted for the standardized ratios. One factor was composed of variance components from the 450 (blue) and 580 nm (yellow) stimuli and accounted for 34% of the variance in standardized ratios. A second factor was composed of variance components from the 560, 580 and 650 nm stimuli and accounted for an additional 20% of variance. Variance

in measures from the 520 nm stimulus did not contribute significantly to either factor for standardized ratios. A single factor was extracted for non-standardized ratios. Each wavelength contributed significantly to the factor and cumulatively explained 50% of the variance.

For item response theory (IRT) analysis, both ratios were scaled from 1.0 to 7.0 then rounded to the nearest integer. A dichotomous response was assigned to each scaled integer response: 0 ("not seen") = 1, 2 and 1 ("seen") = 3, 4, 5, 6, 7. MULITLOG (Scientific Software International, Inc., Lincolnwood, IL) was then used to model these dichotomous data. MULTILOG modeled chromatic contribution to brightness as a latent trait to the probability of endorsing (or "seeing") the stimulus according to the following equation:

$$P_i(\theta) = c_i + (1 - c_i)\frac{1}{[1 + e^{-\alpha_i(\theta - \beta_i)}]}$$

where P = probability of endorsing item, i = item or wavelength, θ = latent trait (chromatic contribution to brightness), α = discrimination of items, β = difficulty of items, and c = guessing parameter of each item. The results from MULITLOG revealed no improvement in model fits using the α and c parameters. This suggested that the probability of guessing correctly was zero ($c = 0$), and each wavelength ideally discriminated whether or not the item was endorsed ($\alpha = 1$). The only difference between the items was the difficulty of the items or where on the continuum of the latent trait the probability functions fell.

As the model pertains to the current experiment, the latent trait represented the chromatic contribution to brightness. Easier items (wavelengths) had higher ratios than more difficult items. The resulting difficulty parameters from MULTILOG were input into a modified Excel spreadsheet (MODFIT, IRT Modeling Lab, University of Illinois) where predicted probability functions were fit against randomly sampled dichotomized ratios used as validation data. The non-standardized ratios fit much more closely to validation data than standardized ratios. The χ^2 values for both ratios are summarized in Table 1, and the cumulative probability functions of observed and validation data are shown in Figure 7.

Table 1 – IRT model fits to validation data (χ^2 results)

Wavelength	Standardized	Non-standardized
450	188	3.31
520	765	0.14
560	189	0.02
580	15.0	0.07
650	51.8	0.35

In general, χ^2 values of < 3.0 indicate that an item is acceptable in the test of a latent trait (Bock, 1972; Mislevy, 1986). Except for 450 nm, χ^2 results for all of the non-standardized ratios were in the acceptable range for use in an overall test of the latent trait. Values for the standardized ratios were not acceptable, ranging from 15 to 188. These results were consistent with those from factor analysis, where the 520 nm stimulus did not contribute to either factor describing the variance in standardized ratios. The χ^2 results for the 520 nm stimulus suggest that the 520 nm stimulus contributed very little information to the overall standardized test of the latent trait. The 580 nm stimulus

contributed significantly to both factors, and its lower χ^2 result suggests that it also contributed the most to the overall standardized model of the latent trait.

Based on the results of factor analysis and IRT, non-standardized ratios were more suitable than standardized ratios for use as a measure of chromatic contribution to brightness. For the rest of this paper, DBM/HFP ratios will refer to log-transformed non-standardized ratios.

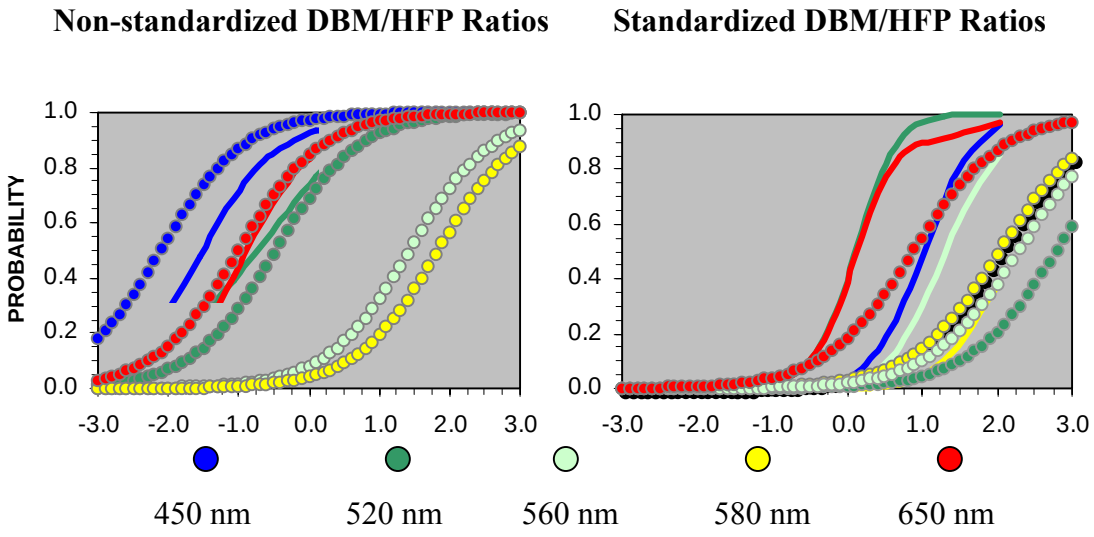


Figure 7 – Cumulative probability plots of standardized and non-standardized ratios. Probability was plotted against the latent trait (chromatic contribution to brightness), and it is easy to see that the observed curves (circles) for non-standardized ratios fit more closely to validation curves (lines) than did standardized ratios. In addition, the non-standardized curves fell along a wider range of the latent trait while the standardized ratios all fell along higher levels of the latent trait. These graphs, combined with the results of χ^2 tests, indicate that non-standardized ratios were more suitable than standardized ratios for use as a measure of chromatic contribution to brightness.

MACULAR PIGMENT OPTICAL DENSITY (MPOD) MEASURES

Males tended to have higher MPOD measures than females ($t = 1.422$, $p = 0.17$). This is consistent with Hammond et al. (1996) who found even greater differences in MPOD measures ($p < 0.001$). In the current study, the distributions of MPOD were also similar to those of Hammond. Distributions in both studies tended to be skewed negatively for females and positively for males. In the current study, MPOD negatively correlated (as expected) with both HFP ($r = -0.62$, $p = 0.003$) and DBM ($r = -0.55$, $p = 0.01$) measures at 450 nm. Both measures were modeled at 450 nm to account for MPOD by multiplying sensitivity by 10^{MPOD}. After adjustment, MPOD correlated neither with HFP ($r = -0.30$, $p = 0.19$), DBM ($r = -0.10$, $p = 0.68$) nor DBM/HFP ratios at 450 nm ($r = 0.14$, $p = 0.55$).

ANTAGONISM OF L- AND M-CONE MECHANISMS AT 580 nm

DBM/HFP ratios drop to unity around 580 nm. This drop in sensitivity is known as the "Sloan notch" after Louise Sloan who first encountered the phenomenon (Sloan, 1928). Due to L- and M-cone antagonism near 580 nm, there is no additional contribution to brightness from chromaticity. In the present experiment, as shown in Figure 8, the antagonism actually subtracts from sensitivity as measured by HFP at 580 nm (i.e. DBM/HFP < 1.0). DBM/HFP ratios at 580 nm were significantly less than 1.0 for both males ($t = -7.432$, $p < 0.001$) and females ($t = -2.814$, $p = 0.01$). In addition, DBM/HFP ratios at 580 nm for males were significantly less than those for females

($t = -3.722$, $p = 0.001$). The antagonism of L- and M-cones has been described as forming a mechanism distinct from that which forms $V(\lambda)$ at wavelengths above 580 nm (Calkins et al., 1992), and this mechanism appears to be gender dimorphic.

ANALYSIS I - BETWEEN-SUBJECTS DESIGN: GENDER COMPARISON

The results of the between-subjects analysis of gender on experimental measures are shown in Figures 8-10 and summarized in Table 2. As shown in Figure 8, female subjects had higher overall DBM/HFP ratios than male subjects ($F = 15.4$, $p = 0.001$), and the effect size (η^2) was large (36%; see Table 2). Multivariate ANOVA (MANOVA) also revealed higher ratios for females at every wavelength except 560 nm. There was no effect of gender on overall HFP measures ($F = 0.32$, $p = 0.58$), nor was there an effect on HFP at any wavelength (Figure 9). Gender also did not have a significant effect on overall DBM measures ($F = 1.27$, $p = 0.27$).

Table 2 – Between-subjects effects of gender on experimental measures

	DBM/HFP RATIO			**HFP**			**DBM**		
Wavelength	**F**	**p**	**η^2**	**F**	**p**	**η^2**	**F**	**p**	**η^2**
ALL	***15.4**	**<0.01**	**0.36**	0.32	0.58	0.02	1.27	0.27	0.04
450	***9.51**	**<0.01**	**0.25**	0.11	0.75	0.01	0.98	0.34	0.05
520	***14.8**	**<0.01**	**0.35**	0.07	0.80	0.00	3.35	0.08	0.15
560	2.44	0.13	0.08	0.60	0.45	0.03	0.38	0.55	0.02
580	***12.3**	**<0.01**	**0.31**	0.45	0.51	0.02	0.03	0.87	0.00
650	***5.95**	**0.02**	**0.18**	0.01	0.91	0.00	1.78	0.20	0.09

* $p < 0.05$

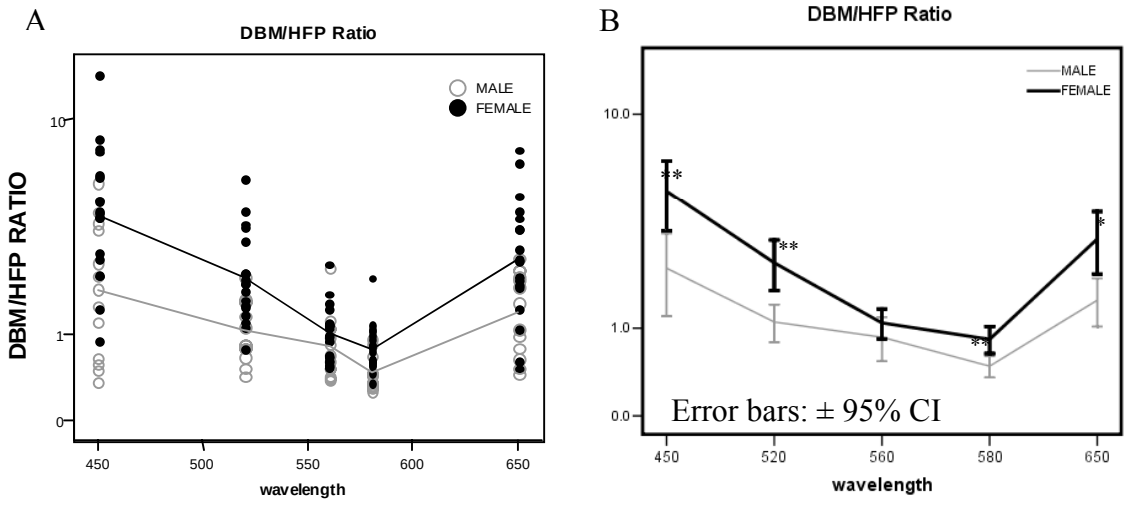


Figure 8 - Gender comparisons of DBM/HFP ratios. Female subjects had higher ratios than males on repeated measures. Multivariate analysis also revealed significantly higher ratios for females at all wavelengths except 560 nm (* $p < 0.05$, ** $p < 0.01$). In Figures 8-10, the scatter data (A) are plotted against *interval* wavelengths while the line data (B) are plotted against *categorical* wavelengths. The different plotting methods account for the different shapes of the trend lines.

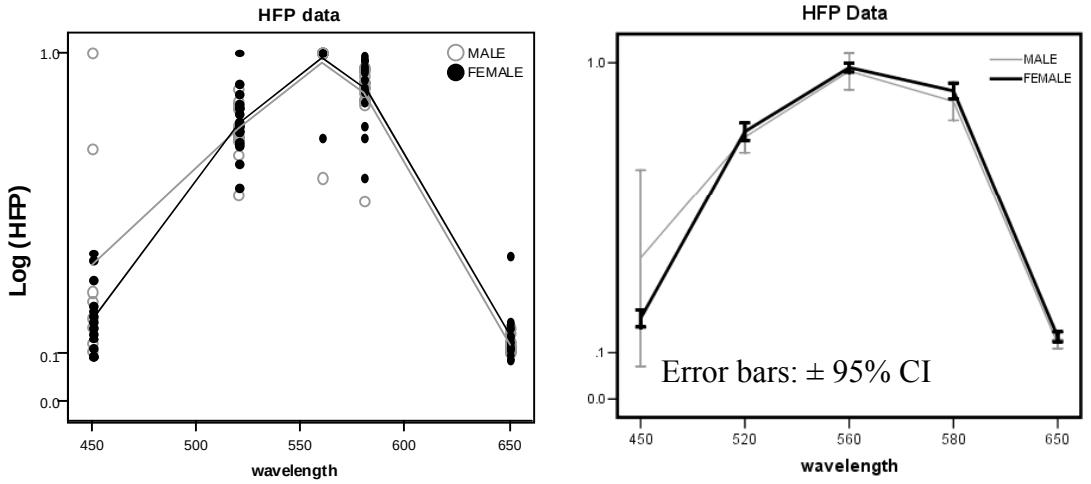


Figure 9 - Gender comparisons of heterochromatic flicker photometry (HFP) data from first experimental sessions. There was not a significant difference between males and females by repeated measures ANOVA ($F = 0.32$, $p = 0.58$), nor were there significant differences at any wavelength on multivariate analysis.

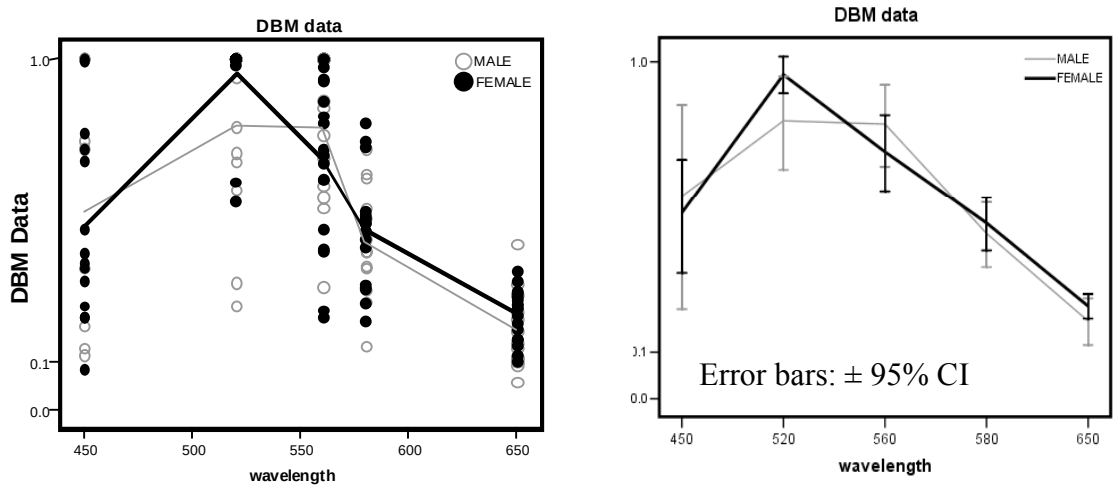


Figure 10 - Gender comparisons of direct brightness matching (DBM) data from first experimental sessions. There was not a significant difference between males and females by repeated measures ANOVA ($F = 1.27$, $p = 0.27$). The greatest difference was at 520 nm ($F = 3.35$, $p = 0.08$).

ANALYSIS I - CONTRACEPTIVE USERS VS. NON-CONTRACEPTIVE USERS

As shown in Figure 6, there were differences in progesterone profiles between contraceptive users and non-contraceptive users across the menstrual cycle. Since a possible mechanism for gender differences involves main and interaction effects of estradiol and progesterone, the between-subjects effects of contraceptive use were examined. Comparisons of DBM/HFP ratios, HFP and DBM measures by contraceptive use are shown in Figure 11, and the results of repeated measures ANOVA are summarized in Table 3. Unlike gender, contraceptive use had no effect on overall DBM/HFP ratios or at any particular wavelength. There was also no main effect of contraceptive use on overall HFP measures ($F = 1.05$, $p = 0.37$), although MANOVA revealed that HFP measures tended to be higher for non-contraceptive users at 450 nm ($F = 3.72$, $p = 0.08$, $\eta^2 = 0.27$).

