Using Electroencephalography as a Method to Determine Blue-Yellow and Red-Green Perceptual Asymmetries in the Human Visual System

A thesis submitted in partial fulfillment of the requirements for the degree of
Bachelor of Science in Neuroscience and the Honors Program

by

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May, 2017
UNIVERSITY OF NEVADA
RENO

THE HONORS PROGRAM

We recommend that the thesis prepared under our supervision by

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entitled

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be accepted in partial fulfillment of the requirements for the degree of

BACHELOR OF SCIENCE, NEUROSCIENCE

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May, 2017
ABSTRACT

Knowledge of color perception in infants and children is limited; in order to develop research methods to gain an understanding of this particular age group, we must first develop objective methods within an adult population that can then be applied to children in future studies. We presented stimuli with a fast-periodic visual stimulation paradigm, and analyzed the resulting steady-state visual-evoked potentials using electroencephalography. We used this technique to directly measure whether a difference response is present between colors defined by two intermediate axes in cone-contrast color space, which elicit equal average responses from the two axes of retinal color coding. Adult human observers are more sensitive to contrast along one of these axes than the other, and a primary motivation of this study is to find a neural correlate for this perceptual asymmetry. We presented adult human observers with blue-yellow (135° - 315°) and red-green (45° - 225°) gratings at an alternation rate of 2.5 Hz, i.e., one presentation every 400ms for each grating. With the same paradigm, we also tested observers with an achromatic condition (i.e. pure luminance gratings) in order to compare difference responses elicited along achromatic axes to those for chromatic axes. Our results show that we created a stimulus design which elicits higher amplitude responses for chromatic stimuli (5.95 µV) compared to achromatic stimuli (4.81 µV). The lowest amplitude difference response between the chromatic axes was 1.72 µV at a contrast ratio of 1.17 for red-green to blue-yellow, and 0.09 µV at a contrast ratio of 1.07 for the achromatic axes. Designing a sensitive measure for comparing responses across chromatic axes that can be used with infants and adults will advance understanding on the developmental trajectory of color perception.
ACKNOWLEDGEMENTS

I would first like to extend my gratitude to my thesis advisor, Dr. Michael Webster, for serving as my mentor for this project. Under his supervision, I was able to contribute to a rigorous and educational project from which I have learned so much. I would also like to thank Kara Emery and Talia Retter, both graduate students in Dr. Webster’s lab, who were essential for the design, implementation, and final analysis of this experiment. Additionally, I am grateful for the contributions to the project made by graduate student Katie Tregillus for her time spent helping with data analysis, and postdoctorate Scott Gwinn for his contributions to the experimental setup. I would also like to acknowledge Alex Aniban, an undergraduate research assistant in Dr. Webster’s lab, who helped to support this project by working diligently to create the stimuli for the experiment. I also would like to thank Dr. Tamara Valentine for her dedication to the students of the Honors Program and her unwavering support of each and every one of our projects. Finally, thank you to my incredible parents, Kate and Bill Tolles, my brother, Tyler Tolles, and my wonderful friends whose love and support motivated me to successfully accomplish my pursuit of an Honors education.
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CHAPTER 1: INTRODUCTION

Colors illuminate and bring perspective to the objects and people in the world around us - they provide helpful cues to our surroundings (i.e. stoplights, road signs, etc.), allow for the successful identification of objects (i.e. rotten vs. ripe fruit), and even provide emotional context to scenes or images (i.e. blue emotes sadness, yellow emotes happiness, etc.) (Conway, 2002). Understanding how our brain processes the sensation of color provides important insight into proper functioning (and therefore, dysfunction) of the human visual system.

Basics of Color Perception

Understanding the basic processes and mechanisms behind color vision is essential for understanding how color vision is adapted and shaped by experience. All light that humans can see is defined by a miniscule sliver of the electromagnetic spectrum in the area termed “visible light.” The visible spectrum encompasses wavelengths of about 400-700 nm, with the shorter wavelengths reflecting deep violet hues and longer wavelengths representing bright red (Conway, 2002). These wavelengths are transmitted into signals in the brain via a series of steps. Rays of light first reflect off a surface and enter the eye through the cornea and the lens, the outermost components of the eye. The amount of light allowed to enter the eye is then regulated by the pupil, which contracts or dilates accordingly (Conway, 2002). Light rays then travel through to the back of the eye to a layer of photoreceptors in the retina, which consists of rods and cones (DeValois & Webster, 2011).
**Receptors: Rods and Cones**

As the human eye receives input in waves of light, the six million cone receptors and 120 million rod receptors in the retina filter this information, which then travels via the optic nerve to the brain (O’Connor, 2015). Cone receptors are densely populated in the fovea, or the area of central “gaze” in the retina (Conway, 2002). Rods, however, are most sensitive to dim light; because all rods use the same photopigment, they do not play a large role in the perception of color (O’Connor, 2015).

