THE EFFECTS OF LUTEIN AND ZEAXANTHIN ON NEURAL PROCESSING SPEED

by

EMILY RENEE BOVIER

(Under the Direction of Billy R. Hammond, Jr.)

ABSTRACT

Lutein and zeaxanthin are found throughout neural tissue and have been shown to promote cellular communication. It has been hypothesized that nutritional modification with the xanthophylls may enhance neural efficiency. The goal of this study was to determine the relationship between macular pigment, a biomarker of total neural lutein and zeaxanthin, and temporal vision and visual motor reaction time. Changes in these outcome variables were also assessed after four months of supplementation with lutein and zeaxanthin. Consistent with past research, macular pigment accounted for a moderate amount of variance in critical flicker fusion (CFF) thresholds, temporal contrast sensitivity, and select measures of coincidence anticipation ability in a sample of college-aged subjects (N = 92). After four months of supplementation, macular pigment significantly increased for 54 subjects and was accompanied by improvements in temporal vision and reduction in visual motor reaction time. Comparatively, subjects in the placebo group (N = 10) did not have significant changes in CFF thresholds or visual motor reaction time, however increases in temporal contrast sensitivity were found. In

general, findings related to CFF thresholds and reaction time were consistent with the predictions of the neural efficiency hypothesis of lutein and zeaxanthin function.

INDEX WORDS: Xanthophylls, Neural efficiency, Temporal processing

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DEDICATION

For my mother, whose unconditional support has been a source of strength in so many ways.

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CHAPTER 1

INTRODUCTION

A hallmark of aging is the slowing of our neural system, with behavioral manifestations in functional domains such as cognition and sensory function. Considerable attention has been given to characterizing losses in neural processing speed in pathologies such as Alzheimer's disease, particularly given its effects on quality of life for patients and their caregivers. Aging in general, however, even in the absence of overt neural pathology, is often accompanied by the decline of neural function related to daily activities that involve reasoning, processing speed, etc. (for an overview of age-related decline in these functions, see Salthouse 2004). This general decline is likely due to reduced neural efficiency, which can be accounted for in part by slower speed of processing. The overall efficiency of the neural system may be more fully described as reflecting a balance between both speed and precision of neural communication. Although age can account for some of the functional changes associated with reduced neural efficiency, lifestyle factors also make a significant contribution to the integrity of the neural system. For example, habits such as exercise and diet could account for functional differences among individuals in a similar age group who are generally in good health. Individual differences in function can also be detected early on, before long-term effects of aging have occurred.

Diet is obviously very influential to general systemic health, and in particular to neural functioning. Nutritional neuroscience is based on the study of dietary components that influence the brain and behavior. This discipline focuses on individual differences in function as they relate to dietary habits and how nutritional modifications impact the central nervous system. For

example, dietary supplementation may be a useful approach to delay the progression of declines in neural efficiency, or potentially even improve performance. Intuitively, any antioxidant that is present in neural tissue could prevent neural degeneration by protecting against oxidative stress. However, it may be that some phytochemicals have properties that influence other aspects of neural function, leading to enhancements as opposed to simply functional maintenance. For example, lutein and zeaxanthin are two dietary carotenoids that are hypothesized to influence neural efficiency and improve function through a variety of mechanisms.

Lutein and zeaxanthin accumulate throughout the body but perhaps are most known for their functional role in the retina, which has been attributed to the pigment's light-absorbing optical (e.g., Wooten and Hammond, 2002; Stringham and Hammond, 2007) and antioxidant and anti-inflammatory effects (for a review see Krinsky et al., 2003). The neural efficiency hypothesis, first posited by Hammond and Wooten (2005), proposed that lutein and zeaxanthin could influence neural processing at the level of the retina and cortex by influencing cellular communication and the structural integrity of neurons. The idea that lutein and zeaxanthin could influence the overall function of the central nervous system, as opposed to simply retina function is supported by the following points, each of which will be elaborated on in upcoming sections:

1) Lutein and zeaxanthin accumulate throughout neural tissue (e.g., retinal tissue and several cortical sites, both in grey and white matter).
2) The amount in cortical tissue is not proportional to the amount of carotenoids found in the diet (relative to beta carotene, for example) which suggests that cortical tissue may accumulate lutein and zeaxanthin actively as opposed to simply by passive diffusion (the selectivity of accumulation by the retina is apparent since lutein and zeaxanthin are the only carotenoids found in the macula).

3) Behavioral evidence relates lutein and zeaxanthin status, both in tissue and serum, to measures of neural processing within the visual and cognitive domains.

Lutein and Zeaxanthin in Neural Tissue

The tissue analysis that first successfully separated retinal lutein and zeaxanthin was conducted by Bone et al. (1985) using high performance liquid chromatography (HPLC). Prior to this, the identification of lutein and zeaxanthin in the retina was based on psychophysical determinations of the pigment's absorption characteristics (Wald, 1945). HPLC has been used extensively to quantify carotenoids throughout human tissue (e.g., Kaplan et al. 1990) and has now been applied to the quantification of lutein and zeaxanthin in the brain (see Table 1).

Specific cortical concentrations have been reported from tissue analyses of elderly humans (Craft et al., 2004), infants (Vishwanathan et al., 2011), and primates (Vishwanathan et al., 2013). These studies formed the basis for the neural efficiency hypothesis since they provide evidence that lutein and zeaxanthin accumulate throughout cortical tissue and are therefore in place to influence neural function. Craft et al. (2004) quantified lutein and zeaxanthin in gray and white matter of the frontal and occipital cortex and reported higher concentrations in gray matter compared to white matter ($p < 0.03$). Vishwanathan et al. (2011) reported regional distribution of lutein and zeaxanthin, specifically in the hippocampus, frontal, auditory, and occipital cortices of infant brains. In general, the average amount of lutein from all four regions was higher than zeaxanthin (48.6 ± 7.2 pmol/g and 13.3 ± 1.5 pmol/g, respectively). Finally, Vishwanathan et al. (2013) quantified lutein and zeaxanthin in the cerebellum, pons, and frontal and occipital cortex of primates fed either a normal stock diet or a semi-purified diet containing either lutein or zeaxanthin.

Interpreting findings from these studies is challenging for several reasons. The ability to evaluate the authors' interpretations by making comparisons between studies is limited. This is particularly true since different methods (subject to different artifacts) and sampling techniques have been used. Furthermore, although there are statistically significant differences in lutein and zeaxanthin across different regions of the brain (see Table 1 for the amounts for each study), it is not clear whether or not these would result in meaningful functional differences. For example, Vishwanathan et al. (2011) make the general argument for the selective tissue accumulation of lutein and zeaxanthin relative to other dietary components. In their study, over half of infant brain carotenoid concentration was lutein and zeaxanthin, compared to one-third beta-carotene, despite evidence that beta-carotene accounted for nearly half of the carotenoids found in a typical infant diet. Craft et al. (2004) specifically suggested a selective uptake of zeaxanthin since the ratio of lutein to zeaxanthin (1.39) was significantly less ($p < 0.0001$) than reports of lutein to zeaxanthin ratios in serum (the authors selected 3 as their comparative and cited reports of ratios in the serum ranging from 3-9). Vishwanathan et al. (2013), on the other hand, stated that the brain selectively accumulates lutein relative to zeaxanthin, since lutein was 10-20 times higher in lutein-fed monkeys compared to stock-fed monkeys, but zeaxanthin was only 2.5-3 times higher in zeaxanthin-fed monkeys compared to stock-fed monkeys. In contrast to the sparse literature on the brain, the functional effects of magnitude differences in retinal lutein and zeaxanthin have been well established. This is likely also methodological: the methods are largely standardized and the tissue sampling is precisely specified.

The functional role of lutein and zeaxanthin in the brain remains speculative. What can be concluded, however, is that these phytochemicals are present throughout neural tissue, even within the first year of life. It has also been well established, ex vivo, that lutein and zeaxanthin

influence cellular function. The inference is transitive: lutein and zeaxanthin are found in neural tissue (A), are known to influence cellular communication/function in a positive manner (B), hence, the likelihood that they are serving similar functions in neural tissue (C).

Proposed Mechanisms of Neural Efficiency

Although it has been formally proposed that lutein and zeaxanthin influence neural efficiency, neural efficiency itself has not been operationally defined. Such a definition should likely include general speed of processing as a central feature. Speed of processing has particular application to the elderly and to individuals in specialized fields, such as athletes, whose success is dependent on the timing of specific actions, often on the order of milliseconds.

Simply characterizing neural efficiency as speed of processing could be misleading, however, since it is important to consider the balance between speed and precision. An example of this type of balance is a complex motor action made in response to a visual stimulus. For example, coincidence anticipation (discussed in more detail later), requires an individual to anticipate the arrival of a moving visual stimulus to some designated area in the environment and complete an appropriate motor action. Baseball players exhibit this skill when a batter has to anticipate the arrival of a pitch in order to swing and make contact. Swinging a bat in order to make contact with a pitch does not only involve a fast reaction to the visual stimulus (i.e., fast visual processing speed), it also involves attentional control in the form of inhibition in order to prevent the motor action from occurring too soon, since the speed of the pitch is variable (e.g., Nakamoto & Mori, 2012).

One central tenet of the hypothesis is inherent in the name; the efficiency of neural communication, whether it be related to speed of communication or signal strength. Efficiency of the neural system could be influenced by several factors:

1) The speed of conduction that leads to neural activation. Slowed neural conduction could be accounted for by the degradation of myelin, for example. This has been a proposed model of cognitive decline in Alzheimer's disease (e.g., Bartzokis, 2004).
2) The number of connections, such as the number of gap junctions or the number of dendritic spines on a neuron, for example, both of which influence signal conduction.
3) Lateral connections, such as gap junctions, which can enhance communication between neurons and influence the neural signal in a way to optimize behavior. For example, lateral connections in the retina serve to enhance contrast differences in the environment via lateral inhibition, thereby improving visibility.
4) The amount of a neural network needed to complete a task. Indeed, elderly individuals have been posited to compensate for age-related degradation of the brain via activation of more neural circuits (e.g., the scaffolding theory of aging and cognition, Park & Reuter-Lorenz, 2009), which results in slowed processing.

