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Developing and Testing a New Technique for Assessing Human Color Acuities

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DEVELOPING AND TESTING A NEW TECHNIQUE FOR ASSESSING HUMAN COLOR ACUITIES

by

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Abstract

This study is a continuation of the "open door" technique of color acuity determination. The open door experiment is a computer based program that tests the subject's ability to discern a continuity break in the outline of a box. When presented with the image of a box on an LED screen, the subject is asked to indicate the location of the open door within three seconds. The addition of a joystick provides subjects with four selection options- top, bottom, left, right- for the location of the open door, as well as a fifth option if they did not believe the open door to be present. Along with varying the colors of the box and the background and the location of the open door, the computer also varied the width of the open door from 1-6 pixels, allowing for an acuity number determination at a defined criterion value within this range. The goal of this study is to determine whether color acuity differences exist among various two color combinations and whether males and females exhibit color acuity differences. This study also examines whether or not background and foreground orientations of two colors affects the ability to discriminate between them. The analyzed results from 12 male and 12 female subjects showed significant color acuity differences among four different color combinations, but suggested no significant differences between genders or background vs. foreground orientation. All subjects experienced great difficulty in discriminating between color combinations including yellow, particularly when the combination of green and yellow.

Acknowledgments

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I. Introduction

A. Vision

Vision, defined as the ability to see, is essentially the perception of objects in the environment as the body converts the light they emit or reflect into chemical and electrical signals. Although the primary anatomical player in this process is the eye, the brain plays important roles as well. After light enters the eye, it is converted into chemical and electrical signals by photoreceptors. Visual information is then transmitted via the optic nerve to the portion of the brain able to decode the information. After directed to the appropriate regions of the brain, from the primary visual cortex located in the occipital lobe, these signals are neutrally translated and interpreted as the images we see.

B. Anatomy of the Eye

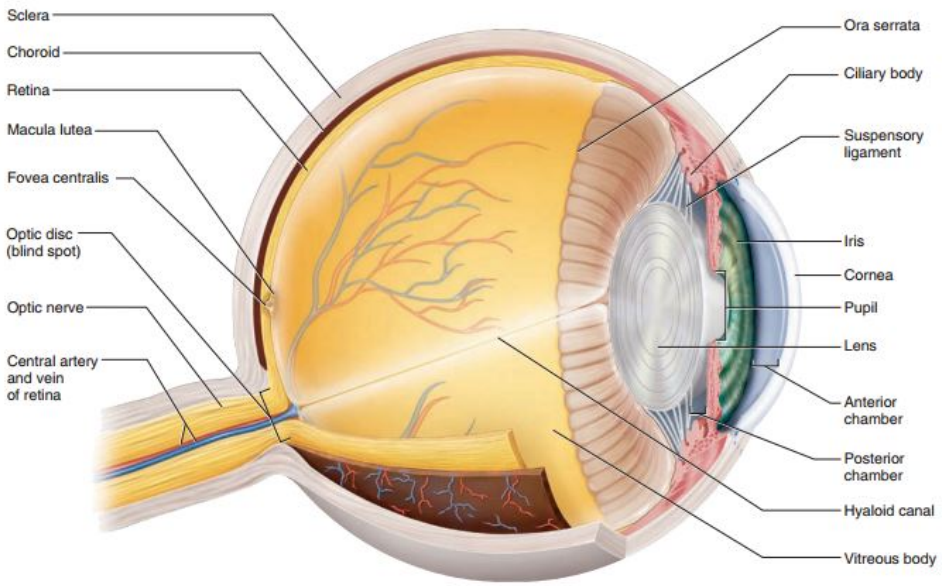


Figure 1.1: The human eye, sagittal section (Saladin, 2011).

The human eye is a complex structure. It is so efficient at facilitating the visual process that similar complex, image-forming structures have evolved in different types of organisms independently some 50-100 times (Land and Nilsson, 2002). Not only does the eye act as the gateway for light entering into the visual system, but it also contains the structure for signal conversion that transforms energy from light into a series of electrical and chemical signals that the brain is able to understand. The outer, most superficial aspect of the eye is a dense layer of vascularized, innervated, collagenous connective tissue called the sclera (Fig. 1.1). The transparent, anterior region of the sclera is called the cornea, and this is the portion of the eye that permits light entry. Because of its transparency and dome shape, the cornea acts as the outermost lens, refracting light back onto a structure actually titled the lens, and contributing 65-75% of the total focusing power of the eye ("Vázquez-Salceda, 2004). The lens, a transparent structure composed of highly compressed and flattened cells known as lens fibers, is another light focusing structure, responsible for refracting and focusing the light from the cornea onto the retina. Between the cornea and the lens is a central opening through which all light must pass called the pupil. The diameter of the pupil is controlled by contractile elements within an adjustable diaphragm-like structure called the iris; this structural duo further regulates the amount of light entering the eye at any given time. Upon arrival at the retina, the innermost layer lining the posterior two-thirds of the eye, the light encounters layers of visual receptors, wherein the process of phototransduction takes place (Saladin, 2011).

C. Retinal Design

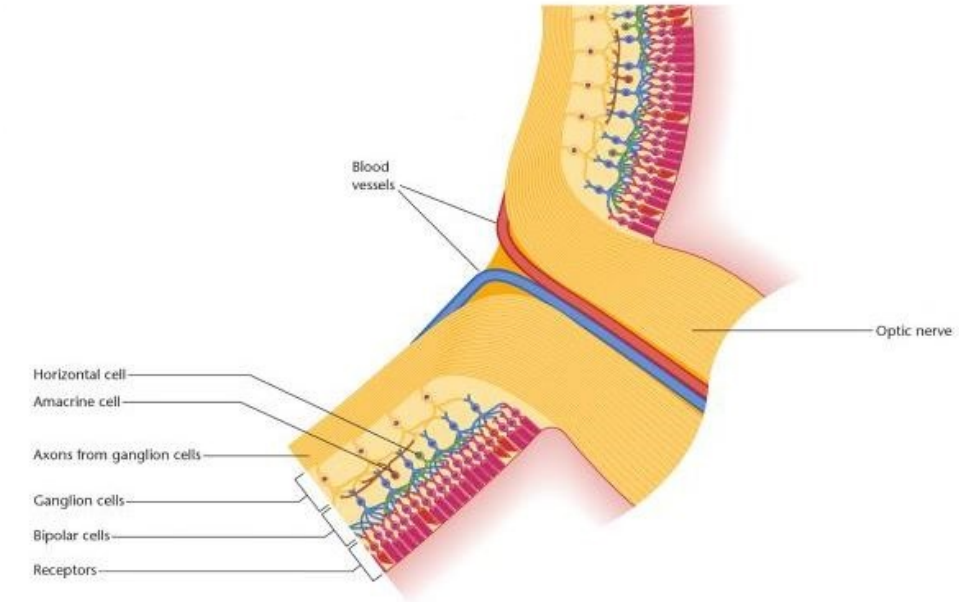


Figure 1.2: Organization of the retina (Kalat, 2009).

Embedded in the retina are six different types of neurons: receptors, Bipolar cells, Ganglion cells, Amacrine cells, Horizontal cells and Interplexiform cells. Collectively, these six cell types are responsible for converting light energy into the action potentials that travel to the brain for decoding via the optic nerve. The arrangement of these neurons facilitates the transmission of signals in a retrograde fashion (Kalat, 2009). This means light must first travel through each layer of cells to reach the photoreceptors, near the posterior aspect of the retina, which then transmit electrical signals back toward the center of the eye (Fig. 1.2). There are two types of photoreceptors located at the back of the eye: Rods and Cones. The differences between the two receptor types will be discussed in further detail later, but for now they are simply referred to as the receptors that are the first players in visual signal transmission. The major signal pathway from photoreceptors to the optic nerve takes the form of a three-neuron chain – photoreceptor to Bipolar cell to Ganglion cell – and is influenced laterally by Amacrine and Horizontal

cells (Fig. 1.3). Amacrine and Horizontal cells play diverse roles in the enhancement of perception of phenomena such as contrast, object edge definition, and light intensity. The Ganglion cells feed information directly into the optic nerve (Saladin, 2011). At every level of the retina, there are reciprocal loops within the circuitry that allow for feed-back laterally within a single layer, vertically between different layers, and from the brain back to the retina. Interplexiform cells are another type of retinal neuron responsible for signal transmission between the inner and outer plexiform layers of the retina, as well as efferent signaling from the brain back to the retina (Kolb, 2011).

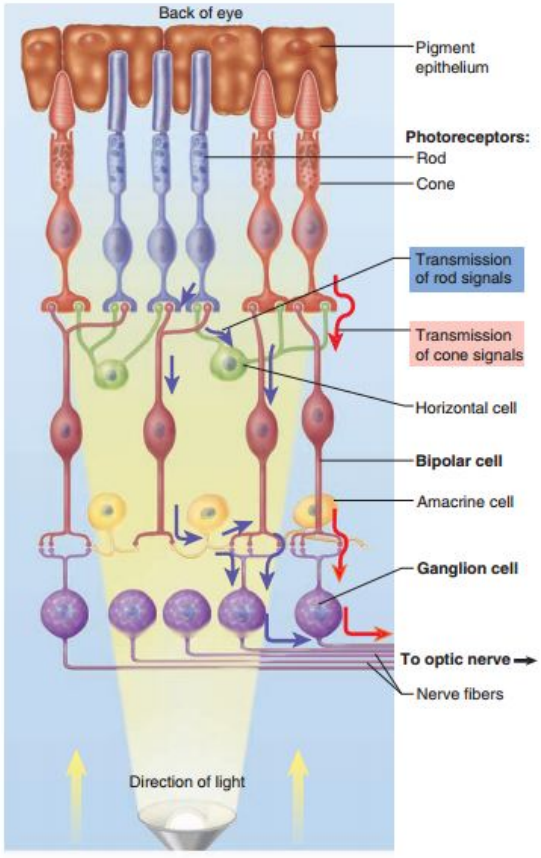


Figure 1.3: Schematic of the retinal cell layers (Saladin, 2011).

D. Phototransduction

Phototransduction is the conversion of light energy to electrical and chemical signals inside the photoreceptor. While both Rods and Cones have light-sensitive photopigments in their outer segments, the most highly studied photopigment is located in Rods, and is referred to as rhodopsin. Absorption of photons by rhodopsin is what initiates a biochemical signal cascade, ultimately leading to the propagation of signals from one neuron to the next. First, rhodopsin absorbs a photon and undergoes a conformation change from the 11-*cis* isomer to all-*trans* retinal, altering the protein component of the molecule known as opsin (Wang et al., 1994). This structural change then begins an activation cascade, activating first an intracellular messenger called transducin, in turn, activating a phosphodiesterase (PDE) that specializes in the hydrolysis of 3',5'-cyclic guanosine monophosphate (cGMP)(Fig. 1.4). The reduced concentration of cGMP within the disk membrane reduces the number of molecules available to bind to the sodium channels in the outer membrane, further resulting in the closure of these ion channels. Finally, this ion channel closure causes a hyperpolarization of the cell membrane in vertebrate photoreceptors and an altered rate of neurotransmitter release to postsynaptic neurons (Purves, 2004).

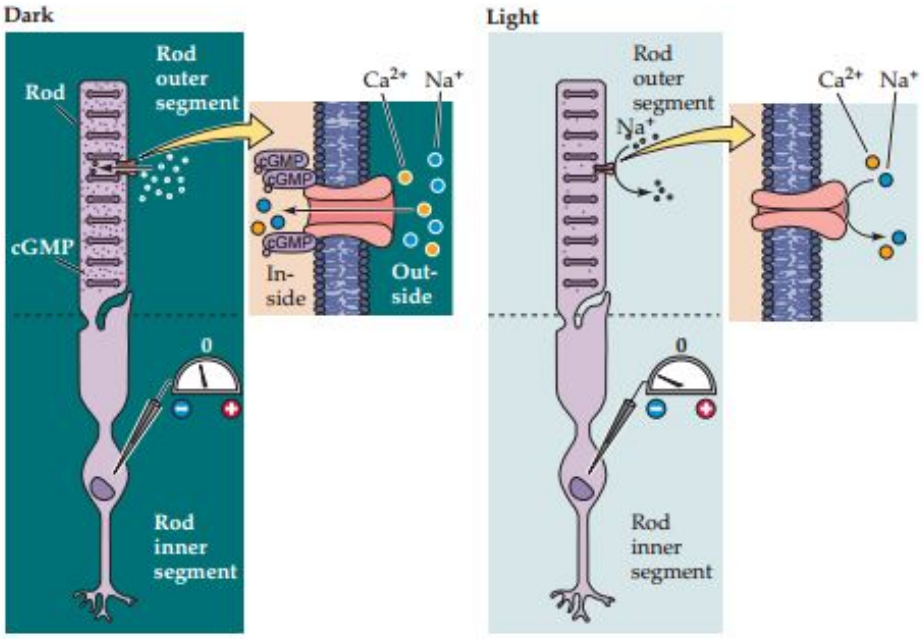


Figure 1.4: Low concentrations of cGMP within the outer segment of Rods during light exposure resulting in ion channel closure and membrane hyperpolarization (Purves, 2004).

The complex and specialized nature of this enzyme cascade affords a high level of signal amplification to the process. Estimates show that a single light-activated rhodopsin molecule triggered by a single photon has the ability to activate approximately 80% of the transducin molecules on the surface of the disk membrane. Despite the 1:1 ratio between transducin molecules and the activation of PDE molecules, each PDE is capable of catalyzing the breakdown of six cGMP molecules and, in turn, closing roughly 2% of the ion channels on the Rod membrane that were previously open in the dark. An equally important phenomenon, also resulting from the complexities of phototransduction, is that of light adaptation. Light adaptation refers to the inverse relationship between light intensity and receptor sensitivity; as levels of illumination increase, receptor sensitivity decreases, preventing complete receptor saturation and extending the range of light intensities over which they are able to maintain operation (Purves, 2004).

E. Visual Receptors: Rods vs. Cones

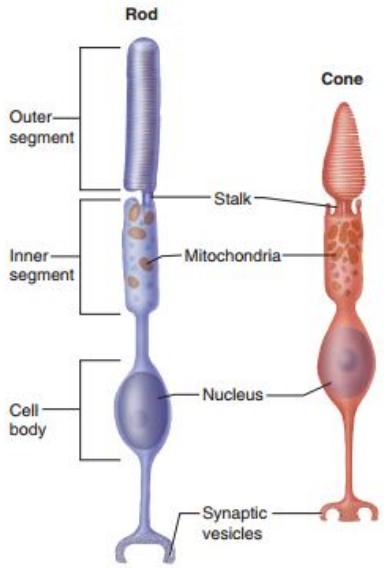


Figure 1.5: Structural differences between Rods and Cones (Saladin, 2011)**.**

Rods and Cones are the two types of photoreceptors located in the retina, differing both in their structure and their functional roles within the visual process. Rods have cylindrical outer segments, containing stacks of approximately 1,000 membranous disks that are densely packed with rhodopsin (Fig. 1.5). Abundant in the periphery of the retina and easily saturated with light, Rods are responsible mainly for night, or scotopic, vision. Rods are also only capable of monochromatic vision, producing images in strictly shades of grey. Cones are much different than Rods for several reasons. Structurally, the outer segments of Cones taper to a point, and the disks are parallel infoldings of the plasma membrane containing not just one, but multiple types of photopigments. Unlike Rods, Cones are relatively insensitive to light and are responsible for high acuity and color vision in variable light conditions, anywhere from dim to bright. The common understanding for why there are two types of photoreceptors is described in the idea known as Duplicity Theory. Duplicity Theory posits that a single type of photoreceptor could simply not be efficient at specializing in both high resolution and high sensitivity,

both of which are necessary for the high quality vision that humans experience. The structure and abundance of Rods, nearly 120 million located in the periphery of the retina, and the system of neural convergence within the retinal circuitry that permits significant signal amplification, allows for highly sensitive night and peripheral vision (Fig. 1.6)(Saladin, 2011).

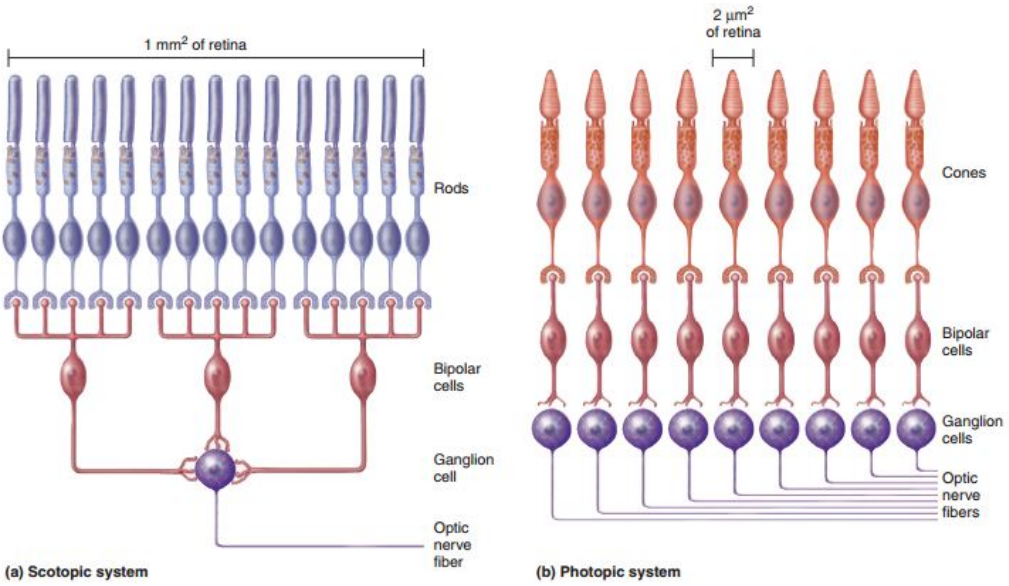


Figure 1.6: Retinal circuitry of Rods compared to Cones illustrating the effects of neural convergence on visual sensitivity (Saladin, 2011).