According to the Young-Helmholtz theory of trichromacy, cone photoreceptors are represented by three peak sensitivities that are responsible for producing the perception of our primary colors: blue, green, and red (Young, 1802). These peak sensitivities of short, medium, and long, or S, L, and M cones, occur at 440nm, 535nm, and 565nm, respectively. These wavelength ranges are distinct, but overlap particularly between the L and M cone regions (Conway, 2002). The spectral sensitivities of the photopigment molecules have been assessed through classical color-matching experiments and, more recently, physiological measures, comparing the responses between photoreceptor types is a necessary substrate for color vision (DeValois & Webster, 2011). Based on these comparisons, all colors represented in a color space that is three-dimensional (DeValois & Webster, 2011; Krauskopf et al., 1982).

**Cone-Opponent Mechanisms**

Studies of human perception of color vision have revealed that color is initially coded by three types of cone receptors and then transformed into opponent mechanisms
that compare the differences in the cone signals (Krauskopf et al., 1982). Though often perceived as a competing theory to trichromacy, the theory of opponency describes how these cone signals are compared. Trichromacy explains which color stimuli on the visible spectrum are encoded by the photoreceptors, but it lacks an account of hue, luminance, and saturation. Hue is defined as what is typically described as the color name, and is represented by the angle around the central axis. Luminance is defined as the achromatic brightness of color, represented by the distance along the central vertical axis, and saturation is defined as the intensity of color, represented by the distance from the central axis (Conway, 2002).

According to the theory of opponency, there are four unique hues: red, green, blue, and yellow (DeValois & Webster, 2011). Signals stemming from opposing S vs. LM cones, or L vs. M cones (Figure 1), represent the axes by which we form a chromatic scale for color coding processes in the early visual system (Webster, Miyahara, Malkoc, & Raker, 2000). In a three-dimensional coordinate system, these hues form opponent axes, with each color of each pair representing a pole of their respective axis (Figure 2). Each pairing represents colors that are impossible to perceive at the same time in the exact same space (Conway, 2002). Based on these cone-opponent mechanisms, researchers can study the relative sensitivity of these cardinal color axes among individuals.

![Figure 1. Cone-opponent mechanisms and how they are represented on an axis in contrasting color space. Diagram representing opponency, showing how the different opponency mechanisms are represented on an axis in contrasting color space.](image-url)
combinations of S, M, and L cones are excited to create color-opponent axes. Excitation of S cones, but not L and M cones leads to the S vs LM opponent axis (left); Excitation of L cones, but not M cones, in the absence of S cones (i.e. the difference between the two) leads to the L vs M opponent axis (middle); Excitation of L and M cones, in the absence of S cones, leads to the L/M non-opponent axis. (Source: DeValois & Webster, 2011)

While the cardinal axes are opponent, the stimuli that isolate them do not correspond to the blue vs. yellow and red vs. green dimensions of classical opponent process theory (Krauskopf, Williams, & Heeley, 1982). To better visualize and examine the blue-yellow and red-green dimensions, an intermediate axis exists halfway between the cardinal axes, allowing for the comparison of colors that are equally excited by S, L, and M cones (Figure 2).

**Figure 2. Cardinal axes and intermediate axes of contrasting color space.** The opponent axes are represented in an axis in a three-dimensional coordinate system (left) and in intermediate axes (right). (Source: DeValois & Webster, 2011)

Examination of the early visual processes shows that the L vs. M and S vs. LM signals operate at sub-cortical levels. Less is known about the visual processing of color
in the cortex. In an fMRI study of neural response to orange-cyan vs. lime-magenta color stimuli, it was found that higher order neurons in the brain could utilize a combination of sub-cortical mechanisms to form a new representation of color (Goddard, Mannion, McDonald, Solomon, & Clifford, 2010).