Mechanisms that may account for the ability of lutein and zeaxanthin to promote neural efficiency are supported by evidence of tubulin as a binding site for lutein and zeaxanthin (e.g., Bernstein et al., 1997). Potential outcomes of the xanthophyll's association with tubulin (which forms microtubules) could be related to the cytoskeleton of dendrites and axonal tracks. Indeed, microtubules have been identified in dendritic spines (e.g., Gu et al., 2008) and implicated in their formation and plasticity. An increase in the length of dendritic spines and total number of spines is one mechanism that could account for enhanced neural efficiency. Microtubules are

also present in the cytoskeleton of axonal tracts. Since lutein and zeaxanthin bind to tubulin and have a horizontal orientation in lipid bilayers (e.g., Pasenkiewicz-Gierula et al., 2012), this may improve the structural integrity of myelin, resulting in preserved or increased axonal conduction speed. One final mechanism by which lutein and zeaxanthin could promote neural efficiency is through increased gap junction communication (e.g., Stahl et al., 1997), which has been demonstrated in somatic tissue. The underlying biology of how exactly this occurs is unknown; however, it has been shown that carotenoids stimulate the production of connexin proteins (e.g., Bertram, 1999), which form gap junctions. It may be that lutein and zeaxanthin's ability to bind to tubulin influences the association between microtubules and ribosomes, resulting in an increased production of connexin proteins. Although reasonable, direct functional studies on neurons themselves are needed in order to adequately evaluate the potential of these carotenoids to promote direct change within nervous tissue.

Testing the neural efficiency hypothesis on a more macro level can be done in a cost and time efficient manner by using behavioral methods and tissue markers of lutein and zeaxanthin. For example, the amount of lutein and zeaxanthin in the retina, referred to as macular pigment, can be assessed noninvasively using psychophysical techniques (e.g., heterochromatic flicker photometry, Wooten et al., 1999). Not only does macular pigment represent a marker of lutein and zeaxanthin in neural tissue, since the retina is part of the central nervous system, but it may also serve as a general proxy for the amount of lutein and zeaxanthin in the brain. Support for the use of macular pigment as a biomarker of cortical lutein and zeaxanthin comes primarily from the primate study conducted by Vishwanathan et al. (2013). Tissue analyses have been conducted on humans, however only preliminary results have been reported. For example, Vishwanathan et al. (2012) reported correlations between lutein and zeaxanthin in the occipital

cortex and the retina (r = 0.87, N =3) in a preliminary sample of deceased individuals over 50 years of age. In Vishwanathan et al.'s primate study, retinal lutein significantly correlated with lutein in the cerebellum, frontal and occipital cortex, and pons. Relationships between retinal zeaxanthin and zeaxanthin in the frontal cortex and cerebellum resulted in similarly high correlations.

Although specific cellular studies linking macular pigment to cortical lutein and zeaxanthin are limited, using macular pigment as an index of total neural concentrations of lutein and zeaxanthin is reasonable. Past studies (Hammond and Wooten, 2005; Renzi and Hammond, 2010; Feeney et al., 2013) have utilized cross-sectional designs simply correlating measures of processing speed with macular pigment as a way of testing the neural efficiency hypothesis. A more direct approach would be to modify dietary intake of lutein and zeaxanthin and assess subsequent changes in neural function and compare those changes to a placebo-control group. However, to date, only one supplementation trial has been conducted (i.e., Johnson et al., 2008, discussed in the following section).

Behavioral Evidence Supporting the Neural Efficiency Hypothesis

Hammond and Wooten (2005) originally determined the relationship between macular pigment and critical flicker fusion (CFF) thresholds. The stimuli for their experiment was expressly chosen to reflect post-receptoral processing presumably localized within the visual cortex (e.g., Powell 1983). Macular pigment was significantly related to CFF in a sample of 134 subjects aged 17-92 years (r = 0.30; $p < 0.001$). This relationship was still significant after statistical adjustment for age and also maintained for stratified age groups (younger: r = 0.33, $p <$ 0.013; middle: r = 0.32, $p < 0.015$; older: r = 0.27, p = 0.04). Based on these results, the authors

suggested that lutein and zeaxanthin could improve the efficiency of neural signals throughout the visual system. This was largely based on evidence from cellular models showing improved gap junction communication between somatosensory cells (Stahl et al., 1997) and reversed declines of neuronal transduction in rat models as result of dietary supplementation with spinach (Joseph et al., 1998). Consistent with this approach, Gutherie and Hammond (2005) measured scotopic sensitivity and found a relationship between macular pigment and intrinsic noise (r = -0.59, $p < 0.05$) for a sample of 13 individuals aged 20-52 years. Their data were later replicated by Zimmer and Hammond (2007) with 39 individuals (r = -0.38, $p < 0.01$). Zimmer and Hammond suggested the effect might reflect activity within the rod pathway (i.e., lutein and zeaxanthin accumulate in rod outer segments and could improve efficiency, thereby decreasing scotopic noise).

Johnson et al. (2008) shifted the focus to the influence of lutein and zeaxanthin on cognition. In humans, vegetable consumption, specifically leafy green vegetables, has been related to slower cognitive decline as reported by Kang et al. (2005). Akbaraly et al. (2007) assessed tissue carotenoids and found the lowest plasma levels of zeaxanthin in subjects with lowest scores on select cognitive measures (plasma lutein did not reach statistical significance). Johnson et al.'s study, however, was the first supplementation trial to investigate cognitive changes as a result of lutein intake in humans.

Cognitive assessments utilized by Johnson et al. were classified into the domains of memory (e.g., verbal fluency, forward and backward digit span, shopping and word list memory tasks) and processing speed (e.g., pattern recognition test, Stroop test). Subjects included 49 women aged 60-80 years who received either placebo (N = 10), DHA (N = 14; 800mg/day), lutein (N = 11; 12mg/day), or a combined dosage of the DHA and lutein supplement (N = 14) for

four months. Significant changes were found for verbal fluency (DHA, lutein, and combined groups, $p < 0.05$), trials to learn a list for the shopping list memory test (DHA + lutein only, $p = 0.03$), and delayed recall for recalling items on an apartment memory test (DHA + lutein only, $p = 0.02$; effect sizes were not reported). Correlations between serum lutein after supplementation and these specific measures of cognition were only significant for the shopping list task (N = 48, $r = 0.30$, $p < 0.05$).

In a similar study, Renzi et al. (2008) reported significant correlations between macular pigment and cognitive performance (i.e., indices related to processing speed and accuracy) in 118 subjects aged 76-85 years (the specific indices were not indicated and no effect sizes were reported). Johnson et al. (2011) linked cortical concentrations of lutein and zeaxanthin to cognitive function in 49 individuals deceased after 98 years of life. Cortical zeaxanthin was reported to significantly correlate with measures of global cognitive function, memory, verbal fluency, and dementia severity. Cortical lutein significantly correlated with measures of verbal fluency and recall. Johnson (2012) further discussed these findings and presented new data from 29 centenarians, however the results did not reach statistical significance at the $p < 0.05$ level and no values of cortical lutein and zeaxanthin were provided.

The available data regarding the relationship between cognition and lutein and zeaxanthin are consistent with the hypothesis that the carotenoids may indeed play a role in cognition. This line of research, however, is relatively new and incomplete. It may be that other measures of neural function, in addition to cognitive assessments, are needed to further understand the role of lutein and zeaxanthin in neural functioning. Using performance on a specific cognitive task as an index of change after supplementation has the potential to be confounded by factors such as ceiling effects. For example, no significant effects of supplementation on measures of

processing speed were reported from Johnson et al.'s 2008 study, which the authors contributed to the high scores at baseline for the treatment group. Another factor to consider is the potential for a restricted range of performance on cognitive indices, particularly in elderly subjects and when investigating cross-sectional relationships. For example, Salthouse (2004) discussed consistent declines in cognitive performance with age accompanied by a restricted distribution of scores.

Visual function measures that reflect neural function, such as CFF thresholds, may be more sensitive to change and individual differences. Indeed, CFF has been associated with cognitive performance, which suggests that relations with lutein and zeaxanthin may reflect a common mechanism related to neural processing speed (Bovier et al., 2013). In addition to CFF, macular pigment has been related to measures of temporal vision and visual motor ability.

Temporal Vision

The most comprehensive way to quantify temporal vision is to measure the complete temporal contrast sensitivity function (Wooten et al., 2010). This function is defined as contrast sensitivity (the inverse of contrast threshold) for sinusoidally modulated light over a wide range of temporal frequencies (see Figure 1). Measuring the entire function is useful because it is thought to be the envelope of multiple temporal filters tuned to different frequencies. In other words, changes in the function could in principle be due to changes at multiple levels of the visual system. For example, critical flicker fusion thresholds, the high frequency cut-off of the temporal contrast sensitivity function, are thought to reflect cortical activity and provide a means of defining the upper limit of an individual's processing capacity.

Several models have been proposed to account for the underlying mechanisms of temporal vision. Wooten et al. (2010) emphasized three components that influence temporal sensitivity, specifically the response time of photoreceptors, the response time of neural inhibition, and the amount of neural noise. Deficits in photoreceptor response time could manifest as high frequency loss; if the photoreceptor response time is too slow, then the luminance change over time for high frequencies occurs at a rate faster than that of the photoreceptor response time (whereas a lower frequency temporally varies at a rate that can be followed by photoreceptors). Low frequency loss is thought to reflect deficits in neural inhibition at the retinal level. Visual perception is driven by contrast, both within the spatial and temporal domain, and lateral inhibition within the retina serves to enhance contrast. A delay in lateral inhibition diminishes contrast detection, thereby resulting in changes in sensitivity. Finally, the presence of neural noise results in losses at all temporal frequencies, since a greater signal will be needed to perceive the stimulus in the presence of increased noise.

Although models of temporal vision are in place, the underlying neurological mechanisms are not well understood. However, using Wooten et al.'s summary of the temporal contrast sensitivity function, it is possible to speculate on the influence of lutein and zeaxanthin on temporal vision given the proposed role that these xanthophylls may have in promoting neural efficiency. At the level of the retina, lutein and zeaxanthin could influence neural processing by promoting gap junction communication, which could influence temporal processing at lower frequencies. At the level of the cortex, if lutein and zeaxanthin influence axonal conduction via the stabilization of myelin, then the upper limit of temporal processing capacity could be affected. Finally, general temporal processing speed could be enhanced by lutein and zeaxanthin if these carotenoids serve to promote a greater signal strength (either by increased gap junction

communication or increased dendritic spine length and density), thereby reducing the influence of neural noise.

Support for the influence of lutein and zeaxanthin on temporal contrast sensitivity comes from a cross-sectional study. Renzi and Hammond (2010) expanded on Hammond and Wooten's (2005) use of CFF thresholds as a measure of temporal vision by assessing the full temporal contrast sensitivity function in sample of 70 subjects aged 15 to 84 years (although the majority of subjects were not elderly - average age was 33 years). They reported a holistic measure of temporal contrast sensitivity in the fovea (i.e., area under a curve generated by plotting log contrast sensitivity as a function of target frequency and fitting a third-order polynomial curve to the data), which was found to relate to macular pigment ($r = 0.29$, $p < 0.01$). Figure 1 shows an example temporal contrast sensitivity function, specifically for two individuals with different macular pigment. Higher temporal contrast sensitivity, which yields a greater area under the curve, is reflective of faster processing speed since it indicates a greater sensitivity to luminance changes over time (i.e., the visual system requires less temporal contrast in order to detect flicker at different frequencies). Renzi and Hammond found that subjects with higher macular pigment had a greater area under the curve, suggesting that these individuals had more efficient neural processing. Relations between individual frequencies and macular pigment were not specifically reported -- a range of *r*-values (0.19 to 0.25) and *p*-values (0.07 to 0.025) were reported for a range of target frequencies (2.5–32 hz).