Table 3 – Between-subjects effects of contraceptive use on experimental measures

	DBM/HFP RATIO			HFP			DBM		
Wavelength	**F**	**p**	**η^2**	**F**	**p**	**η^2**	**F**	**p**	**η^2**
ALL	0.04	0.84	0.00	1.05	0.37	0.11	2.49	0.15	0.20
450	0.04	0.85	0.00	3.72	0.08	0.27	3.11	0.11	0.24
520	0.36	0.56	0.03	0.46	0.51	0.04	0.61	0.45	0.06
560	0.88	0.37	0.06	0.69	0.42	0.07	***7.03**	**0.02**	**0.41**
580	0.14	0.72	0.01	0.36	0.57	0.03	0.67	0.42	0.07
650	1.16	0.30	0.08	0.59	0.46	0.06	0.42	0.53	0.04

* $p < 0.05$

Contraceptive use accounted for 27% of the variance in HFP at 450 nm, but less than 10% at all other wavelengths. Though not significant ($F = 2.49$, $p = 0.15$), the effect size of contraceptive use on overall DBM measures was large ($\eta^2 = 0.20$). DBM measures tended to be higher for non-contraceptive users at 450 nm ($F = 3.11$, $p = 0.11$), while contraceptive users had significantly higher DBM measures at 560 nm ($F = 7.03$, $p = 0.02$).

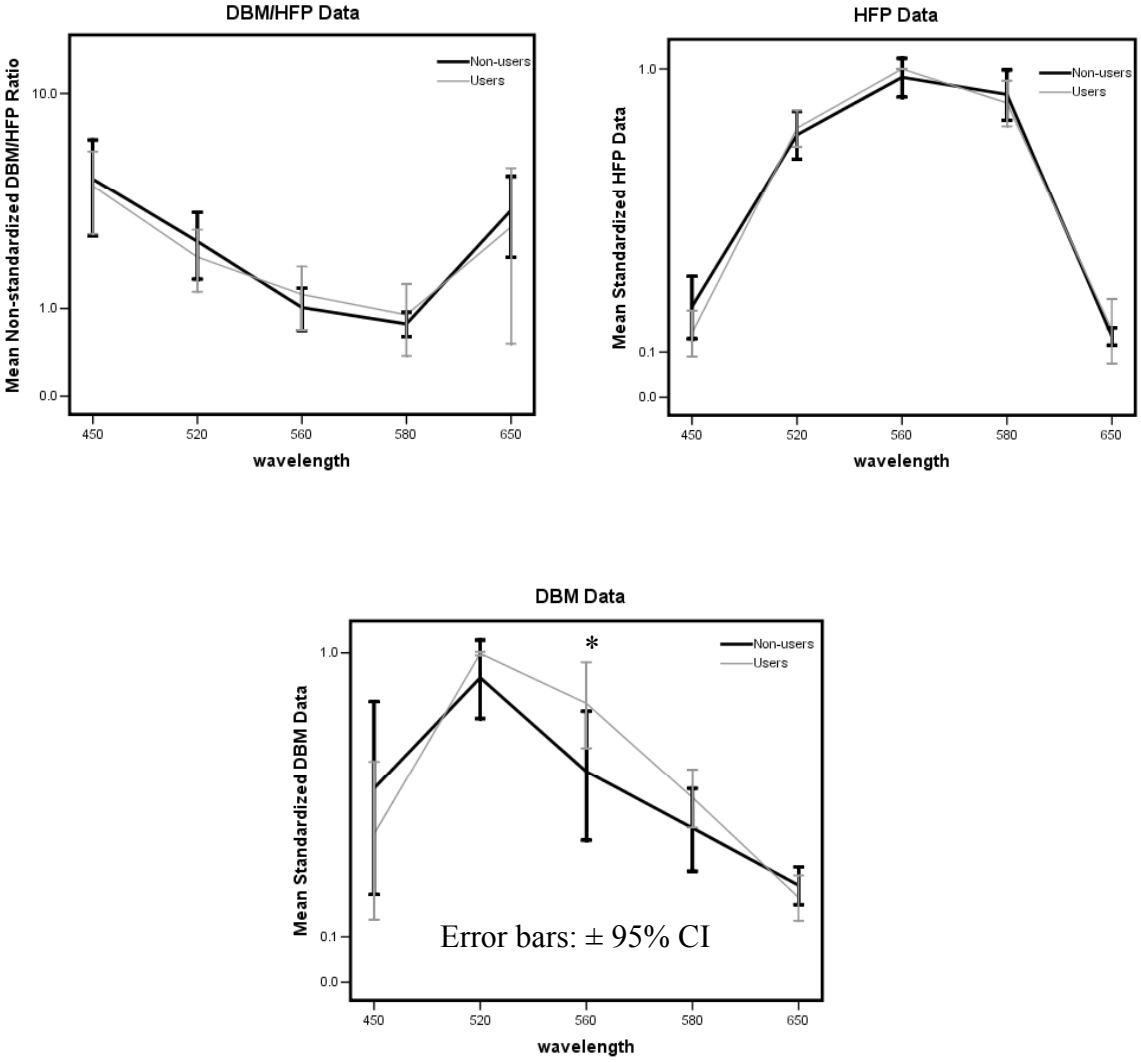


Figure 11 – DBM/HFP, HFP, and DBM measures by contraceptive use. Unlike gender, contraceptive use had no effect on overall DBM/HFP ratios or at any particular wavelength. Contraceptive users actually had higher DBM measures at 560 nm ($F = 7.03$, $p = 0.02$), which accounted for 41% of the variance. (* $p < 0.05$)

ANALYSIS II – CORRELATION AND REGRESSION ANALYSIS OF DBM/HFP RATIOS AND HORMONE MEASURES

For this investigation of the *within females* effects of hormone levels and menstrual phase on DBM/HFP ratios, I predicted a significant positive correlation between DBM/HFP and E2/PG ratios for all four primary wavelengths (450, 520, 580 and 650 nm). All experimental sessions from group II were used, and the correlations of DBM/HFP ratios with hormone measures for all Group II subjects are summarized in Table 4. One-tailed tests were used as I predicted a priori significant *positive* correlations. DBM/HFP ratios are plotted against hormone levels in Figures 12-14. The only significant correlation was at 650 nm (R = 0.33, p = 0.05).

Table 4 – Correlations of DBM/HFP ratios with hormone measures: All subjects

ALL GROUP II SUBJECTS				
MEASURE		**E2**	**PG**	**E2/PG**
RATIO 450	Pearson's R	0.08	0.04	0.21
	Sig (1-tailed)	0.31	0.43	0.16
RATIO 520	Pearson's R	-0.13	-0.14	0.03
	Sig (1-tailed)	0.21	0.26	0.45
RATIO 560	Pearson's R	-0.05	-0.02	0.03
	Sig (1-tailed)	0.41	0.46	0.45
RATIO 580	Pearson's R	-0.08	-0.13	-0.07
	Sig (1-tailed)	0.31	0.27	0.36
RATIO 650	Pearson's R	0.01	0.19	***0.33**
	Sig (1-tailed)	0.48	0.19	**0.05**

* p = 0.05

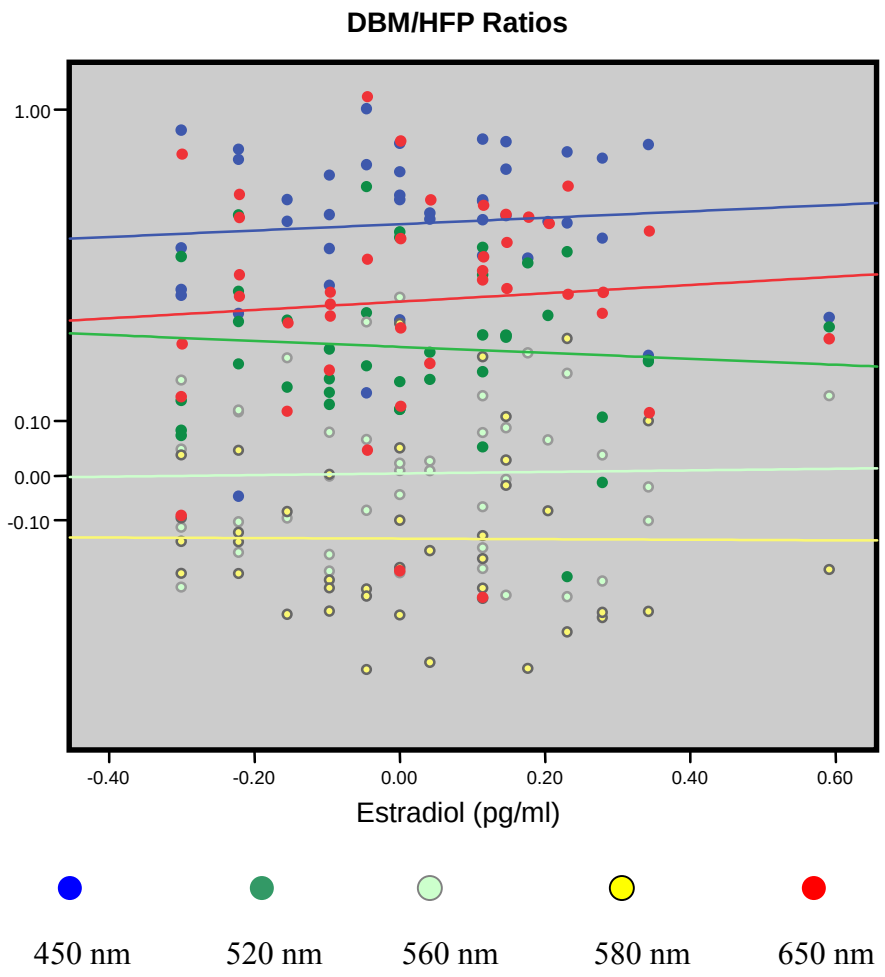


Figure 12 – DBM/HFP ratios plotted against estradiol levels for all Group II subjects. There were no significant relationships. NOTE: Color coding, as shown in the legend, will apply to Figures 12-37.

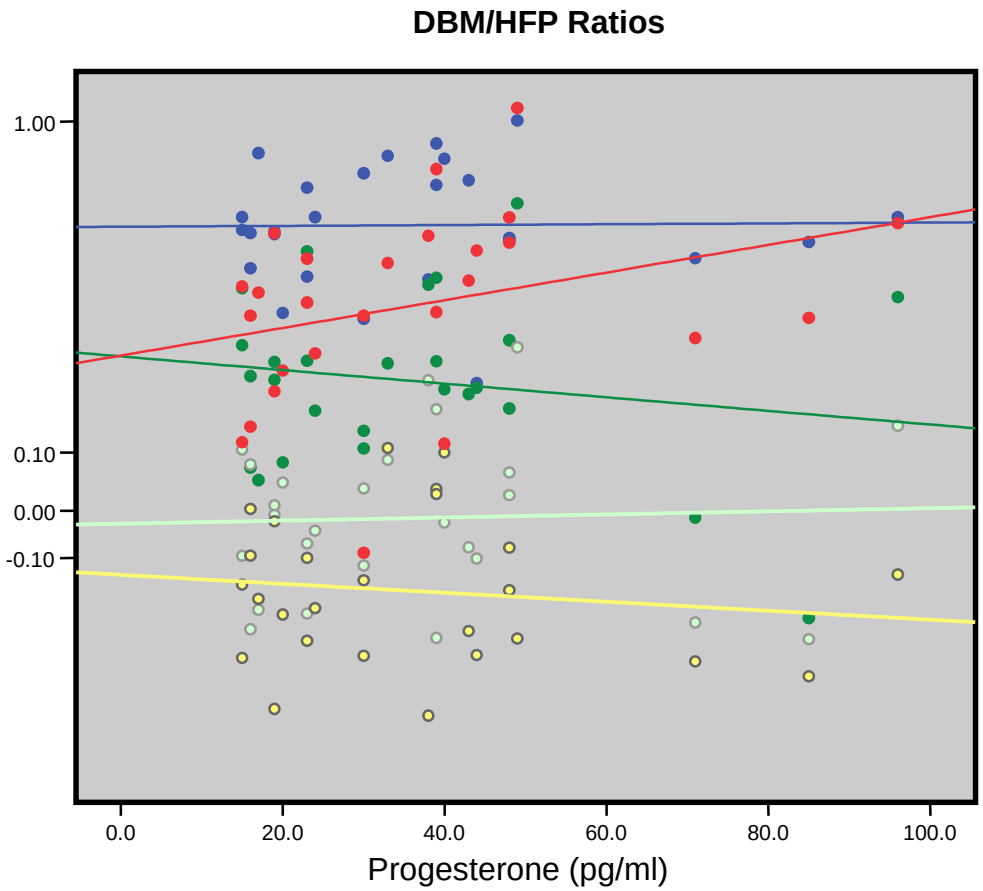


Figure 13 – DBM/HFP ratios plotted against progesterone levels for all Group II subjects. There were no significant relationships.

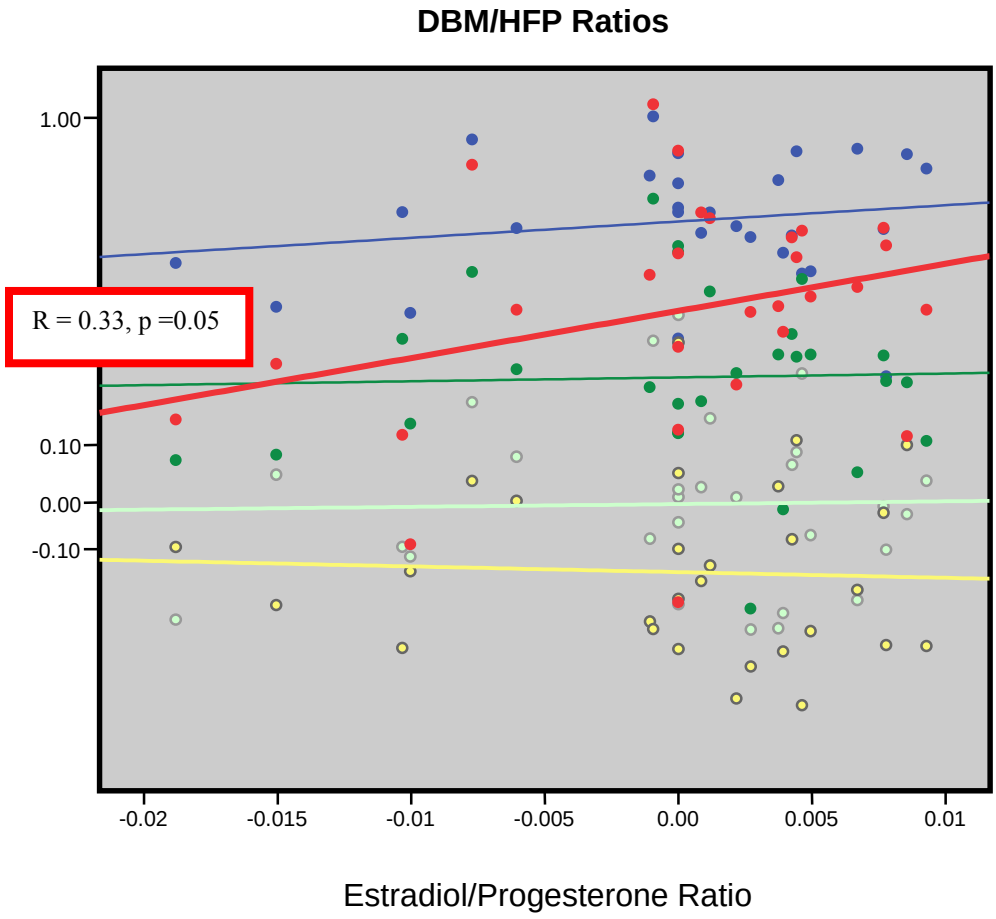


Figure 14 – DBM/HFP plotted against E2/PG ratios for all Group II subjects. There was a significant relationship at 650 nm (R = 0.33, p = 0.05).

As with the between-subjects analysis, data from contraceptive users and non-contraceptive users were analyzed separately. Correlations of DBM/HFP ratios with hormone levels by contraceptive use are shown in Table 5. Two-tailed tests were used as no prediction was made a priori concerning the direction (+/-) of the correlations by contraceptive use. The only correlation approaching significance was between the DBM/HFP ratio at 450 nm and progesterone levels (R = 0.52, p = 0.09) for contraceptive

users. The correlation of DBM/HFP with E2/PG ratios was positive for contraceptive users and negative for non-contraceptive users at every wavelength, as shown in Figure 15.

