RETINAL CAROTENOIDS: RELATIONS TO CONTRAST ENHANCEMENT AND TEMPORAL VISUAL FUNCTION

by

LISA M. RENZI

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

ABSTRACT

Retinal carotenoids lutein and zeaxanthin are found in high concentration in *macula lutea* of the neural retina, where they are termed macular pigment (MP). There are multiple hypotheses for MP's function in retinal tissue, based largely on lutein and zeaxanthin's known antioxidant and light absorption properties. The protective hypothesis, the most widely tested of these hypotheses, suggests that MP is capable of protecting vulnerable retinal tissue from actinic damage and oxidative stress over the lifespan. The two least explored hypotheses, the visual function hypothesis and the neural efficiency hypothesis, suggest that MP is capable of influencing visual function via an optical mechanism and / or via a direct influence on central nervous tissue, both inside and outside the neural retina. In the latter case, MP may be capable of serving as a biomarker of lutein and zeaxanthin embedded in the frontal and occipital cortices. The purpose of the present investigation was to provide further examination of the visual function hypothesis and to provide a first test of the neural efficiency hypothesis.

INDEX WORDS: lutein, zeaxanthin, macular pigment, contrast enhancement, neural efficiency

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LISA M. RENZI

B.S., The University of Georgia, 2003

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LISA M. RENZI

Major Professor:	Billy R. Hammond, Jr.
Committee:	Brett Clementz Jennifer E. McDowell Mary A. Johnson Philip V. Holmes

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
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DEDICATION

For my grandfathers, who were unable to see the conclusion of this project, and for the rest of my family who, despite some of their claims to the contrary, were.

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CHAPTER ONE: GENERAL INTRODUCTION

Overview.

The xanthophyllic carotenoids lutein (L) and zeaxanthin (Z) are known for their ability to serve as active antioxidants and to absorb short-wave light (for review, see Landrum & Bone, 2001). L and Z are distributed ubiquitously in body tissues but exist in highest concentration in the *macula lutea* of the primate retina, so named for the yellow color imparted by L and Z[1] (at this site termed macular pigment, MP) (e.g., Snodderly et al, 1984.; Bone et al, 1985). Multiple hypotheses have been posed for MP's role in the primate retina, based largely on the above-mentioned antioxidant and filtration properties of L and Z. The most widely tested of these hypotheses, *the protective hypothesis* (e.g., Wald, 1945; Wald 1949; Wooten and Hammond, 2002) predicts that MP is capable of protecting retinal tissue from acquired damage in a dose-dependent manner across the lifespan. Given the long time course, and the many mechanisms by which L and Z can promote ocular health (light absorption, antioxidant mechanisms, anti-inflammatory agents, membrane stability, etc., for review, see Zimmer & Hammond, 2007), the role of L and Z in protecting ocular tissues is difficult to study. There are, however, a number of other functions that L and Z might have within the visual system that lead to more immediate changes. For example, a distinct set of optical hypotheses (for a review, see Stringham and Hammond, 2007), here collectively termed *the visual function hypothesis*, predicts that MP is capable of improving visual experience solely via an optical mechanism (i.e., by passively filtering the short-wave component of the visible spectrum).

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[1] As a point of clarification, Z will denote both zeaxanthin and meso-zeaxanthin isomers.

As stated previously, L and Z are primarily known for their antioxidant and filtration properties. L and Z have other properties, however, that have the potential to influence visual system health and function. For example, L and Z are known for their ability to influence oxygen metabolism (Aleman et al, 2001; Liew et al, 2006), structurally support neurons (Crabtree et al, 2001), influence gap junction communication (Stahl and Seis, 2001) and their anti-inflammatory properties (Izumi-Nagai et al, 2007). As mentioned previously, L and Z are not exclusive to retinal tissue; recent research suggests that they are also the dominant carotenoids in the occipital and frontal cortical lobes as well (Craft et al, 2004). Although it is likely that L and Z protect retinal tissue and influence visual function, it is unlikely that, given these additional properties, L and Z serve this function exclusively. As suggested by their *in vitro* properties, it is possible they may also improve neural function, an idea that has been formally advanced as *the neural efficiency hypothesis* (Hammond & Renzi, 2008; Zimmer & Hammond, 2007). Specifically stated, L and Z are thought to directly influence neural function in a variety of central nervous tissues, including visual cortex.