Although the temporal contrast sensitivity function is a very useful index of temporal vision, it does not capture variation in the actual motor response to temporal stimuli. In the real world, timed reactions are often an active process paired with a behavioral response as opposed to a passive perceptual experience. For example, athletes must often match a behavioral

response with temporal variation (e.g. tennis or baseball players hitting a ball). In order to assess visual motor responses, we tested a number of timed behavioral reactions that required both speed and precision.

Visual Motor Reaction Time

One common measure in this category is simple reaction time, in which a subject makes a button press in response to the appearance of a light. Coincidence anticipation timing, or the ability to anticipate the arrival of a moving stimulus at a designated target area, is another measure of visual motor ability. We constructed a device to test coincidence anticipation ability, which was quantified in terms of response error (i.e., time difference between the arrival of the stimulus at the target and the time at which the subject made a response). The relationship between reaction time, coincidence anticipation timing, and macular pigment has been characterized in both elderly and young adults (Renzi et al., 2013). Macular pigment was significantly related to a reaction time task that required subjects to make an accurate button press corresponding to the position of a visual stimulus in one of four quadrants on a computer monitor (N = 49 elderly, $r = -0.25$, $p < 0.05$). Similarly, macular pigment was related to reaction time when subjects (N = 106 young adults) were required to make a button press in response to the illumination of a light occurring either in a fixed ($r = -0.16$, $p < 0.05$) or variable ($r = -0.19$, $p < 0.05$) position along a linear track. In the same sample, macular pigment was related to coincidence anticipation ability, specifically the number of trials in which subjects could not make a response before a 15mph stimulus completed its path along a linear track ($r = -0.22$, $p < 0.05$).

Lutein and zeaxanthin may influence these assessments of visual motor reaction time at the level of the cerebellum. Indeed, these carotenoids have been identified in the gray matter of

cerebellum in primates (Vishwanathan et al., 2013). The cerebellum in particular is involved in the temporal coordination of motor action in response to a visual stimulus (via outputs from purkinje cells), which is precisely the required behavior of a coincidence anticipation timing task (e.g., Fleischer, 2007). In particular, inhibitory projections from purkinje cells are important to timing-related tasks involving visual motor integration; for example, in a standard coincidence anticipation task, two trials in sequence that vary from a fast velocity to a slow velocity requires an inhibition of response in order to accurately respond to the slow velocity (e.g., Nakamoto & Mori, 2012). Lutein and zeaxanthin could influence the conduction of neural signals related to general processing speed and inhibition via promoting gap junction communication and axonal conduction rate in the cerebellum.

Summary and Purpose

The neural efficiency hypothesis posits that lutein and zeaxanthin can influence neural communication. To date, the hypothesis is supported by (1) evidence that lutein and zeaxanthin are located in the neural substrates necessary to influence neural processing, (2) cellular studies that support specific mechanisms of action (e.g., lutein and zeaxanthin bind to tubulin and have been shown to enhance gap junction communication), and (3) behavioral evidence that links lutein and zeaxanthin status (e.g., macular pigment) to measures of neural processing in the cognitive and visual domain. Although consistent with the hypothesis, all of these data are indirect and/or cross-sectional. In recent years, however, purified supplements have become available and past studies have shown that supplementing lutein and zeaxanthin (e.g., Stringham et al., 2008), or carotenoid-rich foods (e.g., Hammond et al., 1997) can increase macular pigment density (and, presumably, central levels of lutein and zeaxanthin as well; Johnson, 2012). The

ability to supplement allows a causal assessment of the hypothesis. To wit, supplementing the xanthophylls, when compared to a placebo, should result in improvements in those measures thought to reflect efficiency of neural processing.

Specific Aims and Hypotheses

Part 1: Cross-Sectional Investigation. The purpose of the cross-sectional investigation was to determine the relationship between macular pigment and measures of temporal vision and visual motor reaction time in a sample of young subjects (ages 18 to 32). Young subjects were recruited to obviate effects of cognitive aging which begins to manifest even in middle-age (for a discussion see Salthouse 2004). Assessments served as baseline measures for a four-month dietary intervention with lutein and zeaxanthin (detailed in part 2).

Macular pigment was measured at different loci across the retina (7.5, 30, 60, and 105 minutes of retinal eccentricity). Temporal vision measures included critical flicker fusion (CFF) thresholds and an abbreviated temporal contrast sensitivity profile (i.e., sensitivity at 0.4, 1.0, and 1.4 log frequency in the fovea and the parafovea). Assessments of temporal contrast sensitivity at three specific frequencies (in addition to CFF thresholds) were selected in order to examine the extent to which lutein and zeaxanthin status is related to different components of the temporal contrast sensitivity function (see Figure 1). Visual motor reaction time measures included variable position reaction time and coincidence anticipation ability, specifically absolute error at 5mph and 10mph and number of missed trials at 15mph and 20mph. The specific hypotheses for Part 1 were as follows:

I. CFF thresholds will positively correlate with macular pigment.

II. Temporal contrast sensitivity at 0.4, 1.0, and 1.4 log frequency in the fovea and the parafovea will positively correlate with macular pigment.

III. Coincidence anticipation measures (i.e., absolute error at 5pmh and 10mph and number of missed trials at 15mph and 20mph) will negatively correlate with macular pigment.

IV. Variable position reaction time will negatively correlate with macular pigment.

Part 2: Dietary Supplementation. The purpose of the dietary supplementation was to assess changes in temporal vision and visual motor reaction time as a result of tissue changes in lutein and zeaxanthin. Subjects consumed a dietary supplement for four months containing either 20mg/day of zeaxanthin (EyePromise Zeaxanthin, provided by ZeaVision, LLC), 26mg/day of zeaxanthin + 8mg/day of lutein + 190mg/day of omega-3 fatty acid (EyePromise vizual EDGE, provided by ZeaVision, LLC; referred to as the multi condition), or a placebo.

This was the first investigation of the effects of supplementing lutein and zeaxanthin on temporal contrast sensitivity, reaction time, and coincidence anticipation ability. Measures of macular pigment served as a proxy of nutrient absorption into neural tissue. Based on the idea that lutein and zeaxanthin may enhance neural efficiency, the specific hypotheses for Part 2 were as follows:

I. CFF thresholds will increase after supplementation (e.g., subjects will be more sensitive to flicker, and therefore less sensitive to perceptual fusion, thereby increasing the frequency at which the target stimulus is perceptually fused).

II. Temporal contrast sensitivity at 0.4, 1.0, and 1.4 log frequency in the fovea and the parafovea will increase after supplementation (i.e., subjects will be more sensitive to

luminance differences over time, and therefore will require less depth of modulation to detect flicker).

III. Coincidence anticipation ability will improve after supplementation, as evidenced by a decrease in absolute error at 5mph and 10mph and a decrease in the number of missed trials at 15mph and 20mph.

IV. Variable position reaction time will decrease after supplementation.

CHAPTER 2

METHOD

Subjects

Subjects were young adults, aged 18-32 years, recruited from the University of Georgia and surrounding Athens community. Exclusion criteria included: corrected visual acuity worse than 20:60, a history of ocular disease, and the use of a lutein or zeaxanthin supplement in the last six months. A total of 92 subjects were recruited for the study in order to over-enroll for each condition in Part 2 as a means of accounting for attrition (36 males, 56 females, average age 21.7 years). For Part 2, the goal was to have 25 subjects in each treatment condition in addition to 10 subjects for a placebo condition (N = 60 total). All participants were included in cross-sectional analyses for Part 1. A total of 29 participants completed the zeaxanthin condition (14 males, 15 females), 25 completed the multi condition (9 males, 16 females), and 10 completed the placebo condition (4 males, 6 females). The remaining subjects either voluntarily dropped from the study (N = 8), were dropped due to noncompliance (e.g., 50% or less compliant according to pill counts half way through the intervention, N = 11), or were lost to follow-up (N = 9).

Procedure

All procedures were approved by the University of Georgia's Institutional Review Board. For Part 1, subjects completed measures of macular pigment, temporal vision, and visual motor reaction time twice within a week's time. The average of the two visits was used for cross-

sectional analyses and as baseline measures for Part 2. At the second baseline visit, subjects received a four month supply of supplements. To promote compliance, phone calls were made to each subject every two weeks over the course of four months. Following supplementation, subjects returned to the lab for a final assessment of macular pigment, temporal vision, and visual motor reaction time.

Assessment of Macular Pigment Optical Density

Macular pigment was assessed with customized heterochromatic flicker photometry using the table-top device described by Wooten et al. (1999). This psychophysical method required the subject to adjust the intensity of a 460nm light (maximally absorbed by macular pigment), which alternated in square-wave at an individually-customized rate with a 570nm light (outside of the absorbance spectrum of macular pigment), until the perception of flicker was eliminated. This was done in the fovea, where macular pigment is present, and the parafovea, where macular pigment is not detectable. The log-difference in the amount of energy required to eliminate the flicker between the fovea and the parafovea was used to derive macular pigment optical density. The size of the foveal stimulus was changed to enable the calculation of macular pigment at different locations across the retina, specifically 7.5, 30, 60, and 105 minutes of retinal eccentricity.

Assessment of Temporal Vision

Temporal vision was assessed by the customized, LED-driven tabletop device described by Wooten et al. (2010). The test stimulus consisted of a 1-degree 660nm target at the center of a 5.5-degree 660nm surround, separated by a 4 arc minute gap (see Figure 2). A fixation point at

the center of the target was used for foveal measurements, and parafoveal measurements were obtained using a 2-degree target and a long-wave fixation point located 7-degrees nasally. Subjects viewed the stimuli through a 3mm artificial pupil. Measurements of temporal contrast sensitivity occurred at 0.4, 1.0, and 1.4 log frequency (i.e, the LEDs were presented in sine-wave at 2.51hz, 10hz, and 25hz, respectively). Temporal contrast sensitivity values were derived from temporal contrast thresholds, or the depth of modulation at which the target first appeared to flicker. Depth of modulation refers to the amplitude modulation of the sine wave, or the difference between the maximum and minimum luminance of the wave. For each frequency setting, the target was initially set at 0% depth of modulation (and therefore perceptually fused) and increased until the subject reported flicker detection, for a total of five ascending trials for each frequency setting. In order to obtain CFF thresholds, depth of modulation was fixed at 100% (i.e., the stimulus varied from completely on to completely off) and the frequency of the target was adjusted. CFF was calculated as the average of an ascending and descending trial (i.e., frequency was either increased until the target perceptually fused or decreased until flicker was first detected, respectively).