Table 5 – Correlations of DBM/HFP ratios with hormone measures by contraceptive use

		NON-CONTRACEPTIVE USERS			*CONTRACEPTIVE USERS*		
MEASURE		**E2**	**PG**	**E2/PG**	**E2**	**PG**	**E2/PG**
RATIO 450	Pearson's R	0.11	-0.14	-0.01	0.08	0.52	0.38
	Sig (2-tailed)	0.60	0.64	0.97	0.77	0.09	0.20
RATIO 520	Pearson's R	-0.15	-0.16	-0.16	0.28	-0.26	0.36
	Sig (2-tailed)	0.47	0.59	0.58	0.26	0.42	0.22
RATIO 560	Pearson's R	-0.18	0.01	-0.30	0.24	-0.03	0.41
	Sig (2-tailed)	0.40	0.98	0.28	0.34	0.94	0.16
RATIO 580	Pearson's R	-0.10	-0.17	-0.32	0.07	0.29	0.17
	Sig (2-tailed)	0.67	0.57	0.25	0.77	0.36	0.59
RATIO 650	Pearson's R	0.05	0.11	-0.04	0.03	-0.02	0.37
	Sig (2-tailed)	0.82	0.72	0.90	0.91	0.95	0.22

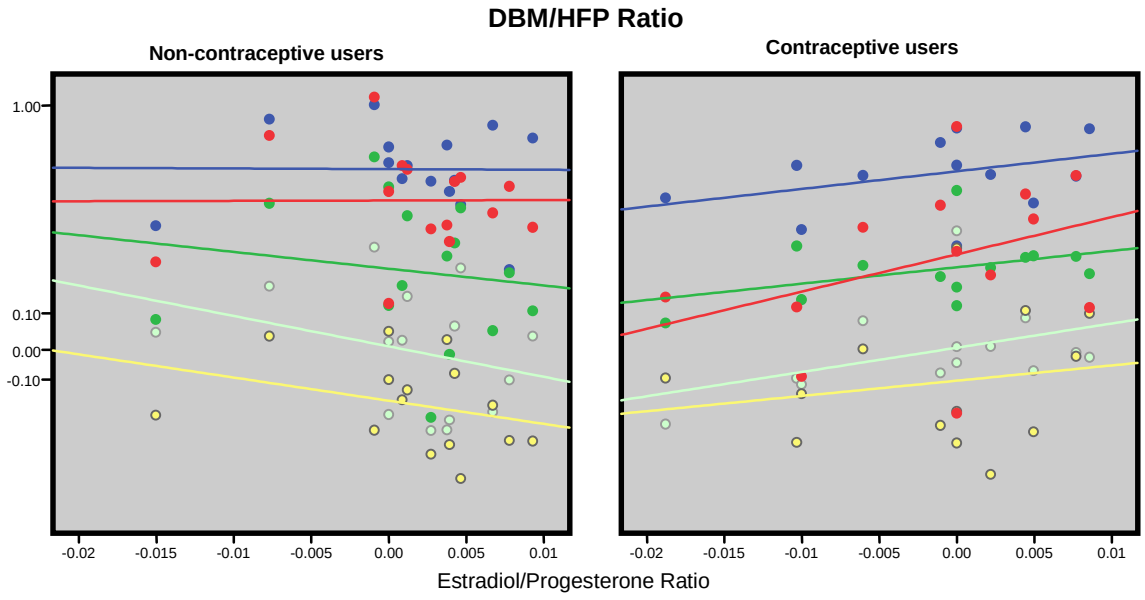


Figure 15 – DBM/HFP plotted against E2/PG ratios by contraceptive use. There were no significant relationships.

In order to further examine the effects of hormone levels and contraceptive use on DBM/HFP ratios, regression analysis of DBM/HFP ratios onto E2, PG and contraceptive use was performed according to the following equation: $DBM/HFP = a_0 + b_1E2 + b_2PG + b_3E2xPG + c_1CONT + c_2CONTxE2 + c_3CONTxPG + c_4CONTxE2xPG$, where E2 = estradiol, PG = progesterone and CONT = contraceptive use. Variables were entered in the order they appear in the regression equation. The final regression model was chosen based on the ANOVA model (i.e. maximum F-value). This method was chosen over maximizing the effect size (R^2), since all added variables will increase the effect size even if they worsen the ANOVA model. The results—with only significant predictors shown—are summarized in Table 6.

Table 6 – DBM/HFP ratios regressed onto hormone levels and contraceptive use

MEASURE	OVERALL MODEL F	p	R^2	PREDICTOR	COEFF	t	p
RATIO 450	**2.33**	**0.07**	**0.49**	CONSTANT (a_0)	0.54	4.82	0.00
				E2	1.28	2.14	0.05
				E2*PG	-0.05	-2.88	0.01
				CONT*E2*PG	0.07	2.20	0.04
RATIO 520	**4.79**	**0.01**	**0.41**	E2	1.34	2.92	0.01
				PG	0.01	2.31	0.03
				E2*PG	-0.05	-3.70	0.00
RATIO 560	**3.52**	**0.03**	**0.33**	CONSTANT (a_0)	-0.15	-2.28	0.03
				E2	0.93	2.49	0.02
				PG	0.01	2.59	0.02
				E2*PG	-0.03	-3.12	0.01
RATIO 580	--	--	--	CONT*E2*PG	(*0.61	2.67	0.01)
RATIO 650	**7.55**	**0.00**	**0.60**	CONSTANT (a_0)	0.29	2.60	0.02
				E2	1.63	3.02	0.01
				PG	0.01	3.59	0.00
				E2*PG	-0.06	-4.11	0.00
				CONT*PG	-0.01	-2.22	0.04

* - standardized coefficient; no predictive value in regression equation

At 580 nm, the three-way interaction of CONT x E2 x PG was significant only when considered as an excluded variable. That is, hormone levels and/or contraceptive use did not combine to significantly predict DBM/HFP ratios at 580 nm. Regression equations can be written to predict DBM/HFP ratios at all other wavelengths.

In matrix form, the equations are:

$$\begin{pmatrix} RATIO@450 \\ RATIO@520 \\ RATIO@560 \\ RATIO@650 \end{pmatrix} = \begin{pmatrix} 0.54 \\ 0.00 \\ -0.15 \\ 0.29 \end{pmatrix} + \begin{pmatrix} 1.28 & 0.00 & (0.07 \times CONT - 0.05) \\ 1.34 & 0.01 & -0.05 \\ 0.93 & 0.01 & -0.03 \\ 1.63 & (0.01 - 0.01 \times CONT) & -0.06 \end{pmatrix} \begin{pmatrix} EST \\ PG \\ EST \times PG \end{pmatrix}$$

The previous regression analysis ignores all information about PG and its interaction with E2 and contraceptive use for nineteen missing PG measures. An alternative approach is to dichotomize progesterone measures based on the median level (i.e. treat progesterone as below or above median) and regress DBM/HFP ratios onto estradiol by dichotomized progesterone levels and contraceptive use. The results of this analysis reveal a positive relationship between E2 and DBM/HFP ratios for non-contraceptive users when PG was below median level with a significant effect at 580 nm ($R^2 = 0.45$, $p = 0.05$). The trend was opposite (negative) for non-contraceptive users when PG was above median level. For contraceptive users, there was a positive relationship between E2 and DBM/HFP ratios when PG was above median level. The results were mixed for contraceptive users when PG was below median levels. There was a positive relationship between E2 and DBM/HFP ratios for 520 nm and 560 nm but

a negative trend at 450, 580, and 650 nm (no significant models). These results are shown in Figure 16.

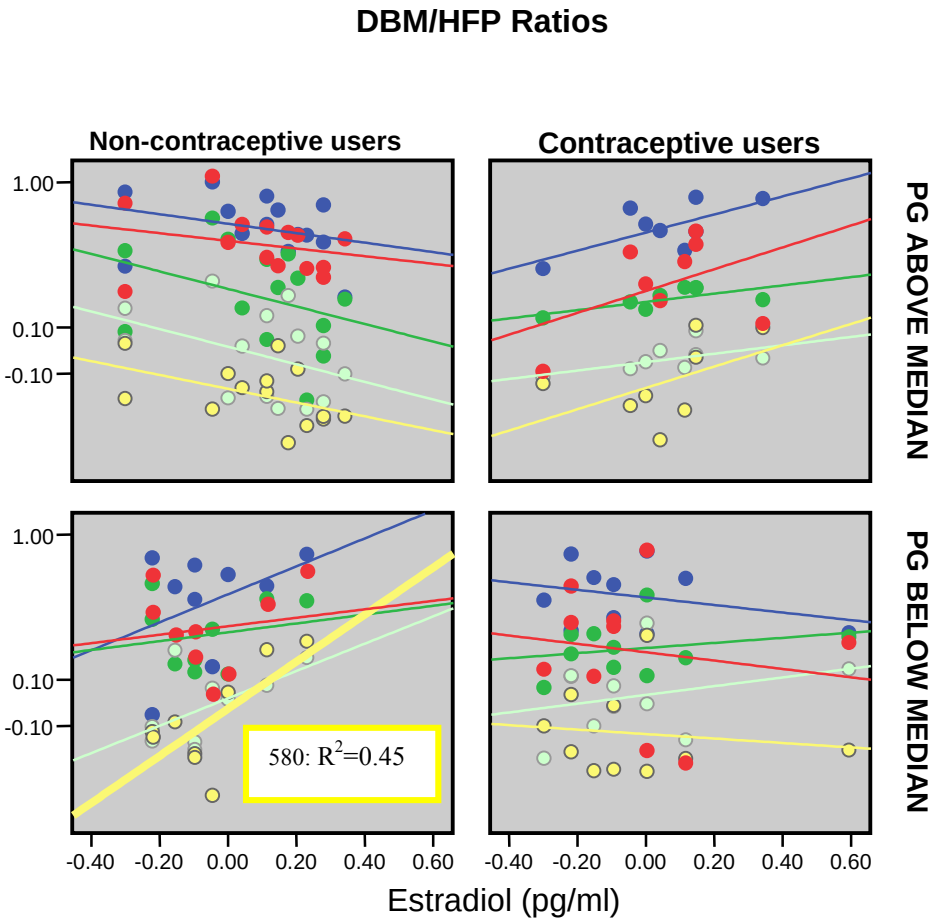


Figure 16 – DBM/HFP ratios plotted against E2 by dichotomized progesterone levels and contraceptive use. The only significant finding is at 580 nm for non-contraceptive users with PG levels below median (plotted in bold). There also appears to be an overall interaction effect of estradiol level with both contraceptive use and progesterone level.

ANALYSIS II – EFFECTS OF MENSTRUAL PHASE AND CONTRACEPTIVE USE ON DBM/HFP RATIOS

An alternate method of analysis is to consider the effect of menstrual phase on DBM/HFP ratios. Univariate ANOVA was used to determine the effects of menstrual phase and contraceptive use on DBM/HFP ratios, and the results are summarized in Table 7. I predicted that DBM/HFP ratios would be at a maximum during the ovulatory phase, intermediate during the menstrual phase and minimum during the luteal phase. However, I made no predictions by contraceptive use.

Table 7 – Effects of menstrual phase and contraceptive use on DBM/HFP ratios

MEASURE	OVERALL MODEL			FACTOR	F	p	η^2
	F	p	R^2				
RATIO 450	0.62	0.69	0.08	PHASE	0.51	0.61	0.03
				CONT	0.22	0.64	0.01
				PHASE * CONT	1.03	0.37	0.05
RATIO 520	0.49	0.78	0.06	PHASE	0.95	0.40	0.05
				CONT	0.66	0.42	0.02
				PHASE * CONT	0.06	0.94	0.00
RATIO 560	0.26	0.93	0.03	PHASE	0.47	0.63	0.03
				CONT	0.21	0.65	0.01
				PHASE * CONT	0.16	0.85	0.01
RATIO 580	0.27	0.93	0.04	PHASE	0.20	0.82	0.01
				CONT	0.00	0.98	0.00
				PHASE * CONT	0.45	0.64	0.02
RATIO 650	**2.56**	**0.04**	**0.26**	PHASE	0.73	0.49	0.04
				CONT	**5.86**	**0.02**	**0.14**
				PHASE * CONT	**2.95**	**0.07**	**0.14**

The only significant findings were at 650 nm where the overall ANOVA model was significant ($F = 2.56$, $p = 0.04$, $R^2 = 0.26$). Non-contraceptive users had higher mean DBM/HFP ratios across the menstrual cycle ($F = 5.86$, $p = 0.02$, $\eta^2 = 0.14$), and there was

an interaction of contraceptive use with menstrual phase ($F = 2.95$, $p = 0.07$, $\eta^2 = 0.14$). These results are shown in Figure 17.

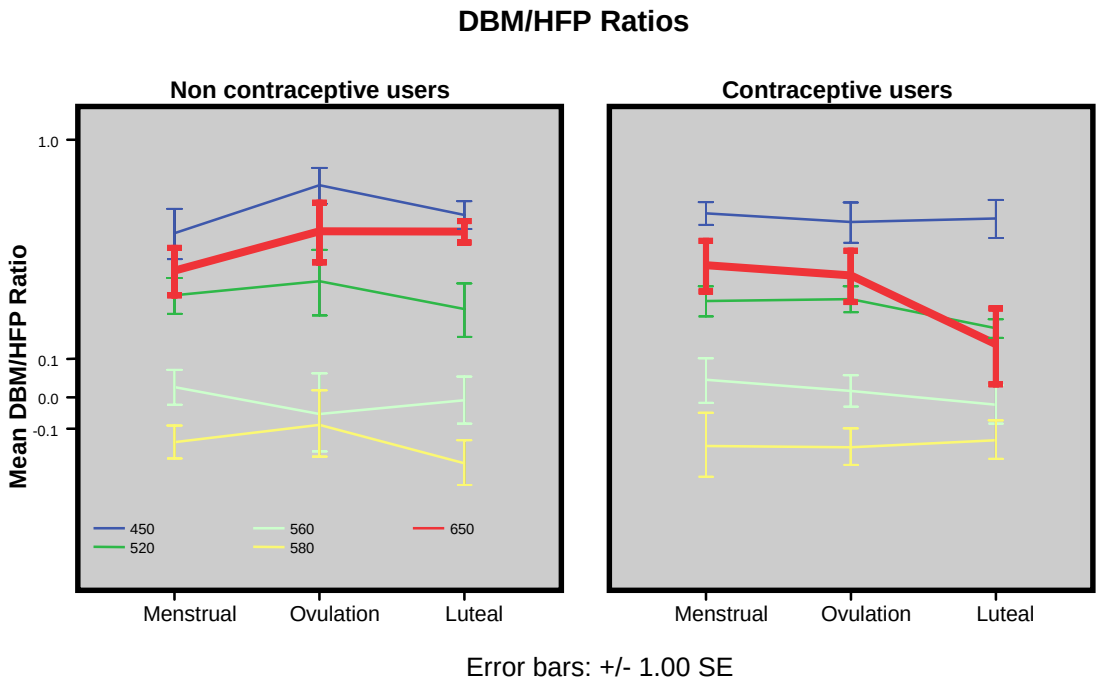


Figure 17 – DBM/HFP ratios plotted against menstrual phase for all five wavelengths. Menstrual phase and contraceptive use combined to predict a significant proportion of the variance ($R^2 = 0.26$) in DBM/HFP ratios at 650 nm ($F = 2.56$, $p = 0.04$). There were no main effects of phase on DBM/HFP ratios at any wavelength, but non-contraceptive users had higher DBM/HFP ratios than contraceptive users at 650 nm ($F = 5.86$, $p = 0.02$, $\eta^2 = 0.14$). The interaction between contraceptive use and menstrual phase approached significance at 650 nm ($F = 2.95$, $p = 0.07$, $\eta^2 = 0.14$). During the luteal phase, non-contraceptive users had significantly higher DBM/HFP ratios than contraceptive users at 650 nm ($t = 3.57$, $p = 0.003$). There were no other simple effects of contraceptive use at any menstrual phase.

There was not a significant change across the menstrual cycle at any wavelength. However, during the luteal phase, non-contraceptive users had higher DBM/HFP ratios than contraceptive users at 650 nm ($t = 3.57$, $p = 0.003$). The significant findings at 650 nm are shown in Figure 18.