Given the growing body of literature supporting *the protective hypothesis* and the relative paucity of research investigating alternative hypotheses, the purpose of the current investigation is: 1) to determine MP's ability to enhance contrast (tests *the visual function hypothesis*) and 2) to relate MP density to changes in temporal visual function (tests *the neural efficiency hypothesis*).

Lutein and Zeaxanthin: Background and Basic Function.

L and Z are members of the xanthophyll family of carotenoids, which are noted for their long, conjugated hydrocarbon chains and terminal hexagonal ring structures (for review, see

Krinsky, 2002; Landrum & Bone, 2001). Xanthophylls, unlike their carotene counterparts, are characterized by hydroxyl functional groups that slightly increase the polarity and biological reactivity of these otherwise hydrophobic molecules. Carotenoids are native to plant species, where they serve a few noteworthy biological functions, such as absorbing short-wave light and serving as active antioxidants. With regard to the former function, L and Z are capable of quenching singlet oxygen species that arise from endogenous sources (e.g., cellular respiration) and from environmental exposures (e.g., actinic damage). With regard to the latter function, L and Z tend to preferentially absorb light in the most highly energetic region of the visible electromagnetic spectrum, peaking at about 460 nm, which is also the peak output wavelength of the sun.

L and Z are also found in, but are not endogenous to, animal tissues in varying concentrations depending on the tissue in question and the species. In humans, L and Z are distributed relatively ubiquitously throughout serum and tissues such as adipose tissue, testes and ovaries, kidneys and skin (e.g., Kaplan et al, 1990; Schmitz et al, 1991). L and Z are also found in relatively high concentrations in human central nervous tissue. In the frontal and occipital cortices, for example, L and Z make up approximately 66-77% of total carotenoid density (Craft et al, 2004). In the neural retina, L and Z exist to the exclusion of all other carotenoids and in higher concentration than any other region of the body (Snodderly et al, 1991) (e.g., approximately 500x greater concentration than serum).

When addressing the question of how L and Z might function in the human body, there are three basic possibilities: 1) that L and Z are vestigial; any former function that they might have had in animal tissue in evolutionary history is no longer retained, that 2) L and Z appear as a mere byproduct of plant consumption and have never retained any function in human tissue,

and 3) L and Z serve some distinct, identifiable function in human tissues. Research suggests that the former two possibilities are unlikely (for review, see Hammond & Renzi, 2008). The question of what these functions are in human tissues is still open, and there are, at least, two nonexclusive possibilities: L and Z may either retain the same functions that they serve in plant species (e.g., antioxidant and light absorption functions), and/or they may present a new set of functions suited specifically to animal tissue.

Hypotheses for L and Z Function in Human Tissues

1. The Protective Hypothesis.

The most widely studied theory regarding MP function states that MP has the ability to protect the retina from damage that accrues across the lifespan. Retinal tissue, like all central nervous tissue, is generally oxygen-rich (although oxygen tension varies somewhat across the layers of the retina) and highly metabolically active, contains high concentrations of transition metals and polyunsaturated fatty acids (PUFA), and is served by an ample dual blood supply consisting of anterior vasculature that supplies the inner retina and a choroidal vascular network that supplies the outer-most portion of the retina (e.g., Alm, 1992; Gariano et al, 1994). Each of these components of the retinal milieu is necessary for visual transduction and overall retinal cell vitality. For example, double-bonds in the PUFA tail result in a cell membrane that is fluid and not easily compressed (e.g., Mitchell et al, 1992).