Assessment of Visual Motor Reaction Time

Reaction time and coincidence anticipation timing were assessed with a linear array of 120 LEDs (broad band white, λmax = 460 nm) placed 2.02 centimeters apart on a 10.1 foot long track mounted at a height of 5.5 feet and a distance of 4 feet from the subject. The variable-position reaction time task required the subject to make a button press in response to the illumination of an LED at a random location along the linear track. Subjects completed a total of 80 trials, 60 of which were used to calculate an average reaction time (the first and last ten trials

were removed – these were in place to allow the subject to become familiar with the task and to account for any trials with an error resulting from a button-press error, respectively). The latency between trials was varied between 1000 and 3000 milliseconds. The coincidence anticipation timing task required the subjects to make a button press in response to the arrival of a moving light bar (created by lighting individual LEDs in a rapid sequence) at a designated point along the track (see Figure 3 for a general configuration). The speed randomly varied between 5, 10, 15, and 20 miles per hour (mph) for 60 trials, 15 trials for each velocity, with a between-trial latency varying randomly between 1000 and 3000 milliseconds. Response error was calculated as the time in milliseconds of a response made before or after the target, and a trial was designated a "missed" trial if a button press did not occur before the light bar arrived at the end of the track. Trial activation, randomization of LEDs, and button presses were controlled and recorded with customized software and custom-designed electronics.

Statistical Analyses

Results were analyzed with SPSS 17.0 and Origins 8.1. Cross-sectional relationships in Part 1 were analyzed with Pearson product-moment correlations (one-tailed, statistical significance set at $p < 0.05$). Changes in dependent variables after supplementation in Part 2 were assessed with paired samples *t*-tests.

CHAPTER 3

RESULTS

Part 1

Table 2 lists the reliability coefficients for measures of macular pigment, temporal vision, and visual motor reaction time. Measures of macular pigment had excellent internal consistency (Cronbach's $\alpha = 0.93$). Assessments of foveal and parafoveal temporal contrast sensitivity, for all three target frequencies, had good internal consistency with a Cronbach's α of 0.80 on average. Good internal consistency (Cronbach's α of 0.80 on average) was also found for measures of variable position reaction time and coincidence anticipation timing error at 10mph and number of missed trials at 15mph and 20mph. Coincidence anticipation timing error at 5mph, however, had poor internal consistency with a Cronbach's α of 0.51.

Table 3 lists the average baseline correlations with macular pigment. Subjects with higher macular pigment had higher CFF (see Figure 4), temporal contrast sensitivity in the fovea (see Figure 5), and parafovea (see Figure 6). Higher macular pigment was also associated with less error at 5mph and fewer missed trials at 15mph for the coincidence anticipation timing task (see Figure 7). Error at 10mph and missed trials at 20mph (see Figure 7), and variable position reaction (see Figure 8) were not significantly related to macular pigment.

Part 2

The descriptive statistics for baseline measures of temporal vision and visual motor reaction time for subjects in each of the three treatment groups are listed in Table 4 and Table 5,

respectively. Differences among the three groups with respect to each variable were analyzed with a one-way ANOVA. No significant differences were found, with the exception of absolute error at 10mph for the coincidence anticipation task ($F = 4.33$, $p = 0.02$); post-hoc comparisons (Tukey's HSD) indicated significant differences between subjects in the zeaxanthin group and the multi group. No significant differences were found between the average macular pigment at 30 minutes retinal eccentricity for each group ($F = 1.55$, $p = 0.22$).

Table 6 lists baseline and final macular pigment values for each group. An aggregate measure was derived for descriptive purposes to account for the spatial distribution of macular pigment and subsequent changes at all retinal eccentricities after supplementation. Macular pigment significantly increased for subjects in the zeaxanthin group (N = 29; 0.40 ± 0.15 to 0.49 ± 0.12 at 30 minutes eccentricity, $t = 4.67$, $p < 0.01$; 1.29 ± 0.52 to 1.56 ± 0.45 aggregate macular pigment change, $t = 5.12$, $p < 0.01$) and for subjects in the multi group (N = 25; 0.33 ± 0.15 to 0.42 ± 0.16, $t = 3.69$, $p < 0.01$ at 30 minutes eccentricity; 1.09 ± 0.50 to 1.37 ± 0.52, $t = 5.14$, $p < 0.01$). Macular pigment significantly decreased at 30 minutes eccentricity for subjects in the placebo group (0.42 ± 0.18 to 0.39 ± 0.19, $t = 2.96$, $p = 0.03$), but no significant changes were found for aggregate macular pigment (1.31 ± 0.59 to 1.27 ± 0.64, $t = 0.82$, $p = 0.43$).

Temporal Vision. Changes in temporal vision after four months of supplementation are listed in Table 7. The data are separated by subjects in the placebo group or an active treatment condition (subjects for the zeaxanthin and multi condition were combined); changes in temporal vision for individual active treatment groups are listed in Table 8. As predicted, critical flicker fusion thresholds significantly increased for subjects in the active treatment condition (N = 54, $t = 2.53$, $p = 0.01$), whereas no significant changes were found for subjects in the placebo

condition (N = 10, t = 0.19, p = 0.86). Statistically significant increases in CFF thresholds were maintained for subjects in the zeaxanthin condition (N = 29, t = 3.02, p = 0.005) but not for subjects in the multi condition (N = 25, t = 0.45, p = 0.66).

Foveal temporal contrast sensitivity significantly increased for subjects in the active treatment condition at each target frequency (1.4 log hz: t = 5.53, p < 0.01; 1.0 log hz: t = 6.44, p < 0.01; 0.4 log hz: t = 4.71, p < 0.01). Significant increases were maintained when data were analyzed by specific treatment group (see Table 8). For the placebo group, no significant changes were found at 1.4 log hz (t = 1.16, p = 0.23) or 0.4 log hz (t = 0.94, p = 0.37), however temporal contrast sensitivity significantly increased at 1.0 log hz (t = 2.58, p = 0.03).

Parafoveal temporal contrast sensitivity significantly increased for subjects in the active treatment condition at each target frequency (1.4 log hz: t = 2.91, p < 0.01; 1.0 log hz: t = 2.94, p = 0.01; 0.4 log hz: t = 3.08, p < 0.01). Significant increases were maintained for subjects in the multi condition, however, for subjects in the zeaxanthin condition, increases in parafoveal temporal contrast sensitivity did not reach statistical significance (see Table 8).

Visual Motor Reaction Time. Changes in visual motor reaction time after supplementation are listed in Table 9 (separated by either active treatment or placebo groups; see Table 10 for changes in these variables for the zeaxanthin and multi group specifically). Coincidence anticipation timing error at 5mph and 10mph did not significantly change for the treatment group (t = 0.99, p = 0.33 and t = 0.32, p = 0.75, respectively). The number of missed trials at 15mph and 20mph did significantly decrease (t = 2.54, p = 0.01 and t = 2.78, p = 0.01, respectively). The absence of a significant change in error at 5mph and 10mph was maintained when data were analyzed by specific treatment group, however significant decreases at 15mph

were only found for the multi group and significant decreases at 20mph were only found for the zeaxanthin group. For the placebo group, no significant changes in coincidence anticipation timing, either error or number of missed trials, were found. Variable position reaction time significantly decreased for subjects in the active treatment condition ($t = 3.60$, $p < 0.01$), however this significant change was only maintained for subjects in the multi condition (see Table 10). Subjects in the placebo group did not have significant changes in variable position reaction time ($t = 0.12$, $p = 0.91$).

CHAPTER 4

DISCUSSION

This study was an investigation of the effects of lutein and zeaxanthin on temporal vision and visual motor reaction time. The goal was to test the neural efficiency hypothesis of lutein and zeaxanthin function by 1) correlating macular pigment, a biomarker of neural lutein and zeaxanthin status, with critical flicker fusion thresholds, temporal contrast sensitivity, coincidence anticipation ability, and reaction time; and 2) assessing changes in temporal vision and visual motor reaction time after four months of xanthophyll supplementation. Assessments of the dependent variables were reliable (Cronbach's alpha between 0.7 and 0.8, with the exception of one coincidence anticipation variable, which will be discussed later). Cross-sectional correlations were consistent with past research: subjects with higher macular pigment had higher critical flicker fusion thresholds and higher temporal contrast sensitivity, along with better performance on certain coincidence anticipation settings. Supplementation with lutein and zeaxanthin lead to improvements on select measures of temporal vision and reaction time. In general, the findings from this study lend support to the neural efficiency hypothesis. Certain considerations, however, should be taken into account when interpreting the data, such as the characteristics of the sample, the utility of macular pigment as an index of neural lutein and zeaxanthin status, and methodological factors related to the outcome variables.

Part 1 Summary

The first part of the study consisted of data from 92 college-aged subjects. The average macular pigment (0.36 at 30 minutes retinal eccentricity) was consistent with what was expected,

however, only three people had macular pigment of 0.1 or less, and three people had macular pigment of 0.7 or more. Despite the limited variability in macular pigment, the results indicated a significant relationship with temporal vision and visual motor reaction time.

Macular pigment accounted for approximately 11.5% of the variance in critical flicker fusion thresholds, which is consistent with Hammond and Wooten's (2005) findings for their younger sample (r = 0.33). Temporal contrast sensitivity varied depending on target frequency in a manner consistent with past research: sensitivity peaked at 1.0 log hertz, was slightly lower at 0.4 log hertz, and was the lowest at the highest target frequency of 1.4 log hertz (see Figures 5 and 6). Macular pigment accounted for 14% of the variance in foveal temporal contrast sensitivity at the highest frequency and approximately 10% of the variance at the lower frequencies. The effect was slightly higher than Renzi and Hammond's (2010) findings, although they used area under the curve as a comprehensive measure of temporal vision (r = 0.29). Parafoveal temporal contrast sensitivity was also related to macular pigment in this sample (approximately 7% of the variance was accounted for).

Macular pigment was significantly related to visual motor reaction time, specifically coincidence anticipation timing error at 5mph and the number of missed trials at 15mph, accounting for approximately 7% of the variance. Error at 10mph and the number of missed trials at 20mph did not reach statistical significance. This could reflect the nature of the task itself. For example, trials at 5mph likely required more precision to accurately time the response compared to higher velocities and therefore resulted in a wider distribution of scores for this sample compared to other velocities. For other tasks, such as reaction time and coincidence anticipation at 20mph (which, given the time window available to make a response, is analogous to another speed-based reaction time task), the restricted range of scores may have limited the

ability to find a relationship with macular pigment. For example, in this sample, macular pigment was not related to variable position reaction time. A significant relationship was found in Renzi et al.'s (2013) sample of young college students, however their average reaction time was 30 milliseconds slower (258ms compared to 228ms) and there was a wider range of performance (187ms – 488ms compared to 201ms – 310ms).