**Visual Adaptation**

Visual adaptation is the concept that the sensitivity and perception of color or other sensory attributes is adjusted because of environmental surroundings and specifically to adjust to the most prevalent stimuli within those surroundings (Webster & Mollon, 1997). Though we adapt to colors based on environmental surroundings, we are also able to consistently recognize colors in all settings, regardless of differences in lighting. This is termed “color constancy” (Goddard et al., 2010b). In a study investigating the role of adaptation in color constancy, participants adapted to either a scene in which illumination was altered, or a scene in which the objects illuminated were changed; it was revealed that there was greater sensitivity to the changing scene than the change in illumination (Goddard et al., 2010b). This further supports the theory that adaptation is involved with color constancy, allowing us to maintain consistent color perception in the ever-changing world.

Adaptation also affects sensitivity to lightness. In a study measuring the sensitivity to lightness measured by brightness, flicker, or motion in color gratings, it was discovered that adaptation can affect each aspect of lightness differently (Webster & Mollon, 1993). Another study investigating the concept of light adaptation utilized electroencephalography, or an EEG, to measure neural response to colored light after adaptation to white light (Münch et al., 2014). This revealed that stronger neural
responses were recorded after adaptation to bright, rather than dim white light in all
colored light stimuli except red light, which showed the greatest overall effect (Münch et
al., 2014). Though we will not be applying short-term adaptation to our experiment, these
findings are significant to our research as we will be employing EEG to measure neural
responses to color stimuli that are useful in determining differences in color processing as
it occurs in the brain.

**Color Statistics: The Blue-Yellow vs. Red-Green Asymmetry**

Neural coding is calibrated by processes of adaptation that adjust sensitivity to the
stimulus the observer is exposed to. For example, the stimulus that appears white is
thought to represent adaptation to the average spectrum we see. Moreover, judgments of
white show more variation in blue-yellow than in red-green (Bosten, Beer, & MacLeod,
2015), and fMRI responses to blue and yellow are weaker than red and green (Goddard,
Solomon, & Clifford, 2010; Mullen, Chang, & Hess, 2015). This is likely due to our
adaptation to the stronger variations in blue and yellow that are common in natural scenes
(Bosten et al., 2015). However, it is not known how long these adaptations take to
develop, and thus at what point in our lives these biases emerge.

It is suggested that the reduced sensitivity in the blue-yellow axis supports the theory
that those colors are more prominent in natural surroundings, as opposed to red-green
variations (Bosten et al., 2015). Sensitivity to blue and yellow due to natural scene
variations are implicated in another study, in which unique colors were perceived during
the different seasons. Unique yellow, for example, was the object of a study theorizing
that there is a shift in the state of adaptation during winter and summer (Welbourne,
Morland, & Wade, 2015). Unique yellow is identified as the midpoint of the red-green
axis, though this study showed support for the hypothesis that the location of unique yellow shifts along this axis per the amount of green light during that time of year (Welbourne et al., 2015). Specifically, the unique yellow position was shown to increasingly shift toward the intermediate blue-yellow axis during summer, because of the greater presence of green surroundings during the summer season. Similarly, it was found that the presence or lack of vegetation during different seasons may account for visual sensitivity variations across the blue-yellow axis (Webster, Mizokami, & Webster, 2007). This shift indicates that during summer, when we are bombarded with increased exposure to green, the receptors will sensitive their sensitivity or adapt, changing our color perception (Welbourne et al., 2015). Similarly, a study on sub-cortical representations of color revealed a preference for the lime-magenta stimuli, which fall along the red-green chromatic scale, indicating that there is an increased response in the red-green axis than in the blue-yellow axis (Goddard, Mannion, McDonald, Solomon, & Clifford, 2010).

To study this asymmetry as a result of a blue-yellow bias at the neurophysiological level, EEG in coordination with SSVEPs were used in this experiment to attempt to find a point at which these opponent contrasts of blue-yellow and red-green hues are equal.

**Developing a Method for Measuring Chromatic Differences: SSVEP and EEG**

Electroencephalography (EEG) is a useful tool for measuring the neural processes underlying brain function and dysfunction, in addition to cognitive processes. EEG allows the benefits of a non-invasive form of brain imaging that records specific responses called event-related potentials (ERPs). When paired with fast periodic visual
stimuli, these responses are modeled by steady-state visual evoked potentials (SSVEPs). These electrophysiological responses occur in the brain as a result of a specific sensory or motor stimulus; electrical responses form peaks, which occur at the same rate as information being processed through the brain. (Luck, 2005; Woodman, 2010). Electrical activity recorded across electrodes at any given time during an EEG recording is a collection of electrical activity across neuron populations that is summed and averaged in a given time period. Collecting these averaged responses to specific events is useful because it allows for the identification of mechanisms underlying neuro-cognitive processes (i.e. memory, attention, visual sensation) (Luck, 2005).