One potential problem arising from the anatomical configuration and metabolic demands of retinal tissue is the fact that this tissue is at high risk for oxidative stress (Porter et al, 1995). This problem is amplified by the fact that light is incessantly focused on retinal tissue by the cornea and crystalline lens. The potential of such light to damage retinal tissue varies depending

on wavelength. Ultra-violet light is largely absorbed by the anterior media (e.g., cornea and lens) and does not reach retinal tissue in appreciable quantity (Boettner & Wolter, 1962). Long-wave light is capable of damaging retinal tissue via thermal mechanisms, but exposure must be prolonged and of high intensity to initiate such damage (e.g., Okuno, 1991). Short-wave light is, however, able to reach the retina in appreciable quantities and energetic enough to catalyze a variety of reactions distinct from bleaching visual pigment, such as free radical formation in the presence of oxygen (for review, see Ham, 1983). PUFA are one of the most common targets for oxidative damage from free radical formation (e.g., Porter et al, 1995). When retinal tissue is in a state of minimal damage, lysosomes of the retinal pigment epithelium (RPE) are able to digest oxidized PUFA. As oxidative stress increases, however, the RPE is unable to perform its digestive function adequately and instead sequesters oxidized PUFA, forming drusen (e.g., Sarks et al, 1980; Sarks et al, 1994; Spraul & Grossniklaus, 1997; Guymer & Bird, 1998; Sarks, et al, 1999; Curcio et al, 2005). Lipid peroxidation also follows a self-perpetuating cascade pattern; consequently, a relatively small amount of oxidative stress can result in larger scale damage. Over time, continued RPE insufficiency and continued accrual of oxidative stress causes vascular and geographic changes to retinal tissue that can result in bleeding, scarring and a loss of sight (e.g., Freund et al, 1993; CNPTRG, 1998). Once advanced to this state, this condition meets diagnostic criteria for exudative age-related macular degeneration (AMD) and is the leading cause of blindness in developed countries and is among the leading causes of blindness worldwide (Resnikoff et al, 2004; for review see Gehrs et al, 2006).

Oxidative stress cannot, unfortunately, be eliminated, as it also arises from non-pathological cellular processes, such as cellular respiration and immune response. In order to prevent retinal degeneration, oxidative stress must, at least, be controlled. In addition to support

from endogenous antioxidants, (e.g., glutathione S-transferase; Maeda et al, 2005), exogenous antioxidants like L and Z are thought to serve this function. In addition to antioxidant functions, L and Z selectively absorb short-wave light, thus reducing the amount of potentially harmful light available to catalyze oxidation reactions (for review, see Krinsky, 2002). This *protective hypothesis* for MP function predicts that as MP density increases, risk for AMD and other retinal degenerative diseases decreases. A growing body of literature supports this hypothesis. For example, Bone and colleagues (2001) found that individuals with higher MP density had an 82% lower chance of developing AMD when analyzing clinical changes to the retina and L and Z concentrations in post-mortem tissue. This result was confirmed *in vivo* by Beatty et al (2001). Emerging evidence from epidemiology, biochemical analyses and randomized controlled trials also suggest that MP is able to protect against AMD in a variety of subjects with varying disease progression states (e.g., Chucair et al, 2007; SanGiovanni et al, 2007; Robman et al, 2007; Parisi et al, 2007; Tan et al, 2007; Wang et al, 2007; Cangemi, 2007; Moeller et al, 2006).

2. The Visual Function Hypothesis.

Walls and Judd (1933) originally proposed a set of hypotheses for intraocular yellow filters that was later adapted to MP (also, of course, an intraocular yellow filter) by Nussbaum et al (1981). Included in these hypotheses, here collectively referred to as *the visual function hypotheses*, are predictions that MP is capable, exclusively via its optical properties, of influencing visual acuity by reducing the effects of chromatic aberration, of reducing glare discomfort and disability, of improving visibility by the absorption of atmospheric haze, and by enhancing contrast. Preliminary models (e.g., Wooten & Hammond, 2002) suggest that many of these predictions are possible. Despite the long history of many of these hypotheses (e.g., Shultze

proposed the Acuity hypothesis in 1866), and the fact that they are often cited as fact, it has only been quite recently that these ideas have been empirically tested. Moreover, only the first two predictions, that MP can improve acuity and reduce glare dysfunction, have been evaluated and one had proven true while the other has not been supported.