In general, the strength of the significant relationships is moderate and likely captures the effect accurately when considering the multifaceted nature of temporal vision and visual motor reaction time. It is doubtful that lutein and zeaxanthin status could account for a large amount of variability in processing speed, particularly in young individuals from a relatively homogeneous sample. These tasks are inherently dynamic as well, and could change as a function of daily fluctuations in alertness, for example. This is likely the reason why the reliability data for temporal vision and visual motor reaction time assessments were lower than assessments of macular pigment, which is typically stable.

Part 2 Summary

The goal of the second part of this study was to determine if supplementation with lutein and zeaxanthin lead to improvements in temporal vision and visual motor reaction time. Change in lutein and zeaxanthin status was quantified by macular pigment, under the assumption that macular pigment represents a marker of xanthophylls throughout neural tissue. Since lutein and zeaxanthin may improve neural efficiency via mechanisms such as enhanced gap junction communication, it was hypothesized that an increase in lutein and zeaxanthin status from four months of dietary supplementation (as indicated by higher macular pigment) would result in increased critical flicker fusion thresholds and temporal contrast sensitivity, decreased

coincidence anticipation error and fewer missed trials, and decreased variable position reaction time.

Macular pigment significantly increased across the retina for those subjects who completed four months of supplementation with either zeaxanthin or a combined dose of zeaxanthin, lutein, and omega-3 fatty acid. Macular pigment stayed the same for subjects in the placebo group, with the exception of a slight decrease at 30 minutes retinal eccentricity. For the treatment group, the average increase in retinal lutein and zeaxanthin was close to a tenth of a log unit for the most central retinal eccentricities. Comparisons to other supplementation studies are somewhat challenging given that most trials differ with respect to the dosage or the duration of the intervention. However, the magnitude of change in macular pigment is close to that of Stringham et al. (2008), who supplemented 40 individuals with 12mg/day of lutein and zeaxanthin (primarily lutein) for six months and reported a 0.16 log unit change in macular pigment. Supplementation with the 20mg/day of zeaxanthin formula used in this project has also lead to a tenth of a log unit change in macular pigment for 10 females supplemented for three months (unpublished data).

After supplementation, subjects in the treatment groups had higher critical flicker fusion thresholds and temporal contrast sensitivity in the fovea and the parafovea. Improvements reflected an increase in processing speed of approximately one cycle per second and a reduction in temporal contrast required for target detection by approximately 10% (this contrast reduction, resulting in better sensitivity, was greatest for high-frequency targets). However, these findings should be interpreted with caution since the placebo group also had significant changes in temporal contrast sensitivity in the fovea and the parafovea, though these were for the lower target frequencies as opposed to higher target frequencies. Furthermore, although CFF

thresholds did not change for the placebo group, four of the ten participants had a higher CFF threshold after supplementation. The lack of a significant change could have been driven by two individuals who had drastic decreases in CFF. More definitive interpretations could be made with more subjects in the placebo group and if the sample sizes of the placebo and treatment groups were the same.

Coincidence anticipation timing error at 5pmh and 10mph did not statistically change for either the treatment or placebo groups. Although subjects with higher macular pigment had less error at 5mph at baseline, supplementation with lutein and zeaxanthin did not improve performance on this task. Coincidence anticipation ability is a complex skill that can be influenced not only by factors related to the individual performing the task (e.g. motor speed, inhibition, etc.) but also by the way in which the task itself is presented. The design for this study included randomization of four velocities across sixty trials. This was done in part to control for potential learning effects that could occur if fifteen trials of a single velocity were presented in succession. However, in doing so, a new element of variability was introduced. Each subject received a different pattern of trial-to-trial speed variations, which, as discussed, can influence the response (e.g., greater anticipation occurs when a slow velocity trial follows a fast velocity trial). It could be that methodological factors limited the ability to find a significant effect of supplementation on coincidence anticipation error, or that quantifying visual motor ability by absolute error for these velocities is not the most accurate way to define the skill. The number of missed trials for the faster velocities, on the other hand, did significantly decrease for the treatment group and remained the same for the placebo group. Variable position reaction time also improved for the treatment group, with an average decrease in reaction time of nearly seven milliseconds. This significant reduction in the number of missed trials at fast velocities

and overall reaction time for the treatment group is consistent with increases in critical flicker fusion thresholds, which indicated that subjects could process an additional luminance cycle per second.

Although changes in temporal vision and visual motor reaction time were also analyzed separately for the two individual treatment groups, it is hard to deduce differences in outcome variables as a function of the treatment condition (i.e., pure zeaxanthin versus a combined dose of zeaxanthin, lutein, and omega-3 fatty acids, see Tables 8 and 10). Each sample on its own has limited statistical power, and any observed differences between the two treatment groups could be a function of error and the small sample sizes. A larger number of people in each condition, and perhaps a longer supplementation duration, may allow for a more accurate comparison between the effectiveness of different supplementation regimes. At this stage, it is more worthwhile to consider both treatment groups comprehensively, since the primary goal was to increase xanthophyll concentration throughout neural tissue.

Since retinal lutein and zeaxanthin correlated with amounts in cortical tissue and xanthophyll-rich diets lead to concurrent increases in cortical amounts in primates (Vishwanathan et al., 2013), it was assumed that the observed changes in macular pigment reflected an increase in cortical lutein and zeaxanthin as well. However, as mentioned, it is unknown how increases in macular pigment optical density translate to specific amounts in cortical tissue. Despite this limitation, changes in retinal lutein and zeaxanthin status were accompanied by functional differences in visual processing speed. The magnitude of improvements may be more prominent for individuals in a deficient state, and individual differences may be more apparent when comparing people with low lutein and zeaxanthin status compared to high lutein and zeaxanthin status. These particular subjects, however, were

relatively similar with respect to macular pigment. A larger sample size with a wider range of macular pigment would likely elucidate individual differences and allow for a greater response to nutritional modification in order to compare changes between individuals with and without an increase in neural lutein and zeaxanthin status.

The specific mechanisms of enhanced processing speed remain speculative. At this stage, it is not possible to definitively say whether or not the observed changes in temporal vision and visual motor reaction time reflect changes in neural efficiency at the level of the retina or the cortex specifically. It is likely that cortical changes in neural communication lead to improvements in the outcome variables, particularly given the increase in critical flicker fusion thresholds and the reduction in reaction time, along with uniform increases in different components of the temporal contrast sensitivity function in both the fovea and the parafovea. However, it is not possible to rule out the influence of changes at the level of the retina, such as the potential for an increase in retinal response time as a function of enhanced gap junction communication among retinal cells.

General Discussion

Individual variation in temporal vision and visual motor reaction time was in part accounted for by lutein and zeaxanthin status, as determined by macular pigment. Supplementation with xanthophylls lead to changes in macular pigment and select outcome variables related to temporal processing speed and reaction time. The magnitude increase in cortical lutein and zeaxanthin relative to the change in macular pigment optical density is not known. However, this study represents a starting point in deducing functional differences associated with variation in xanthophyll status, both cross-sectionally and after a dietary

intervention, in order to test the effects of lutein and zeaxanthin on temporal vision and visual motor reaction time. Considering these variables in isolation and drawing conclusions regarding the efficiency of the neural system simply from these two domains, however, has its limitations. Like lifestyle in general, which encompasses several factors such as diet, exercise, stress levels, etc., the efficiency of the neural system is not accurately defined by one or two variables in isolation. For example, an individual could be considered healthy based on his or her diet and exercise regime, but be a life-long smoker. Similarly, individuals with slower temporal processing may still excel at cognitive tasks that are also considered to reflect an efficient neural system. The goal of this study was to begin to test the neural efficiency hypothesis using select outcome variables, and the results lend support to additional work that can more comprehensively quantify neural efficiency and assess changes after supplementation with lutein and zeaxanthin.

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Table 1: Overview of studies that report cortical concentrations of lutein and zeaxanthin [reported as means (standard deviations)].

Study	**Subjects**	**Brain Region**	**Lutein (pmol/g)**	**Zeaxanthin (pmol/g)**
Craft et al., 2004	Non-diseased elders deceased ages 67-90 years; N = 5.	Frontal Cortex (Gray Matter)	11.8 (2.6)	9.2 (2.3)
		Frontal Cortex (White Matter)	8.7 (3.2)	7.8 (2.8)
		Occipital Cortex (Gray Matter)	8.3 (2.3)	6.7 (2.4)
		Occipital Cortex (White Matter)	2.8 (1.2)	1.8 (0.6)
Vishwanathan et al., 2011	Infants deceased < 1 year of age; N = 30.	Frontal Cortex (N = 29)	41.33 (16.94)	11.98 (3.22)
		Hippocampus (N = 24)	30.28 (9.42)	12.21 (25.2)
		Auditory Cortex (N = 11)	53.43 (18.93)	18.51 (4.28)
		Occipital Cortex (N = 28)	47.0 (18.57)	12.60 (3.42)
Vishwanathan et al., 2013*	Primates fed stock diet, deceased ages 9-13 years; N = 5.	Cerebellum	20.65	20.00
		Frontal Cortex	40.1	30.6
		Occipital Cortex	50.65	50.1
		Pons	10.3	10.4
	Primates fed pure lutein or zeaxanthin diet, deceased ages 9-15 years; N = 6 per group.	Cerebellum	250	75
		Frontal Cortex	425	100
		Occipital Cortex	1225	150
		Pons	150	25

*Approximation of L and Z; values derived from Figure 1 in Vishwanathan et al., 2013. Standard errors not included in the figure.

Table 2: Descriptive statistics (mean ± standard deviation) for each baseline measure and associated reliability estimates (Cronbach's alpha).

	Baseline 1	Baseline 2	N[1]	α
Macular Pigment (30 eccentricity)	0.36 ± 0.16	0.37 ± 0.17	86	0.93
Critical Flicker Fusion Thresholds	26.14 ± 2.95	26.95 ± 2.18	83 [2]	0.71
Foveal Temporal Contrast Sensitivity				
1.4 log hertz	0.17 ± 0.17	0.18 ± 0.17	75 [2, 3, 4]	0.83
1.0 log hertz	1.23 ± 0.16	1.23 ± 0.14	82 [2,3]	0.82
0.4 log hertz	1.18 ± 0.17	1.16 ± 0.15	82 [2,3]	0.84
Parafoveal Temporal Contrast Sensitivity				
1.4 log hertz	0.34 ± 0.20	0.35 ± 0.20	78 [2, 3, 5, 6]	0.81
1.0 log hertz	1.09 ± 0.14	1.07 ± 0.14	81 [2, 3, 6]	0.81
0.4 log hertz	0.99 ± 0.13	0.98 ± 0.15	81 [2, 3, 6]	0.78
Coincidence Anticipation Timing				
Error - 5MPH	75.21 ± 38.93	69.58 ± 35.65	76 [3, 7]	0.51
Error - 10MPH	71.79 ± 33.85	67.41 ± 35.67	76 [3, 7]	0.82
Missed - 15MPH	2.66 ± 2.76	1.48 ± 2.38	76 [3, 7]	0.70
Missed - 20MPH	6.75 ± 3.82	5.20 ± 3.47	76 [3, 7]	0.80
Variable Position Reaction Time	231.01 ± 21.55	225.88 ± 21.85	77 [7]	0.80

[1] Out of 92 subjects, 6 did not complete a second baseline (2 drops and 4 time restrictions during over-enrollment). Total N for measurement reliability is 86.
[2] Only one baseline for 3 subjects as a result of equipment maintenance.
[3] First baseline visit excluded for 1 subject who had difficulty with the task.
[4] A total of 8 subjects could not detect flicker for at least one baseline visit.
[5] A total of 3 subjects could not detect flicker for at least one baseline visit.
[6] First baseline visit excluded for 1 subject as a result of equipment malfunction for parafoveal measures.
[7] Only one baseline for 9 subjects as a result of equipment maintenance.