EEG records two specific types of responses: action potentials (APs) and postsynaptic potentials (PSPs). APs occur when voltage spikes during brain activity, causing the release of neurotransmitters, which, when bound to postsynaptic cell membranes, emit PSPs. This causes ion channels within the postsynaptic cell membranes to open and close to allow for graded potential changes (Luck, 2005). Because PSPs are difficult to isolate, but not APs, single-unit recordings are used to measure and record AP activity, while local field potentials gather PSP recordings from a mass of neurons (Luck, 2005). Furthermore, as APs rarely occur simultaneously, and require strategic placement of high impedance electrodes that only record the electrical activity of neurons nearby; this makes APs less plausible for spatial considerations of electrical activity. Therefore, PSPs are reflected in responses used in EEG. When PSPs accumulate as a response to the release of neurotransmitters, current flows through pyramidal cells, which are cells whose location lies close to the skull (Luck, 2005). During this current flow, an unequal charge is accrued in pyramidal cells, called a dipole, and as these dipoles are laterally summated
among cells across the surface of the skull, responses become measurable (Luck, 2005). Though EEG provides reliably accurate temporal resolution and is advantageous in determining the activity of neuron populations, it is limited in that it cannot reliably measure the activity of single neurons or neurons that do not fire simultaneously (Cohen, 2017). However, EEG remains a powerful tool for measuring the responses of populations of neurons.

In a study of the discrimination of faces, EEG was used in combination with fast periodic visual stimulation to record brain frequencies as a function of the frequency of images with faces as stimuli (Retter & Rossion, 2016). Faces were shown at a rate of six images per second, or at 6 Hz. Additionally, the identities were alternated at a rate of 3 Hz. Prior exposure to one of the faces reduced sensitivity to it, leading to a 3 Hz response in the EEG (Retter & Rossion, 2016). This study shows how EEG can be used to reveal the relative strength of different stimuli, and we will use the same procedure for comparing the neural responses to different colors.

One study investigated the frequency rate of faces using the SSVEP paradigm (Alonso-Prieto, Van Belle, Liu-Shuang, Norcia, & Rossion, 2013). The results of this study suggested that SSVEP responses could differ in potency among stimuli; therefore, it was revealed that when the same face was repeated, the SSVEP response was significantly lower than it was when different faces were shown (Alonso-Prieto et al., 2013). This can be applied to our study, in which we hope to find responses that decrease toward a minimum as the stimulus approaches white.

In periodic visual stimulations, presentation rates occur at designated frequencies, which lock the SSVEP responses in the brain to a frequency that matches that of the
visual stimulus presentation. This paradigm is particularly useful with EEG, as it evokes responses in the brain that can then be recorded, filtered, and analyzed. For example, the theory behind using SSVEP as an experimental measure is that if a visual stimulus is represented on a screen at a frequency of 2.5 Hz (i.e. 2.5 cycles per second), the oscillations in electrical brain activity should reliably match that 2.5 Hz frequency (Alonso-Prieto et al., 2013). Furthermore, repeated presentation of one visual stimulus produces a time-locked neural response to that stimulus (2.5 Hz), while alternating between two stimuli produces a difference response at half the frequency (1.25 Hz) if one stimulus is stronger. The amplitude of the response of the base frequency (2.5 Hz) provides a quantitative measure of what the stimuli have in common, while the amplitude at half the base frequency (1.25 Hz) provides a quantitative measure of what's different between them (Alonso-Prieto et al., 2013; Luck, 2005). Therefore, the fast periodic visual stimulation paired with the measure of SSVEPs is a useful technique for measuring the extent to which two stimuli produce similar or different responses in the brain.

**Contributions to the Field**

In this study, we use these methods to design an experiment that measures the relative sensitivity to blue-yellow (135° - 315°) gratings vs. red-green (45° - 225°) gratings, to measure the contrast ratio (i.e. blue-yellow contrast/red-green contrast) at which the 1.25 Hz response amplitude is minimized. We utilize fast periodic visual stimulation, SSVEP, and EEG to monitor these symmetrical and asymmetrical responses due to their reliability of providing time-locked responses to visual stimuli. In this task, two stimuli of contrasting gratings are alternated at certain rate (e.g. 2.5 Hz), leading to a 2.5 Hz signal in the EEG response to each stimulus presentation. If one of the stimuli is
stronger, a signal is also found at the alternation frequency (1.25 Hz). The SSVEP can therefore be used to measure the relative strength of two stimuli, by finding the levels at which the 1.25 Hz signal is minimized (Retter & Rossion, 2016). This helps to quantitatively characterize the intermediate cone-opponent axes, elucidating mechanisms underlying visual processes in adults, using techniques that can be used in later studies to characterize and compare the color perception of infants and children. The specific objectives in this study are to: (1) Identify the contrast balance for chromatic gratings by measuring steady-state visual-evoked potential responses (SSVEPs) to find the minimum difference frequency, and to (2) Create a stimulus design which favors the chromatic processing system, i.e., higher amplitude responses for chromatic stimuli versus achromatic stimuli.