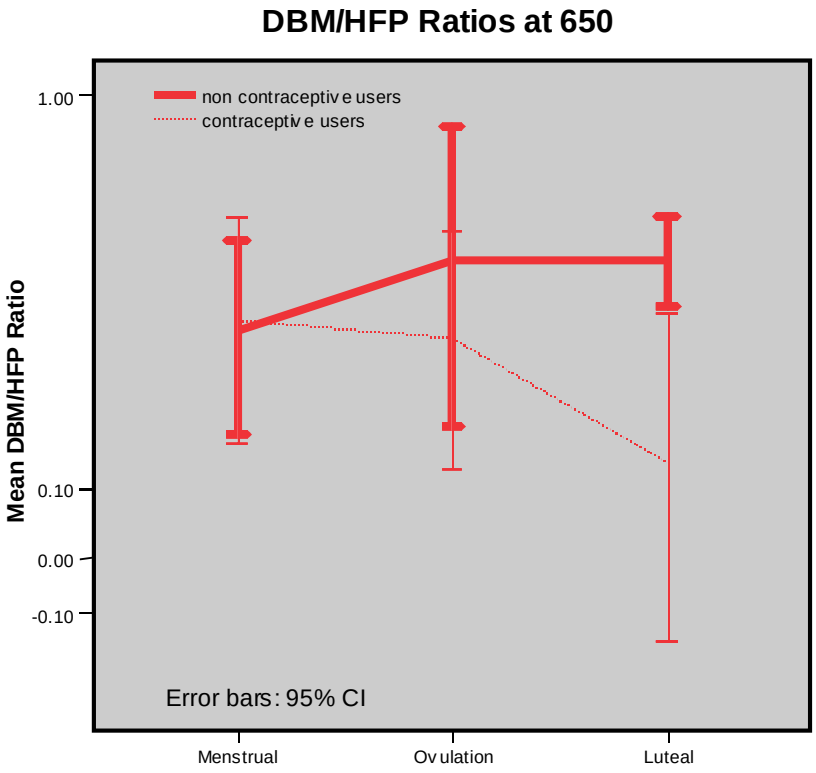


Figure 18 – DBM/HFP ratios at 650 nm plotted against menstrual phase. Menstrual phase and contraceptive use combined to predict a significant proportion of the variance ($R^2 = 0.26$) in DBM/HFP ratios ($F = 2.56$, $p = 0.04$). Non-contraceptive users had higher DBM/HFP ratios than users ($F = 5.86$, $p = 0.02$, $\eta^2 = 0.14$), and the interaction between contraceptive use and menstrual phase approached significance ($F = 2.95$, $p = 0.07$, $\eta^2 = 0.14$). During the luteal phase, non-contraceptive users had significantly higher DBM/HFP ratios than contraceptive users ($t = 3.57$, $p = 0.003$).

As in previous studies of menstrual cycle effects on visual function (Akar et al., 2005; Yucel et al., 2005), the menstrual cycles was divided into two halves. The *follicular phase* represents days 1-14 when both mean estradiol and progesterone are low, and the *luteal phase* represents days 15-28 when mean estradiol and progesterone are generally higher. Figure 19 shows DBM/HFP profiles for all wavelengths across dichotomized menstrual phase by contraceptive use.

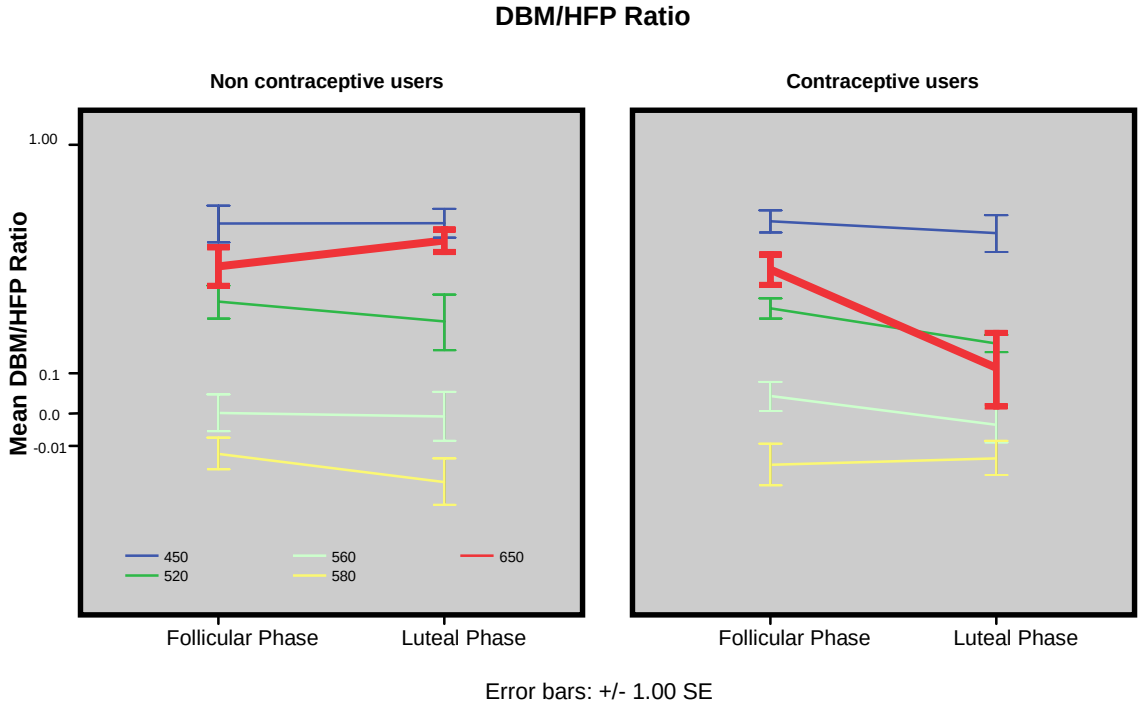


Figure 19 – DBM/HFP ratios plotted against dichotomized menstrual phase for all five wavelengths. Non-contraceptive users had higher DBM/HFP ratios than contraceptive users at 650 nm ($F = 9.36$, $p = 0.004$, $\eta^2 = 0.19$). There was also a significant interaction between contraceptive use and menstrual phase at 650 nm ($F = 8.36$, $p = 0.01$, $\eta^2 = 0.18$). During the luteal phase, non-contraceptive users had significantly higher DBM/HFP ratios than contraceptive users at 650 nm ($t = 3.97$, $p = 0.001$). There were no other simple effects of contraceptive use at either dichotomized phase.

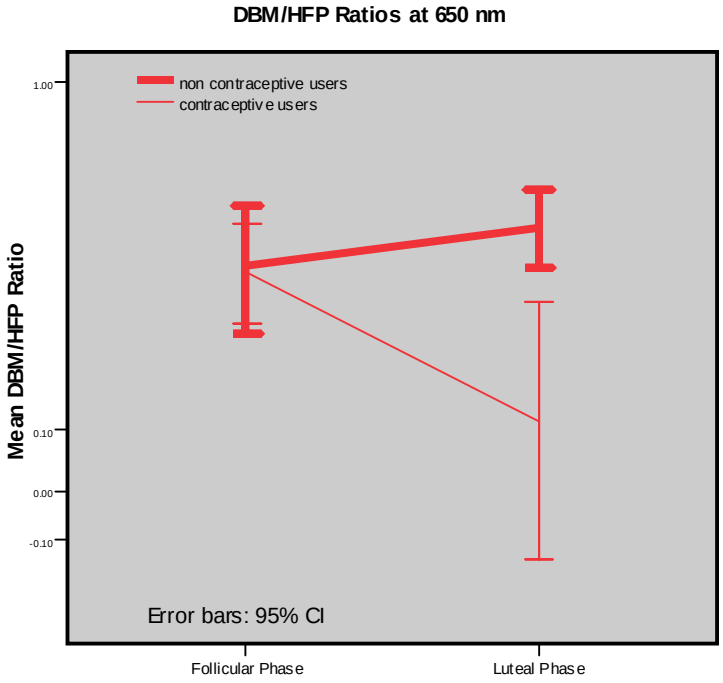


Figure 20 – DBM/HFP ratios at 650 nm plotted against dichotomized menstrual phase. Overall, menstrual phase and contraceptive use combined to predict a significant proportion of the variance ($R^2 = 0.30$) in DBM/HFP ratios ($F = 5.51$, $p = 0.003$). There was a significant difference between contraceptive users and non-contraceptive users ($F = 9.36$, $p = 0.004$, $\eta^2 = 0.19$). There was also a significant interaction between contraceptive use and menstrual phase ($F = 8.36$, $p = 0.01$, $\eta^2 = 0.18$). During the luteal phase, non-contraceptive users had significantly higher DBM/HFP ratios than contraceptive users ($t = 3.97$, $p = 0.001$).

Univariate ANOVA was used to determine the effects of dichotomized phase and contraceptive use on DBM/HFP ratios. As with scaled phase, the only significant findings were at 650 nm where the overall ANOVA model was significant ($F = 5.51$, $p = 0.003$, $R^2 = 0.30$). Non-contraceptive users again had higher mean DBM/HFP ratios across the menstrual cycle at 650 nm ($F = 9.36$, $p = 0.004$, $\eta^2 = 0.19$). There was also a significant interaction ($F = 8.36$, $p = 0.006$, $\eta^2 = 0.18$) of contraceptive use with dichotomized menstrual phase at 650 nm as shown in Figure 19. Although there was not

a significant change across the dichotomized menstrual cycle for any wavelength, DBM/HFP ratios at 650 nm for contraceptive users tended to be lower during the luteal phase. In addition, during the luteal phase, non-contraceptive users did have higher DBM/HFP ratios than users at 650 nm ($t = 3.97$, $p = 0.001$). The significant findings at 650 nm are shown in Figure 20.

ANALYSIS II – CORRELATION AND REGRESSION ANALYSIS OF HFP WITH HORMONE MEASURES

Correlations of HFP with hormone measures for all group II subjects are shown in Table 8. HFP measures are plotted against hormone levels in Figures 21-22. As predicted, none of the correlations between HFP and hormone measures were significant at the $p < 0.05$ level.

Table 8 – Correlations of HFP measures with hormone levels: All subjects

		ALL GROUP II SUBJECTS		
MEASURE		**E2**	**PG**	**E2/PG**
HFP 450	Pearson's R	-0.10	0.32	-0.22
	Sig (2-tailed)	0.51	0.11	0.26
HFP 520	Pearson's R	-0.08	-0.04	-0.23
	Sig (2-tailed)	0.62	0.84	0.24
HFP 560	Pearson's R	0.15	0.17	0.26
	Sig (2-tailed)	0.35	0.40	0.18
HFP 580	Pearson's R	0.12	0.07	0.25
	Sig (2-tailed)	0.43	0.74	0.19
HFP 650	Pearson's R	0.09	0.20	0.04
	Sig (2-tailed)	0.57	0.34	0.84

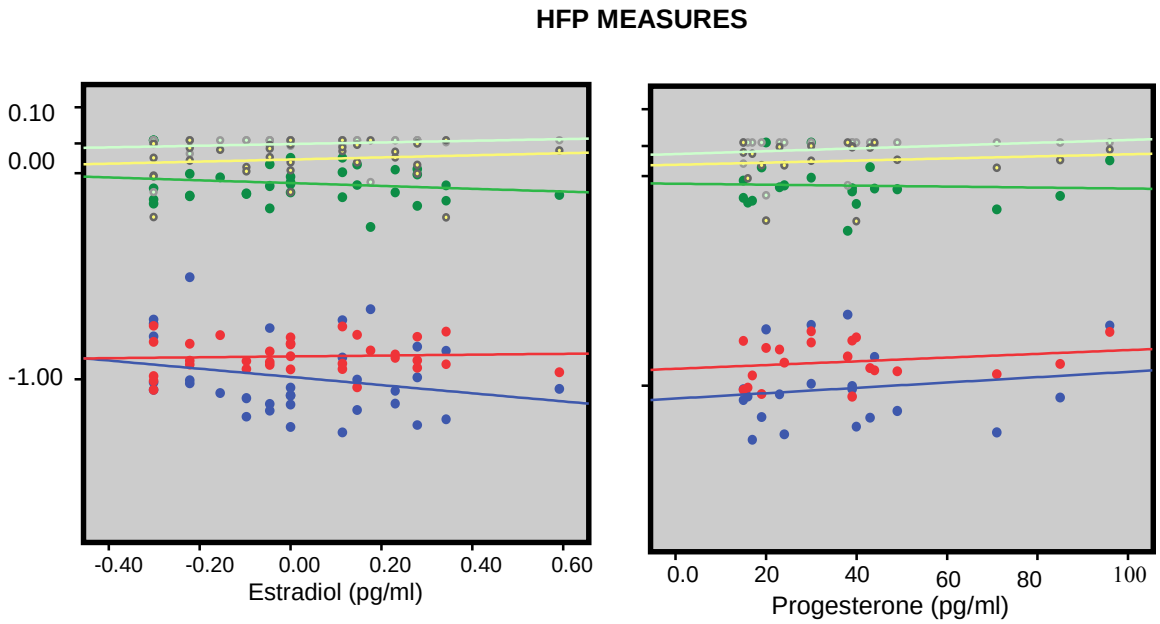


Figure 21 – HFP measures plotted against estradiol and progesterone levels for all Group II subjects. There were no significant relationships.

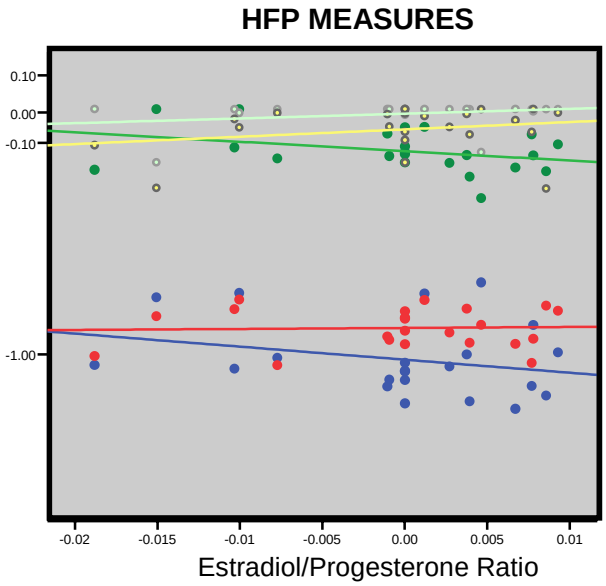


Figure 22 – HFP measures plotted against E2/PG ratios for all Group II subjects. There were no significant relationships.

Correlations of HFP measures with hormone levels by contraceptive use are shown in Table 9. For non-contraceptive users, HFP measures significantly correlated with E2 measures at 580 nm ($r = 0.41$, $p = 0.05$) and with E2/PG ratios at 560 nm ($r = 0.51$, $p = 0.05$) and 580 nm ($r = 0.70$, $p < 0.01$). For contraceptive users, HFP measures did not correlate significantly with hormone levels at any wavelength. The effects by contraceptive use are shown in Figure 23.

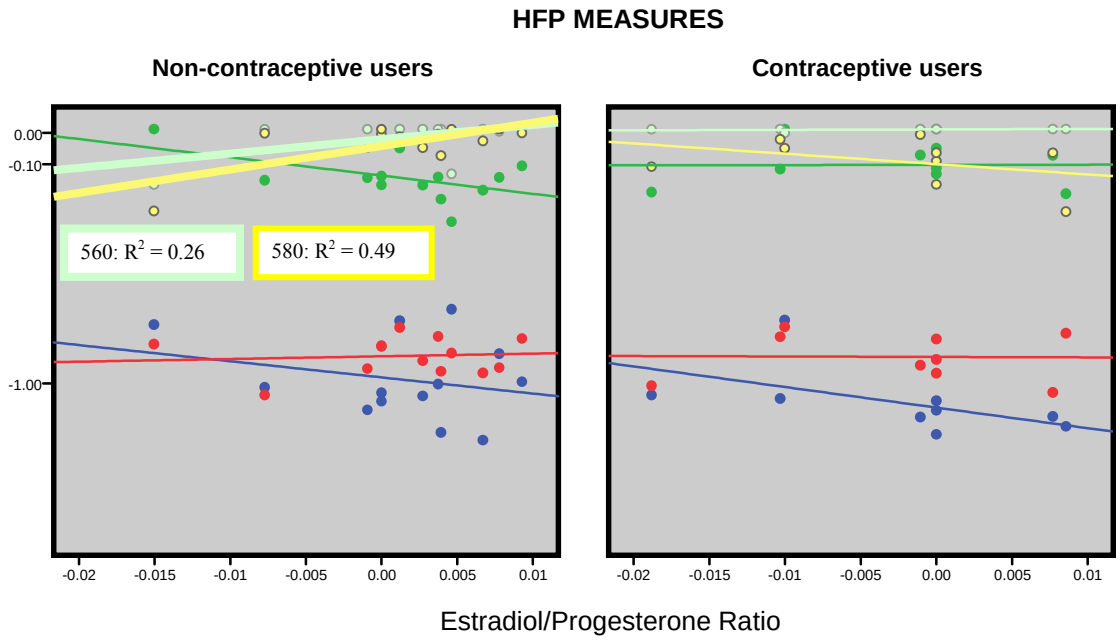


Figure 23 – HFP measures plotted against E2/PG ratios by contraceptive use. E2/PG ratios significantly predicted HFP measures for non-contraceptive users at 560 nm ($F = 4.56$, $p = 0.05$, $R^2 = 0.26$) and 580 nm ($F = 12.61$, $p = 0.004$, $R^2 = 0.49$), both plotted in bold. There were no significant relationships for contraceptive users.