Table 3: Baseline correlations between macular pigment and measures of temporal vision and visual motor reaction time (N = 92).

	Macular Pigment Optical Density (30´ eccentricity)	
	r-value	*p*-value
Critical Flicker Fusion Thresholds	0.34	< 0.01
Foveal Temporal Contrast Sensitivity		
1.4 log hertz	0.38	< 0.01
1.0 log hertz	0.32	< 0.01
0.4 log hertz	0.31	< 0.01
Parafoveal Temporal Contrast Sensitivity		
1.4 log hertz	0.25	< 0.01
1.0 log hertz	0.27	< 0.01
0.4 log hertz	0.29	< 0.01
Coincidence Anticipation Timing		
Error - 5MPH	-0.28	< 0.01
Error - 10MPH	-0.11	0.15
Missed - 15MPH	-0.26	< 0.01
Missed - 20MPH	-0.07	0.26
Variable Position Reaction Time	-0.07	0.25

Table 4: Descriptive statistics [mean ± standard deviation (range)] for measures of temporal vision for each treatment group at baseline.

	Placebo (N = 10)	Zeaxanthin (N = 29)	Multi (N = 25)	*F*-value	*p*-value
Critical Flicker Fusion Thresholds	28.50 ± 3.76 (23.35 – 35.37)	26.59 ± 2.37 (22.82 – 30.68)	26.60 ± 1.65 (23.87 – 28.92)	2.68	0.08
Foveal Temporal Contrast Sensitivity					
1.4 log hertz	0.24 ± 0.20 (0.0 – 0.54)	0.18± 0.19 (0.0 – 0.60)	0.17 ± 0.10 (0.0 – 0.32)	0.79	0.46
1.0 log hertz	1.28 ± 0.14 (1.06 – 1.51)	1.25± 0.17 (0.95 – 1.71)	1.24 ± 0.07 (1.10 – 1.37)	0.36	0.70
0.4 log hertz	1.20 ± 0.16 (1.01 – 1.50)	1.19 ± 0.19 (0.89 – 1.81)	1.17 ± 0.12 (1.02 – 1.26)	0.23	0.79
Parafoveal Temporal Contrast Sensitivity					
1.4 log hertz	0.37 ± 0.23 (0.0 – 0.58)	0.34 ± 0.22 (0.0 – 0.69)	0.38 ± 0.12 (0.11 – 0.59)	0.39	0.68
1.0 log hertz	1.17 ± 0.10 (1.04 – 1.27)	1.08 ± 0.16 (0.72 – 1.40)	0.12 ± 0.06 (0.99 – 1.25)	0.52	0.60
0.4 log hertz	1.07 ± 0.09 (0.93 – 1.18)	0.99 ± 0.14 (0.66 – 1.36)	0.99 ± 0.15 (0.39 – 1.11)	0.04	0.96

Table 5: Descriptive statistics [mean ± standard deviation (range)] for measures of visual motor reaction time for each treatment group at baseline.

	Placebo (N = 10)	Zeaxanthin (N = 29)	Multi (N = 25)	*F*-value	*p*-value
Coincidence Anticipation Timing[1]					
Error - 5MPH	77.71 ± 47.11 (31.2 – 195.22)	60.71 ± 25.39 (20.15 – 149.76)	77.47 ± 28.16 (29.23 – 129.64)	2.40	0.10
Error - 10MPH	75.51 ± 32.81 (41.39 – 144.16)	59.09 ± 29.5 (22.09 – 132.59)	83.69 ± 32.08 (39.33 – 151.22)	4.33	0.02[2]
Missed - 15MPH	2.0 ± 2.37 (0.0 – 8.0)	2.16 ± 2.45 (0.0 – 9.5)	2.44 ± 2.51 (0.0 – 11.5)	0.15	0.86
Missed - 20MPH	5.35 ± 2.86 (2.0 – 10.5)	5.95 ± 3.99 (0.5 – 13)	7.42 ± 3.16 (1.5 -15)	1.73	0.19
Variable Position Reaction Time	219.63 ± 14.17 (196.82 – 238.79)	232.13 ± 25.92 (201.23 – 310.38)	227.62 ± 19.56 (201.43 – 281.22)	1.22	0.30

[1]Error is expressed in milliseconds (ms); Missed is expressed in total number of trials.
[2]Post-hoc comparisons indicate significant differences between subjects in the zeaxanthin group and subjects in the multi group.

Table 6. Macular pigment at baseline and after four months of supplementation for each group.

Group	Retinal Eccentricity	Baseline	Final	Change	t-value
Placebo (N = 10)	7.5´	0.49 ± 0.18	0.47 ± 0.19	- 0.02	1.02
	30´	0.42 ± 0.18	0.39 ± 0.16	- 0.03	2.69[1]
	60´	0.28 ± 0.15	0.28 ± 0.09	0.00	0.10
	105´	0.12 ± 0.12	0.14 ± 0.59	+ 0.02	0.47
	Aggregate	1.31 ± 0.22	1.27 ± 0.63	- 0.04	0.82
Zeaxanthin (N = 29)	7.5´	0.49 ± 0.17	0.57 ± 0.18	+ 0.08	3.69[2]
	30´	0.40 ± 0.15	0.49 ± 0.16	+ 0.09	4.44[2]
	60´	0.27 ± 0.13	0.33 ± 0.12	+ 0.07	3.43[2]
	105´	0.14 ± 0.08	0.18 ± 0.10	+ 0.04	3.33[2]
	Aggregate	1.29 ± 0.50	1.56 ± 0.45	+ 0.27	5.14[2]
Multi (N = 25)	7.5´	0.43 ± 0.15	0.51 ± 0.18	+ 0.08	3.58[2]
	30´	0.33 ± 0.15	0.42 ± 0.16	+ 0.09	4.67[2]
	60´	0.22 ± 0.15	0.28 ± 0.12	+ 0.06	3.75[2]
	105´	0.10 ± 0.09	0.17 ± 0.10	+ 0.07	3.43[2]
	Aggregate	1.09 ± 0.50	1.37 ± 0.52	+ 0.28	5.12[2]

[1] $p = 0.03$
[2] $p < 0.01$

Table 7: Changes in measures of temporal vision for subjects either in an active treatment group (N = 54) or the placebo group (N = 10).

Variable	Group	Baseline	Final	Change	t-value	p-value
Critical Flicker Fusion Thresholds	Treatment	26.60 ± 2.04	27.34 ± 2.25	+ 0.74	2.53	0.01
	Placebo	28.50 ± 3.76	28.61 ± 2.11	+ 0.11	0.19	0.86
Foveal Temporal Contrast Sensitivity						
1.4 log hertz	Treatment	0.17 ± 0.15	0.25 ± 0.14	+ 0.08	5.53	< 0.01
	Placebo	0.24 ± 0.20	0.26 ± 0.23	+ 0.02	1.16	0.28
1.0 log hertz	Treatment	1.24 ± 0.13	1.37 ± 0.16	+ 0.13	6.44	< 0.01
	Placebo	1.28 ± 0.14	1.37 ± 0.12	+ 0.09	2.58	0.03
0.4 log hertz	Treatment	1.18 ± 0.15	1.27 ± 0.13	+ 0.09	4.71	< 0.01
	Placebo	1.19 ± 0.16	1.24 ± 0.12	+ 0.05	0.94	0.37
Parafoveal Temporal Contrast Sensitivity						
1.4 log hertz	Treatment	0.36 ± 0.18	0.42 ± 0.17	+ 0.06	2.91	0.01
	Placebo	0.37 ± 0.23	0.40 ± 0.22	+ 0.03	1.88	0.09
1.0 log hertz	Treatment	1.10 ± 0.13	1.17 ± 0.21	+ 0.07	2.94	0.01
	Placebo	1.10 ± 0.13	1.20 ± 0.13	+ 0.10	2.74	0.02
0.4 log hertz	Treatment	0.99 ± 0.14	1.07 ± 0.19	+ 0.08	3.08	< 0.01
	Placebo	1.01 ± 0.13	1.10 ± 0.10	+ 0.09	2.97	0.02

Table 8: Changes in measures of temporal vision for subjects in different active treatment groups.

Variable	Group[1]	Baseline	Final	Change	t-value	p-value
Critical Flicker Fusion Thresholds	Zeaxanthin	26.59 ± 2.37	28.00 ± 2.16	+ 1.41	3.02	< 0.01
	Multi	26.60 ± 1.65	26.43 ± 2.01	- 0.17	0.45	0.66
Foveal Temporal Contrast Sensitivity						
1.4 log hertz	Zeaxanthin	0.18± 0.19	0.25 ± 0.17	+ 0.07	3.51	< 0.01
	Multi	0.17 ± 0.10	0.24 ± 0.10	+ 0.07	4.61	< 0.01
1.0 log hertz	Zeaxanthin	1.25± 0.17	1.42 ± 0.17	+ 0.17	6.27	< 0.01
	Multi	1.24 ± 0.07	1.30 ± 0.11	+ 0.06	3.09	< 0.01
0.4 log hertz	Zeaxanthin	1.19 ± 0.19	1.29 ± 0.15	+ 0.10	3.12	< 0.01
	Multi	1.17 ± 0.07	1.24 ± 0.09	+ 0.07	4.52	< 0.01
Parafoveal Temporal Contrast Sensitivity						
1.4 log hertz	Zeaxanthin	0.34 ± 0.22	0.38 ± 0.18	+ 0.04	1.25	0.22
	Multi	0.38 ± 0.12	0.46 ± 0.15	+ 0.08	3.36	< 0.01
1.0 log hertz	Zeaxanthin	1.08 ± 0.16	1.15 ± 0.26	+ 0.07	1.67	0.11
	Multi	1.12 ± 0.06	1.19 ± 0.10	+ 0.07	3.90	< 0.01
0.4 log hertz	Zeaxanthin	0.99 ± 0.14	1.05 ± 0.23	+ 0.06	1.54	0.13
	Multi	0.99 ± 0.15	1.09 ± 0.09	+ 0.10	3.17	< 0.01

[1] Zeaxanthin Group, N = 29; Multi Group, N = 25.