Engles et al (2007) found that MP and both resolution acuity and hyperacuity were not related when assessed psychophysically in young, healthy adults. In contrast, others (e.g., Richer et al, 2004; Olmedilla et al, 2003) found an improvement in visual acuity with increased MP density. At first blush, the results of these studies could be seen as contradictory. On closer analysis, however, the studies are not inconsistent. Engles et al. (2007) tested young subjects only. They also used stimuli that were carefully characterized to test only the optical effects associated with chromatic aberration (i.e., they actually tested the predictions of the acuity hypothesis). Richer et al. (2004) and Olmedilla et al. (2003) tested elderly subjects. The sample tested by Richer et al. (2004), for example, were elderly Veterans with atrophic AMD (a group probably suffering from some nutritional deficiencies) and he supplemented them with an antioxidant cocktail including L and Z. Hence, in the Richer et al. (2004) and Olmedilla et al. (2003) studies, it is impossible to disentangle the biological effects of L and Z from the optical effects. This is particularly true since L and Z are likely to have distinct effects upon the disease process itself (e.g., as noted anti-inflammatory effects).

Probably the best-tested of the visual function hypotheses is the prediction that MP can reduce both discomfort glare and photostress recovery time. Richer et al. (2004) and Olmedilla et al. (2003), each suggest that L supplementation can improve visual performance under glare conditions in AMD and cataract patients respectively. Unfortunately, as noted, neither study enabled assessment of the mechanism (i.e., optical or biological) by which increased MP was

able to attenuate glare disability. For example, Richer and colleagues (2004) used a subjective questionnaire to assess improvements in function under glare conditions. Research by Stringham & Hammond (2007), in contrast, using young, healthy subjects was designed to specifically test whether MP improved visual function under glare conditions through an optical mechanism. These researchers created stimuli that varied in wavelength content and found that MP improved glare as a direct function of absorption. Hence, when subjects were tested using monochromatic glare sources not absorbed by MP, there was no relation. When, however, subjects were tested using light absorbed by MP (e.g., broad-band white light as is common in everyday situations), there was a strong relation (r = 0.76) (Stringham & Hammond, 2007). In addition, individuals with higher MP density and were able to regain sight of a 100% contrast grating after a 5-second exposure to a relatively intense (2.5 $\mu W/cm^2$) stimulus more rapidly than individuals with lower MP density (r = -0.79) (i.e., the photostress test; Stringham & Hammond, 2007). Again, however, this effect was wavelength dependent as the optical hypotheses would predict. Perhaps more importantly, increasing MP density via a daily 12 mg L+Z supplement over a 6-month period, improved function under glare conditions and reduced photostress recovery times (n = 40, Stringham & Hammond, 2008). Taken together, the strong correlations, the straightforward mechanistic explanations, and the fact that intervention could change the visual outcomes support the conclusion that MP does strongly reduce glare disability.

The two remaining predictions for *the visual function hypotheses* are that MP is capable of improving visibility and of enhancing contrast. Although neither prediction has been empirically tested, Wooten & Hammond (2002) modeled MP's putative impact on visibility. Unlike the acuity and glare hypotheses, which are based on MP's ability to filter short-wave light and, consequently, to reduce the effects of chromatic aberration and intraocular scatter, the

visibility component of *the visual function hypothesis* addresses MP's ability to attenuate the impact of potential aberrations that arise from environmental conditions. Short-wave light in the atmosphere scatters more readily than does longer wavelength light (according to the simple Rayleigh equation, λ^{-4}); consequently, objects viewed through an atmosphere containing high quantities of short-wave light are more difficult to see than objects viewed in an atmosphere that does not contain so-called blue haze aerosol (for review, see Wooten & Hammond, 2002). This reduced ability to see objects through blue haze aerosol is exacerbated as a direct function of distance of that object from the viewer. This is one reason why visibility is often expressed as visual range (i.e., how far an object can be from an individual and still be visible given a certain set of environmental conditions). MP, by absorbing this veiling haze, would allow an individual to essentially see through it. Although extensively modeled by Wooten and Hammond (2002), and feasible, the visibility hypothesis has not been empirically tested.