Cones, on the other hand, are much less abundant. Numbering only about 6 million compared to the 120 million Rods, Cones reside mainly in the fovea and give rise to an area of high resolution (Kalat, 2009). As the distance away from the fovea centralis increases, the concentration of Cones decreases and, consequently, the potential for precisely acute vision decreases (Fig. 1.7). For this reason, and because Cones are the primary photoreceptor responsible for color vision, it makes sense that the fovea would be the area of interest for studying color acuity.

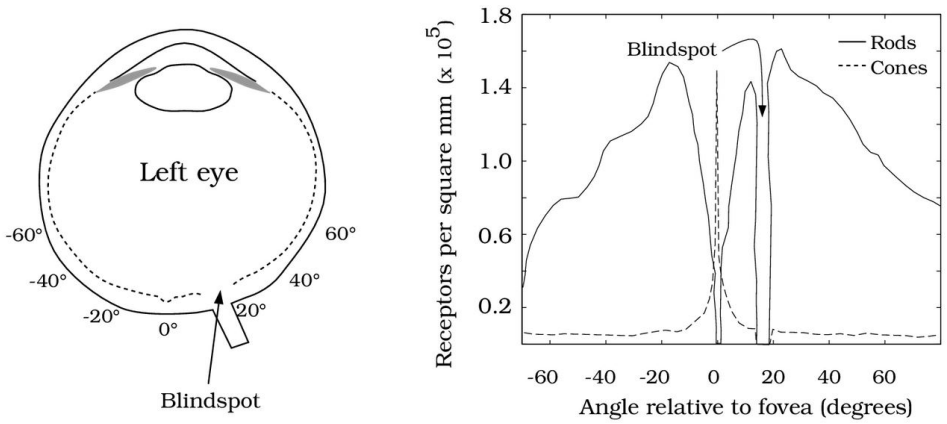


Figure 1.7: Distribution of Rods and Cones in the retina, relative to the fovea (Wandell, 2012).

F. Trichromatic Theory of Color

In the visual system of the average human, there are three different types of Cones based on the type of photopigment contained in their outer disks. Each of these three photopigments is maximally sensitive to a particular set of light wavelengths, giving rise to three different types of Cones: Short (S) wavelength cones, Medium (M) wavelength cones, and Long (L) wavelength cones, with respective peak absorptions of 419, 531, and 559 nanometers (Fig.1.8). According to the Trichromatic Theory, humans are able to perceive a full spectrum of color based on the relative response rates of the three types of Cones. The three Cones have previously been referred to as blue, green, and red, based on the wavelengths of light they absorb, however, this can be misleading due to the fact that the perception of color is ultimately dependent on the stimulation of two or more types of Cones in a particular ratio (Conway et al. 2010).

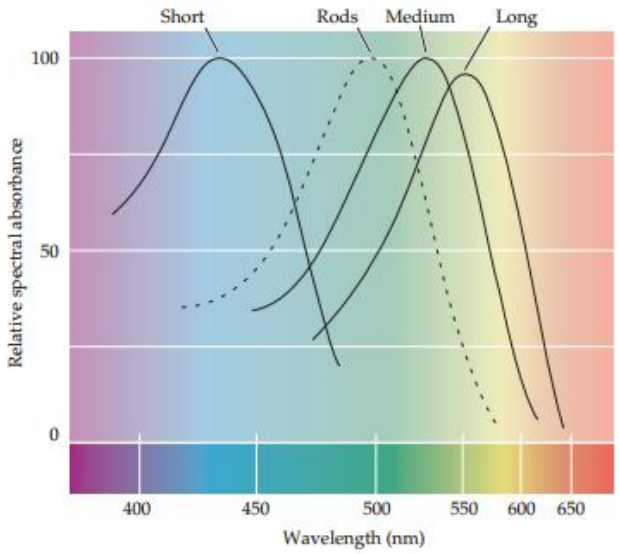


Figure 1.8: Absorption spectra of Rods and three types of Cones (Purves, 2004).

The three Cone types are not equal in abundance. On average, approximately 64% of the Cones in the retina are L-cones, 32% are M-cones, and a mere 2% are S-cones. The distribution of each Cone type in the retina varies among individuals, but generally speaking the M- and L-cones are most highly concentrated in the fovea centralis while the S-cones, with the highest sensitivity, are positioned peripherally to the fovea (Nave, 2014). Figure 1.9 shows an example of how the three Cone types might be arranged in the fovea, with an overall ratio of M- and L-cones to S-cones of 100:1.

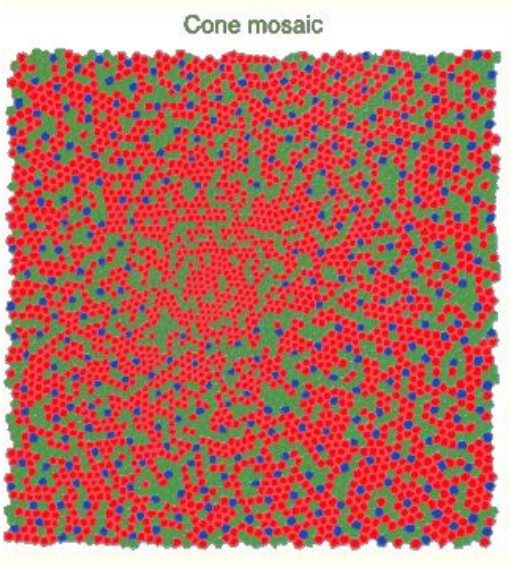


Figure 1.9: Sample distribution of Cones in the retina based on actual histological sections from a human eye (Montag).

G. Opponent-Process Theory

In 1878, Edward Hering proposed the Opponent-Process Theory, suggesting that humans perceive color in terms of opposites. This hypothesis is based on the idea that color perception is controlled by the activity of three independent receptor types that respond to opposing color pairs: white and black, blue and yellow, and green and red. The antagonistic manner in which these opponent color pairs excite and inhibit each receptor explains why humans cannot perceive two opponent colors simultaneously; for example, there is no such thing as "greenish red" or "yellowish blue". The Opponent Process Theory relies not only on the rate and ratio of stimulation among the three different Cone types, as is outlined in the Trichromatic Theory of Color, but also on the synaptic layout of Bipolar and Ganglion cells. Bipolar cells receive both excitatory and inhibitory stimuli from synapses with Horizontal cells and the three types of Cones, depending on the wavelengths of light that are present and being absorbed, and the total sum of this synaptic stimulation determines whether the Bipolar cell will be stimulated or inhibited

(Kandel et al., 2000). Figure 1.10 displays an example of what the wiring of one Bipolar cell might look like.

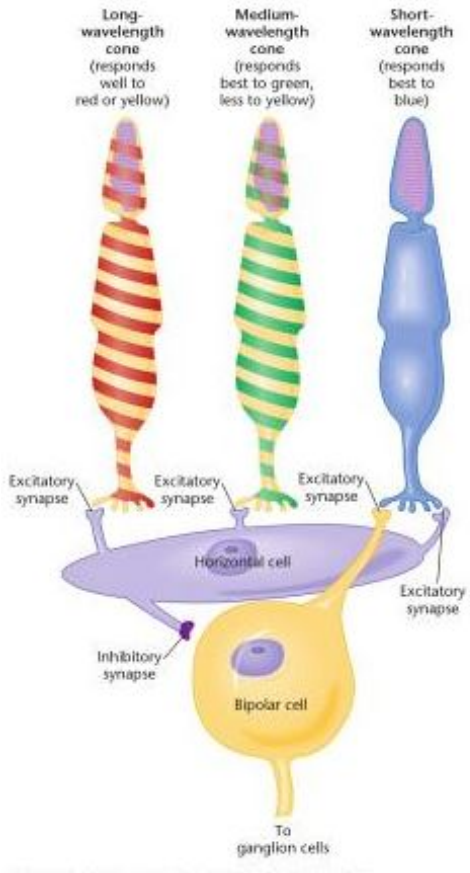


Figure 1.10: Possible synaptic wiring of one Bipolar cell (Kalat, 2009).

Bipolar cells proceed to transmit signals to Ganglion cells, where the bulk of the opponent process comes into play. The Ganglion cells responsible for handling the majority of color discrimination are called Parvocellular cells, or P cells. These P cells can be classified further into two subtypes, one subtype dealing with red-green differences and the other dealing with yellow-blue differences (Dubuc, 2014). Figure 1.11 illustrates patterns of excitation and inhibition of these two types of Ganglion cells that lead to the perception of the each of the four colors red, green, yellow, and blue.

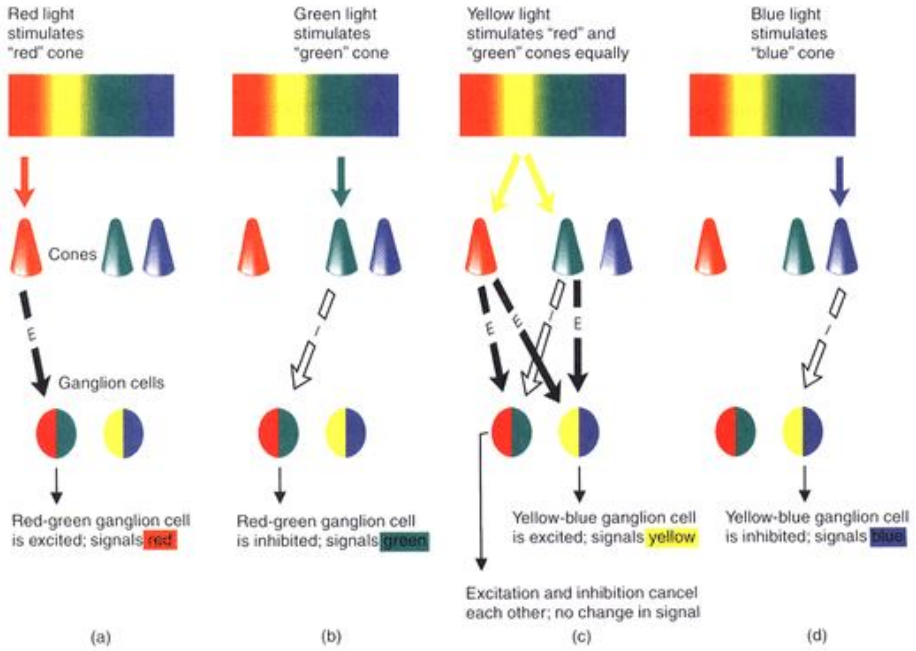


Figure 1.11: Color opponency in the retina (Carlson, 2011).

H. Visual Projection Pathway

The axons of retinal Ganglion cells are what make up the optic nerve- the direct carrier or signals from the retina to the brain. The optic nerve exits the retina through a region called the optic disk, colloquially referred to as the "blind spot" due to the lack of photoreceptors in this region. On the way to the lateral geniculate nucleus of the thalamus (the LGN), the optic nerves of both eyes converge and then immediately undergo hemidecussation at the optic chiasm where two separate optic tracts emerge, one leading to each hemisphere of the brain. At the LGN, a group of third-order neurons arise and function to project signals to the primary visual cortex of the occipital lobe where conscious image perception occurs (Saladin, 2011).

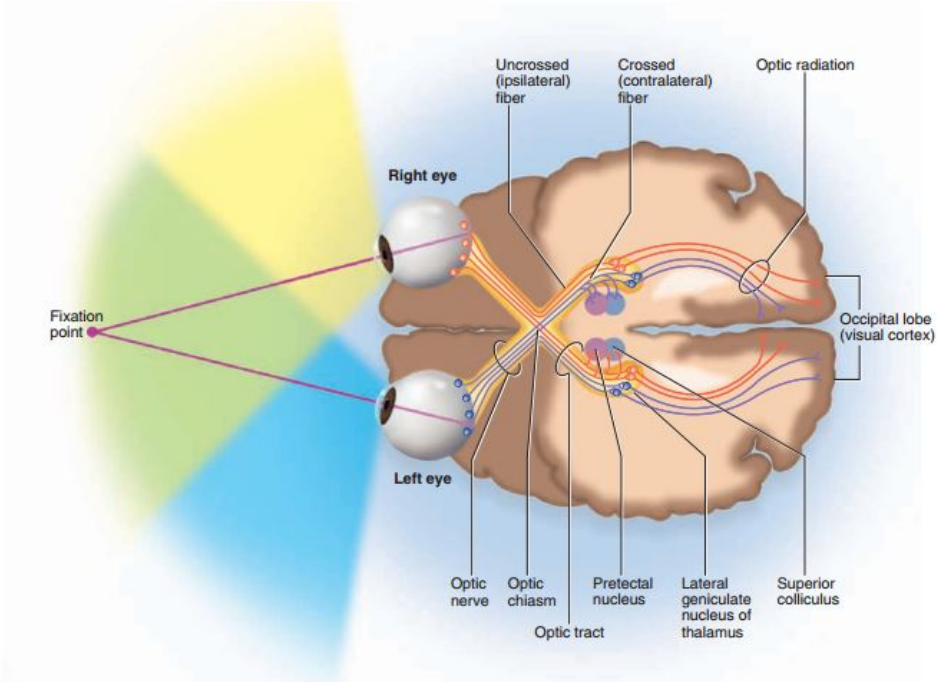


Figure 1.12: Visual Projection Pathway (Saladin, 2011).

Within the LGN, there are multiple layers of Parvocellular cells, Koniocellular cells, and Magnocellular cells that correspond to the red-green color vision and blue-yellow color vision signals from the retinal ganglia, and transmit the signals to organized columns of the primary visual cortex. The actual processing of color and color opposition is thought to occur in areas called “blobs” within the organized columns of the primary visual cortex (Fig. 1.13). Blobs are circularly symmetric and some contain the same color opposition structure as the P-ganglion cells that originate the visual pathways leading up to them (Dubuc, 2014).

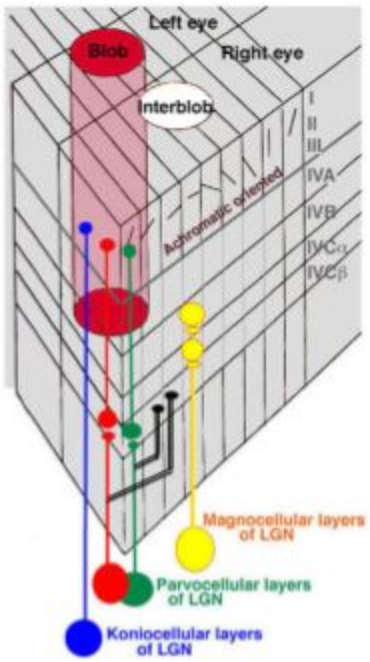


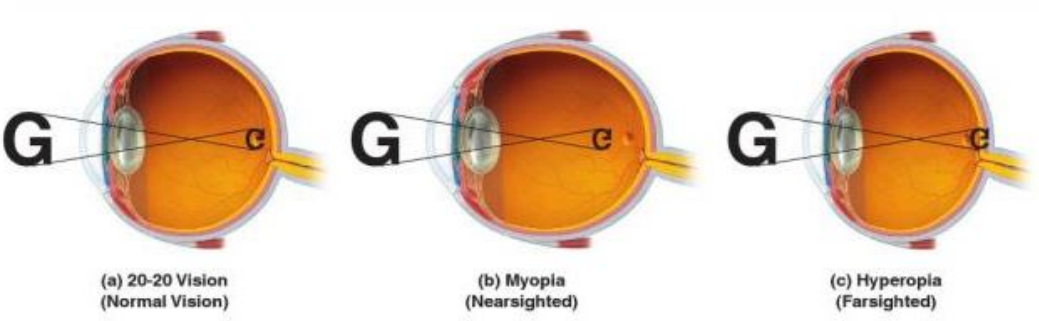
Figure 1.13: Blob located within the columns of the primary visual cortex (Kolb, Fernandez, and Nelson, 2007).

I. Visual Acuity

Visual acuity is defined as the clarity or clearness of vision, or how well a person sees. Acuity depends on many physiological factors such the sharpness of the retinal focus within the eye, the sensitivity of the nervous elements, and the interpretative abilities of the brain. The most common modern day tests for visual acuity employ the use of black and white eye charts containing letters, patterns, or shapes, depending on the test. The different types of acuity tests are classified by task, such as recognition, detection, or localization. Recognition tests, such as the Snellen Test, are the most common in today's clinical setting and they require the subject to identify the target image by name. Figure 1.14 shows a sample Snellen chart from which the subject must recognize and name letters.

Figure 1.14: Snellen Test (Kolb, Fernandez, and Nelson, 2007).

Detection tests are a simpler, less common acuity test in which the subject must indicate the presence or absence of a perceived stimulus, such as a dot or a line. Lastly, localization tests involve an object containing some sort of break or discontinuity in form that the subject must be able to identify. The measurement in this test is known as Vernier acuity, or "hyperacuity," because its resolution is markedly higher than a normal acuity test (Kolb, Fernandez, and Nelson, 2007).There are many factors that can affect one's visual acuity such as refractive error, pupil size, levels of illumination, time of exposure of the target, retinal area stimulated, eye movement, and the state of adaptation of the eye. Refractive errors such as myopia (short-sightedness) and hyperopia (far-sightedness) result in images being focused either in front or behind the retina (Fig.1.15). These two conditions can be combatted with the use of corrected lenses. (Kalloniatis and Luu, 2007).



Figrue 1.15: Eyeball shape affects visual acuity (Freberg, 2015).

A condition known as astigmatism is another case of refractive error where an imperfection of the cornea prevents it from properly focusing light onto a part of the retina, and causes a blurred area in an otherwise clear image (Heitig, 2015).