CHAPTER 2: MATERIALS AND METHODS

Participants

Three healthy participants (age range 21-27; three female) were recruited within the UNR Visual Perception Lab and tested independently. All participants reported normal or corrected to normal vision. Eligibility for participation required providing informed consent, in accordance with IRB policy.

Materials

This experiment measured color contrast sensitivity using visual stimuli of alternate gratings composed of blue-yellow or red-green contrasts. To control for responses elicited specifically to color, as opposed to luminance, the alternate gratings were adjusted to remove luminance contrast. These stimuli were created through Visual Basic and Matlab, and were presented on a high-resolution Display ++ LCD computer.
monitor for precise timing and consistency between stimulus presentation rate and external EEG recording. A BioSemi EEG system was used to record electrical responses in the brain via the placement of 128 electrodes across an EEG skull cap with a conductive gel. The EEG signals were analyzed using a Fourier transform to measure the responses at different frequencies. The data were analyzed to look for responses at both the presentation frequency (2.5 Hz) and the alternation frequency (1.25 Hz) in order to identify the stimulus levels at which the difference frequency is smallest.

**Stimuli Presentation**

The control stimuli consisted of gratings of alternating achromatic visual stimuli (Figure 1), while the experimental stimuli consisted of alternating contrasts of red-green and blue-yellow hues (Figure 2). Grating stimuli in each condition were presented on a gray background and individualized for each participant’s isoluminance settings. The blue-yellow gratings were held at a fixed contrast of 0.3655, while red-green grating contrast levels were varied to act as a point of comparison from which to measure the relative sensitivities to blue-yellow versus red-green hues. In the achromatic condition, the medium contrast level was held constant while contrast levels of the same gratings increased and decreased. Gratings were presented at a 20% duty cycle with a frequency resolution of 0.0833 Hz and presented at a visual angle of 25°, with 1 cycle per degree accounted for. Using an SSVEP method, each trial consisted of 30 flickering grating stimuli presented on the screen at a frequency rate of 2.5 Hz (or images per second) for a total of 12 seconds per trial. A total of 300 trials were performed, and each trial consisted of either the achromatic condition or red-green vs. blue-yellow condition. Per trial, each grating stimulus stayed on screen for 80 milliseconds, with a 320 millisecond break
between each of the 30 stimuli. Each condition was tested for a total of 4 minutes, with randomized saturations alternating with the respective reference contrast. The reference for the red-green vs. blue-yellow condition was a medium saturation of blue-yellow gratings, while 5 randomized saturation levels of the red-green gratings alternated with this reference. The reference for the achromatic luminance control was a medium contrast of grating that alternated with five randomized levels of contrast of the same gratings (since there is no color in this condition).
Figure 3. Achromatic grating stimuli presented at a frequency of 2.5 images per second (Hz). The medium of 5 contrast levels was held constant and used as a reference (right). Each of the 5 contrast levels was divided by the reference contrast to calculate contrast ratios (left). The ratios increase from top to bottom with the following values: 0.56, 0.75, 1, 1.33, 1.78
Figure 4. Chromatic grating stimuli presented at a frequency of 2.5 images per second (Hz). The blue-yellow gratings at the medium contrast level was held constant and used as a reference (right). Each of the 5 contrast levels of red-green gratings was divided by the reference contrast to calculate contrast ratios (left). The ratios increase from top to bottom with the following values: 0.56, 0.75, 1, 1.33, 1.78.
Procedure

Prior to the experiment, a minimum-motion test was performed by each participant to calculate each individual’s isoluminance settings. This step is important to adjust for individual differences in the perception of luminance. Using the average of 2-3 minimum-motion tests, the luminance mean of S and LM stimuli was individualized for the creation of experimental stimuli. Doing this allows us to stimulate color-sensitive mechanisms, and not luminance-sensitive mechanisms, so we can clearly determine that recorded neural responses are a result of identifying hue (color), not just changes in brightness or intensity of the colored gratings (Lu, Lesmes, & Sperling, 1999). The experiment was conducted in a quiet, darkened room in the Mack Social Sciences building at UNR. After consenting to the experiment, each participant sat in front of the monitor and a computer keyboard at a viewing distance of 57cm – measured by distance from eye to screen. Overall, this study lasted roughly 3 hours, consisting of EEG setup and electrode placement, stimuli presentation, and cleanup. There were 300 trials, each with timing between trials left up to the participant based upon keyboard response. Participants were offered a break at any time and were informed of their progress at the halfway point.