Contrast enhancement is based on a different optical mechanism. Although posited (e.g., by Nussbaum et al., 1981), this hypothesis has been neither modeled nor tested with respect to MP, but has been addressed with regard to extraocular yellow filters in general (e.g., Luria, 1972; Wolffsohn et al, 2000). Contrast can be defined as the degree of difference between a given target and its immediate surround (e.g., Schwartz, 1994). In natural visual scenes, an object must differ enough on some parameter (e.g., luminance, wavelength) from its background (e.g., the sky) in order to be seen as a distinct object. The anatomy of the neural retina makes it particularly apt at highlighting these differences via detection of (and, at times, creation of) edges. For example, it is commonly known that bipolar cells in the retina alter firing rates depending on which portion of the receptive field is activated, with maximal firing rates being achieved when a luminance difference occurs over only part of the receptive field. Given the fact

that the neural retina relies heavily upon the existence of edges in the visual field, any mechanism that optically enhances the appearance of edges (i.e., via filtering a portion of the visible spectrum at an edge) should amplify the difference between target and background and, consequently, enhance contrast. As stated previously, MP is capable of absorbing a relatively narrow bandwidth of light peaking at about 460 nm. Consequently, it is predicted that individuals with higher MP passively filter a larger proportion of the short-wave component of a broad-band light source (like the sun). If that light source surrounds a longer wavelength target (e.g., viewing an airplane on the background of the blue sky), the difference in absorption between the target and surround may result in an enhanced edge between target and surround and, consequently, a target (e.g., the airplane) that is more readily detectable. It is important to stress that contrast enhancement differs subtly from other components of the visual function hypotheses, despite the fact that each component is based on MP's ability to absorb short-wave light, thus allowing the differential absorbance of components of a visual scene. As stated previously, contrast enhancement predicts improvement in visual function in cases where absorbing short-wave light can lead to enhancement of an edge.

3. The Neural Efficiency Hypothesis.

L and Z have been largely described in the literature as being short-wave light filters and active antioxidants. Such characterizations ignore that carotenoids are not exclusive to retinal tissue, nor are their functions likely to be solely optical or protective in nature (especially given the fact that acquired diseases of later life may not have been as problematic in evolutionary history as they are presently). Anatomical research, for instance, suggests that although L and Z are concentrated most highly in the macula, they are found in significant quantities in other

regions of the body (e.g., Kaplan et al, 1990; Schmitz et al, 1991). Moreover, their positioning within neural structures is not limited to the retina; L and Z make up approximately 66-77% of the total carotenoid concentration in both the frontal lobe and in the visual processing regions of the brain, such as the striate cortex and visual association cortices (Craft et al, 2004). L and Z are, consequently, positioned in regions of the central nervous system that are critical for cognitive function and visual processing.

Once embedded into neural tissues, L and Z tend preferentially to situate within microtubules, which form the cytoskeletons of retinal axons (e.g., Bernstein et al, 1997; Crabtree et al, 2001). Historically, microtubules have been thought of only as structural elements; however, recent research suggests that microtubules also tend to influence neural second messenger systems and gap junction communication (Stahl & Seis, 2001; Vaney et al, 1998). In addition, tubulin is also thought to serve as a binding protein for L and Z (e.g., Crabtree et al, 2001). Consequently, L and Z uptake into retinal microtubules is likely governed by tubulin. L and Z are, therefore, optimally placed to regulate microtubule dynamic instability (e.g., Crabtree et al, 2001) and, by extension of their position, to further influence inter-neuronal communication. In addition to structural and neuronal communicative functions, L and Z are thought to aid in oxygen respiration. For example, Liew et al (2006) and Aleman et al (2001) found relations between MP density and retinal thickness, which is a marker of hypoxia in the retina. Moreover, L is thought to aid in blood flow via anti-inflammatory (e.g., Izumi-Nagai et al, 2007) and anti-atherosclerotic (e.g., Dwyer et al, 2001) mechanisms.