J. Gender Differences in Color Vision

Genetically speaking, there exists great potential for differences in male and female color vision. The potential for color blindness in males and females is a perfect example of a genetically based gender difference in color vision. As discussed previously, the average human possesses three types of Cones in their retina with three different types of light wave specific photoreceptors. Because of genetic defects, some people have only one or two pigment types, or possess all three with one type being abnormal. The most common color vison deficiency is red-green color blindness; due to a defect on the X-chromosome, where the genes coding for the pigments of L- and M-cones are located, sometimes these two Cones contain the same photopigment, resulting in an inability to distinguish between the colors red and green (Murray et al. 2012). The fact that this condition arises from a defect on the X-chromosome, means males are much more likely to inherit the color deficiency than females- approximately 8% of males are known to have the condition compared with less than 1% of females (Kalat, 2009).

Another X-linked visual condition, resulting in the potential for male and female color vision differences, is known as tetrachromacy. Tetrachromacy refers to a condition where a female exhibits higher color distinguishing capabilities than average due to the presence of a fourth photopigment. The appearance of this condition is explained by the genetic mechanism known as X-chromosome inactivation. The gene for L-cone pigment is located on the X-chromosome, so men only have the potential for producing one type of L-cone receptor. Women generally only express the genes from one X-chromosome per cell, a random process of X-chromosome activation and inactivation, but because they have two different X-chromosomes, the potential exists for the expression of two slightly different L-cone receptor genes and the production of a slightly different fourth photoreceptor. An estimated 15% of the female population act are carriers of X-linked color vision abnormalities (Jordan and Mollon, 1993). The greater prevalence of genetically based red-green color blindness in males and the potential for tetrachromacy in females suggests that the two gender populations may display inherent differences in color vision abilities.

II. Materials and Methods

A. Subjects

All of the subjects in this experiment were volunteer, undergraduate students from the University of Maine. Over 200 volunteers completed the experiment and out of these, 24 were selected for data analysis- 12 males and 12 females. The number of male subjects was a limiting factor, due simply to a lesser number of male volunteers. Since one of the primary goals of this thesis was to compare visual acuities between male and females, equal numbers of each gender were studied. The 12 female subjects were semi-randomly selected so as to match the general age demographic and average black and white acuity number of the male subject group. The final pool of selected subjects ranged in age from 18-28 years old, possessed neither color blindness nor any other known visual abnormalities, and most were enrolled in Bio 208 (Anatomy and Physiology), Bio 377 (Medical Physiology), or Bio 474 (Neurobiology) academic classes. Each subject received extra credit as a reward for participation.

B. Pre-Tests

Before participating in the experiment, subjects were first informed about the goals and specific details of the experiment before being asked to sign or agree to an informed consent form. Subjects were then taken through a series of pre-tests, both to rule out any existing visual abnormalities, potentially presenting as confounding variables, and to determine a baseline visual acuity for each subject so as to normalize the groups on the basis of black and white acuity. The pre-tests included a radial and a grid astigmatism test, the Ishihara Test for Color Blindness, and the Landolt C vision chart, testing for

black and white acuity (see Appendices E, F, G, and I). The subjects were also asked to fill out a confidential questionnaire, providing information about age, sex, health history, known visual impairments, and other personal information. All of the personal information gathered from these questionnaires was referenced using only each subject's designated identification number.

C. Determining Black and White Acuity

The Landolt C test was the chosen method for determining a baseline black and white acuity number for each subject, which would in turn be used to select subjects with similar acuities for analysis (Table 2.1). This was because it most closely resembles the computer generated and screen displayed open door acuity testing which was the result examined in this thesis. In the Landolt C test, the subjects were asked to sit in line with the eye chart, a controlled distance of 10ft. away, designated with a mark of tape on the floor of the lab. From this distance, the subject was asked to choose the first line of the chart in which the letters became difficult to read (for most subjects, this was somewhere between lines 9-11) and then proceed to read each subsequent line, from left to right, giving one of the following four responses to indicate the orientation of the opening of the letter C: "up", "down", "left", or "right". To match subjects based on the results of this test, I developed a single number score based on the percentage of correct responses for each line. For example, an acuity number of 12.4 means the subject correctly identified the side of the opening on all five of the 'C's in line 12, and then only correctly identified 40%, or 2 out of the 5, in line 13. By selecting subjects with comparable visual acuity numbers, using the black and white Landolt C acuity test, I was able to control for

potential inherent differences in normal acuities that would compromise my ability to draw conclusions specific to color acuity from the computer-generated open door experiment.

Gender	Age	Acuity Number		Gender	Age	Acuity Number
M	28	10.8		F	27	12.8
M	19	11.8		F	19	11.8
M	27	12.8		F	20	12.6
M	22	12.2		F	20	12.8
M	19	13.2		F	18	12.4
M	24	11.2		F	22	12.4
M	19	11.8		F	19	12.2
M	20	13.6		F	24	12.2
M	21	13.4		F	22	11.8
M	18	11.2		F	20	12.6
M	20	13.2		F	21	12.2
M	24	10.8		F	22	10.4
Average	21.8	12.2		Average	21.2	12.2
Standard Deviation	3.31	1.04		Standard Deviation	2.48	0.65
Range	18-28	10.8-13.6		Range	18-27	10.4-12.8

Table 2.1: Male and female age and acuity number data for all 24 subjects used in this study.

D. Experimental Setup

After obtaining informed consent and completing all of the preliminary testing, each subject was brought into the testing room to begin the open door computer-generated experiment. At the experimental station, the subject was seated behind a desk, 10ft. away from a flat, LED computer monitor from which the open door images were displayed (Figure 2.1). Located on the desk was a keyboard for navigating through the different sections of the experiment, a joystick for entering responses, an instruction sheet providing step by step instructions on how to proceed through the experiment, and a

couple of small, round, battery powered push lights for referencing the keyboard or the instruction sheet during the experiment since the experiment was performed in the dark.

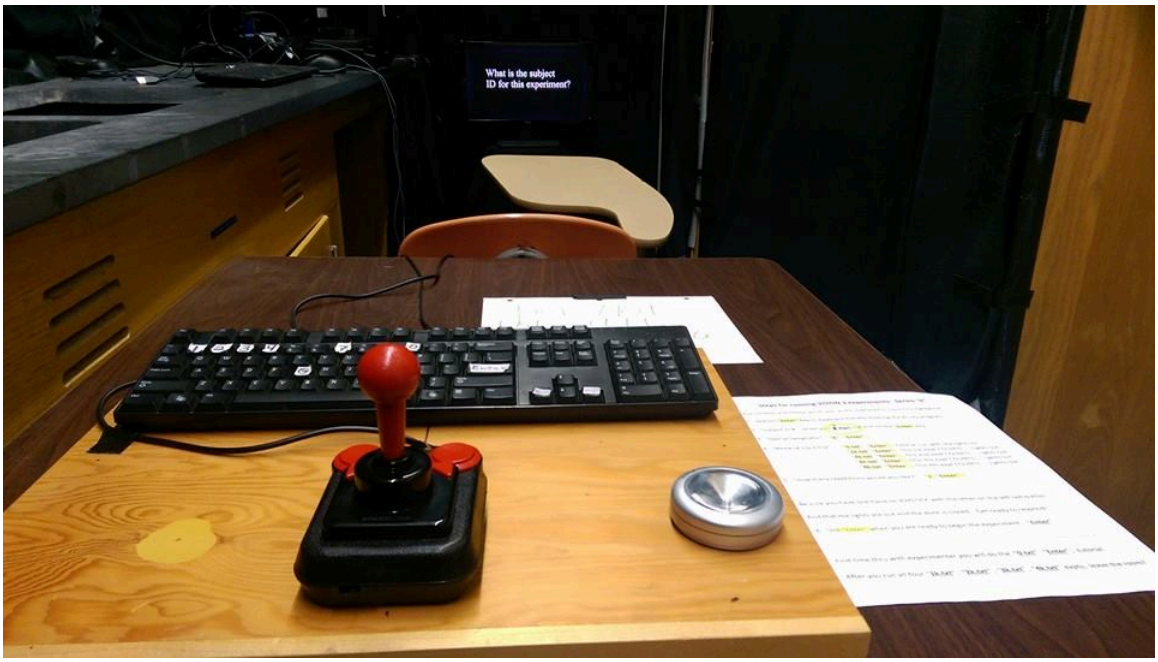


Figure 2.1: The experimental station.

Upon sitting down, the subject was given detailed verbal instructions on how to complete the experiment and walked through a practice tutorial to get a feel for the program and the responses required. After ensuring that the subject was comfortable with the experiment and had no further questions, the experimenter turned off the lights and left the room, closing the door on the way out to ensure the same level of darkness for all subjects. The following is an example of the verbal instructions dictated to the subject by the experimenter:

> What you are going to see during this experiment is a series of colored screens, each with a box in the center. What you will be looking for is a break in one of the four sides of this box- this is called the "open door". What you are asked to do each time you see a new screen with a new box, is to denote if and where you see this break in the box, using the joystick in front of you. There are four possible

> sides of the box on which the open-door may appear, therefore, four directions in which the joystick can be moved. For example, if you see a break on the left side of the square, move the joystick to the left, etc. It is also possible that you may not see a break at all; if this is the case, and the box appears to be complete on all sides, press the round, red button on the top left side of the joystick base. This gives you a total of five selection options. Keep in mind that each box will only remain on the screen for three seconds, so please make a selection within this time frame, even if you are unsure. I will stay here and walk you through a practice run so that you can see what you are looking for but afterwards, I will be leaving the room and turning off the lights while you complete the other four sections of the experiment. If you need light to navigate the keyboard between trials or refer to the directions, please use the push lights provided. Each section will contain a series of different screens, where the color of the background and the color of the box will change each time, and will take approximately 8 minutes, resulting in a total of just over 30 minutes required to complete the whole experiment. Feel free to take a break in between sections if needed. Once you have finished all four trials, you may turn on the light and open the door. Any questions? Good luck and have fun!

After completing the experimental portion, the subject was asked to fill out a post-test survey in which they rated certain aspects of the testing experience. The post-test was short, requiring 5-10 additional minutes to complete, and included questions on clarity of instruction, difficulty of the experiment, any blurriness, dizziness, fatigue, discomfort, or other unusual or unexpected feelings experienced, as well as an overall rating of his or her personal experience.

E. Experimental Design

The computer program used to conduct this experiment was created by Mike Murphy, a computer programmer with Sensory Cyber Systems, LLC (Orono, ME). This acuity program displays a box, centered on the screen, with a small break in the middle of one of four sides, referred to as the “open door” (Figure 2.2). The location of the open door, in the center of any one of the four sides of the displayed box, and the width of the

opening, ranged from 1-6 pixels. The subject was asked to identify, with an appropriate movement of the joystick, which side of the box contained the opening, as the openings themselves varied from 1-6 pixels. The other two variables altered were the color of the box outline and the background color of the screen. The colors used in this experiment were black, grey, green, red, and yellow.

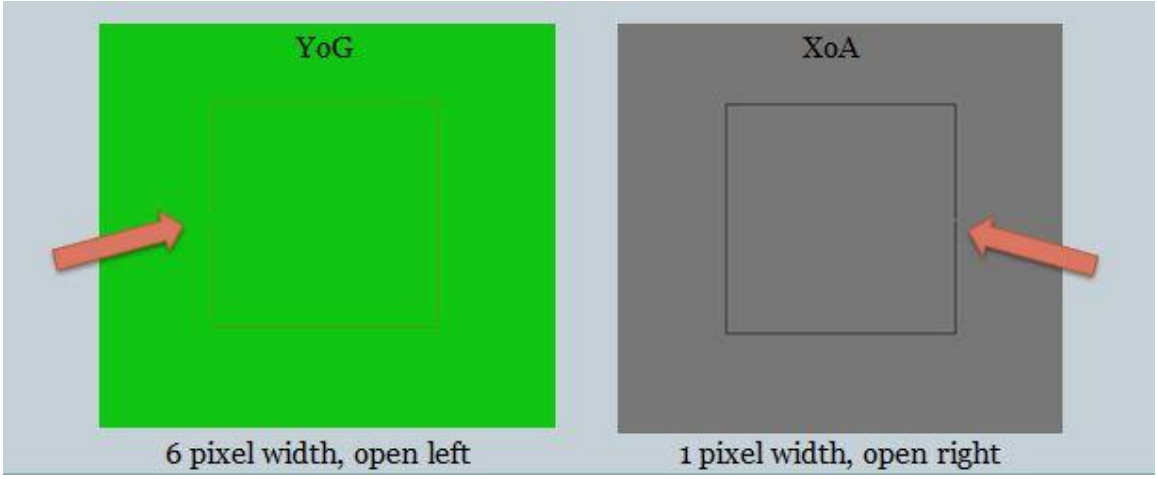


Figure 2.2: Sample trial screens as seen in the experiment. "YoG" denotes a yellow box on a green background; "XoA" denotes a black box on a grey background. Arrows indicate location of open door. Image courtesy of Lindsey Gori.

For my experiment, I used what is called the "A" series, designated as such based on the color combinations included. The "A" series contained a total of 14 different color combinations, eight of which I chose to analyze (Table 2.2). Each of the four colors, namely red, green, yellow, and grey, were displayed as either background or foreground (i.e. box) colors, resulting in 12 combinations. Both combinations of black and grey were used as well, making a combined total of 14 color combinations. The abbreviations for the colors used are as follows: red (R), green (G), yellow (Y), grey (A), black (X). The notation denotes the letter of the box color first and the letter of the background color last. For example, a red box on a green background would be denoted RoG.

		Color of Box (Foreground)				
		Red	Green	Yellow	Grey	Black
Color of Background	Red	--	GoR	YoR	AoR	--
	Green	RoG	--	YoG	AoG	--
	Yellow	RoY	GoY	--	AoY	--
	Grey	RoA	GoA	YoA	--	XoA
	Black	--	--	--	AoX	--

Table 2.2: All 14 color combinations seen in the "A" series; the eight highlighted combinations represent those chosen for analysis.

Each of the three second screens that appear in the experiment, containing one colored box on a single, different colored background, is considered one trial. For each of the 14 color combinations, there were 24 trials- one for each of six pixel widths paired with each of four open door locations (6 pixel widths x 4 locations = 24 trials per color combination). This means that there were a total of 336 trials in the entirety of the "A" series (14 color combos x 24 trials each = 336 total trials). Between the three seconds for each trial and the countdown in between, these 336 trials took approximately 32 minutes to complete. Because this amount of time is lengthy to maintain complete concentration, the 336 trials were divided into four different sections, designated 1a, 2a, 3a, and 4a, in hopes of reducing the effects of fatigue as it would provide intervening breaks.

In order to analyze the multiple manipulated variables, there were a couple of other variables that needed to be kept constant; these variables have to do with light. The amount of light a subject is exposed to can have significant effects on the visual process, due to the differing numbers of photons that bombard the retina under different intensities of light. To control for ambient light in the room, the room lights were turned off and the

door was closed for the duration of the experiment. There were no windows in the room, so sunlight was not a factor. The light emitted from the LED computer monitor, also needed to be controlled. The same monitor was used for all subjects, and they were all seated at the same distance away from the screen, therefore the light emitted was the same for all subjects. The intensity of light emitted from the LED monitor, as it was displaying different colors, was also measured, using a photodiode and a voltmeter, to ensure matching intensities for all colors except for black. This measure was taken to ensure that subjects would not be influenced by higher and lower light intensities associated with particular colors, and that the only distinguishing factor between screens would be the specific wavelength of light (i.e. color) displayed.

The data collected in this experiment were a series of "true" or "false" answers to each trial and were saved in a text file on the computer. These files were later converted into an Excel worksheet for further analysis. These results were compiled into a percent correct response for each pixel width and color combination. With each trial, the subject was given only three seconds to make a decision and enter an answer. If the subject correctly identified the side on which the open door was located, then the computer logged the answer as "true". If the subject chose any of the other three sides, pressed the button signaling that they could not see a break, or neglected to provide an answer at all, the computer logged this as a "false" answer. The purpose of the time limit and stressing to the subject the importance that they enter a response, even if they had to guess, was to impose a forced choice that would allow us to distinguish between times when the subject could clearly see the gap and times when they were only guessing. The subjects were told

they had five selection options, so despite the fact that there were only four possible locations for the open door, the presence of an implied fifth option technically reduced the odds of choosing correctly due to random chance from 25% to 20%. Conversely, if the subject could clearly see the gap in every trial, and chose correctly every time, the percent correct response would be 100%. I define the halfway mark between these two extremes as the subject's acuity for that color combination. By graphing the percent correct response against the varying pixel widths for any given color combination, I was able to determine the acuity value. For example, if subject X has a curve that shifts to the left, with a 60% criterion point that lies somewhere between 1-3 pixels, this subject would be considered to have a higher acuity than subject Y, with a right-shifted curve and a 60% criterion point occurring between 4-6 pixels. In this example, subject X was able to discern, with a higher rate of success, the location of a smaller break in the box, than could subject Y.

F. Distinguishing Foreground from Background

Another one of the goals of this study was to determine the effects of foreground and background colors on the measured acuities. The color of the box and the color of the surrounding screen were designated foreground (the box) and background, respectively, and as mentioned before, each two color combination was tested both ways with both colors in both locations. For example, when looking at the color combination of red and green, there were both trials with a red box on a green screen, a situation referred to with the notation "RoG", as well as trials with a green box on a red screen, given the opposite notation of "GoR". In separating the two scenarios, the goal was to determine whether or

not the foreground and background orientations were a factor in the subject's ability to differentiate. In most cases, the finding was that the orientation was not a factor in color acuity determination, meaning the results were similar for a particular color combination despite which color was in the foreground and which was in the background. Because of this finding in the majority of the color combinations analyzed, I decided to construct another group of data sets for analysis in which a single graph would represent the subject's color acuity for adding together the two color combination, regardless of orientation. In this case, the curve would not be notated with GoR or RoG, but rather G&R. These graphs were shaped with twice the number of subject data points, making the curves a little cleaner and more reliable when attempting to interpret acuity differences from one color combination to the next. The only color combination that was unable to be combined in this manner was the black and grey combination. Due to a programming error, the XoA data was unreadable at the third pixel width. The graph of the black on grey data was relatively comparable to the grey on black data, minus of course the disappearance of the curve at the third pixel width, but for the sake of preserving the integrity of the AoX data, I decided not to combine the two. For all comparisons of the black and grey combination with the other color combinations, I simply used the AoX results.