Table 9: Changes in measures of visual motor reaction time for subjects either in an active treatment group (N = 54) or the placebo group (N = 10).

Variable	Group	Baseline	Final	Change	t-value	p-value
Coincidence Anticipation Timing[1]						
Error - 5MPH	Treatment	68.47 ± 27.77	73.39 ± 37.79	+ 4.92	0.99	0.33
	Placebo	77.71 ± 47.12	83.94 ± 47.43	+ 6.23	0.73	0.49
Error - 10MPH	Treatment	70.48 ± 32.85	69.42 ± 33.82	- 1.06	0.32	0.74
	Placebo	75.51 ± 32.81	69.70 ± 44.63	- 5.81	0.76	0.47
Missed - 15MPH	Treatment	2.29 ± 2.46	1.59 ± 2.42	- 0.70	2.54	0.01
	Placebo	2.00 ± 2.37	1.20 ± 1.55	- 0.80	1.84	0.10
Missed - 20MPH	Treatment	6.63 ± 3.67	5.52 ± 3.75	- 1.11	2.78	< 0.01
	Placebo	5.35 ± 2.86	4.70 ± 4.30	- 0.65	0.75	0.47
Variable Position Reaction Time (ms)	Treatment	229.94 ± 23.30	223.36 ± 21.64	- 6.58	3.60	< 0.01
	Placebo	219.63 ± 14.17	220.07 ± 20.44	+ 0.44	0.12	0.91

[1]Error is expressed in milliseconds (ms); Missed is expressed in total number of trials.

Table 10: Changes in measures of visual motor reaction time for subjects in different active treatment groups.

Variable	Group[1]	Baseline	Final	Change	t-value	p-value
Coincidence Anticipation Timing[2]						
Error - 5MPH	Zeaxanthin	60.70 ± 25.39	65.39 ± 32.37	+ 4.69	0.75	0.45
	Multi	77.47 ± 28.16	82.05 ± 42.26	+ 4.58	0.63	0.53
Error - 10MPH	Zeaxanthin	59.09 ± 29.5	55.39 ± 24.27	- 29.59	1.02	0.31
	Multi	83.70 ± 32.08	85.68 ± 36.40	+ 1.98	0.34	0.74
Missed - 15MPH	Zeaxanthin	2.15 ± 2.44	1.44 ± 2.16	- 0.71	1.62	0.11
	Multi	2.44 ± 2.51	1.76 ± 2.73	- 0.68	2.16	0.04
Missed - 20MPH	Zeaxanthin	5.94 ± 3.98	4.31 ± 3.24	- 1.63	2.82	0.01
	Multi	7.42 ± 3.16	6.92 ± 3.86	- 0.50	0.95	0.35
Variable Position Reaction Time (ms)	Zeaxanthin	232.13 ± 25.19	228.07 ± 22.53	- 4.06	1.59	0.12
	Multi	227.28 ± 19.91	217.66 ± 19.48	- 9.62	3.82	< 0.01

[1] Zeaxanthin Group, N = 29; Multi Group, N = 25.
[2]Error is expressed in milliseconds (ms); Missed is expressed in total number of trials.

Figure 1. The temporal contrast sensitivity function for two subjects with different macular pigment (MP).

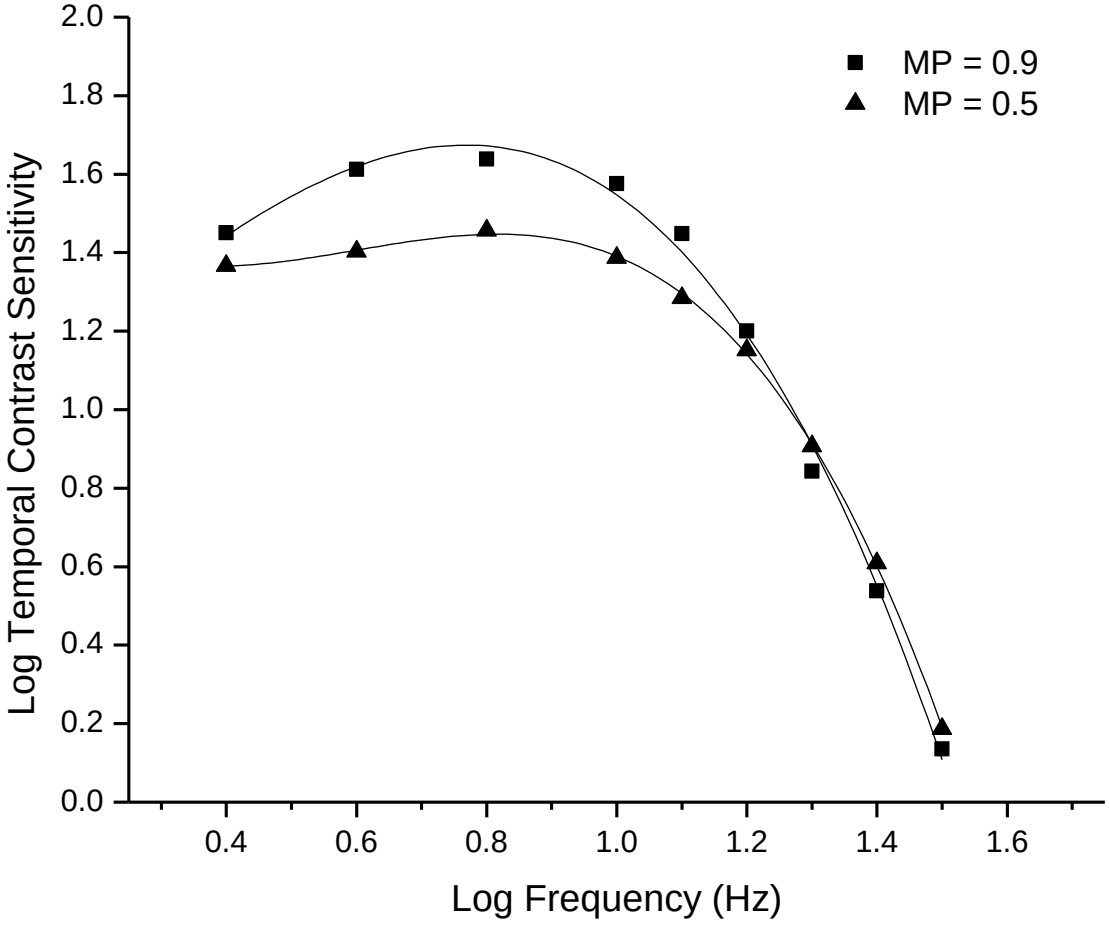


Figure 2. Stimulus configuration for assessing temporal vision (not to scale).

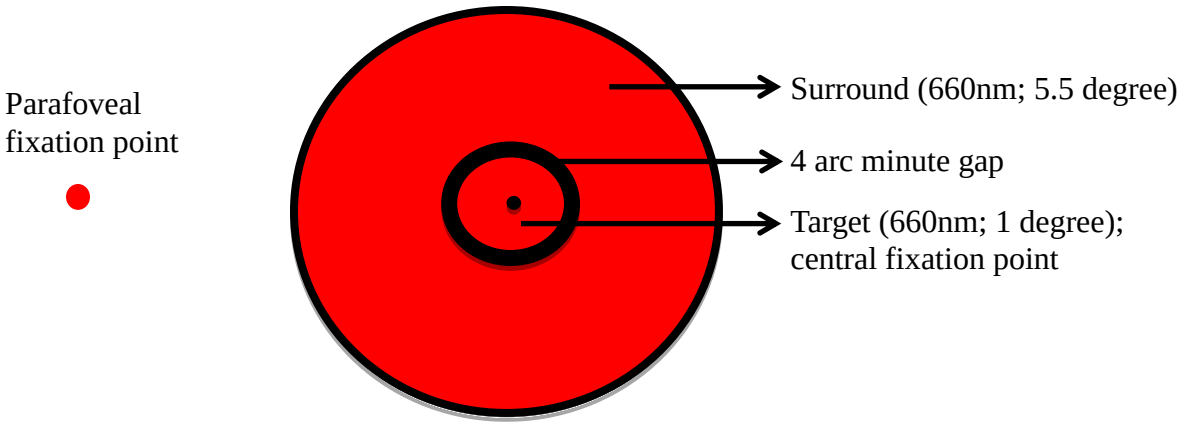
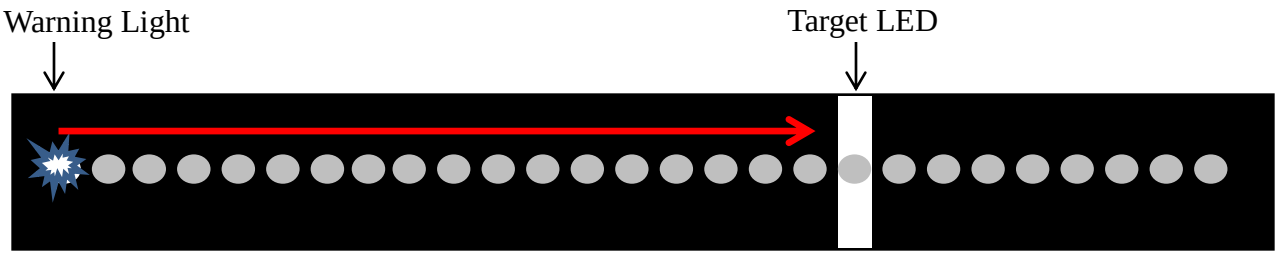


Figure 3. Stimulus configuration for assessing coincidence anticipation timing.



Speed	Perfect Anticipation	Total Track Time
5MPH	932 ms	1377 ms
10MPH	466 ms	689 ms
15MPH	311 ms	459 ms
20MPH	233 ms	344 ms

Figure 4: Baseline correlations between macular pigment and critical flicker fusion thresholds.