During the presentation of visual stimuli, grating stimuli were presented at a rate of 2.5 images per second (Hz) with a contrast range from 0.2-0.6, equating to ratios (contrast level : reference contrast) of 0.56-1.78. In each control trial, during a period of 12 seconds, 30 images of alternating stimuli of the reference grating and a random contrast grating were presented, with a pause at the end of each trial. In each experimental trial, the same presentation times and frequencies were presented, but with a
set blue-yellow reference alternating with a red-green grating of randomly selected saturations. During each trial, participants were instructed to stare at a fixation cross in the center of the screen, which occasionally would change from a cross to the shape of a circle; when this occurred, participants were instructed to press the spacebar. This served two purposes: to ensure the participant remained fixated at the center (to minimize eye movements and stimulate peripheral vision) and to ensure that the participant is paying attention to the screen. After the completion of all the trials, electrodes were removed from the cap, and the cap removed from the participant. The skull cap was cleaned between each participant.

CHAPTER 3: RESULTS

Data Acquisition

Data from the BioSemi EEG recording system was gathered and imported as BDF files and filtered to eliminate unnecessary high and low frequency noise (below 0.1 Hz; above 120 Hz) and artifacts that may have been produced by small, involuntary

![Figure 5. Pictorial (left) and graphical (right) representation of electrodes on EEG skull cap used in this experiment. The shaded blue area (bottom) represents the region](image-url)
of interest, as this covers the area of the brain where the visual cortex is located. (Source: https://www.biosemi.com/headcap.htm)

movements (i.e. blinking, swallowing, etc.). Filtering these extraneous signals allows for the elimination of excess noise without compromising the overall shape of the response (Luck, 2005). Initially gathered at a recording frequency of 2,048 Hz, the data was downsized to a sample of 512 Hz for better visualization of the activity of interest, and was spliced together to collect a unified data sample eliminating between-trial pause.

A fast Fourier transform (FFT) was used to convert responses from the time domain, represented by amplitude vs. time, to a frequency domain, in terms of amplitude vs. temporal frequency (see Figures 7, 10) (Luck, 2005).

**Topography of responses to Achromatic Stimuli**

To visualize the EEG data, topographical maps illustrating average levels of brain activity along the back side of the head – the area surrounding the visual cortex – at both the presentation frequency (2.5 Hz) and alternation frequency (1.25 Hz) were constructed. According to the topography, the data for the achromatic gratings indicates high activity across all saturation levels at the 2.5 Hz presentation frequency, with the overall highest recorded response at an amplitude of 10 μV. In contrast, brain activity at the 1.25 Hz frequency varies systematically with changes in relative contrast, and shows a clear minimum when the two gratings have the same contrast (Figure 6).
Figure 6. Topographical map of responses to achromatic stimuli. *Left:* Achromatic gratings of increasing contrast ratio (0.56-1.33), with reference contrast to the left (0.3655 RMS). *Right:* Topographical maps showing posterior brain region to indicate activity level of responses to luminance stimuli at corresponding saturation levels. This visual representation indicates the averaged activity among the three participants at both the 2.5 Hz presentation frequency (left) and the 1.25 Hz alternating frequency of interest (right), summed with their harmonic frequencies. The color scale (bottom right) represents levels of brain activity, with red indicating high activity, and blue indicating low activity. 10μv represents the amplitude of the overall highest response within the presentation frequency, and 5μv represents the amplitude of the overall highest response at the alternate frequency, for the responses to achromatic gratings.
Analysis of Asymmetry in Achromatic Contrast

Data was analyzed on the frequency domain (i.e. frequency vs. amplitude scatterplot) (Figure 7), and the 2.5 Hz and 1.25 Hz responses were compared across the different contrast levels (Figure 8, Table 1).

Figure 7 illustrates brain responses as points on a scatterplot representing responses averaged across the three participants. Each dot represents each of the five contrast conditions. The strongest response, as indicated by the highest amplitude (~2.5 μV) in this specific time scale, occurs at the 2.5 Hz presentation frequency, with the next most responsive points following along the harmonics of this frequency. Responses are also visible at the 1.25 Hz alternating frequency and its harmonics, with amplitudes around 0.5 μV).