G. Statistical Analysis

Microsoft Excel was the analytical tool used to determine statistically significant differences within the data. Tables 3.2, 3.3, 3.4, and 3.9 were compiled using p-values of the data in the figures directly above them. Because each of these figures contained only

data from a single subject group, either all humans, male, or females, the p-values were obtained through a series of paired, two-tailed t-tests. Tables 3.5, 3.6, 3.7, and 3.8 were also constructed using p-values of the data in the figures directly above them; however, a different t-test had to be used to look to statistical significance. These figures represented a particular color combination, containing data on the color acuities of multiple subject groups, so the p-values displayed in these four tables were obtained through a series of two-sample equal variance two-tailed t-tests.

III. Results

A. Color Acuity Differences for All 24 Subjects

When comparing the average color acuities for all subjects and for all color combinations (except for XoA), I found that each line followed a general upward trend from 1-6 pixels (Figure 3.1). The lines all begin at approximately 10% at one pixel width and trend upward at different rates. The starting percentage of 10%, which is even less than a random chance of 20%, signifies that the subjects occasionally failed to respond during the three second time frame for each trial or that they did end up pressing the joystick button indicating the suggested, but never occurring, possibility of the box having no open door. The grey on black (AoX) color combination displayed the highest acuity, reaching 60% correct response at an estimated pixel width of only 1.97. The only other color combination that actually reached the inflection point on the graph was the green and red (G&R) color combination, with a 60% correct response at an interpolated pixel width of 4.91. The color acuities of the other two color combinations, both containing yellow (R&Y and Y&G), were too low to even reach the 60% criterion value on the graph. Estimated inflection points were extrapolated for the R&Y combination, using the slope of the line between the 5^{th} and 6^{th} pixel widths (Table 3.1). The Y&G inflection point could not be calculated with any reliability due to the unpredictable shape of the lines and the fact that the data never reached above 30% correct response.

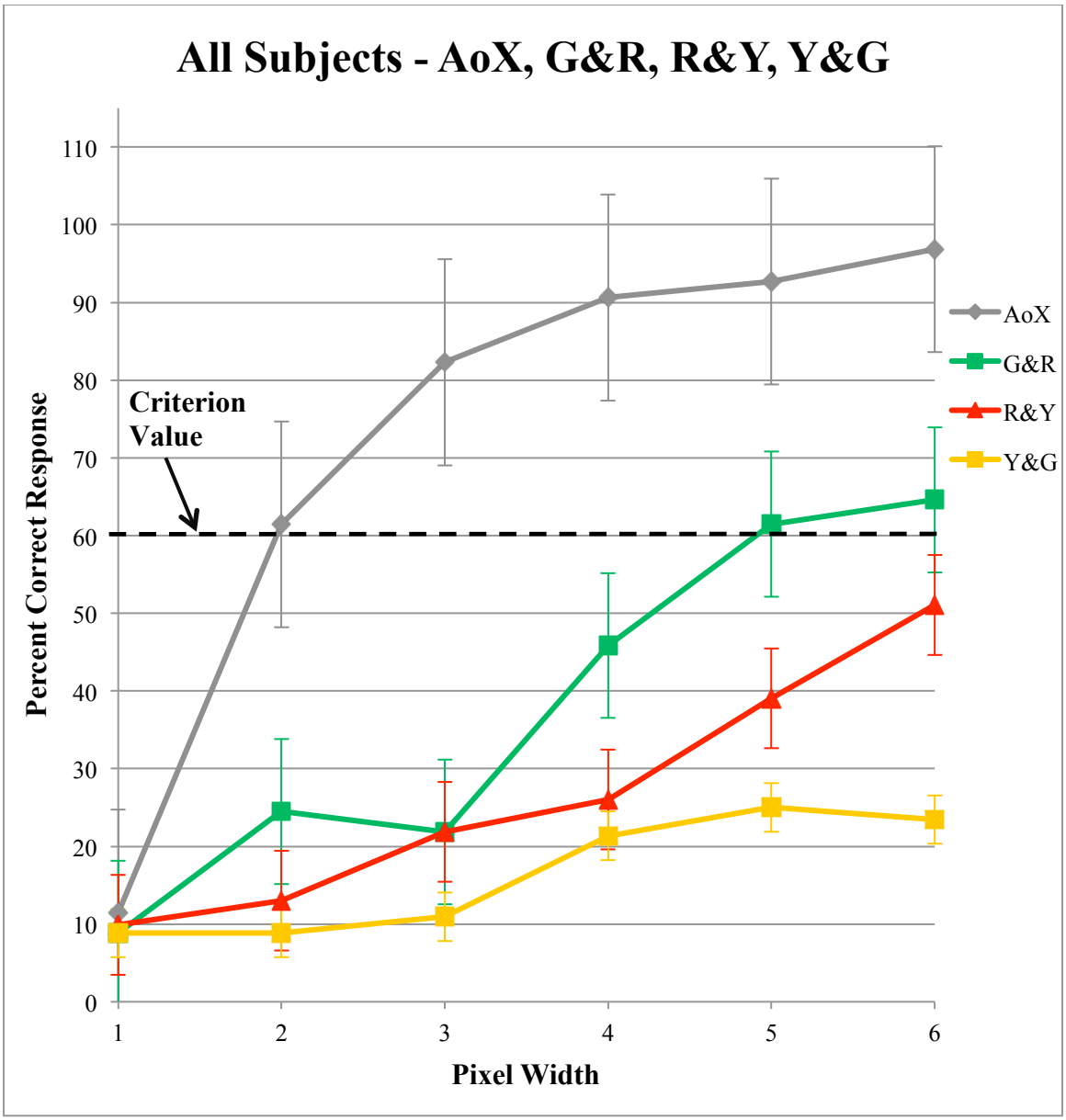


Figure 3.1: Differences in color acuities for all 24 subjects. Error bars represent standard error for each point.

Estimated Inflection Points (60%)				
Color Combination		Humans	Males	Females
	AoX	1.97	1.83	2.32
	G&R	4.91	4.91	4.9
	R&Y	6.74*	6.97*	6.46*
	Y&G	---	---	---

Table 3.1: Inflection points for all subjects and all color combinations. Asterisk indicates extrapolated inflection points not appearing on graph.

Standard error bars were added to each point in Figure 3.1 in order to compare statistically significant differences in average percent correct response at each pixel width, for each color combination. Overall, I found that the subjects demonstrated the highest acuity when viewing the grey on black color combination. This color combination resulted in significantly greater correct response rates, from 2-6 pixels, than any other color combination (Table 3.2). The next highest human color acuity was found for the green and red color combination. This combination was significantly higher than both combinations containing yellow at the 2nd, 4th, 5th, and 6th pixel widths. Subjects demonstrated the lowest color acuity when viewing the color combination of green and yellow.

P-values for All Subjects (t-test)		Pixel Width				
		2	3	4	5	6
Color Combination	AoX/G&R	<.001*	<.001*	<.001*	<.001*	<.001*
	AoX/R&Y	<.001*	<.001*	<.001*	<.001*	<.001*
	AoX/Y&G	<.001*	<.001*	<.001*	<.001*	<.001*
	G&R/R&Y	0.007*	1.00	<.001*	<.001*	0.021*
	G&R/Y&G	<.001*	0.021*	<.001*	<.001*	<.001*
	R&Y/Y&G	0.118	0.001*	0.322	0.007*	<.001*

Table 3.2: P-values of human color acuities for all different color combinations at pixel widths of 2, 3, 4, 5, and 6. Asterisk indicates statistical significance (p<.05).

B. Male Color Acuity Differences

Figure 3.2 displays the average percent correct response rate among male subjects for each of the four color combinations analyzed. The error bars represent the standard error at each pixel width. The male subject group displays similar results as the human subject group. Males displayed the highest correct response rate when viewing the grey on black (AoX) color combination, at all pixel widths of two or more (Table 3.3). The second highest correct response rate is associated with the green and red (G&R) color combination, as can be seen in the significant differences between this color combination and the two containing yellow at the 4^{th}, 5^{th}, and 6^{th} pixel widths. The lowest male color acuities were associated with those combinations containing yellow (R&Y and Y&G); whether or not there is a significant difference between the two is slightly more unclear from the data. The red and yellow combination (R&Y) results in a greater percent correct response rate than the yellow and green (Y&G) combination, at the 3^{nd} and 6^{th} pixel widths, but the data does not appear to be significantly different at the pixel widths in between. While neither of these lines reaches the 60% criterion value for the pixel widths tested, it would appear that a distinction between the two color combinations begins to appear at the sixth pixel width, as the R&Y line approaches 50% correct and the Y&G line hovers just slightly above 20% correct, signifying random chance.

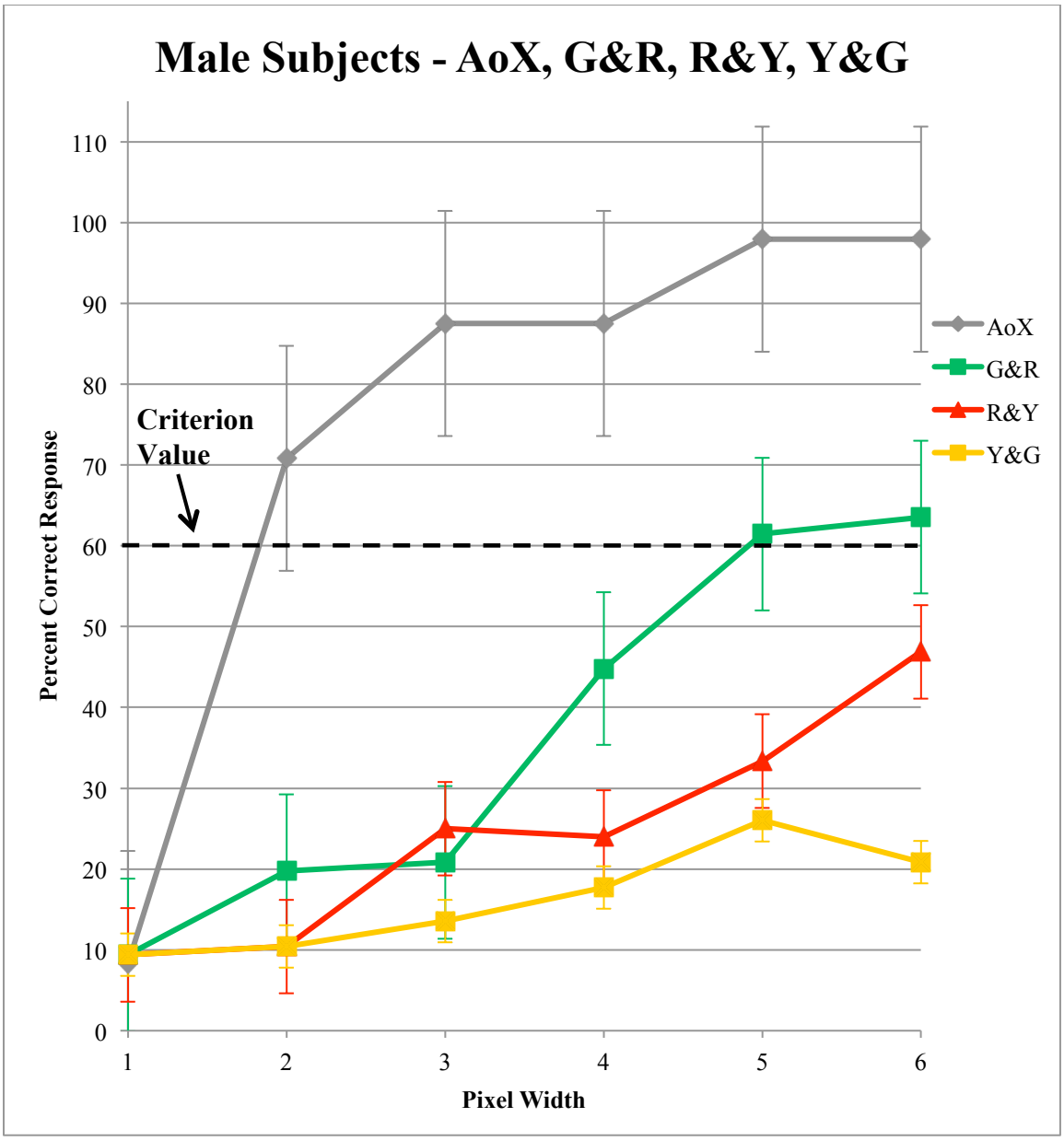


Figure 3.2: Differences in color acuities for all 12 male subjects. Error bars represent standard error for each point.

P-values for Male Subjects (t-test)		Pixel Width				
		2	3	4	5	6
Color Combination	AoX/G&R	0.002*	<.001*	0.005*	<.001*	0.001*
	AoX/R&Y	<.001*	<.001*	<.001*	<.001*	<.001*
	AoX/Y&G	<.001*	<.001*	<.001*	<.001*	<.001*
	G&R/R&Y	0.142	0.357	0.009*	<.001*	0.050*
	G&R/Y&G	0.095	0.166	0.001*	<.001*	<.001*
	R&Y/Y&G	1.00	0.013*	0.354	0.258	<.001*

Table 3.3: P-values of male color acuities, for all different color combinations, at pixel widths of 2, 3, 4, 5, and 6. Asterisk indicates statistical significance (p<.05).

C. Female Color Acuity Differences

Figure 3.3 displays the average percent correct response rate among female subjects for each of the four color combinations analyzed. The error bars represent the standard error at each pixel width. The female subject group displays markedly similar results as both the male, and logically, the human subject group. Females, like males, displayed the highest correct response rate when viewing the grey on black (AoX) color combination, at all pixel widths of three or more (Table 3.4). The second highest correct response rate is again, associated with the green and red (G&R) color combination, as can be seen in the significant differences between this color combination and the two containing yellow. The lowest female color acuities were associated with those combinations containing yellow (R&Y and Y&G); unlike males however, the distinction between the two is more clear. The red and yellow combination (R&Y) results in a significantly greater percent correct response rate than the yellow and green (Y&G) combination, at the 2^{nd}, 3^{rd}, 5^{th} and 6^{th} pixel widths. Like the males, neither of these lines reaches the 60% criterion value for the pixel widths tested, but at the sixth pixel width, the R&Y has a clear positive slope and surpasses the 50% correct line while the Y&G line is nearly horizontal and hovers

around a mere 25% correct line. This suggests that females were ultimately unsuccessful in distinguishing between the colors yellow and green.

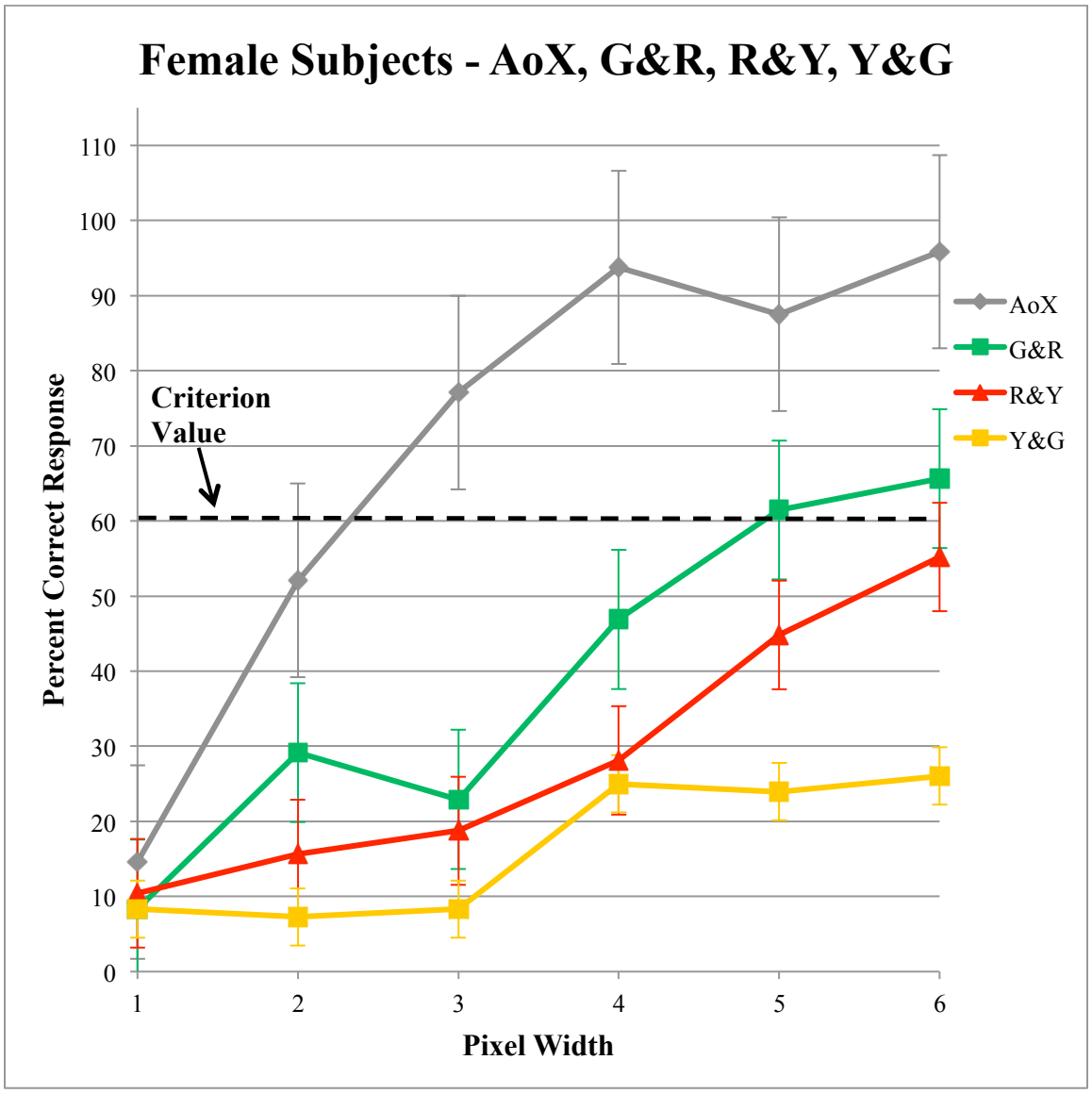


Figure 3.3: Differences in color acuities for all 12 female subjects. Error bars represent standard error for each point.