Table 9 – Correlations of HFP measures with hormone levels by contraceptive use

MEASURE		*NON-CONTRACEPTIVE USERS* E2	PG	E2/PG	*CONTRACEPTIVE USERS* E2	PG	E2/PG
HFP 450	Pearson's R	-0.10	0.16	-0.16	-0.18	0.19	-0.47
	Sig (2-tailed)	0.63	0.59	0.56	0.48	0.55	0.11
HFP 520	Pearson's R	0.01	-0.01	-0.49	-0.16	0.36	0.11
	Sig (2-tailed)	0.98	0.98	0.07	0.53	0.25	0.73
HFP 560	Pearson's R	0.16	0.33	***0.51**	0.31	0.24	0.17
	Sig (2-tailed)	0.43	0.25	**0.05**	0.22	0.44	0.57
HFP 580	Pearson's R	***0.41**	0.15	****0.70**	-0.20	-0.38	-0.21
	Sig (2-tailed)	**0.05**	0.61	**0.00**	0.43	0.22	0.50
HFP 650	Pearson's R	0.19	0.09	0.09	0.03	0.46	-0.01
	Sig (2-tailed)	0.36	0.76	0.75	0.92	0.14	0.97

* $p < 0.05$, ** $p < 0.01$

The main and interaction effects of continuous hormone levels and contraceptive use on HFP measures were then examined using multiple regression. The regression results, with significant predictors shown, are summarized in Table 10.

Table 10 – HFP measures regressed onto hormone levels and contraceptive use

MEASURE	OVERALL MODEL F	p	R^2	PREDICTOR	COEFF	t	p
HFP 580	**6.42**	**0.00**	**0.68**	CONSTANT	-0.13	-4.29	0.00
				E2	0.81	5.57	0.00
				PG	0.002	3.23	0.01
				E2*PG	-0.02	-4.85	0.00
				CONT	0.11	2.08	0.05
				CONT*E2	-0.43	-4.24	0.00
				CONT*PG	-0.004	-2.21	0.00

Hormone levels and/or contraceptive use only combined to significantly predict HFP measures at 580 nm ($F = 6.42$, $p < 0.001$, $R^2 = 0.68$), and the regression equation can be written as: HFP 580 = - 0.013 + 0.81 x E2 + 0.002 x PG + 0.02 x E2 x PG + CONT x (0.11 + 0.43 x E2 – 0.004 x PG).

The results of regressing HFP measures onto estradiol, dichotomized progesterone levels, and contraceptive use reveal mixed effects of E2 on HFP measures. For non-contraceptive users, when PG was below median level, E2 significantly predicted HFP only at 520 nm ($R^2 = 0.44$, $p = 0.03$). There were no other significant effects for non-contraceptive users. For contraceptive users, there was a negative trend of E2 on HFP measures when PG was above median level, although there were no significant effects. There appeared to be a negligible effect of E2 at any wavelength for contraceptive users when PG was below median level (see Figure 24).

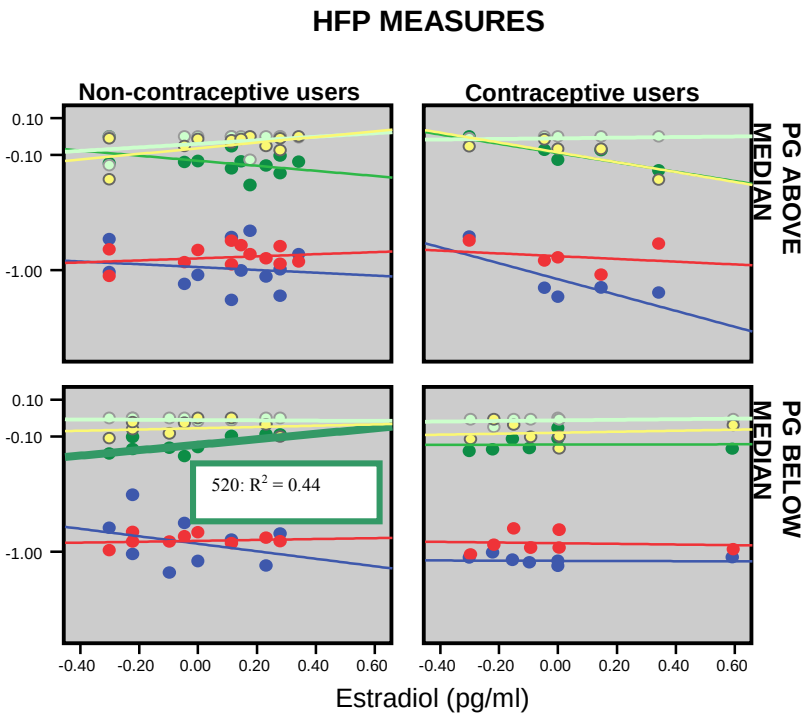


Figure 24 – HFP measures plotted against E2 by dichotomized PG levels and contraceptive use. For non-contraceptive users, E2 significantly predicted HFP measures at 520 nm (plotted in bold) when PG was below median. There were no significant relationships for contraceptive users, and estradiol appears to have had no effect at all on HFP measures when PG was below median levels.

ANALYSIS II – EFFECTS OF MENSTRUAL PHASE AND CONTRACEPTIVE USE ON HFP MEASURES

Figure 25 shows HFP profiles for all wavelengths across the menstrual cycle by contraceptive use. Univariate ANOVA was used to determine the effects of menstrual phase and contraceptive use on HFP ratios. The only notable trend was the main effect of contraceptive use at 450 nm ($F = 3.48$, $p = 0.07$, $\eta^2 = 0.12$). No overall ANOVA model was significant, and there was no main effect of menstrual phase at any wavelength.

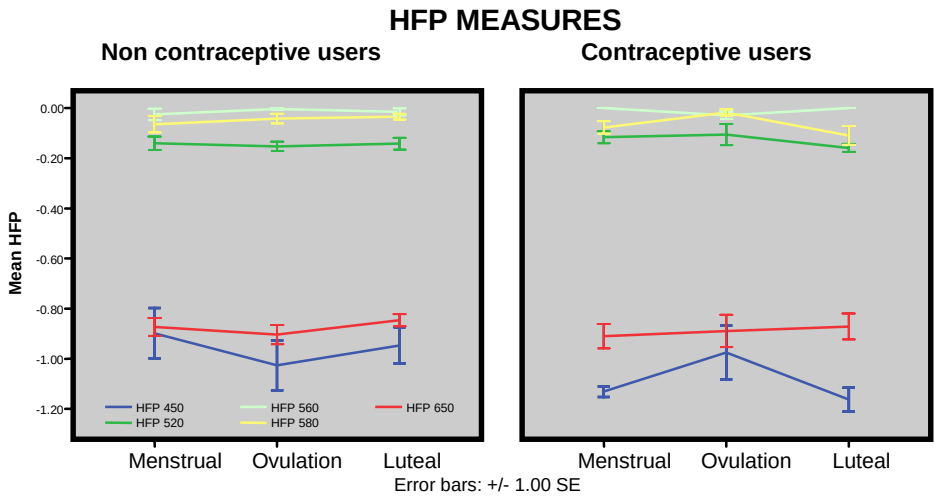


Figure 25 – HFP measures plotted against menstrual phase by contraceptive use. No effects were significant at $p < 0.05$ level, but HFP measures tended to be higher for non-contraceptive users at 450 nm ($F = 3.48$, $p = 0.07$). In addition, HFP measures tended to be higher for non-contraceptive users at 450 nm during both the menstrual phase ($t = 2.28$, $p = 0.06$) and the luteal phase ($t = 2.07$, $p = 0.06$). There were no other trends or simple effects of contraceptive use at any menstrual phase.

Analysis of the simple effects of contraceptive use revealed that HFP measures tended to be higher for non-contraceptive users at 450 nm during both the menstrual

phase (t = 2.28, p = 0.06) and the luteal phase (t = 2.07, p = 0.06). These trends at 450 nm are shown in Figure 26.

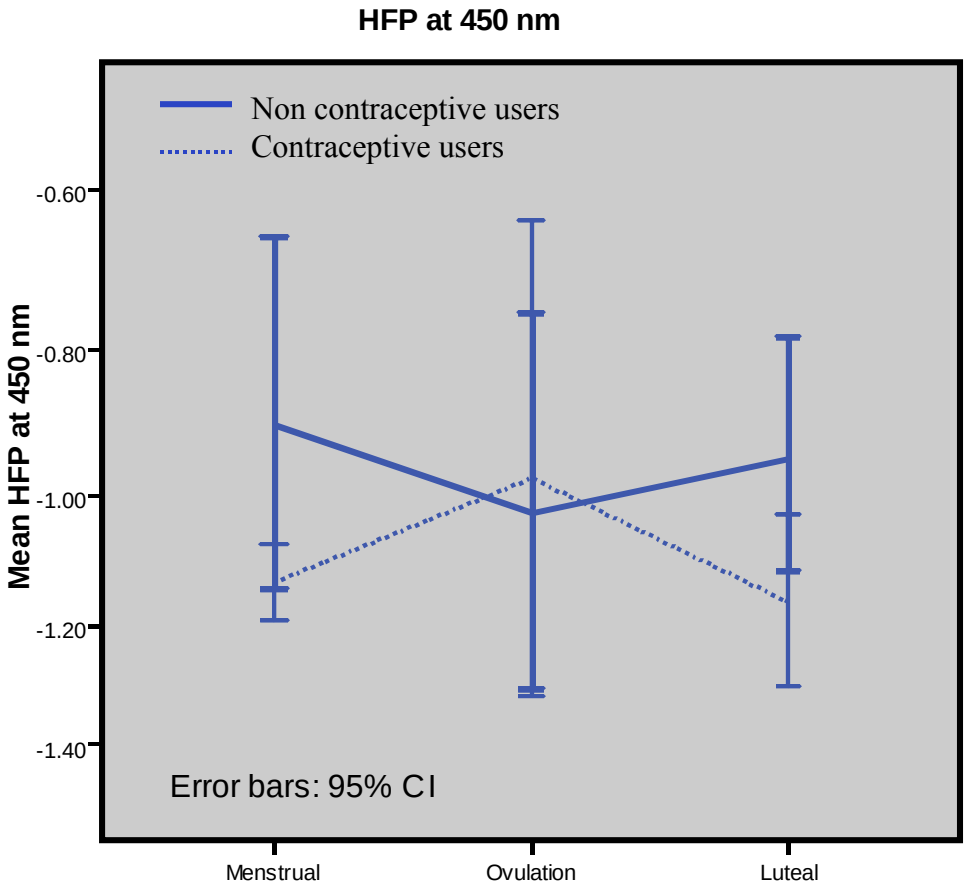


Figure 26 – HFP measures at 450 nm plotted against menstrual phase by contraceptive use. Although not significant at $p < 0.05$ level, HFP measures tended to be higher for non-contraceptive users ($F = 3.48$, $p = 0.07$). In addition, HFP measures tended to be higher for non-contraceptive users at 450 nm during both the menstrual phase ($t = 2.28$, $p = 0.06$) and the luteal phase ($t = 2.07$, $p = 0.06$).

HFP profiles for all wavelengths across dichotomized menstrual phases by contraceptive use are shown in Figure 27. Univariate ANOVA was used to determine the effects of dichotomized phase and contraceptive use on HFP ratios. Non-contraceptive users had higher HFP measures than contraceptive users at 450 nm ($F = 4.18$, $p = 0.05$, $\eta^2 = 0.12$). There were no main or interaction effects of dichotomized menstrual phase on HFP measures at any wavelength.

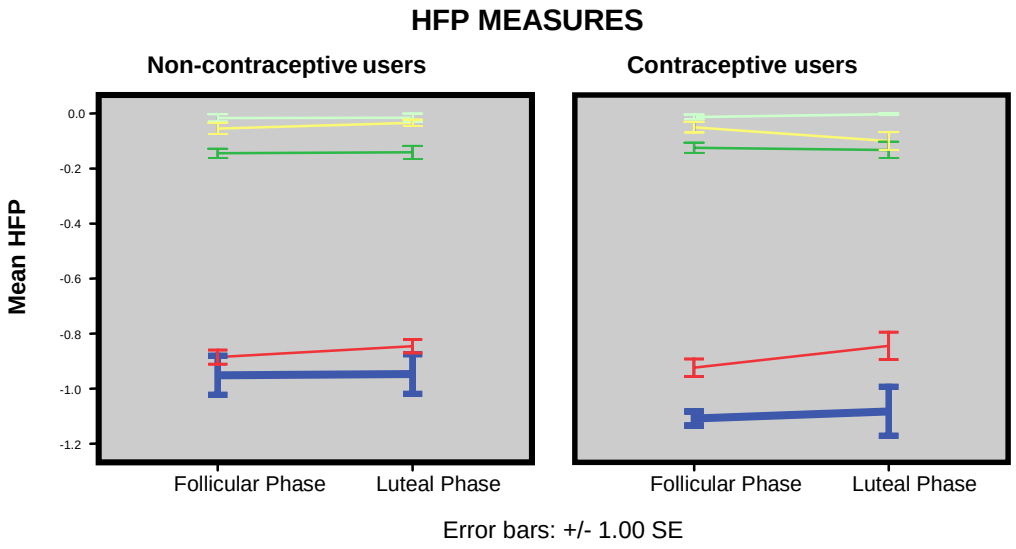


Figure 27 – HFP measures plotted against dichotomized menstrual phase by contraceptive use. Non-contraceptive users had higher HFP measures at 450 nm ($F = 4.18$, $p = 0.05$; plotted in bold on both graphs for comparison) and a trend toward higher HFP measures at 650 nm ($F = 3.48$, $p = 0.07$). There was also a trend toward higher HFP measures for non-contraceptive users at 450 nm during the follicular phase ($t = 2.09$, $p = 0.06$). There were no other simple effects of contraceptive use on HFP measures at any wavelength.

During the follicular phase, HFP measures tended to be higher for non-contraceptive users at 450 nm ($t = 2.09$, $p = 0.06$). This trend is shown in Figure 28.

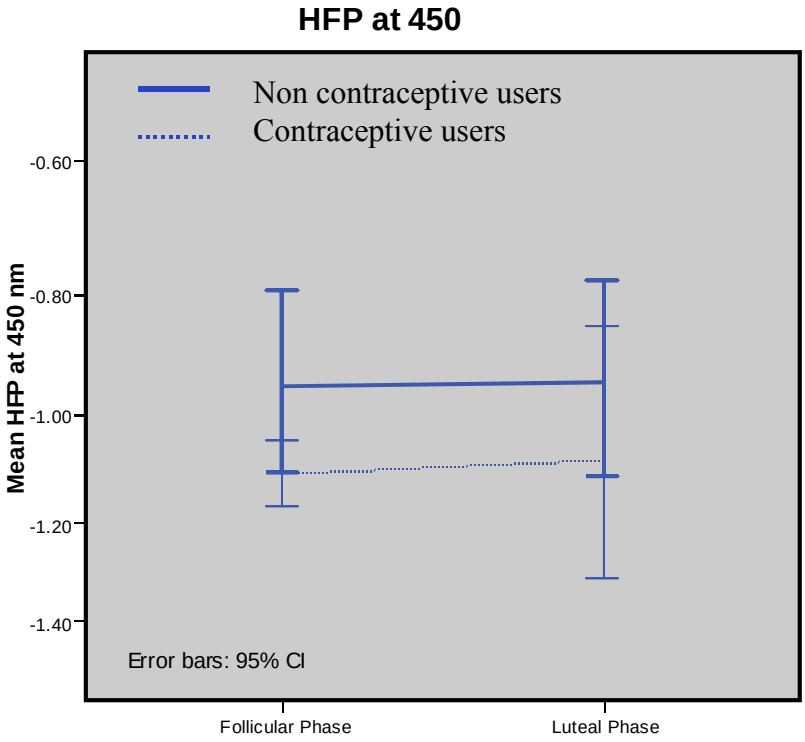


Figure 28 – HFP measures at 450 nm plotted against dichotomized menstrual phase by contraceptive use. Non-contraceptive users had higher HFP measures ($F = 4.18$, $p = 0.05$). There was also a trend toward higher HFP measures for non-contraceptive users during the follicular phase ($t = 2.09$, $p = 0.06$).

ANALYSIS II – CORRELATION AND REGRESSION ANALYSIS OF DBM WITH HORMONE MEASURES

Correlations of DBM with hormone measures for all group II subjects are shown in Table 11. DBM measures are plotted against hormone levels in Figures 29-30.