At the 2.5 Hz presentation frequency in Figure 8, data from the contrast ratio vs. amplitude plot indicates a consistent amplitude response (~4-5 μV). At the 1.25 Hz alternating frequency, the contrast plot shows a value of 0, or very close to 0, at the equal contrast levels between reference and stimulus. This means that there is little to no response at the difference frequency. This result is as expected, and shows that the method can reliably measure the point of equal effective contrast, at least when the gratings both vary in luminance.
Figure 7. Scatterplot of amplitude plotted against frequency in the achromatic luminance condition. Points on this plot represent averaged responses from the three participants, with different colors indicating the five levels of saturation. Points of interest include amplitude values at 2.5 Hz frequencies and its harmonics, as well as responses at 1.25 Hz frequencies.
Electrical Responses to Achromatic Stimuli at 1.25 Hz

\[ y = 3.6917x^2 - 7.8861x + 4.3009 \]
Vertex = (1.07, 0.09)

**Figure 8.** Bar graphs of amplitude responses at (a) 2.5 Hz and (b) 1.25 Hz frequencies to achromatic gratings. The amplitude is plotted against the ratio of contrast level to reference contrast, with equivalent contrast levels represented in the middle (1:1). The trendline was used to solve for the x and y coordinates of the vertex of the parabola of the sinusoidal wave at the highest response.

**Table 1**

*Contrast Ratios and Amplitudes*

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>Red-Green vs. Blue-Yellow</th>
<th>Achromatic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency (Hz)</td>
<td>Contrast Ratio</td>
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*Note.* Contrast ratios measured by dividing contrast step (5 levels) by reference contrast (0.3655).
Topography of responses to Red-Green vs. Blue-Yellow Chromatic Stimuli

Figure 9 shows topographical maps of the responses for the red-green and blue-yellow condition. According to the topography, there are relatively high-amplitude responses to chromatic stimuli at both the 2.5 Hz presentation frequency and the 1.25 Hz alternation frequency. Although the responses at 2.5 Hz are higher, with an amplitude of 18μV as the overall highest response, there remains a highly consistent level of activity at the 1.25 Hz frequency, with the overall highest amplitude at 6μV (Figure 5).

At an equal contrast ratio (1:1) between the reference blue-yellow grating and the red-green grating, there is a medium-high level activity at both 2.5 Hz and 1.25 Hz. In the data at 2.5 Hz frequencies, the level of brain activity, as indicated by orange-red areas, increases in accordance with increasing red-green saturation levels. Alternatively, the 1.25 Hz data shows that the strongest brain responses occurred at the lowest (0.2055) and highest (0.6500) contrast levels, while responses decrease moving toward the reference point but importantly, do not drop to zero as they did for the achromatic control (Figure 9).
Figure 9. Topographical map of responses to chromatic stimuli. Left: Red-green chromatic gratings of increasing contrast, with blue-yellow reference contrast indicated to the left. Right: Topographical maps showing posterior brain region to indicate activity level of responses to hue at corresponding saturation levels. This visual representation indicates the averaged activity among the three participants at both the 2.5 Hz presentation frequency (left) and the 1.25 Hz alternating frequency of interest (right), summed with their harmonic frequencies. The color scale (bottom right) represents levels of brain activity, with red indicating a lot of activity, and blue indicating very little activity. 18μV represents the amplitude of the highest response at the presentation frequency, and 6μV represents the amplitude of the highest response at the alternate frequency, for responses to chromatic grating stimuli.
Analysis of Red-Green vs. Blue-Yellow Responses

Data was again analyzed in the frequency domain (i.e. frequency vs. amplitude scatterplot) (Figure 10), and the 2.5 Hz and 1.25 Hz responses were compared across the different contrast levels (Figure 11, Table 1).

Figure 10 illustrates brain responses as points on a scatterplot representing responses averaged among the three participants. Each spike also represents a summed representation of all five contrast levels. The strongest response, as indicated by the highest amplitude (~4.5 μV) in this specific time scale, occurs at the 2.5 Hz presentation frequency, with the next most responsive points following along the harmonics of this frequency; this is almost twice the amplitude of the highest response in the achromatic condition. A reliable asymmetric response is also visible at the 1.25 Hz alternating frequency, with amplitudes reaching up to 2 μV; this value is near the highest response elicited in the achromatic condition.