P-values for Female Subjects (t-test)		Pixel Width				
		2	3	4	5	6
Color Combination	AoX/G&R	0.152	<.001*	<.001*	0.032*	0.020*
	AoX/R&Y	0.011*	<.001*	<.001*	0.001*	0.004*
	AoX/Y&G	0.002*	<.001*	<.001*	<.001*	<.001*
	G&R/R&Y	0.020*	0.610	0.017*	0.057	0.211
	G&R/Y&G	0.001*	0.070	0.003*	<.001*	<.001*
	R&Y/Y&G	0.008*	0.038*	0.649	0.012*	<.001*

Table 3.4: P-values of female color acuities for all different color combinations at pixel widths of 2, 3, 4, 5, and 6. Asterisk indicates statistical significance (p<.05).

D. Male vs. Female Color Acuity Differences

Figures 3.4, 3.5, 3.6, and 3.7 illustrate comparisons of average male and average female color acuities for the following color combinations, respectively: grey on black (AoX), green and red (G&R), red and yellow (R&Y), and yellow and green (Y&G). Error bars on all four graphs display standard error at each pixel width. Tables 3.6, 3.7, 3.8, and 3.9 display p-values for each data set comparison illustrated in the figures directly above them. Based on the values in these tables, there are no significant differences in male and female color acuities for any of the four color combinations. The male AoX line has a lower value at the 60% criterion value, which suggests that male color acuity is greater for this color combination, but the insignificance shown in the statistical tests does not support this claim. As stated before, both genders displayed extremely poor acuities when discriminating between green and yellow. Neither the male nor the female line displays a consistently positive trend for this color combination, and both fail to ever reach above a 30% correct response rate.

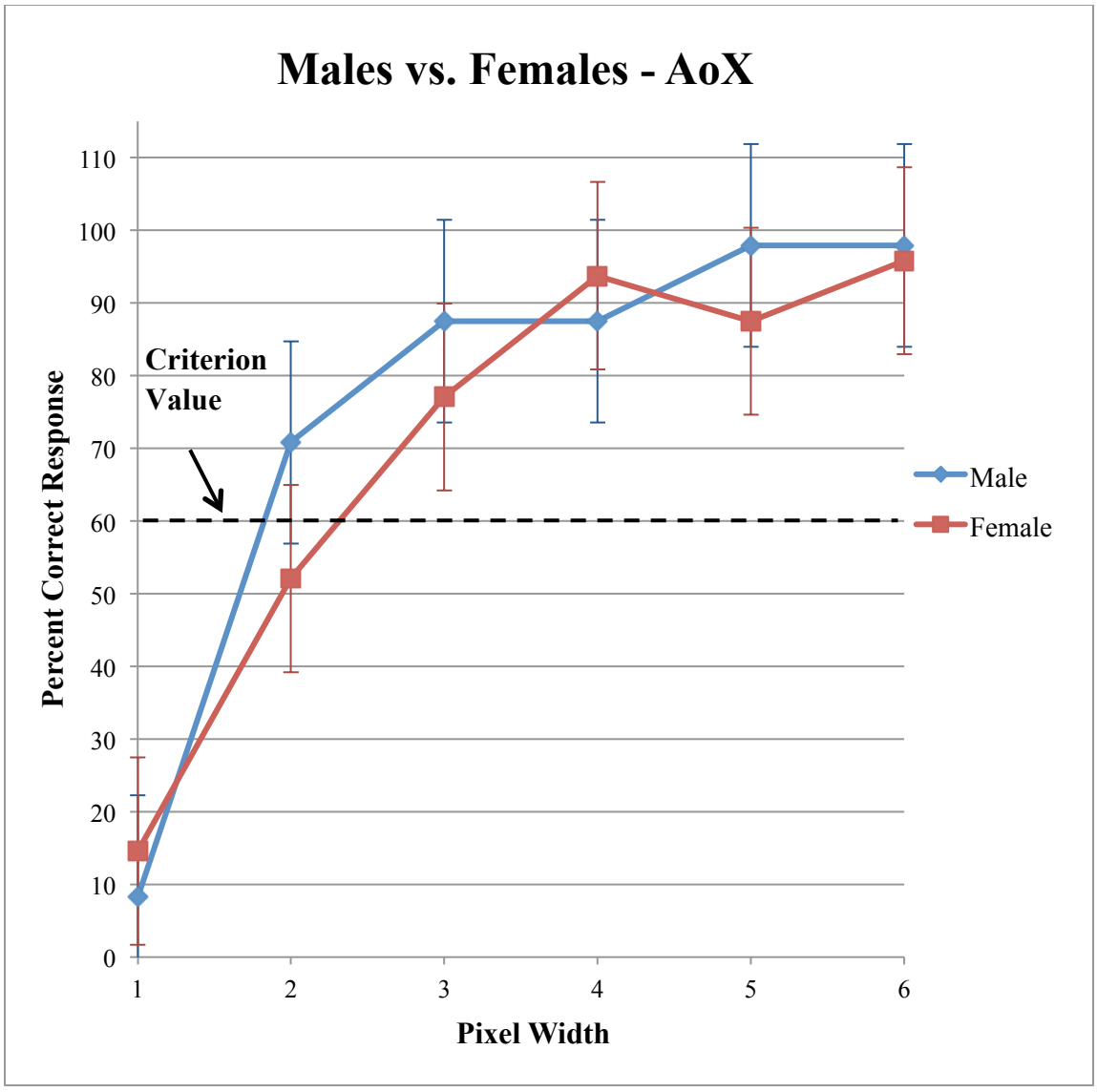


Figure 3.4: Average male and average female grey on black color acuities. Error bars display standard error for each percent correct response point.

AoX (two-sample variance t-test)	Pixel Width				
	2	3	4	5	6
P-values	0.264	0.375	0.519	0.143	0.659

Table 3.5: P-values comparing male and female color acuities for the color combination grey on black, from 2-6 pixel widths. No statistically significant differences found.

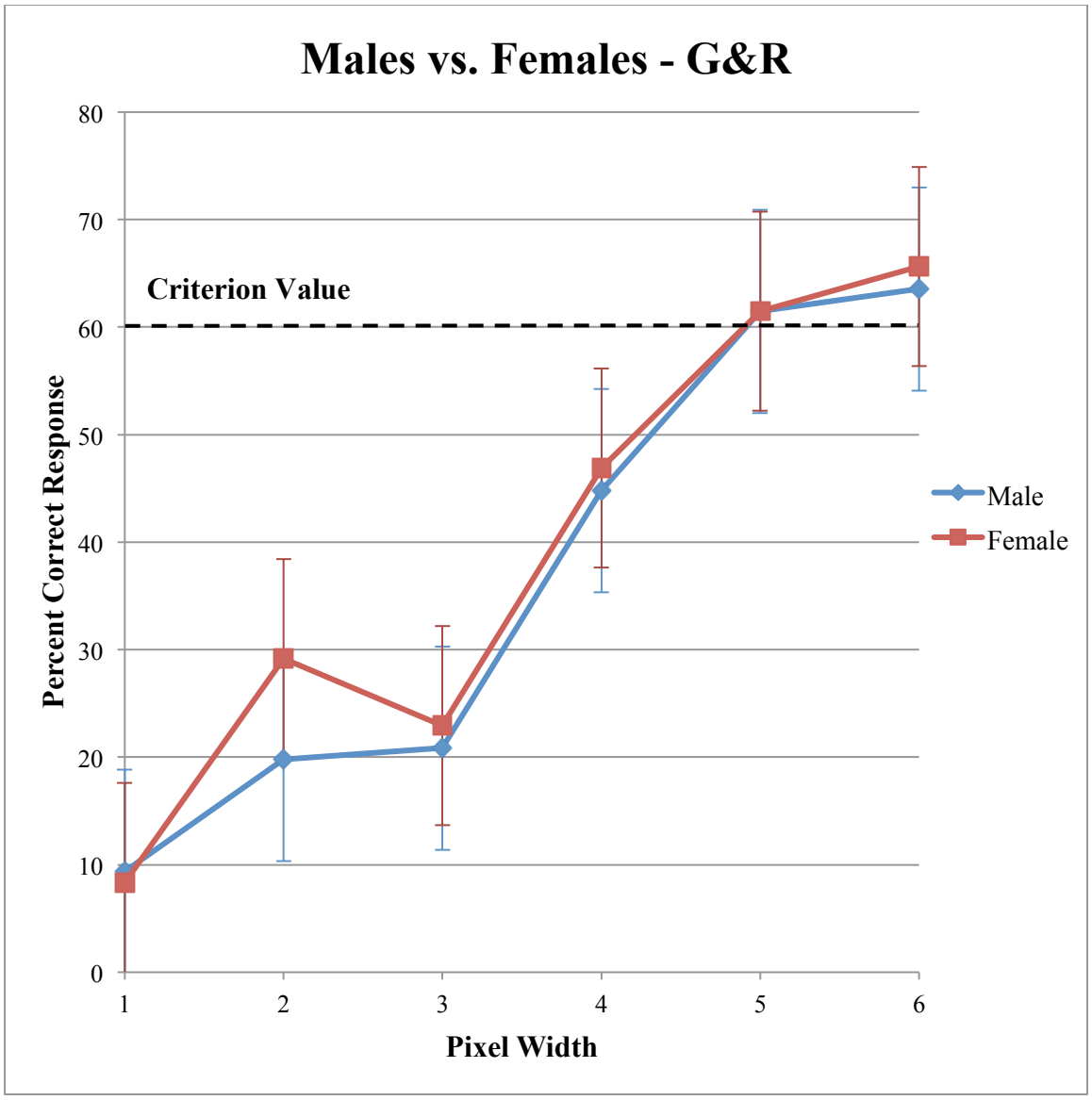


Figure 3.5: Average male and average female green and red color acuities. Error bars display standard error for each percent correct response point.

G&R (two-sample variance t-test)	Pixel Width				
	2	3	4	5	6
P-values	0.242	0.812	0.840	1.000	0.813

Table 3.6: P-values comparing average male and female color acuities for the color combination green and red, from 2-6 pixel widths. No statistically significant differences found.

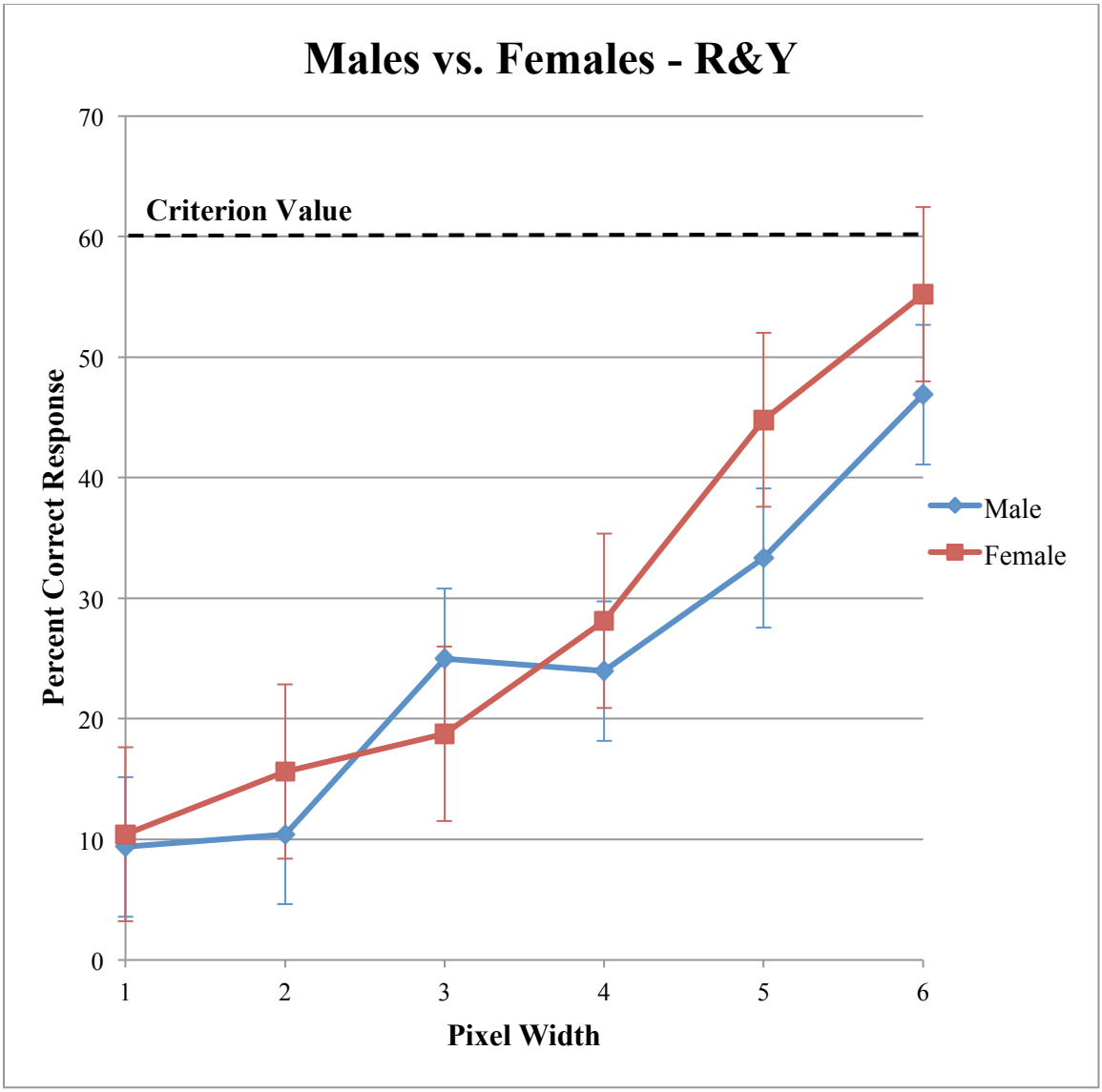


Figure 3.6: Average male and average female red and yellow color acuities. Error bars display standard error for each percent correct response point.

R&Y (two-sample variance t-test)	Pixel Width				
	2	3	4	5	6
P-values	0.296	0.383	0.636	0.290	0.452

Table 3.7: P-values comparing average male and female color acuities for the color combination red and yellow, from 2-6 pixel widths. No statistically significant differences found.

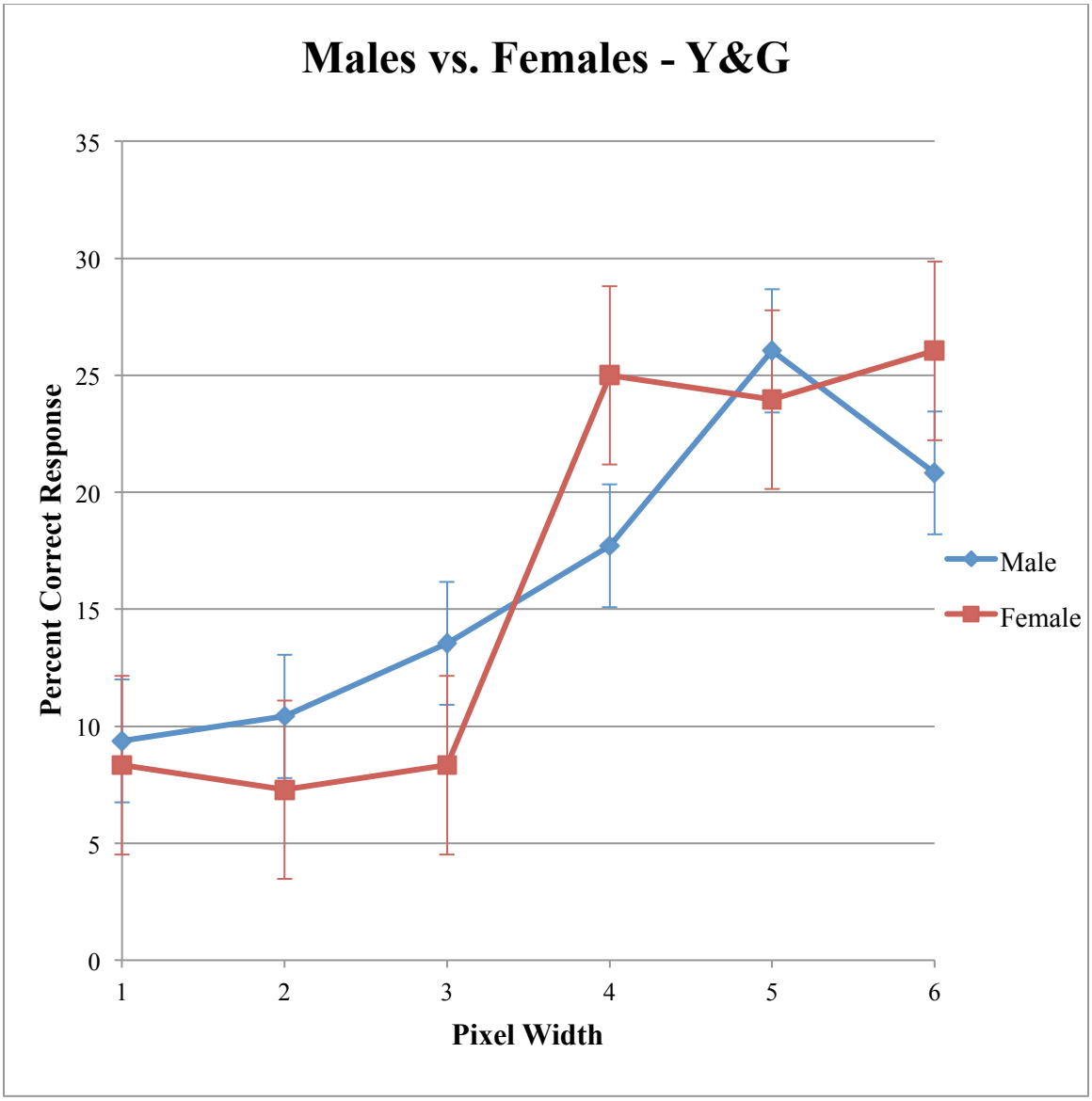


Figure 3.7: Average male and average female yellow and green color acuities. Error bars display standard error for each percent correct response point.