Table 11 – Correlations of DBM with hormone measures: All subjects

ALL GROUP II SUBJECTS				
MEASURE		**E2**	**PG**	**E2/PG**
DBM 450	Pearson's R	0.06	0.34	0.00
	Sig (2-tailed)	0.70	0.09	0.99
DBM 520	Pearson's R	-0.08	-0.22	-0.25
	Sig (2-tailed)	0.62	0.28	0.21
DBM 560	Pearson's R	0.12	0.19	0.09
	Sig (2-tailed)	0.43	0.35	0.65
DBM 580	Pearson's R	0.06	0.01	-0.07
	Sig (2-tailed)	0.73	0.94	0.74
DBM 650	Pearson's R	0.25	****0.71**	***0.40**
	Sig (2-tailed)	0.11	**0.00**	**0.04**

* $p < 0.05$, ** $p < 0.01$

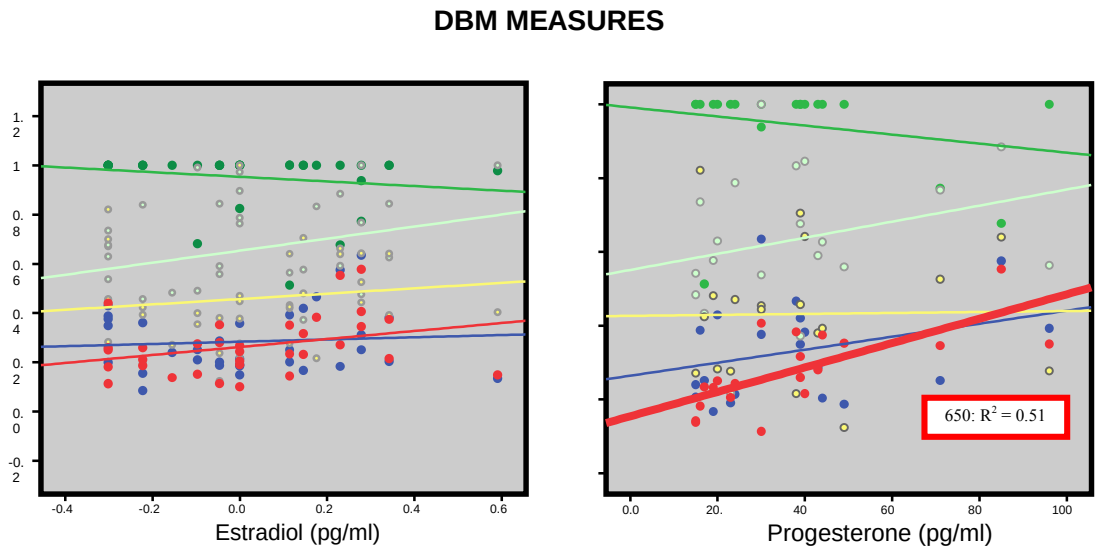


Figure 29 – DBM measures plotted against estradiol and progesterone levels for all Group II subjects. There was a positive relationship between DBM measures and progesterone levels at 650 nm ($R^2 = 0.51$, $p < 0.001$; plotted in bold). There was also a positive trend at 450 nm ($R^2 = 0.11$, $p = 0.09$)

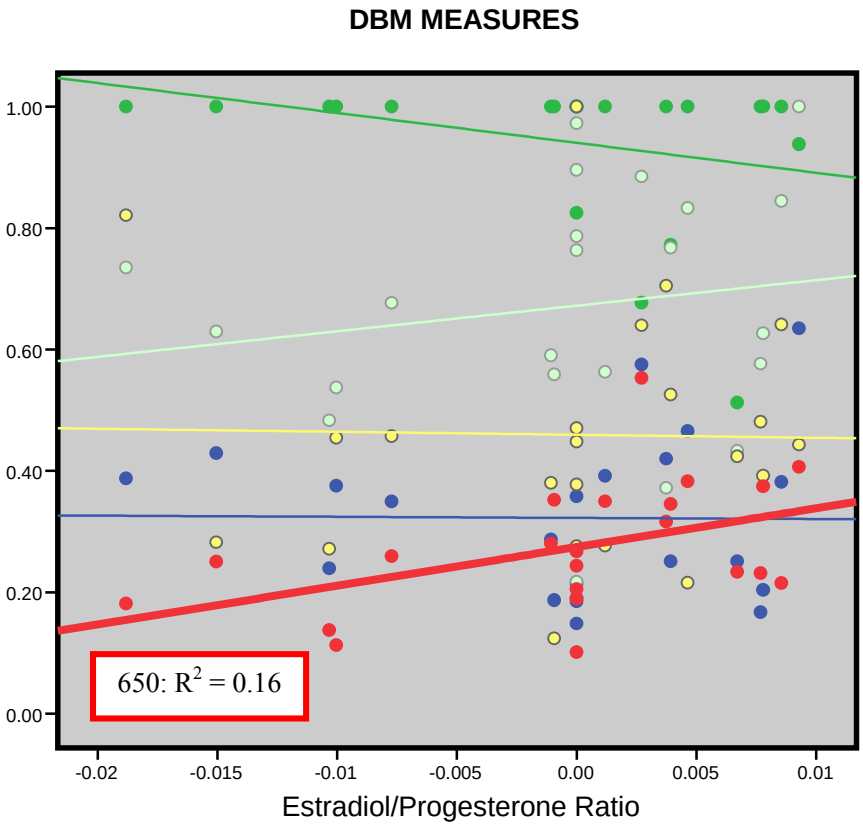


Figure 30 – DBM measures plotted against E2/PG ratios for all Group II subjects. There was a significant positive relationship between DBM measures and E2/PG ratios at 650 nm ($R^2 = 0.16$, $p = 0.04$).

Although I predicted there would be no significant relationships, DBM measures correlated significantly at 650 nm with PG measures ($R = 0.71$, $p < 0.01$; see Figure 29) and E2/PG ratios ($R = 0.40$, $p = 0.04$; see Figure 30). DBM measures at 450 nm tended to positively correlate with PG levels ($R = 0.34$, $p = 0.09$; see Figure 29).

Correlations of DBM measures with hormone levels by contraceptive use are shown in Table 12. For non-contraceptive users, DBM measures were significantly positively correlated with both E2 (R = 0.44, p = 0.03) and PG (R = 0.60, p = 0.02) at 650 nm. For contraceptive users, the only significant correlation was between DBM measures at 450 nm and PG measures (R = 0.60, p = 0.02).

Table 12 – Correlations of DBM with hormone measures by contraceptive use

		NON-CONTRACEPTIVE USERS			***CONTRACEPTIVE USERS***		
MEASURE		**E2**	**PG**	**E2/PG**	**E2**	**PG**	**E2/PG**
DBM 450	Pearson's R	0.17	0.15	0.08	-0.18	****0.60**	-0.23
	Sig (2-tailed)	0.41	0.62	0.77	0.48	**0.04**	0.46
DBM 520	Pearson's R	-0.07	-0.06	-0.29	----	----	----
	Sig (2-tailed)	0.74	0.83	0.29	----	----	----
DBM 560	Pearson's R	0.15	0.29	0.13	0.15	0.03	0.10
	Sig (2-tailed)	0.47	0.32	0.65	0.57	0.92	0.74
DBM 580	Pearson's R	0.22	0.19	0.14	-0.20	0.08	-0.28
	Sig (2-tailed)	0.28	0.52	0.62	0.43	0.80	0.35
DBM 650	Pearson's R	****0.44**	****0.60**	0.35	-0.14	0.45	0.43
	Sig (2-tailed)	**0.03**	**0.02**	0.20	0.58	0.14	0.14

* p < 0.1, ** p < 0.05, ---- unable to compute correlation due to constant DBM 520

The main and interaction effects of continuous hormone levels and contraceptive use on DBM measures were then examined using multiple regression. The regression results, with only significant predictors shown, are summarized in Table 13. When considering the effects on DBM measures at 580 nm, the three-way interaction of CONT x E2 x PG was significant as an excluded variable (t = 2.67, p = 0.01). However, hormone levels and/or contraceptive use only combined to significantly predict DBM

measures at 650 nm ($F = 13.70$, $p < 0.001$, $R^2 = 0.27$). The regression equation can be written as: DBM 650 = 0.27 + 0.27 x E2.

Table 13 – DBM measures regressed onto hormone levels and contraceptive use

MEASURE	OVERALL MODEL			PREDICTOR	COEFF	t	p
	F	p	R^2				
DBM 580				CONT*E2*PG	(*0.61	2.67	0.01)
DBM 650	13.70	<0.001	0.27	CONSTANT	0.27	14.77	0.00
				E2	0.27	2.95	0.01
				PG	(*0.58	3.72	0.00)
				CONT*PG	(*-0.49	-3.26	0.00)

* - standardized coefficient; no predictive value to regression equation

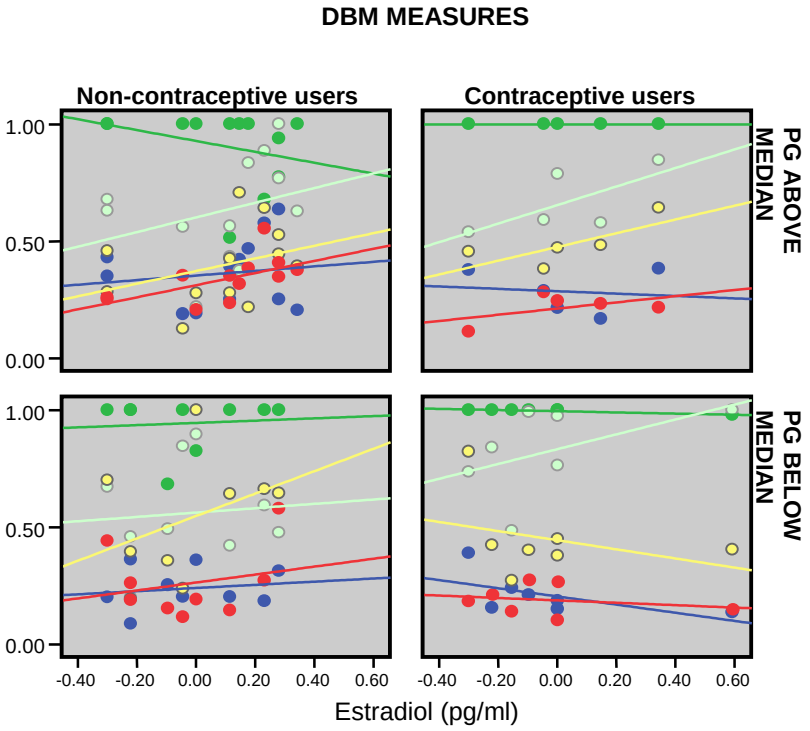


Figure 31 – DBM measures plotted against E2 by dichotomized progesterone levels and contraceptive use. There were no significant findings at any wavelength for any condition, nor was there a systematic interaction of estradiol level with PG level or contraceptive use.

The results of DBM measures regressed onto estradiol, dichotomized progesterone levels, and contraceptive use are shown in Figure 31. For non-contraceptive users, there was a positive trend of E2 on DBM measures at all wavelengths when PG was below median level, but there were no significant models. When PG was above median level, there was a negative trend at 520 nm, but trends were positive at all other wavelengths. For contraceptive users, E2 had mixed effects on DBM measures when PG was above or below median level; no relationships were significant.

ANALYSIS II – EFFECTS OF MENSTRUAL PHASE AND CONTRACEPTIVE USE ON DBM MEASURES

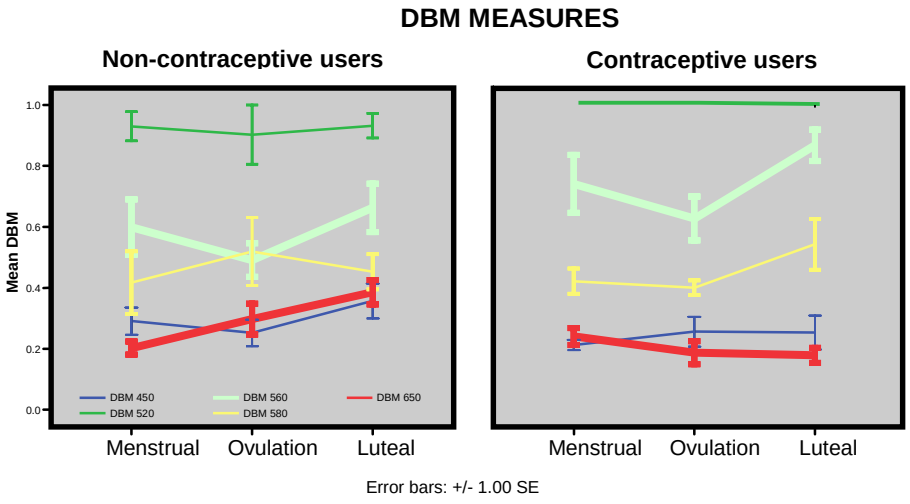


Figure 32 – DBM measures plotted against menstrual phase by contraceptive use. DBM measures were higher for non-contraceptive users at 650 nm ($F = 9.73$, $p = 0.00$) but higher for contraceptive users at 560 nm ($F = 4.20$, $p = 0.05$), plotted in bold on both graphs. Mean DBM measures also tended to be higher for contraceptive users at 520 nm ($F = 3.37$, $p = 0.08$). During the luteal phase, DBM measures were higher for non-contraceptive users at 650 nm ($t = 3.41$, $p = 0.004$), but there were no other simple effects at any wavelength.

Figure 32 shows DBM profiles for all wavelengths across menstrual cycle by contraceptive use. Univariate ANOVA was used to determine the effects of menstrual cycle phase and contraceptive use on DBM measures, and the ANOVA results are summarized in Table 14.

Table 14 – Effects of menstrual phase and contraceptive use on DBM measures

MEASURE	OVERALL MODEL			FACTOR	F	p	η^2
	F	p	R^2				
DBM 450	0.61	0.69	0.10	PHASE	0.29	0.75	0.02
				CONT	2.36	0.14	0.08
				PHASE * CONT	0.01	0.99	0.00
DBM 520	0.69	0.63	0.11	PHASE	0.02	0.98	0.00
				CONT	3.37	0.08	0.10
				PHASE * CONT	0.04	0.96	0.00
DBM 560	1.56	0.20	0.21	PHASE	1.29	0.29	0.08
				CONT	**4.20**	**0.05**	**0.13**
				PHASE * CONT	0.62	0.55	0.04
DBM 580	0.42	0.83	0.07	PHASE	0.49	0.62	0.03
				CONT	0.00	0.95	0.00
				PHASE * CONT	0.66	0.53	0.04
DBM 650	**5.32**	**0.001**	**0.48**	PHASE	1.23	0.29	0.08
				CONT	**9.73**	**0.00**	**0.25**
				PHASE * CONT	**5.56**	**0.01**	**0.28**

There was no main effect of menstrual phase for all subjects at any wavelength, but there were main effects of contraceptive use at 560 nm ($F = 4.20$, $p = 0.05$; see Figure 33), and 650 nm ($F = 9.73$, $p = 0.004$; see Figure 33). In addition, the interaction of menstrual cycle phase and contraceptive use was significant ($F = 5.56$, $p = 0.01$, $\eta^2 = 0.28$), as was the overall ANOVA model at 650 nm ($F = 5.32$, $p = 0.001$, $R^2 = 0.48$). For non-contraceptive users, DBM measures at 650 nm were higher during the luteal phase

compared to the menstrual phase ($t = 4.297$, $p < 0.001$). For contraceptive users, DBM measures at 560 nm were higher during the luteal phase compared to the ovulatory phase ($t = 2.955$, $p = 0.013$). The only simple effect of contraceptive use was during the luteal phase, when DBM measures were higher for non-users at 650 nm ($t = 3.41$, $p = 0.004$).

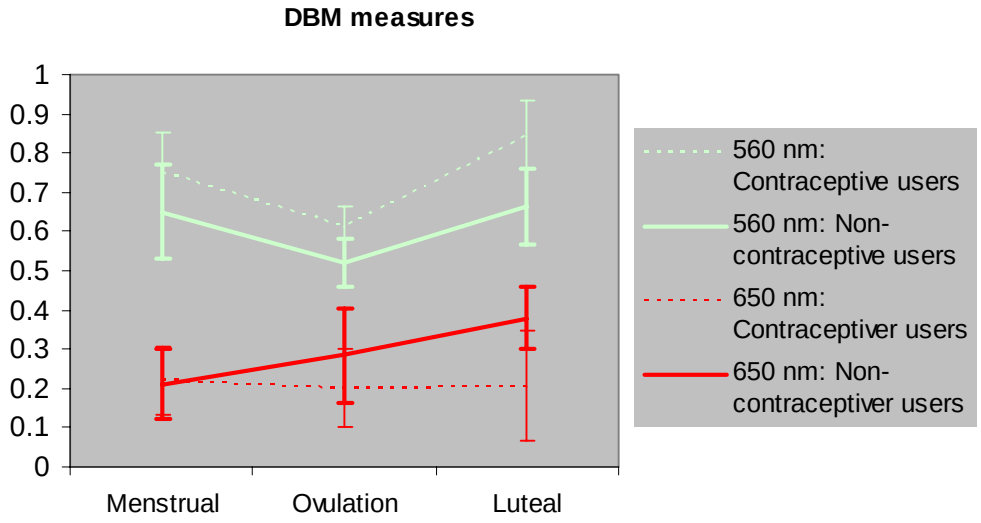


Figure 33 – DBM measures at 560 nm and 650 nm plotted against menstrual phase by contraceptive use. DBM measures at 560 nm were higher for contraceptive users ($F = 4.20$, $p = 0.05$), but there were no simple effects of contraceptive use or menstrual cycle phase at 560 nm. DBM measures were higher at 650 nm for non-contraceptive users ($F = 9.73$, $p = 0.00$). For non-contraceptive users, DBM measures at 650 nm were higher during the luteal phase than the menstrual phase ($t = 4.297$, $p < 0.001$). During the luteal phase, DBM measures were also higher for non-contraceptive users at 650 nm ($t = 3.41$, $p = 0.004$). Error bars represent 95% confidence intervals.