At the 2.5 Hz presentation frequency in Figure 9, data from the contrast ratio vs. amplitude plot indicates an increasing response as contrast ratio increases, ranging from ~5-7 μV. These amplitude values are relatively close, but increase toward the highest contrast. At the 1.25 Hz alternating frequency, the contrast plot shows the lowest response near the 1:1 contrast ratio, with increasing responses toward increasing contrast; this mirrors the data in the topographical maps. Thus in this condition there is a shallow minimum and substantial responses at all contrast ratios.
Figure 10. Scatterplot of amplitude plotted against frequency in the red-green vs. blue-yellow condition. Points on this plot represent averaged responses from the three participants, with different colors indicating the five levels of saturation. Points of interest include amplitude values at 2.5 Hz frequencies and its harmonics, as well as responses at 1.25 Hz frequencies.
Electrical Responses to Chromatic Stimuli at 1.25 Hz

\[ y = 1.9136x^2 - 4.4603x + 4.3113 \]

Vertex = (1.17, 1.71)

**Figure 11.** Bar graphs of amplitude responses at (a) 2.5 Hz and (b) 1.25 Hz frequencies to achromatic gratings. The amplitude is plotted against the ratio of contrast level to reference contrast, with equivalent contrast levels represented in the middle (1:1). The trendline was used to solve for the x and y coordinates of the vertex of the parabola of the sinusoidal wave at the highest response.

**CHAPTER 4: DISCUSSION**

**Conclusions**

We conducted a high-density EEG experiment involving participants who were shown randomized trials of alternating blue-yellow and red-green gratings, as well as alternating contrast levels of achromatic gratings. While viewing the flashing stimuli, presented at a rate of 2.5 Hz, or images per second, the EEG recorded SSVEPs, as electrical responses of the brain. Quantitative data, including frequency domain plots and bar graphs of contrast ratio vs. amplitude, in addition to qualitative data gathered in the
form of topographical heat maps of the brain were used to analyze the neural responses to luminance and chromatic contrast.

Averaged data for the chromatic gratings of blue-yellow vs. red-green indicate an increased level of activity as the contrast ratio increases. Additionally, there are strong responses elicited at the alternation frequency of 1.25 Hz in chromatic gratings, indicating the presence of an asymmetry in responses to red-green and blue-yellow hues. Therefore, our chromatic responses do reflect an asymmetry and support prior research indicating that the locus of sensitivity is correlated with the locus of natural daylights; furthermore, neural responses are particularly receptive to increased contrasts deviating from the blue-yellow locus (Bosten et al., 2015).

This study accomplished four objectives that are novel to the field of color perception research. First, we developed a viable method to measure the relative contrast of stimuli, as demonstrated by the luminance control. Second, we applied this method to test the relative sensitivity to blue-yellow versus red-green hues. Third, we found the minimum difference frequency value near the nominal threshold balance; thus, we were unable to support the theory that humans are less sensitive to blue-yellow, according to the EEG method. Lastly, the minimum did not go to zero, even though the stimuli were equivalent in LM and S cone contrast; thus, our results suggest some re-organization of color coding higher up in the human visual cortex.

Limitations and Future Directions

Limitations of this study include a low sampling size of 3 participants. In order to be able to state more conclusive results about brain patterns that can be generalized
across the human visual system, and even the visual systems of other animals, more participants are necessary. Nevertheless, the data shows promise in helping support our hypotheses about chromatic asymmetries among red-green vs. blue-yellow hues, and in identifying EEG as a potentially reliable method for determining this. Furthermore, other experiments involving more intermediate contrast levels or a larger range of contrast values may be investigated to investigate if a finer minimum contrast may elicit a stronger or weaker asymmetry.

The future goals of this project include testing enough participants in order to make more specific conclusions about the characteristics of the asymmetries, and once it is (or is not) established that EEG can be used to understand color perception at a neurophysiological level, this may be applied to the study of color perception in infants. Mechanisms underlying visual perception in adults are unclear, and even more so in infants. It is nearly impossible to obtain subjective responses to color perception from infants, as they do not have the attention span or verbal abilities required for this sort of judgement. Therefore, establishing EEG use as an objective method to discover the underlying mechanisms for perception in infants can open the door to future research questions, such as those proposed for the future of this experiment: how experience shapes color sensitivity, the timescale underlying these properties, the amount of time necessary to form higher order mechanisms of color, and whether infants will indicate similar blue-yellow biases in color perception (Emery et al., 2016).
REFERENCES