Y&G (two-sample variance t-test)	Pixel Width				
	2	3	4	5	6
P-values	0.477187	0.3705	0.322542	0.794622	0.544567

Table 3.8: P-values comparing average male and female color acuities for the color combination yellow and green, from 2-6 pixel widths. No statistically significant differences found.

E. Foreground vs. Background Acuity Differences

I found one case in which there appears to be a difference in color acuity when distinguishing between the same two colors in different orientations. For the color combination red and yellow, females had a greater ability to distinguish the RoY orientation than the YoR, signified by the RoY line reaching the inflection point and not the YoR line (Figure 3.8). The graph displays error bars to show standard error for each point. A series of paired two-tailed t-tests on the data at the 2nd, 3rd, 4th, 5th, and 6th pixel widths resulted in a significant difference in the two orientations at the 6th pixel width (Table 3.9). This was the only case observed in which the orientation of the two colors in a particular combination appeared to make a reliable difference in acuity abilities.

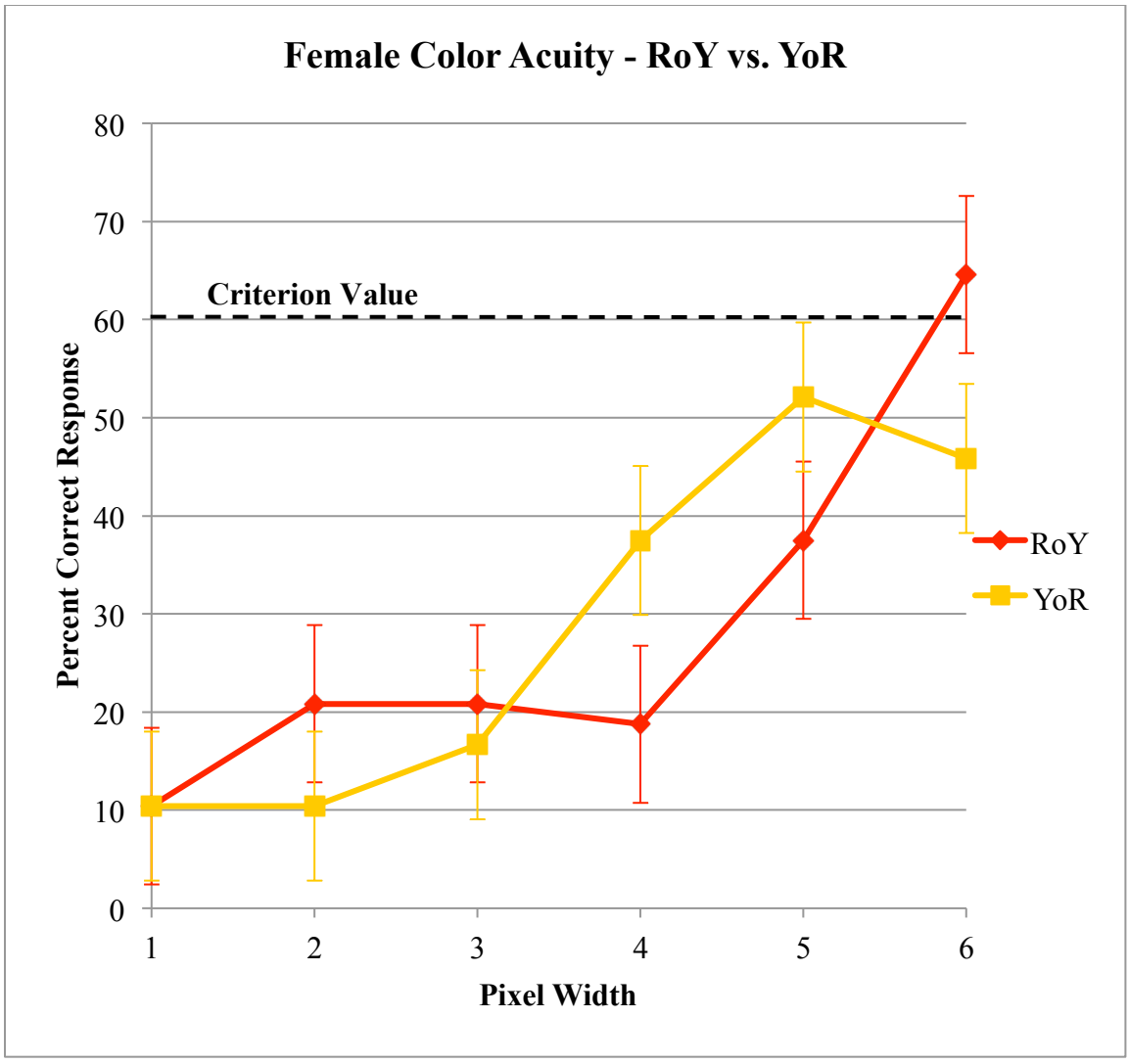


Figure 3.8: Differences in female color acuity based on the orientation of the two colors, RoY vs. YoR. Error bars display standard error for each point.

P-values for Female Subjects	Pixel Width				
	2	3	4	5	6
Color Combination RoY/YoR	0.054	0.615	0.069	0.067	0.043*

Table 3.9: P-values of female color acuities comparing RoY vs. YoR orientations at pixel widths of 2, 3, 4, 5, and 6. Asterisk indicates statistical significance (p<.05).

IV. Discussion

The "open door" technique for measuring visual acuity, employed in this study, is a relatively new concept. In 2014, Lindsey Gori, who was also working with Dr. Leonard Kass on the collaborative vision study known as "The Vision Project," conducted the pilot study for this test that uses pixel width of the open door as a measure of human visual acuity. Much of the data from Gori's study was suggestive of color acuity differences between males and females, but lacked the statistical significance to support meaningful conclusions. For this reason, I decided to conduct a similar study using different equipment and modified techniques in an attempt to improve the clarity of the data and be able to draw statistically significant conclusions. I compared my results to Gori's from last year to see if the added advantage of a joystick with five selection options would provide a better view of acuity differences than the previous two selection options. I also asked the same questions of "Are there significant differences in human color acuity in regard to different color combinations?" and "Are there significant color acuity differences between males and females?" A third question I posed was "Does the background vs. foreground orientation of two colors affect the subject's ability to distinguish between them?" Finally, I examined potential flaws in the "open door" test and ways to improve the technique in the future, as well as how an improved method might allow this procedure to evolve and become useful in a clinical setting as a universal test for color acuity determination.

A. Does the joystick addition provide different results than last year's experiment?

The five selection options offered by the joystick used in this version of the "open door" experiment allowed for a much different interpretation of the percent correct response curves this year as opposed to last. In Gori's 2014 study, the experiment allowed for only two selection options for the location of the "open door"- either on the left or right side of the box. The limitation of two options meant that the subject had a 50/50 chance of answering correctly, even when the open door could not be discerned. With a 50% random chance, the defined acuity criterion was at 75% correct response. This year's modification of a joystick provided the subject with five different selection options, reducing the odds of choosing correctly by random chance from 50% to 20%. This reduction lowered the defined acuity number to 60% and lessened the likelihood of false positives in the results, meaning that the subjects' results were less likely to suggest a higher acuity when in fact they were unable to discern the location of the open door. I think the strongest indicator that the joystick is advantageous in this way, lies in the data on the G&Y color combination. With the joystick method, and a random chance of correct response at 20%, it is clear that humans have significant difficulty in distinguishing between green and yellow because the percent correct response never reaches above 30% and the response curve displays no positive linear trend that would indicate an increased ability to distinguish the open door at higher pixel widths. This finding was not evident in last year's study, where the G&Y data still displayed a positive trend. My hypothesis for this discrepancy is that the data was much more likely to contain false positives in last year's study because the subjects still had a 50% chance of

choosing correctly, whereas this year, the presence of guessing is indicated more clearly by a percent correct response that is consistently below 30%.

B. Are there significant differences in human color acuities for different color combinations?

In looking at Table 3.1 and 3.2, we are able to see clear differences in acuity among the different color combinations for all subjects. Table 3.1 shows the pixel widths at which the average correct response for each subject group reached the acuity criterion value of 60%. Subjects were able to locate the open door when viewing grey on black at a much lower pixel width than any other combination, as is supported by the significant p-values displayed in Table 3.2. The next highest human color acuity is for the color combination of green and red, followed by both color combinations containing yellow, R&Y and Y&G. Subjects displayed the lowest color acuity when viewing the yellow and green combination. As is seen in Table 3.1, I was unable to extrapolate a theoretical pixel width at which this curve would reach the acuity criterion value because the percent correct response was so low and did not follow a consistent positive trend.

The presence of significant differences in color acuity when viewing different color combinations is a logical discovery when thinking about the concentration and arrangement of Cones within the retina. The high ratio of L- and M-cones to S-cones might explain why humans are better able to distinguish between green and red. The varying distributions of these two types of Cones in human retinas, however, might explain why the distinguishing ability is not quite as high as the grey on black color combination that involves differing light intensities (Fig. 4.1).

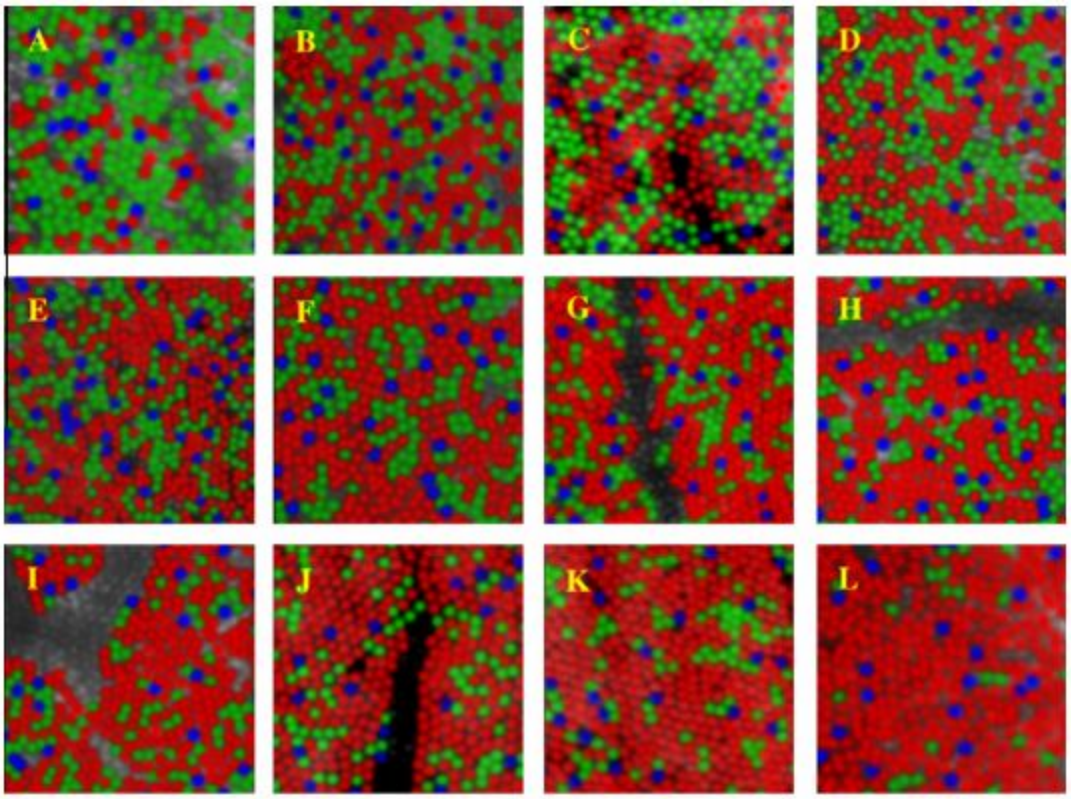


Figure 4.1: Cone mosaics of 10 different subjects with normal color vision (Willims, 2011).

The perception of yellow relies on an equal stimulation of green and red Cones, which may also contribute to the explanation of why humans experience a decreased ability to differentiate between combinations of yellow with either red or green (Fig. 1.11). When discriminating between green and red, the visual system is only dealing with input from the color-opponent fields of the green-red Ganglion cells. Seeing yellow relies on the processing of information from the color-opponent field of blue-yellow Ganglion cells. The processing of information from both color-opponent fields from both Ganglion cells might be a reason for why color acuity is lower for these color combinations. To test this hypothesis in the future, I would consider including color combinations of blue with each other color. By the logic of the color-opponent process, I would hypothesize that humans have a blue and yellow color acuity that is comparable to their green and red color acuity,

and that they would have an equally great difficulty in discriminating between blue when it is juxtaposed with green or red. The only finding that could not be explained by this hypothesis is the discovery of significant color acuity differences between the R&Y and the G&Y combinations, suggested by the p-values at three of the five pixel widths examined in Table 3.2.

C. Are there significant differences in male vs. female color acuities?

Similar to Gori's 2014 study, the results of this study suggest that there are not significant differences in color acuity between males and females. As can be seen visually in Figures 3.4-3.7 and statistically in the accompanying Tables 3.5-3.8, the data comparing male and female color acuities for all color combinations analyzed is not statistically different. There was one case, for the color combination of grey on black, in which the male color acuity curve reaches the defined acuity criterion of 60% before the females, but there is a lack of statistical evidence to back up the claim that males have better color acuity. Despite a lack of evidence supporting the claim that male and females have differing color acuities in this study, past experiments have found differences among the sexes. A study conducted by Israel Abramov in 2012 found that women had better acuity than men when discerning shades of green, blue, and yellow. The same study suggested that males had better visual abilities when viewing fast-moving objects and were better at discerning details of objects from afar. The reason behind these differences is thought to be attributed to evolutionary differences stemming back to prehistoric gender roles. In prehistoric civilization, men were the hunters of society and were reliant on their strength and detection of predators and prey for survival. Because of the need to detect both

predators and prey, men evolved visual systems adept at tracking rapidly moving stimuli and discerning fine details of objects at longer distances away. Women, on the other hand, maintained roles as gatherers and caretakers. In order to recognize the differences between dangerous and safely edible foliage and berries, women evolved visual systems that were able to discriminate better between different shades of color. Women also needed to be able to detect subtle changes in the facial expressions and overall appearance of their children, mainly for health purposes, so their visual systems are more attuned to objects that are stationary and near to the eye (Owen, 2012).

Physiological differences have also been found in male and female visual systems. A previous study on visual cortex development in rats has shown that postnatal and pubescent hormone-related cell death appears to play a role in the density of neurons present in male vs. female visual cortices. Postnatal apoptosis in the visual cortex is reduced by the presence of androgens, and ovarian hormones abundant during puberty increase rates of cell death in the visual cortices of females- two observed phenomena that result in approximately 20% more neurons in the visual cortices of males than females (Schulz et al., 2009). Abramov's team states that the development of the human visual cortex is also susceptible to the influence of masculine hormones, particularly testosterone. The prenatal exposure of males to testosterone results in 25% more neurons in the primary visual cortices of males as opposed to females, at birth (Owen, 2012).

D. Does the foreground vs. background orientation affect the subject's ability to differentiate between two colors?

There was a single occurrence in this study of a case in which the different foreground and background orientations of a particular color combination resulted in different color acuities. Figure 3.8 shows the average female color acuities when viewing the combination of red and yellow in two orientations: RoY and YoR. Table 3.9 displays the p-values for the data and indicates that females have a significantly higher percent correct response rate for the RoY orientation at the sixth pixel width. This one significant p-value is not indicative of an overall statistical difference in the two orientations, especially since the lines overlap on occasion and this is the only color combination that displayed any significance. This result suggests that the potential exists for orientation specific color acuity differences, but the data from this study is not strong enough to support any such conclusions.

E. Future Studies and Ways to Improve

The open door experiment has undergone multiple improvements since the pilot study was conducted in 2014, however, there are still many area on which the technique can be improved to increase the scope and accuracy of results, as well as the practicality of application. One of the main problems with the experiment as it stands now is the lengthy duration and the limitations this imposes. In order to obtain data for the eight color combinations I chose to analyze in this experiment, the subjects were required to commit to approximately 32-40 minutes of testing time (depending on whether or not they took short breaks in between sections). This length of time has multiple effects on the

experiment including increased risk of subject fatigue, a limit to the number of color combinations any one subject can be tested on, and an inability to test over a wide enough range of pixel widths to reach a defined acuity criterion for all different color combinations.