DBM profiles for all wavelengths across dichotomized menstrual phases by contraceptive use are shown in Figure 34. Univariate ANOVA was used to determine the effects of dichotomized phase and contraceptive use on DBM measures, and the results are summarized in Table 15. Contraceptive users had higher overall DBM measures at

560 nm (F = 4.67, p = 0.04), but non-contraceptive users had higher DBM measures at 650 nm (F = 13.31, p < 0.001). In addition, for non-contraceptive users, DBM measures at 650 nm were higher during the luteal phase compared to the menstrual phase (t = 4.297, p < 0.001). When examined for simple effects of contraceptive use on DBM at particular phases, contraceptive users had higher DBM measures at 520 nm during the follicular phase (t = 2.10, p = 0.05) but lower at 650 nm during luteal phase (t = 3.81, p = 0.002). The significant findings are shown in Figures 35-37.

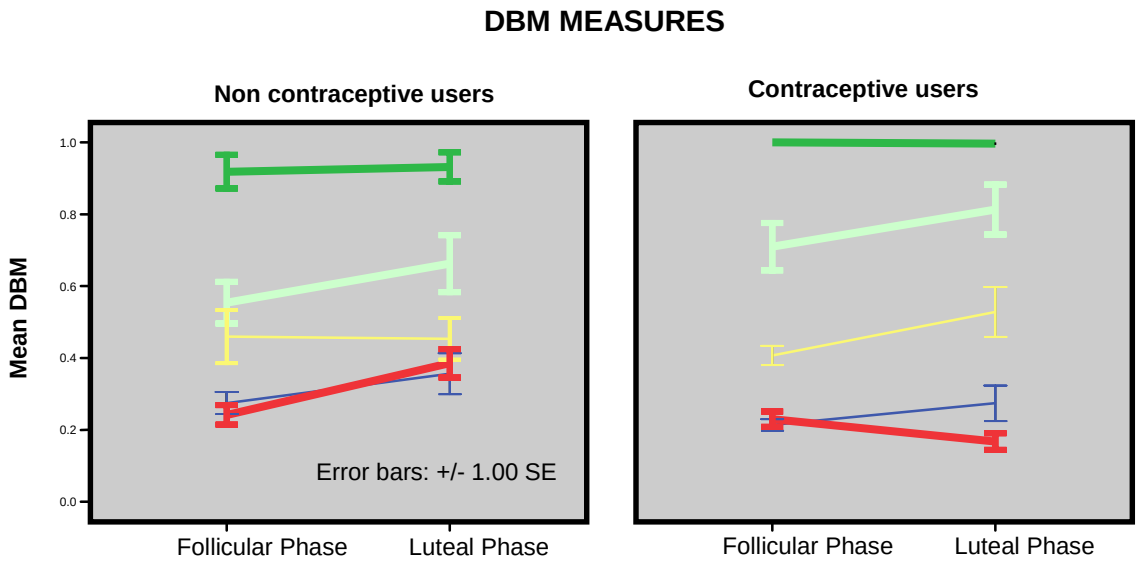


Figure 34 – DBM measures plotted against dichotomized menstrual phase by contraceptive use. Contraceptive users had higher DBM measures at 560 nm (F = 4.67, p = 0.04), but non-contraceptive users had higher DBM measures at 650 nm (F = 13.31, p < 0.001). In addition, contraceptive users had higher DBM measures at 520 nm during follicular phase (t = 2.10, p = 0.05) but lower at 650 nm during luteal phase (t = 3.81, p < 0.01). The only simple effect of phase on DBM measures was at 650 nm. For non-contraceptive users, DBM measures at 650 nm were higher during the luteal phase compared to the follicular phase (t = 4.297, p < 0.001).

Table 15 – Effects of dichotomized menstrual phase and contraceptive use on DBM

MEASURE	OVERALL MODEL			FACTOR	F	p	η^2
	F	p	R^2				
DBM 450	2.05	0.13	0.17	LUTEAL PHASE	2.89	0.10	0.09
				CONT	2.94	0.10	0.09
				PHASE * CONT	0.07	0.80	0.00
DBM 520	1.20	0.33	0.10	LUTEAL PHASE	0.02	0.90	0.00
				CONT	3.34	0.08	0.10
				PHASE * CONT	0.05	0.83	0.00
DBM 560	**2.38**	**0.09**	**0.19**	LUTEAL PHASE	2.22	0.15	0.07
				CONT	**4.67**	**0.04**	**0.13**
				PHASE * CONT	0.00	0.97	0.00
DBM 580	0.45	0.72	0.04	LUTEAL PHASE	0.73	0.40	0.02
				CONT	0.03	0.87	0.00
				PHASE * CONT	0.90	0.35	0.03
DBM 650	**8.301**	**<0.001**	**0.445**	LUTEAL PHASE	1.67	0.21	0.05
				CONT	**13.31**	**0.00**	**0.30**
				PHASE * CONT	**10.63**	**0.00**	**0.26**

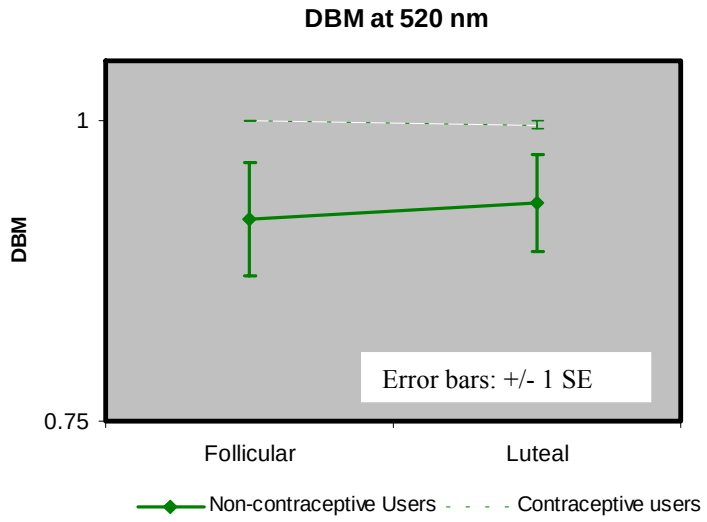


Figure 35 – DBM measures at 520 nm plotted against dichotomized menstrual phase by contraceptive use. There were no main effects of contraceptive use or menstrual phase, but contraceptive users had higher DBM measures at 520 nm during the follicular phase ($t = 2.10$, $p = 0.05$).

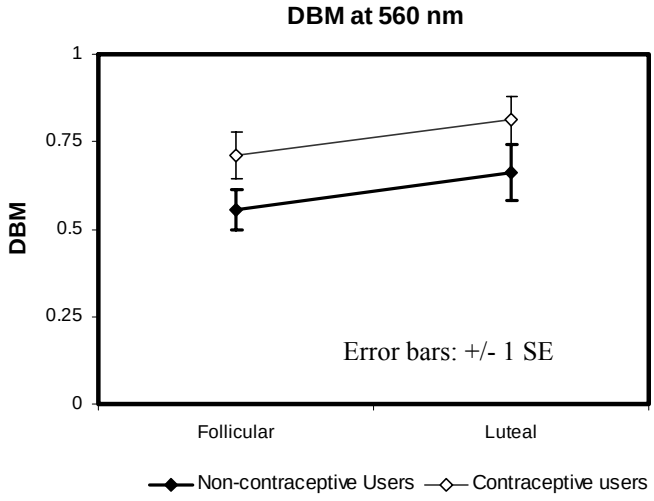


Figure 36 – DBM measures at 560 nm plotted against dichotomized menstrual phase by contraceptive use. Contraceptive users had higher DBM measures at 560 nm ($F = 4.67$, $p = 0.04$), but there were no simple effects of contraceptive use or menstrual cycle phase.

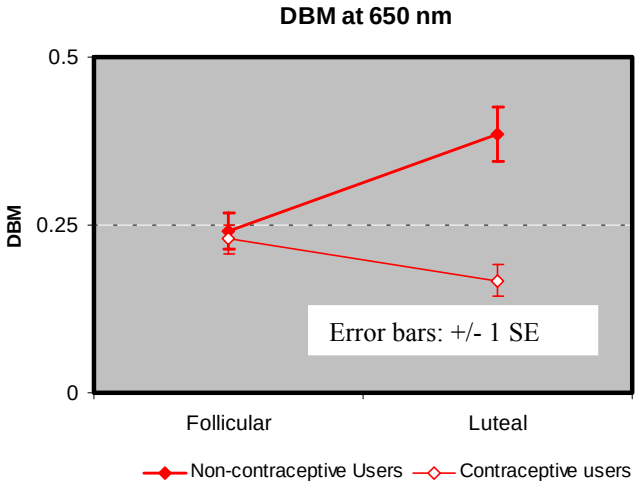


Figure 37 – DBM measures at 650 nm plotted against dichotomized menstrual phase by contraceptive use. For non-contraceptive users, DBM measures at 650 nm were higher during the luteal phase compared to the follicular phase ($t = 4.297$, $p < 0.001$). Contraceptive non-users had higher DBM measures at 650 nm ($F = 13.31$, $p < 0.001$). Contraceptive users had lower DBM measures during the luteal phase ($t = 3.81$, $p < 0.001$).

SUMMARY RESULTS

As predicted, overall DBM/HFP ratios were higher for female subjects than male subjects. Females also had higher DBM/HFP ratios at every wavelength except 560 nm. Unlike gender, contraceptive use had no effect on overall DBM/HFP ratios nor did contraceptive use have a significant effect on DBM/HFP ratios at any particular wavelength. There was no main effect of contraceptive use on overall HFP measures or on overall DBM measures, but contraceptive users did have significantly higher DBM measures than non-contraceptive users at 560 nm.

The only significant relationship between DBM/HFP ratios and E2/PG was at 650 nm. There were no significant relationships between HFP measures and hormone levels at any wavelength. DBM measures at 650 nm significantly positively correlated with both PG and E2/PG. When the correlations were analyzed by contraceptive use, there was an interesting interaction of E2/PG ratios and contraceptive use on DBM/HFP ratios. Although there were no significant findings, the correlation of DBM/HFP with E2/PG ratios was positive for contraceptive users and negative for non-contraceptive users at every wavelength. For non-contraceptive users, HFP measures positively correlated with E2 levels at 580 nm and with E2/PG ratios at both 560 nm and 580 nm. In addition, DBM measures positively correlated with both E2 and PG levels at 650 nm. For contraceptive users, the only significant finding was the positive correlation between DBM measures and PG levels at 650 nm.

Regression equations could be written to predict DBM/HFP ratios based on continuous hormone levels and contraceptive use at all wavelengths except 580 nm. The

results were not as broad for HFP and DBM measures. HFP measures were only predicted by hormone levels and contraceptive use at 580 nm, while E2 predicted DBM measures at 650 nm. When the effect of E2 on DBM/HFP ratios was examined by dichotomized PG level and contraceptive use, E2 significantly predicted HFP measures at 520 nm for non-contraceptive users when PG was below median level.

There were limited effects of menstrual phase and contraceptive use on DBM/HFP ratios. At 650 nm, non-contraceptive users had higher mean DBM/HFP ratios across the menstrual cycle. There was also an interaction of contraceptive use with menstrual phase. During the luteal phase, non-contraceptive users had higher DBM/HFP ratios than contraceptive users at 650 nm. Non-contraceptive users also had higher HFP measures than contraceptive users at 450 nm, and there was a non-significant trend for higher HFP measures during the follicular phase. DBM measures were higher for contraceptive users at 520 and 560 nm but higher for non-contraceptive users at 650 nm. The only simple effect of menstrual cycle was at 650 nm where DBM measures were higher during the luteal phase.

DISCUSSION

SUMMARY OF PURPOSE

Visual perception is a complex response resulting from genetics, experience, gender, hormone levels, learning and social influences. It is therefore methodologically challenging to establish significant gender differences in human perception. The results of this study add to the body of evidence that suggests a female advantage in chromatic processing. The effect size of the overall between-gender comparison was very large, with gender accounting for 36% of the variance in chromatic contribution to brightness. This effect is on the order of the effect size (44%) of the well-accepted male advantage in spatial processing (Linn and Peterson, 1985). The within-female effects of contraceptive use, hormone levels and menstrual cycle phase were not as robust and were limited to certain conditions and stimulus wavelengths.

What follows is an examination of chromatic processing within the human visual system. Current thought on organization and activation effects at each level of visual transformation will be applied to account for significant findings. Novel approaches ranging from the treatment of the initial visual sensation as a purely physical problem to the role of family dynamics in perception will also be applied.

VARIABILITY IN HORMONE AND EXPERIMENTAL MEASURES

The within-female analysis depended on valid hormone measures. Although care was taken to properly collect, store and deliver saliva samples (see Dabbs, 1991), there was considerably more variation in PG measures than in E2 measures. This is in agreement with Chatterton et al. (2005) who found that standard errors in saliva measures of PG were an order of magnitude greater than standard errors in E2. In addition, the expected peak in E2 levels near ovulation was not observed. In the present study, observing more robust cyclical effects based on short-term activation influences of female steroid hormones might depend on making experimental measurements near peak E2 levels and would have required more frequent saliva sampling (see review by Kelly et al., 1999). If females differ significantly from males during a short period of time during the cycle, there might be both a large between-gender effect and a cyclical effect that was missed in this analysis. The limited hormone effects on experimental measures might also be due to large individual differences in hormone profiles across the menstrual cycle as demonstrated by Alliende (2002).

There was greater variability in DBM measures than HFP measures, consistent with previous work (Meyer et al., 1978; Yaguchi et al., 1993). Variability in DBM/HFP ratios was greater at short and long wavelengths relative to the more sensitive portion of the visual spectrum. This is also consistent with previous findings (Guth and Lodge, 1973; Yaguchi et al., 1993). In the present study, I found that variability in DBM/HFP ratios was not significantly different between males and females.

ENDOGENOUS VS. EXOGENOUS HORMONE EFFECTS

While the effects of contraceptive use on visual measures were limited in the present study, it is worthwhile to mention that synthetic progestin can affect the central nervous system differently than endogenous progesterone (reviewed by Glick and Bennett, 1981; Martin and Buono, 1997). Mechanisms for the limited effects of contraceptive use in the present study may involve the different actions of endogenous and exogenous progesterone on target tissues. The goal of most hormonal contraceptive devices is to inhibit ovulation. When ovulation does not occur, the corpus luteum—a temporary organ responsible for progesterone production—stops secreting progesterone and decays (Bengtsson, 1971). This reduction in progesterone could be responsible for some of the effects of contraceptive use found in the present study.

GENDER AND HORMONE EFFECTS THROUGHOUT THE VISUAL SYSTEM

Anterior segment

There is evidence in the literature for gender differences in anterior segment structures and organization. In their study of visual acuity as a function of gender, hue and age, Kelton et al. (1978) found an interaction of gender with hue on visual acuity. They suggested that their findings were, in part, due to chromatic aberration. However, I am unaware of any studies suggesting a directional difference in refractive error that could account for significant chromatic aberration differences between males and females. In a large-scale study involving 2583 school-aged children, girls had more powerful corneas and lenses as well as deeper vitreal chamber depths while boys had

deeper anterior chambers and overall longer axial lengths (Zadnick et al., 2003). There were, however, no significant gender differences in refractive error.

There were strict visual acuity requirements in the present study (20/25 each eye), and all subjects had received a complete ophthalmic evaluation within the last 12 months. This requirement ruled out uncorrected hyperopia or accommodative problems that could lead to significant individual differences in chromatic aberrations. These requirements, combined with the results of Zadnick et al., make it highly unlikely that organizational differences in the anterior segment could have significantly contributed to the large gender differences found in the current study.

In her review of ocular and visual changes during the menstrual cycle, Guttridge (1994) concluded there was at least "reasonable evidence" that the cornea was sensitive to female hormones. The human cornea swells an average of 4.5% overnight, while the average post-ovulatory overnight change is 5.6% (Mertz, 1980). It is possible that corneal swelling produced aberration effects responsible for a small portion of the limited cyclical effects. However, a small difference in corneal swelling is not likely to have contributed significantly to the large between-gender difference in chromatic contribution to brightness found in the present study.

Macular pigment optical density (MPOD)

Based on the low correlations between DBM/HFP ratios and MPOD measures, it is reasonable to assume little to no contribution of MPOD to the large gender effect on DBM/HFP ratios. However, Hammond et al. (1996) suggested cyclical changes in

MPOD measures. In the present study, HFP measures at 450 nm did not change across the menstrual cycle. MPOD was only measured once for each subject, so the possible effect of cycling MPOD cannot be evaluated.