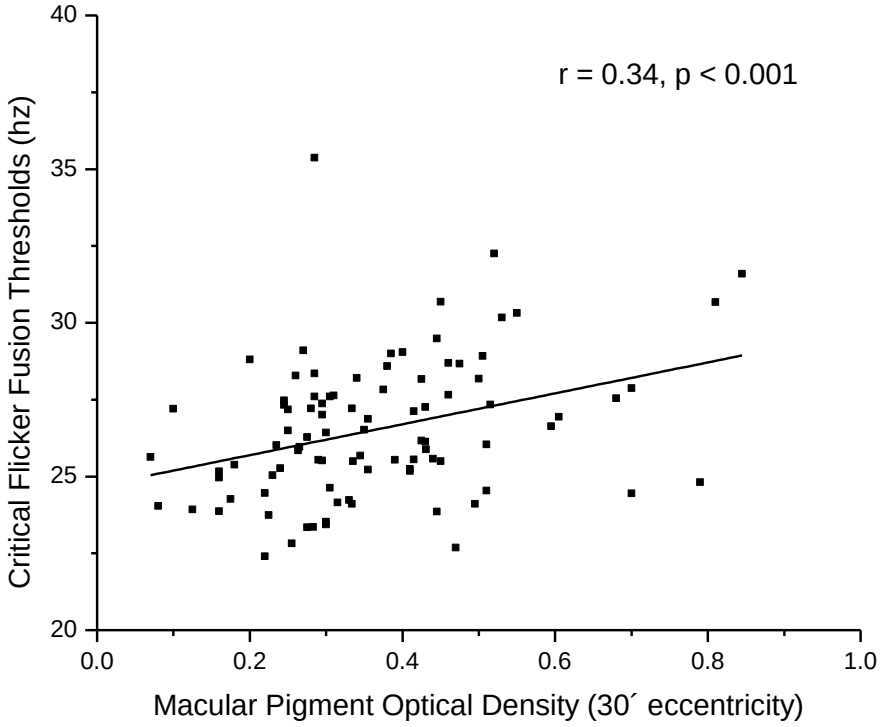


Figure 5: Baseline correlations between macular pigment and foveal temporal contrast sensitivity.

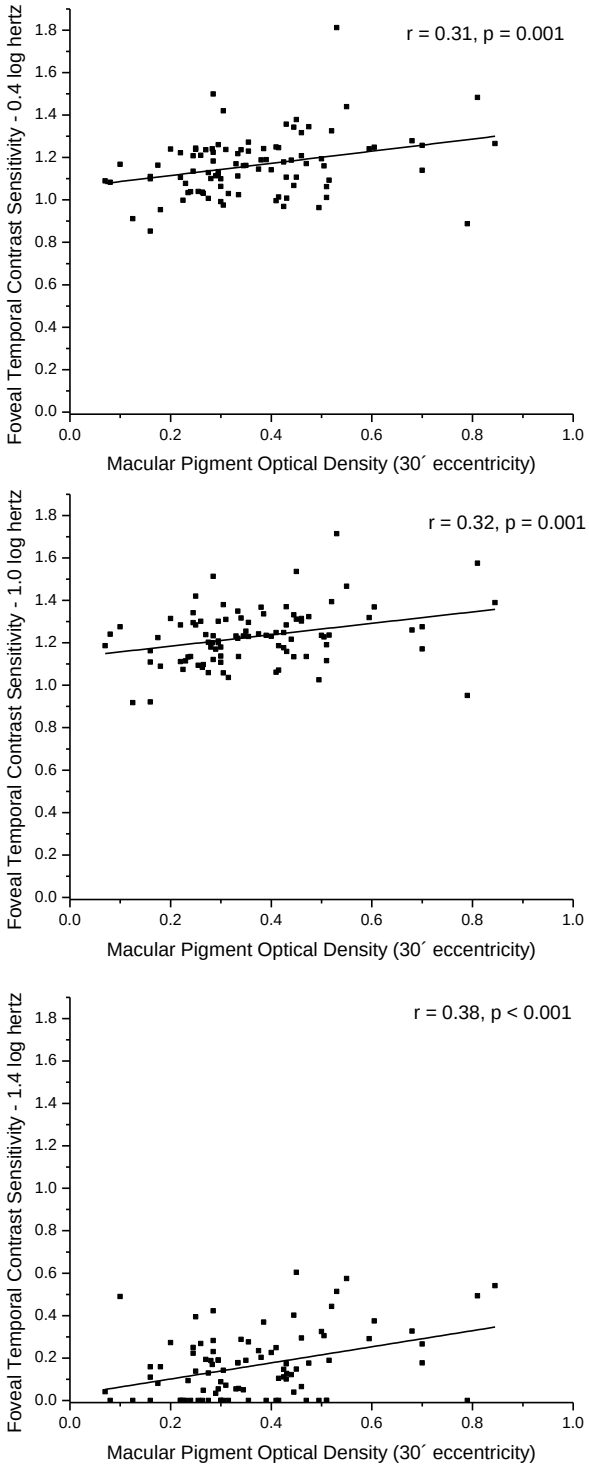


Figure 6: Baseline correlations between macular pigment and parafoveal temporal contrast sensitivity.

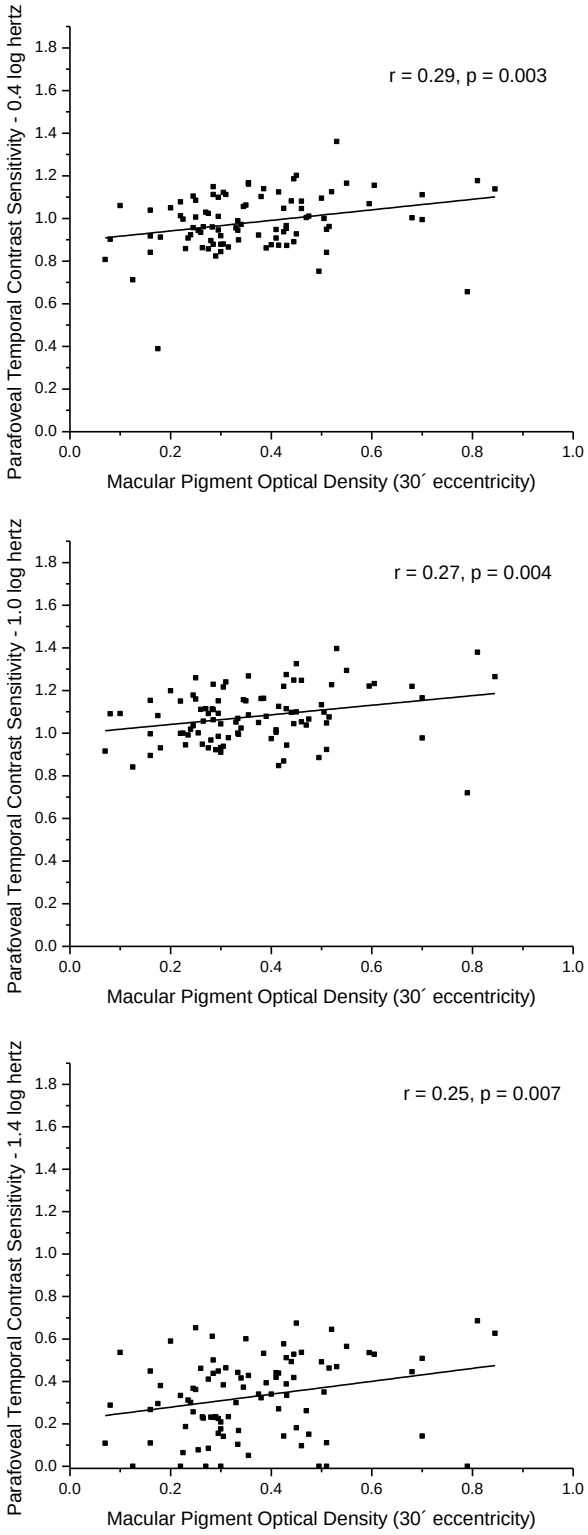


Figure 7: Baseline correlations between macular pigment and coincidence anticipation variables.

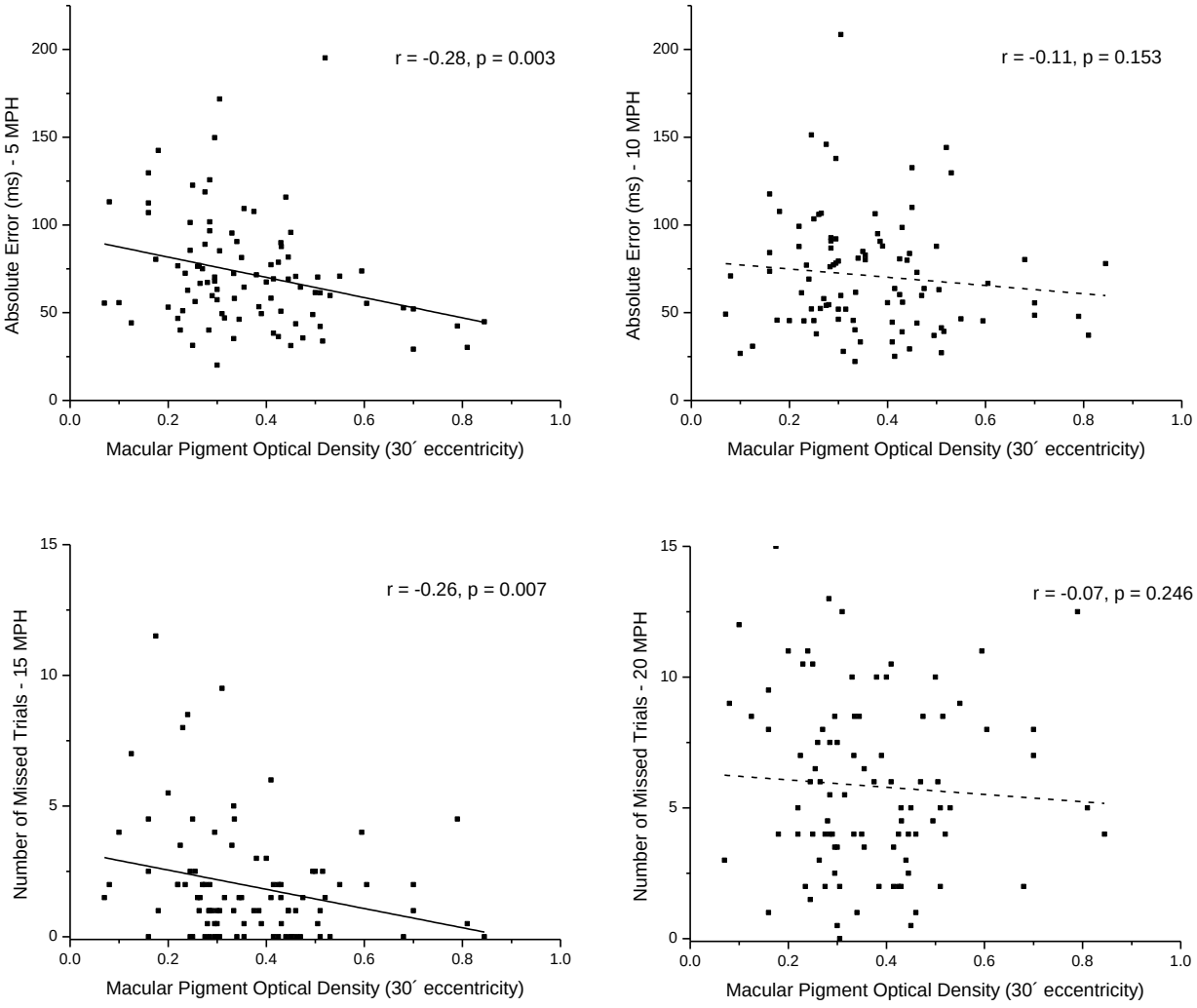


Figure 8: Baseline correlations between macular pigment and variable position reaction time.