A major improvement in the experiment that would eliminate many of these problems and increase the scope of future studies is automation of the computer program. Currently in the process of being developed, an automated program would be an invaluable advancement in the open door technique, allowing the experiment to adapt to a particular subject's responses and quickly calculate an acuity number for any desired color combination. For example, if the subject were to view a red box on a green background and correctly identify the open door at a pixel width of three, then the next time this color combination appeared, the program would decrease the pixel width. By adjusting in this way to each correct or incorrect subject response, the program would be able to accurately identify the exact point at which a subject can begin to identify the open door and eliminate unnecessary trials. Automating the program would cut down on the amount of time required to find the acuity number, broadening the range of color combinations and pixel widths that could be analyzed for one subject in the same amount of time. Ultimately, the greatest advantage to automation would be the ability to receive an instant color acuity number which would be useful when trying to employ the open door technique in a clinical, diagnostic setting.

One other setback in this study was the potential for procedural inconsistency due to a team of researchers administering the same test without a standard script for instructing the subjects. This brought my attention to a potential issue with continuity of responses among subjects using the joystick. As mentioned in the methods section, the experiment only contained four locations for the open door- any of four sides of the box- but the subjects were told they had a fifth option of pressing a button on the joystick if they did not believe the open door was present at all. In the reality of the program, this fifth option was nonexistent, but because the program only records a response as true or false, the imaginary fifth option was given to subjects to lower the odds of guessing correctly when they were unable to see the open door. There was no standardized script for introducing the fifth option, so it is possible that some subjects used the fifth option and some did not. The capabilities of the computer program did not allow for the recording of actual responses (instead of just true or false) but I think an interesting future study might involve looking at how many times the subjects choose the fifth option, a response that would be indicative of a subject's complete inability to see the open door.

Finally, there is one other interesting study in which the open door technique could be employed to test, and that is the effects of age on color acuity. Some well-known visual impairments that have been linked with the natural aging process include presbyopia, a hardening of the lens causing the inability to focus on objects up close, macular degeneration, glaucoma, cataracts, and diabetic retinopathy, a condition that affects up to 40% of diabetics over the age of 40 to some degree. Color vision has also been known to deteriorate with age, as the photoreceptors in the retina lose sensitivity (Heitig, 2014). My

experiment examined the average color acuities of 24 subjects of subjects between the ages of 18-28. By widening this age bracket, gathering more subjects, and averaging the acuities of subjects who are closer in age, we would be able to examine the exact effects that age has on color acuity and at what stage a decreased acuity might begin to appear. I think the open door experiment, especially an automated version in the future, has great potential for further studies and for practical color acuity assessment in a clinical setting.

V. References

Carlson, Neil R. *Foundations of Behavioral Neuroscience*. Eighth ed. Boston: Allyn & Bacon, 2011. Print.

Conway, B. R., S. Chatterjee, G. D. Field, G. D. Horwitz, E. N. Johnson, K. Koida, and K. Mancuso. "Advances in Color Science: From Retina to Behavior." *Journal of Neuroscience* 30.45 (2010): 14955-4963. Web. 9 Apr. 2015.

Dubuc, Bruno. "The Retina." Web log post. *The Brain from Top to Bottom*. McGill University, 2014. Web. 9 Apr. 2015.

Freberg, Laura. *Discovering Behavioral Neuroscience: An Introduction to Biological Psychology*. Third ed. Boston: Cengage Learning, 2015. Print.

Gori, Lindsey, "A New Method for Measuring Color Acuity in Humans: A Pilot Study" (2014).*Honors College*.Paper 179.

Heitig, Gary, OD. "Astigmatism." *All About Vision*. Access Media Group, LLC, 23 Mar. 2015. Web. 9 Apr. 2015.

Heitig, Gary, OD. "How Your Vision Changes as You Age." *All About Vision*. Access Media Group, LLC, May 2014. Web. 10 Apr. 2015.

Jordan, G., and J.D. Mollon. "A Study of Women Heterozygous for Colour Deficiencies." *Vision Research* 33.11 (1993): 1495-508. Web. 10 Apr. 2015.

Kalat, James W. *Biological Psychology*. Tenth ed. Belmont, CA: Wadsworth, Cengage Learning, 2009. Print.

Kalloniatis, Michael, and Charles Luu. "Visual Acuity." *Webvision: The Organization of the Retina and Visual System*. University Of Utah, 5 June 2007. Web. 9 Apr. 2015.

Kandel, Eric R., James H. Schwartz, and Thomas M. Jessell. *Principles of Neural Science*. Fourth ed. New York: McGraw-Hill, 2000. 577-80. Print.

Kolb, Helga, Eduardo Fernandez, and Ralph Nelson. *Webvision: The Organization of the Retina and Visual System*. Bethesda, MD: National Library of Medicine (US), 2007. Web. 9 Apr. 2015.

Kolb, Helga. "Feedback Loops." *Webvision: The Organization of the Retina and Visual System*. University of Utah, July 2011. Web. 14 Apr. 2015.

Land, Michael F., and Dan-Eric Nilsson. *Animal Eyes*. New York: Oxford UP, 2002. Print.

Montag, Ethan D., Ph.D. "Rods & Cones." *Center for Imaging Science*. Rochester Institute of Technology, n.d. Web. 13 Apr. 2015

Murray, I. J., N. R. A. Parry, D. J. Mckeefry, and A. Panorgias. "Sex-related Differences in Peripheral Human Color Vision: A Color Matching Study."*Journal of Vision* 12.1 (2012): 1-10. Web.

Nave, Carl R. "The Color-Sensitive Cones." *HyperPhysics*. Georgia State University, 2014. Web. 9 Apr. 2015.

Owen, James. "Men and Women Really Do See Things Differently." *National Geographic*. National Geographic Society, 6 Sept. 2012. Web. 10 Apr. 2015.

Purves, Dale. *Neuroscience*. Third ed. Sunderland, MA: Sinauer Associates, 2004. Print.

Saladin, Kenneth S. *Human Anatomy*. Third ed. New York: McGraw-Hill, 2011. Print.

Schulz, Kalynn M., Heather A. Molenda-Figueira, and Cheryl L. Sisk. "Back to the Future: The Organizational–Activational Hypothesis Adapted to Puberty and Adolescence." *Hormones and Behavior* 55.5 (2009): 597-604. *National Center for Biotechnology Information*. Web. 10 Apr. 2015.

Vázquez-Salceda, Javier. *The Role of Norms and Electronic Institutions in Multi-agent Systems: The HarmonIA Framework*. Basel: Birkhäuser, 2004. Print.

Wandell, Brian A. "Chapter 3: The Photoreceptor Mosaic." *Foundations of Vision*. Stanford University, 12 Feb. 2012. Web. 12 Apr. 2015.

Wang, Q., R. Schoenlein, L. Peteanu, R. Mathies, and C. Shank. "Vibrationally Coherent Photochemistry in the Femtosecond Primary Event of Vision."*Science* 266.5184 (1994): 422-24. *JSTOR*. Web.

Williams, David R. "Imaging Single Cells in the Living Retina." *Vision Research*51.13 (2011): 1379-396. *Elsevier*. Web. 10 Apr. 2015.

VI. Appendices

Appendix A

MEMORANDUM

TO: Leonard Kass
100 Murray Hall

FROM: Gayle Jones
Assistant to the Institutional Review Board for the Protection of Human Subjects (IRB)

SUBJECT: "The PC Monitor as a Visual Stimulator for Educational Studies," #2008-02-06

DATE: April 3, 2015

The Institutional Review Board for the Protection of Human Subjects (IRB) conducted its continuing review of the above referenced project in an expedited review on 6/23/2014. The IRB approved renewal, and the approval period is now through 6/22/2015. The next continuing review of this project must be conducted by the IRB before the end of the approval period. Although you will receive a request for review information approximately 6-8 weeks before that date, it is your responsibility to submit review information before the approval period expires.

Enclosed are approved copies of the consent documents for this project. These consent forms are approved for use through 6/22/2015. **These approved copies must be duplicated and used when enrolling subjects during the approval period.**

Please remember that each subject must be given a copy of the informed consent document. Any unanticipated problems or injury to the subject must be reported to the IRB. Any proposed changes to the research must be approved by the IRB **prior** to implementation. Any significant new findings must be reported to the subject.

If you have any questions, please contact me at 1-1498. Thank you.

Appendix B

-

UMaine Institutional Review Board
Approved for Use Through:

JUN 17 2014

Informed Consent Form

For Subjects 18 years or older participating in this study

You are invited to take part in a research project. It is conducted by or under the direction of Dr. Len Kass, a vision scientist in the School of Biology and Ecology, at the University of Maine.

Purpose of study: To examine the way the eye works and to develop a school science exercise.

What you will be asked to do: You will be asked to look at objects on a wall, several sheets of paper, and on a computer screen. You will be asked to report what you see by responding orally, by marking on papers, or by pressing keys on a keyboard.

Time it takes to complete study: This will take about an hour of your time. After that you have the option to continue with an additional 10-30 minute test. ***You may stop at any time or for any reason.*** This is completely voluntary on your part.

Risks: Except for your time and inconvenience, there are no risks involved with participating in this experiment.

Benefits: You may find this study interesting and educational because we will be testing your very own visual system! The results will help us understand how we use our eyes to see things. Eventually, we also think this project would be of interest for use in Science as well as Science Education classes in various K-16 classroom settings.

Compensation: When applicable, course or educational credit will be given for participation.

Confidentiality: Your identity will be kept confidential. This consent form will be shredded at the end of this study. A number will be assigned to your file containing all of your responses. The results from these studies will always remain confidential, and will be stored indefinitely in the office of Dr. Len Kass, 104a Murray Hall, School of Biology & Ecology, University of Maine.

Contact Information: Contact Dr. Len Kass (ph: 581-2567; email: Len.Kass@umit.maine.edu), 100 Murray Hall, School of Biology and Ecology, University of Maine, Orono, ME 04469 with any questions concerning this research and educational project.

Other Contact Information (and for any other concerns or questions):
Contact Gayle Jones (ph: 581-1498; email: Gayle.Jones@umit.maine.edu), Assistant to the Protection of Human Subjects Review Board, University of Maine, 5703 Alumni Hall, Room 114, Orono, ME 04469-5703, with any questions about all rights as a research participant.

Appendix C

Confidential Questionnaire for Vision Tests

Subject ID #: _____ **Age:** _____

Are you (circle one) Right handed or Left handed

Gender: Male or Female

Do you wear corrective lenses? Y or N

If yes, about when did you obtain your most recent prescription lenses? _____

Are you currently wearing (circle one) contact lenses or glasses

Have you ever had corrective eye laser surgery? Y or N

Do you have glaucoma? Y or N

Do you have any of the follow?

Nearsightedness (can see objects up close clearly) Y or N

Farsightedness (can see objects from a distance clearly) Y or N

Astigmatism Y or N

Color-blindness Y or N

If yes:

Red Green Color Blindness (difficulty distinguishing greens vs. reds) Y or N

Blue Yellow Color Blindness (difficulty distinguishing blues vs. greens or yellows vs violets) Y or N

Do you have any other visual limitations or impairments you know of? Y or N

If yes, what are they? _____

Do you have diabetes? Y or N

Do you have a seizure disorder? Y or N

Do you smoke? Y or N

Do you on average drink alcohol more than once a week? Y or N

Do you take any medications? Y or N

If yes, which ones? _____

How many hours on average would you estimate you spend in front of a computer screen each day? _____

Are there any health factors that may affect your performance for this experiment?
Y or N

If yes, what are they? _____

_____

_____

TURN PAGE OVER

Appendix D

Confidential Questionnaire for Vision Tests

Name: _____ Age: _____
(please print)

Do you wear corrective lenses? Y or N

Are you currently wearing those lenses? Y or N

Have you ever had corrective eye laser surgery? Y or N

Do you have any visual limitations or impairments (e.g. color blindness)? Y or N

If so, please explain: _____

Are you (circle one) Right handed or Left handed?

Are you (circle one) Male or Female?

Fitzpatrick Category A (please circle all that apply)

Score	0	1	2	3	4
What are the color(s) of your eyes?	Light blue, grey, or green	Blue, Grey, or Green	Blue	Dark Brown	Brownish Black
What is the natural color of your hair?	Sandy Red	Blond	Chestnut/ Dark Blond	Dark Brown	Black
What is the color of your skin? (Non-exposed areas)	Reddish	Very Pale	Pale w/Beige Tint	Light Brown	Dark Brown
Do you have any freckles on unexposed areas of skin?	Many	Several	Few	Incidental	None

Fitzpatrick Category B (please circle all that apply)

Score	0	1	2	3	4
What happens when you stay in the sun too long?	Painful Redness, Blistering, Peeling	Blistering Followed by Peeling	Burn Sometimes Followed by Peeling	Rare Burns	Never Burned
To what degree do you turn brown?	Hardly or not at all	Light Color Tan	Reasonable Tan	Tan Easily	Turn Dark Brown Quickly
Do you turn brown after sun exposure?	Never	Seldom	Sometimes	Often	Always
How does your face react to sun exposure?	Very Sensitive	Sensitive	Normal	Very Resistant	Never had a Problem

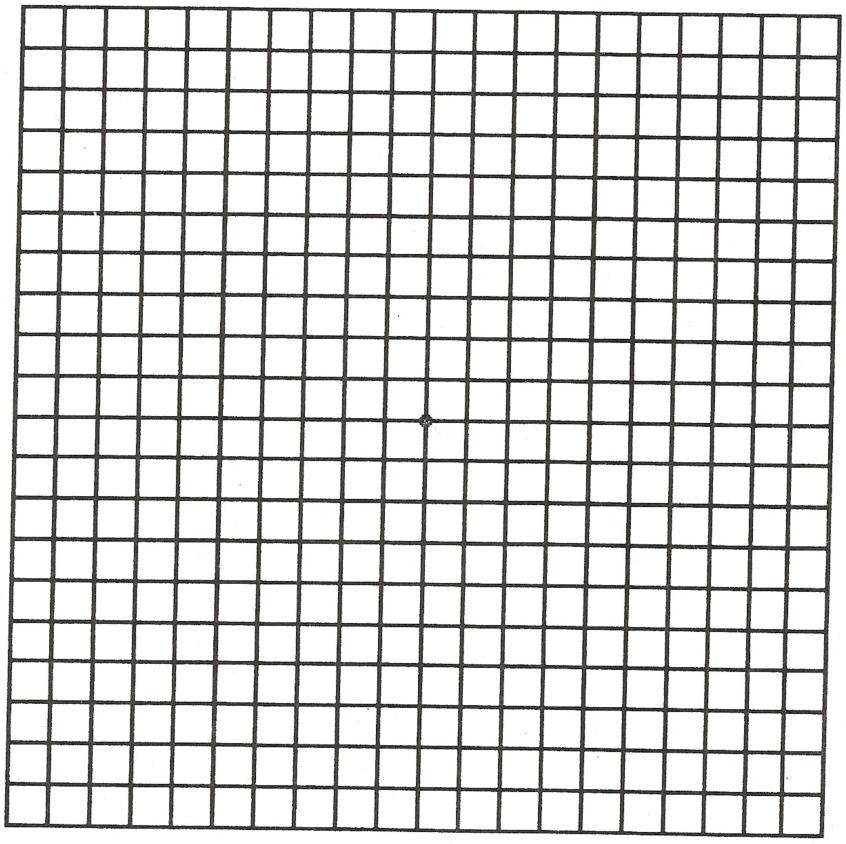
---

For office use only:

Category A Subtotal: _____ Category B Subtotal: _____ Total Score: _____

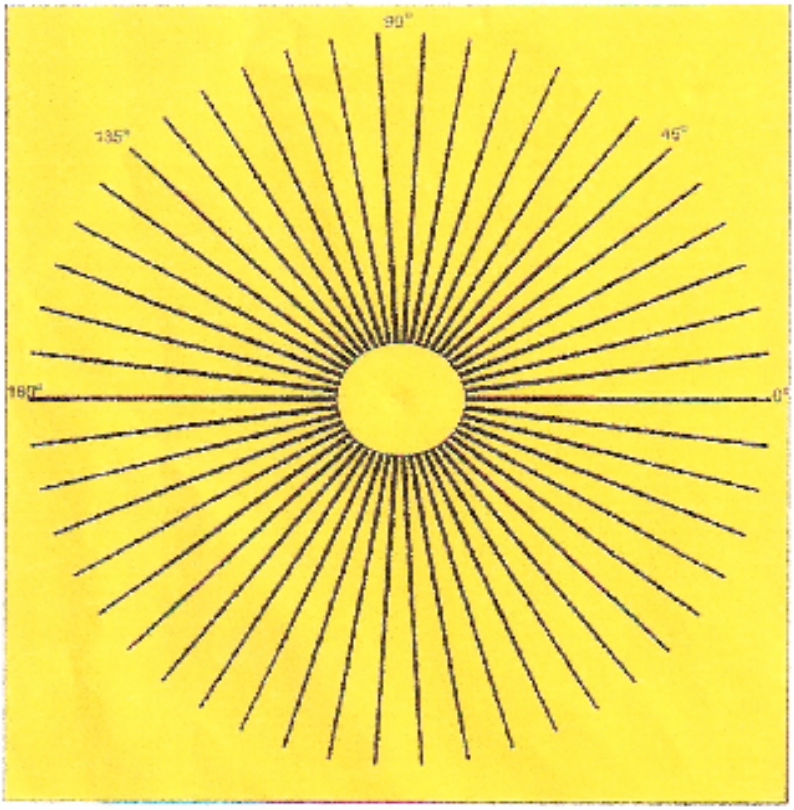
Appendix E

AMSLER GRID



1. Test vision with one eye at a time, and use normal glasses for reading.
2. Hold chart at normal reading distance.
3. Stare at central dot and look for distortion or blind spots in the grid.