Photopigments and photoreceptors

In the introduction, I discussed the possibility that inclusion of heterozygous carriers for protan or deutan color vision defects (CVD) might contribute to measured gender differences in chromatic sensitivity. While it is possible that my screening regimen was insufficiently sensitive to exclude all carriers, it did detect three carriers out of twenty-one female volunteers. This is consistent with the percentage of carriers (15%) in the female population (Neitz and Jacobs, 1986). Even if carriers are present in the study, it is doubtful that the small advantage afforded them in color discrimination could significantly contribute to the overall gender effect on DBM/HFP ratios (36%). Considering that L- and M- cone mechanisms are minimally sensitive to short wavelengths, the effect of gender on DBM/HFP ratios at 450 nm (25%) can not be accounted for by L- and M- photopigment variations in CVD carriers. It is also difficult to imagine how polymorphism of M- and L-cone photopigments could account for the *within-females* findings at 520, 560 and 650 nm.

It is generally accepted that the cone photoreceptor mosaic varies a great deal among genotypically normal individuals (Kimble and Williams, 2000). However, Brainard et al. (2000) found that L/M cone ratios ranging from 0.25 to 9.0 resulted in virtually no differences in color perception. Neitz et al. (2002) also found an "enormous

difference in estimated cone ratio, but no corresponding variation in color perception." In addition, the results of Kimble and Williams (2000) revealed no gender differences in L/M organization. Overall, there is a lack of support for the role of photoreceptor organization in the robust gender differences found in the present study.

However, it may be reasonable to attribute some of the *cyclica*l effects found in the present study to photoreceptor mechanisms. Non-contraceptive users had significantly higher DBM/HFP ratios and DBM measures than contraceptive users at 650 nm during the luteal phase. In addition for non-contraceptive users, DBM measures at 650 nm were significantly higher during the luteal phase than during the follicular phase. DBM measures at 650 nm were also positively correlated with PG levels and with E2/PG ratios. These findings are in agreement with those of Diamond et al. (1972) who found that non-contraceptive users had higher sensitivity to a small (subtending 1 minute of arc) white stimulus during the luteal phase compared to the follicular phase.

It is possible that the presently observed effects at 650 nm may result from increased body temperature caused by the synergism of E2 with PG during the luteal phase (Forman et al., 1987). In their treatment of visual sensations as the consequence of the physical properties of photoreceptors, St. George (1952) and Lewis (1955) reasoned that sensations to stimuli at wavelengths greater than 590 nm depended on temperature as well as stimulus lightness. Although he studied rod photoreceptors, Lewis concluded there was a "fairly good" extension of the model at photopic levels. On the premise that small amounts of long-wavelength light could only decompose thermally excited photopigment, de Vries (1948) also demonstrated that a 1° increase in core body

temperature (due to increases in ambient temperature) increased an observer's threshold sensitivity to both 660 nm and 730 nm light.

Jordan and Mollon (1993) argued that the results of Richter (1951), suggesting temperature-related seasonal changes in anomaloscope matches, were "wholly artefactual" as the findings could be accounted for by changes in instrument temperature. Instrument effects could not, however, explain the results of Kim and Tokura (1998) who used reflective color chips and found that during the luteal phase "redder" colors were preferred over "cooler" (blue and green) colors. Jordan and Mollon's criticism would also have little impact on the present results, as the instrumentation is not as susceptible to temperature changes. However, since I did not measure body temperature, it is not possible to evaluate its effect on the results of the present study.

In the chain of events leading to visual perception, beyond photopigment decomposition, are ionic channels in the photoreceptor surface membrane that close and hyperpolarize the cell (see Baylor, 1996). It is then possible that ionic changes in the photoreceptor environment could affect visual responses. Increased absorption theoretically results in increased photoreceptor excitation, leading to increased release of neurotransmitter from photoreceptors. This enhances the probability of a visual signal and decreases detection threshold. Knowles (1980) found that the relative absorption of long wavelengths in chicken photoreceptors increased as serum concentrations of chloride ion increased. Venkatesh et al. (2002) suggested that ion level changes during the menstrual cycle could be sufficient to affect visual sensitivity. It is possible to

attribute a portion of the present findings at 650 nm to either cyclical temperature or ionic changes at the level of the photoreceptors.

Toker et al. (2003) revealed an influence of sex hormones on blood flow in retrobulbar arteries. Blood flow resistance (BFR) decreased and peak systolic velocity (PSV) increased with increased estrogen levels. The opposite occurred with increased testosterone levels (increased BFR and decreased PSV). Estrogen was therefore beneficial to ocular hemodynamics, while testosterone antagonized the benefits of estrogen. In a study designed to investigate the effects of blood flow on the suppression of L-cone flicker responses by an intense ($10^{3.8}$ retinal Trolands) 640 nm stimulus, Eisner and Samples (2003) found that flicker suppression was directly related to heart rate and inversely related to blood pressure. It is then possible that gender differences in blood flow could have influenced the present findings.

Post-receptor mechanisms

HFP can be modeled with the *non-opponent* addition of L+M cone mechanisms (see Cavanagh et al., 1987), and the present findings at 580 nm could result from indirect cyclical changes in dopaminergic action on diffuse bipolar cells or their synapses with magnocellular ganglion (parasol) cells. Such an idea would be an extension of previous studies linking dopamine with changes in luminance processes (Masson et al., 1993). It has also been suggested that contrast sensitivity is different for cycling vs. non-cycling women (Johnson and Petersik, 1987). In the present study, it is possible then that E2 and/or PG acted directly or indirectly on luminance only (L+M) mechanisms.

For non-contraceptive users, there was a dependence of DBM/HFP ratios at 580 nm on E2 levels when PG levels were low. This result makes sense in the context of neurotransmitter release and function. It is known that bipolar cells with combined L and M inputs release glutamate onto bistratified ganglion cells (Calkins and Sperling, 1999; Lee, 2004). Since estrogens augment glutamate release (Smith, 1989), an increase in E2 would increase the opponent L+M response. This would particularly be the case when glutamate is not antagonized by PG (Smith et al., 1987).

In long-term users of contraceptives, Marre et al. (1974) found tritan (blue/yellow) defects on FM-100 testing. These results are in agreement with those of Eisner et al. (2004) who suggest that changes in S-cone pathways are associated with estrogen receptor activity in normal, cycling female subjects. It is known that certain estrogen receptors, ER_{α}, exist only in the retinal epithelium of pre-menopausal women (Ogueta et al., 1999). It is possible that, as Eisner (2004) speculates, different subtypes of estrogen receptors affect different visual functions. In the retina, S and L+M bipolar cells release different forms of glutamate (excitatory vs. inhibitory) onto synapses with bistratified ganglion cells, helping to establish +S-(L+M) or blue-yellow opponency. In the present study, E2 levels affected HFP measures *only* at 580 nm. However, E2 levels had a significant effect on DBM/HFP ratios at all wavelengths *except* 580 nm. Therefore, it is possible that estrogen receptors are differentially distributed on ganglion cells and result in a "trade-off" between luminance and chromatic systems at this level.

Dorsal lateral geniculate nucleus (dLGN) of the thalamus

It is well established that the thalamus is the primary color relay in the human visual system. The robust gender differences of the present study are in agreement with the psychophysical work of Jacobs (1983a) who found that increment threshold differences between green (540 nm) and red (640 nm) stimuli were gender dimorphic in light-adapted squirrel monkeys. In effect, male monkeys behaved in a protanomalous fashion when light-adapted. Subsequent results of Jacobs (1983b) revealed a significantly higher proportion of spectrally opponent cells in dLGN of female monkeys than of male monkeys. In addition, in male monkeys virtually every spectrally opponent cell (85 of 86) was coded for blue-yellow. Jacobs's findings may not extend beyond the studied population or to humans. However, the results of factor analysis by gender in the present study, where a second factor in DBM/HFP ratios composed of variance components from the 520 nm ("green") and 650 nm ("red") stimuli was extracted for females but not males, are in agreement with the findings of Jacobs.

Visual cortex: Primary (V1), Secondary (V2) and Visual area 4 (V4)

While it has been suggested that there is color processing specialization within V1 and V2 (Livingstone and Hubel 1987; 1988), the majority of these cells also respond to luminance differences (reviewed by Gegenfürtner and Kiper, 2003). It is probably most accurate to describe the set of chromatic-sensitive cells in V1 and V2 as ranging in function from strict luminance processing to strict color opponency. It is not difficult, then, to imagine a gender difference in the relative number of luminance vs. color-

opponent cells in visual cortex. Although no such evidence exists in the current literature, a possible explanation for the robust gender findings of the present study might be that there is a gender dimorphic balance of luminance and chromatic processing in visual cortex.

There may be cyclical effects on many brain areas, including primary (V1) and secondary (V2) visual cortex similar to those discussed for the retina and thalamus (see Smith and Zubieta, 2001). After all, estrogen can act directly and quickly on nervous cell membranes to increase cerebral blood flow, change regional activation patterns, or modulate neurotransmitter release or production (reviewed by Markou et al., 2005; Smith and Zubieta, 2001). Due to infrequent sampling, some hormone effects may have been missed in the present study. It is therefore possible that short-term changes in both estradiol and progesterone contributed to the robust between-gender findings.

We know that there are organizational effects of sex hormones on the mammalian brain, but it is difficult to make a clear connection with visual processes. There are few human studies comparing the structure and function of visual cortical areas between genders. Luders et al. (2004) found no gender difference in cortical complexity in occipital or temporal regions. However, Levin et al. (1998) found in a study comparing male and female visual responses to light stimulation that males actually had significantly higher BOLD responses to a red stimulus than females. The results of Cowan et al. (2000) revealed higher BOLD signal changes to red light in females as compared to males. This finding was not significant ($p = 0.15$), but female signal changes were higher at every red light intensity. BOLD signal changes to blue light were significantly higher

for males, and did not saturated as intensity increased while the signal became saturated for females at about 12 lux. Taken together, these studies do not provide an explanation for the present results, as they do not firmly establish a male or female advantage. This suggests either that gender differences do not exist in the cortical response to red or blue light stimulation or that BOLD signals are highly dependent on methodology and individual differences.

In cortical area V2 there persists an organization for wavelength sensitivity much like that of the retina. However, just as the ability to discriminate between similar hues improves from retina to V1, it becomes further improved from V1 to V2 where the ability to make local spatial comparisons is present in approximately 1/3 of color coded V2 cells (Moutoussis and Zeki, 2002). I am not aware of studies that would implicate V2 specifically in the present between-gender or cyclical findings.

Visual area 4 (V4) lies on the fusiform gyrus at the junction of the temporal and occipital lobes. Zeki (1993) hypothesized that, while color cells in V1 and V2 follow a retinotopic organization and are primarily coded for luminance, V4 is organized in a chromatic plan. To test this, Zeki altered the spectral reflectances from a geometrical ("Mondrian") display and found that V1 cells had identical responses to stimuli of different colors provided they reflected the same amount of energy. Conversely, he found that V4 cells had identical responses to different stimulus energies provided the chromaticity was unchanged. Later work revealed a visual area, V4α, which is activated by viewing color in natural scenes (Bartels and Zeki, 2000). Activation of this area would be minimal with the simple targets used in the present study.

I know of no studies indicating gender differences in V4. However, V4 has been implicated as an attention "gate" for visual stimuli (Moran and Desimone, 1985). If two stimuli lie within a V4 receptive field, responses to unattended stimuli are dramatically reduced. Changes in hormone levels across the menstrual cycle have been associated with changes in arousal and possibly attention states as measured by two-flash fusion thresholds (Kopell et al., 1969; Braier and Asso, 1980). In both of these studies, critical two-flash fusion flicker thresholds were increased during the luteal phase when compared to other menstrual cycle phases. These results, taken together with Moran and Desimone (1985), loosely implicate the menstrual cycle with changes in V4 function.

Other central nervous system structures

Prefrontal cortex is believed to help orchestrate thought in accordance with internal goals (Miller and Cohen, 2001). It is also known that the hippocampus is involved in memory (Brodal, 1969). On proton magnetic resonance spectroscopy (H MRS), higher baseline metabolite concentrations have been observed in the prefrontal cortex of females compared to males (Grachev and Apkarian, 2000). An increase in the number of rat hippocampal dendritic spines has been demonstrated in the presence of estrogen in cycling females (Gould et al., 1990). However, given the minimal memory requirements of the present experiment, it would be difficult to imagine how gender dimorphism in prefrontal cortex or estrogen-related changes in hippocampus would affect the present findings.

OTHER EFFECTS

Learning effects

In an editorial on gender brain differences, Jones (1998) wrote, "In short, our brains are what we teach them to be given their genetically defined templates." It is possible that all learning or improvement in ability represents neural plasticity (reviewed by Tsodyks and Gilbert, 2004). It is also possible that overall gender differences in life experiences may cause a difference in visual system organization. Munroe and Munroe (1971) explored this idea in a study of the male advantage in spatial orientation in a primitive culture. They found that boys tended to be better at orienting themselves to their environment without instruction. However, they also found large within-male differences and that boys who had greater spatial abilities were more apt to wander far from home.

Parke and Sawin (1976) found that variations in fathers' behavior were related to their male infants' behavior. The suggestion was that there was an environmental influence of same sex parenting on child behaviors. Greene and Gynther (1995) suggested that gender differences in color perception "may be a function of differential socialization for women and men." Since they found perceptual differences (women identified significantly more colors than men) and not cognitive differences (no differences in vocabulary), they were basically suggesting that environmental influence could feedback on to a child's *perception*. It is possible then, that the large between-gender findings of the present study are in part due to differences in chromatic "experiences" afforded boys and girls by caregivers.

Mood

It has been argued that many women have fluctuations in mood, affect, body systems and behavior that occur across the menstrual cycle (Moos et al., 1969). It could be further argued that a portion of the present results is attributable to reduced task performance at certain menstrual cycle phases. However, Guttridge (1996) found that mood was not a covariate of standard achromatic visual field performance, and Slade and Jenner (1980) found that poor performance on a perception task only correlated with mood when the task was very difficult. Since the tasks involved were very straightforward, mood effects do not seem to apply to the present findings.

EXTENSIONS TO FURTHER STUDIES

There was a large effect of gender in the present study on the sensitivity to chromatic contribution to brightness. This result combined with the limited effects of hormones and the menstrual cycle hint at an innate or genetic mechanism for the gender difference. This would be in agreement with the findings of Iijima et al. (2001). They found that the free drawings of girls affected with congenital adrenal hyperplasia (CAH) were more like the drawings of boys than unaffected girls. A reasonable follow-on to the present study could be to compare boys with girls affected and unaffected by CAH. The present study could also be repeated in girls exposed prenatally to higher levels of androgens by the presence of a twin brother or exogenous androgenic substances.

PHILOSOPHY OF FINDINGS

Oen et al. (1994) concluded, "Sex is an important cofactor of contrast sensitivity functions that should be controlled for in future studies." In spite of this and a myriad of results suggesting gender differences in visual function, there seems to be reluctance to accept them as real or incorporate gender norms into research protocols or clinical practice. A decade ago, an anonymous editorial in The American Journal of Psychiatry (N.C.A., 1997) called for "unbiased opinions" and "careful science" in the design and interpretation of studies involving gender as a predictor of behavior or function. I agree but would call for unbiased, careful science in *all* areas of research.

Using a systematic and rigorous approach, I have concluded that females have a profound advantage in chromatic contribution to brightness. This does not mean that females necessarily see more color, or that they can discriminate better between colors. It is more related to the findings reviewed by Alexander (2003), suggesting that the relative contributions of parvocellular processes are greater in females than males. I am admittedly disappointed by the limited findings of hormone and menstrual cycle effects. I do remain convinced that more careful sampling of hormone levels would reveal more within-female differences with potential applications to both vision and clinical science.

APPLICATIONS TO VISION SCIENCE AND CLINICAL PRACTICE

Gender norms for vision science and clinical practice would not be straightforward to develop, as there is evidence in the present study that some of the gender differences would be modified by sex hormone levels or contraceptive use.

Individual differences in menstrual cycle profiles would make modeling gender norms even more difficult; however there are a few straightforward recommendations.

Males and females need to be analyzed separately whenever possible in all color research. There is too much evidence in the present study that females and males differ in chromatic processing. It is also possible that the limited nature of the menstrual cycle effects was due to small sample sizes. With this in mind, careful tracking of the menstrual cycle and contraceptive use for large samples might elucidate the effects of female sex hormones on chromatic processing. When these effects are better understood, it should be a priority to make the models available for clinical use.

CONCLUSIONS

1. Females have higher chromatic contribution to brightness than males. The findings supporting this conclusion were robust; however, establishing the exact mechanisms responsible for the effect of gender was well beyond the scope of this study.
2. Hormone level and menstrual cycle phase had limited effects on chromatic contribution to brightness across tested wavelengths. Significant findings at 650 nm may result from physiological changes (body temperature, ionic environment and blood flow) and agree with previous findings on S-cone mechanisms.
3. These findings are relevant to both vision research and clinical practice. Developing tools to further model gender and hormonal effects on chromatic processing relies on collecting large sample data and should be a priority for the vision science community.

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