Appendix F



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if you have astigmatism then the lines will show up as distortion in one or several of the rays of the Astigmatic Mirror above. The angle of the major distortion is indicated on the chart.

People with natural clear vision see all the lines in the same thickness and exactly the same spacing between the lines.

Look at the Astigmatism chart from several distances and notice where the distortion begin and ends. It is possible to have astigmatism only at the near, or only at the distance, or at any place in between.

Download the Acrobat file and print a chart in with the background colour you like the best. Set your printer to the highest print quality as possible

The Astigmatic Mirror provide an accurate feedback concerning your astigmatism.

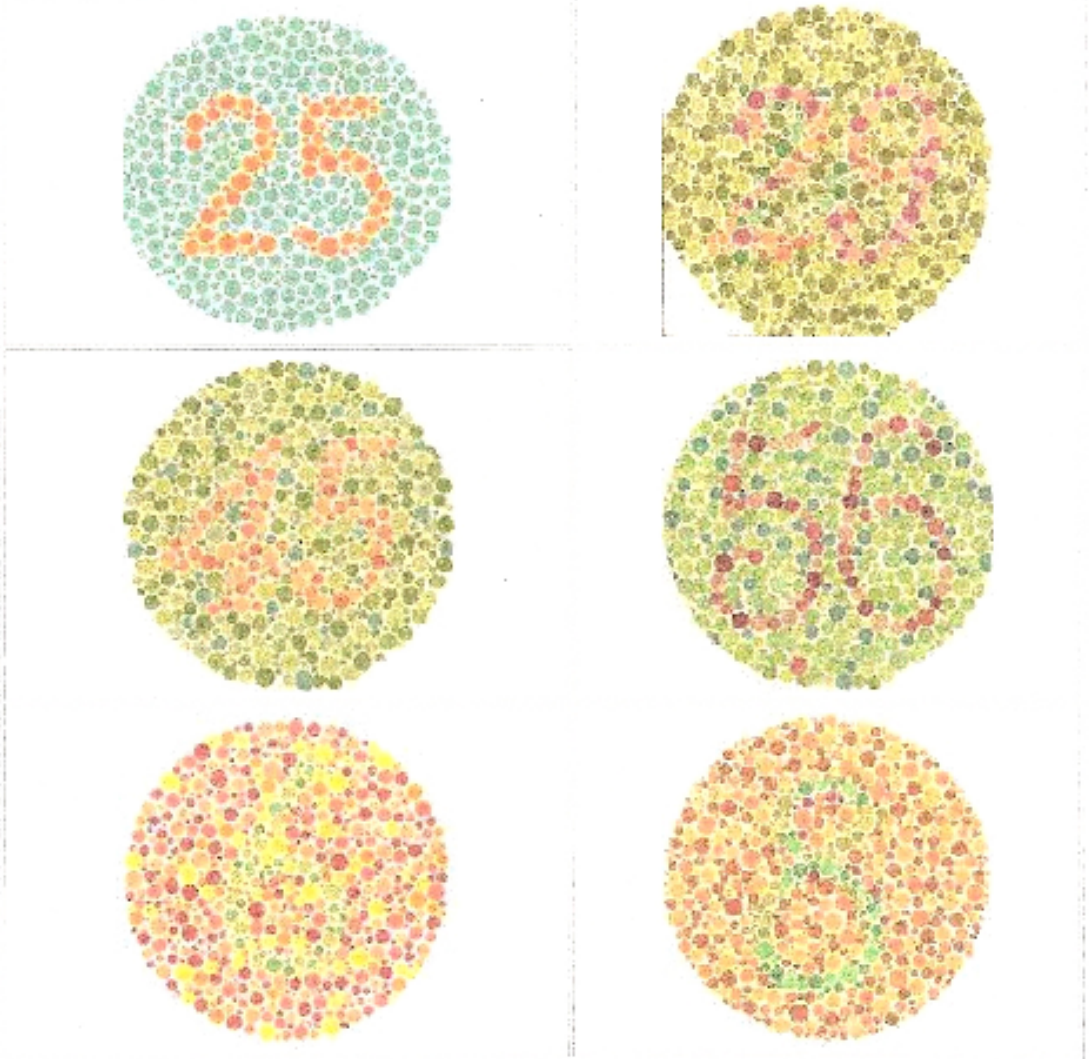
3/24/2008 8:51 PM

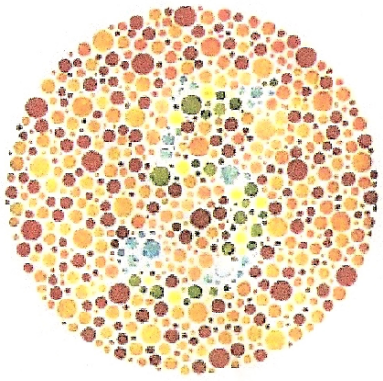
Appendix G

Ishihara Test for Color Blindness

Main Page About Color Deficiency Tests for Color Blindness
Shareware software for the color blind About the Color Charts
Basic Black/Grays Basic Blues Basic Browns Basic Greens
Basic Oranges Basic Purples Basic Reds Basic Off-Whites Basic Yellows
Hex Color Chart Links to other Sites about Color Deficiency

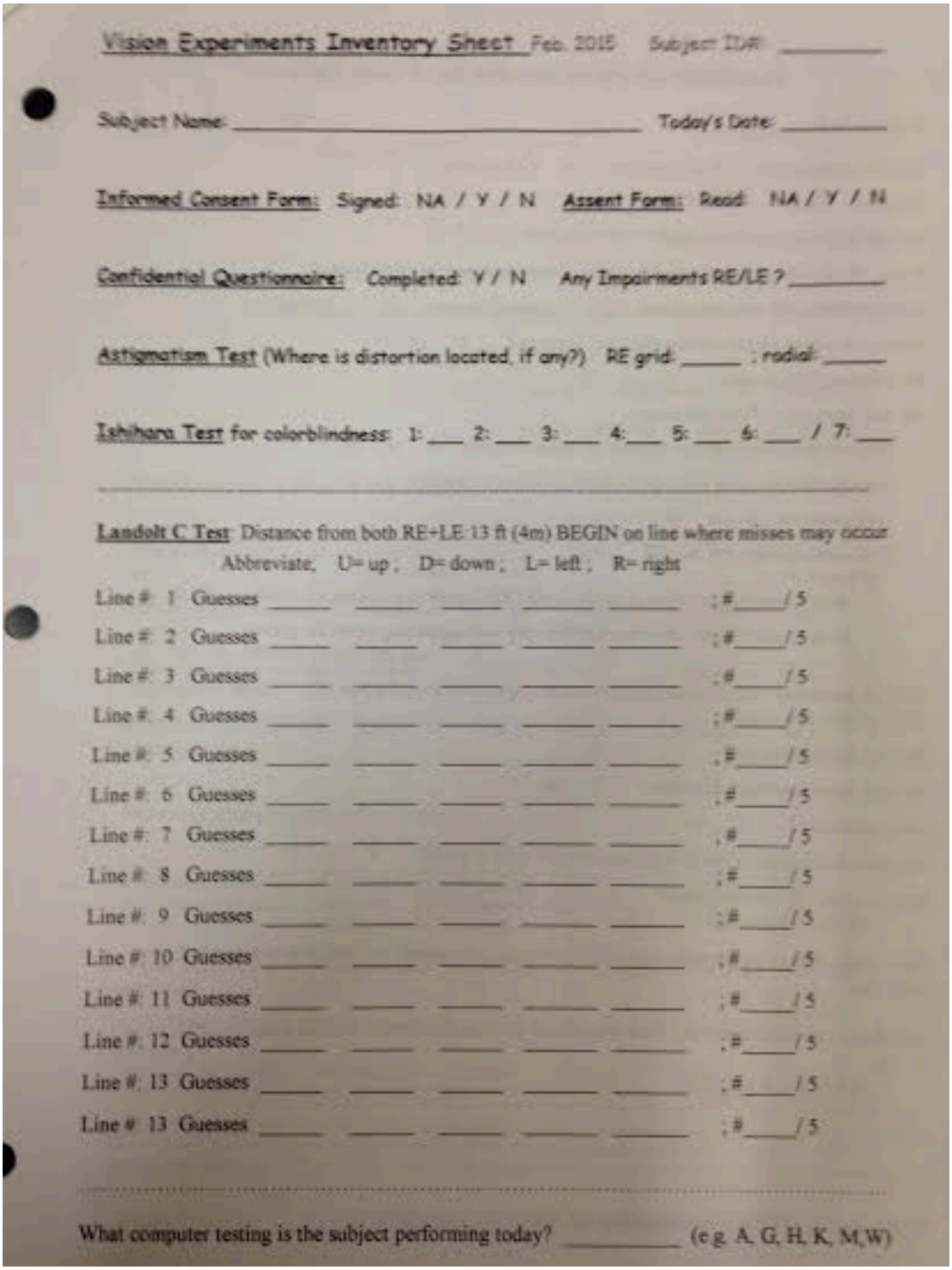
What numbers do you see revealed in the patterns of dots below?





The test to the left is simpler. The individual with normal color vision will see a 5 revealed in the dot pattern. An individual with Red/Green (the most common) color blindness will see a 2 revealed in the dots.

Appendix H



Vision Experiments Inventory Sheet Feb. 2015 Subject ID#: _____

Subject Name: _____ Today's Date: _____

Informed Consent Form: Signed: NA / Y / N Assent Form: Read: NA / Y / N

Confidential Questionnaire: Completed: Y / N Any Impairments RE/LE ? _____

Astigmatism Test (Where is distortion located, if any?) RE grid: _____ ; radial: _____

Ishihara Test for colorblindness: 1: ___ 2: ___ 3: ___ 4: ___ 5: ___ 6: ___ / 7: ___

Landolt C Test: Distance from both RE+LE 13 ft (4m) BEGIN on line where misses may occur

Abbreviate; U= up ; D= down ; L= left ; R= right

Line #: 1 Guesses _____ _____ _____ _____ _____ ; #____/ 5

Line #: 2 Guesses _____ _____ _____ _____ _____ ; #____/ 5

Line #: 3 Guesses _____ _____ _____ _____ _____ ; #____/ 5

Line #: 4 Guesses _____ _____ _____ _____ _____ ; #____/ 5

Line #: 5 Guesses _____ _____ _____ _____ _____ ; #____/ 5

Line #: 6 Guesses _____ _____ _____ _____ _____ ; #____/ 5

Line #: 7 Guesses _____ _____ _____ _____ _____ ; #____/ 5

Line #: 8 Guesses _____ _____ _____ _____ _____ ; #____/ 5

Line #: 9 Guesses _____ _____ _____ _____ _____ ; #____/ 5

Line #: 10 Guesses _____ _____ _____ _____ _____ ; #____/ 5

Line #: 11 Guesses _____ _____ _____ _____ _____ ; #____/ 5

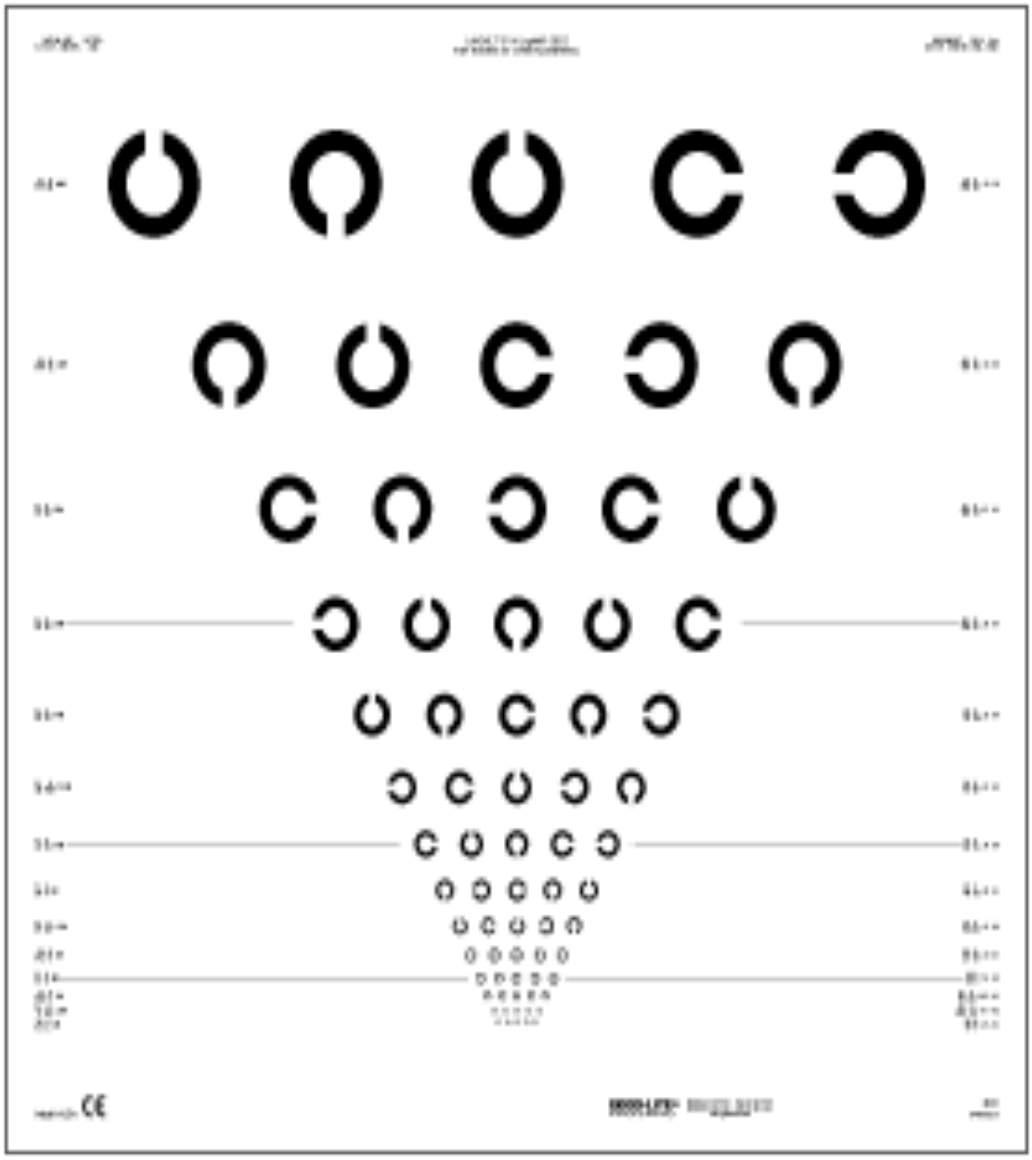
Line #: 12 Guesses _____ _____ _____ _____ _____ ; #____/ 5

Line #: 13 Guesses _____ _____ _____ _____ _____ ; #____/ 5

Line # 13 Guesses _____ _____ _____ _____ _____ ; #____/ 5

What computer testing is the subject performing today? _____ (e.g. A, G, H, K, M, W)

Appendix I



Appendix J

Steps for running VISION 3 experiments: Series "a"

Once computer and monitor are on, and "acuity" experiment in Vision3.0 is highlighted:

1. depress "Enter" key on keyboard thereby entering the Acuity program

2. "Subject ID #": enter your **2 digit** ID# and hit the "Enter" key

3. "Special Designator": "a" "Enter"

4. "Name of Input File": "0.txt" "Enter" ; tutorial run with the lights on
   → "1a.txt" "Enter" ; This 1st expt COUNTS ... Lights out
   "2a.txt" "Enter" ; This 2nd expt COUNTS ... Lights out
   "3a.txt" "Enter" ; This 3rd expt COUNTS ... Lights out
   "4a.txt" "Enter" ; This 4th expt COUNTS ... Lights out

   (wait for a few seconds for the file to Load!)

5. "How many repetitions would you like?": "1" "Enter"

.....

Be sure you have one hand on JOYSTICK with the other on the left red button.

And that the lights are out and the door is closed. Get ready to respond!

6. "Hit "Enter" when you are ready to begin the experiment: "Enter"

.....

First time thru with experimenter you will do the "0.txt" "Enter" ; tutorial

After you run all four "1a.txt" , "2a.txt" , "3a.txt" , "4a.txt" expts, leave the room!

Appendix K

Vision Experiments 10 Post-Test Questions Feb. 2015

Subject ID#: ___ Subject Name: _____ Today's Date: _____

1. Which expt series did you just complete? A? G? H? K? M? W? Other: _____

2. Was the expt: Way too long?__ Bit too long?__ About right?__ Bit too short?__

3. How clear were the directions provided (0-9)? ___ (0=totally unclear; 9=totally clear)

4. Did you experience any blurriness during the experiment? Yes / No

5. Did you experience any eye or vision discomfort during the experiment? Yes / No

6. If "Yes", what kind of discomfort did you experience? _____

7. How many hours today did you use a computer before doing this experiment? _____

8. Were those number of hours of computer use typical of your average day? Yes / No

9. Was there anything unusual or unexpected that you experienced? _____

_____

_____

_____

_____

10. On a scale of 1-10, rate your experience: _____

Note: 1=Totally bored out of my mind; 5= OK as expected; 10=most fun I've had ever]

VII. Author's Biography

Jordan D. Servetas was born in Blue Hill, Maine on July 30th, 1993. She grew up in the small town of Hancock, Maine and graduated from Mount Desert Island High School in 2011 as the valedictorian of her class. Jordan is pursuing a Bachelor of Science degree in Biology with a concentration in pre-medical studies. She is also a member of Golden Key International Honor Society, serving as vice-president of the UMaine Chapter during her senior year.

Upon graduation, Jordan plans to work as an emergency medical technician to gain practical experience in the medical field before committing to a path of graduate level education.