In order to function efficiently throughout the lifespan, neural tissue must be able to maintain its ability to change oscillatory patterns in a synchronous manner with changes in sensory stimuli. In addition to this ability, it must be able to minimize input from irrelevant

stimuli, and it must be able to institute these changes rapidly without increasing noise levels in the system. Given the above-described metabolic, structural and biochemical functions of L and Z in central nervous tissue, it is conceivable that L and Z could acutely influence neural efficiency (Hammond & Renzi, 2008; Zimmer & Hammond, 2007).

Although there has been no test of this hypothesis directly, ancillary data are consistent with this interpretation. For example, Zimmer and Hammond (2007) provide data showing the relation between MP density and rod-mediated noise. Noise was defined in their study as threshold variability in scotopic thresholds (average deviation from a psychometric function) and was interpreted as reflecting the efficiency of rod photoreceptor function in determining absolute thresholds.

Perhaps one reason that the neural efficiency hypothesis has not been studied is that two major problems must be overcome prior to doing so: 1), relatively few techniques exist for directly assessing L and Z content *in vivo*, and 2), that the bulk of topically relevant literature has focused on markers of the disease process. With regard to the former problem, commonly used methods of determining L and Z status (e.g., serum analysis and dietary recall methods) are not able to assess L and Z concentration *in* the tissue of interest (i.e., central nervous tissue). With regard to the latter problem, a focus on the disease process makes it difficult to disentangle protective effects of L and Z from purely functional effects. Addressing these issues was a major impetus of the present study.

Summary.

L and Z have multiple functions in plant tissue that have extended to human tissue as a result of consuming L- and Z-rich foods. The most commonly noted of these functions, short-

wave light absorption and their antioxidant properties, are most likely protective for retinal tissue, but are likely not *exclusively* protective. As stated previously, the bulk of research conducted to-date has been focused on the protective hypothesis of MP, and a relative paucity of research exists to identify other viable hypotheses. L and Z have other properties (e.g., the ability to influence interneuronal communication, the ability to influence metabolism) that may lead to other functions. Given the fact that changes in visual and neural function have often been confounded with protective effects in past literature, research that disentangles possible effects for MP is warranted. Consequently, the present investigation is focused on alternative hypotheses of MP function, namely the visual function hypothesis and the neural efficiency hypothesis.

CHAPTER TWO: CONTRAST ENHANCEMENT ARM

Overview.

The effects of extraocular chromatic filters on visual function have been assessed previously, with mixed results depending on the visual function parameter tested, the type and density of filter used and the population investigated (for review, see Wolffsohn et al, 2000). Yellow filters (peak absorption from approximately 400-500 nm) in particular have received attention because they have the ability to filter short-wave light that is both highly prevalent in our atmosphere and is most highly prone to chromatic aberration and scatter (e.g., Gilmartin & Hogan, 1985; Hemenger, 1992; Wooten & Hammond, 2002). The majority of studies investigating the effects of chromatic filters on visual function have assessed contrast sensitivity (generally via CRT monitors and printed grating targets) and aberrations in color vision caused by the addition of the filter (Wolffsohn et al, 2000). With regard to contrast sensitivity, Wolffsohn et al (2000) and others (e.g., Luria, 1972; Yap, 1984; Hovis et al, 1989; Leat et al, 1990; Leguire & Suh, 1993) have found improvements in contrast sensitivity with the addition of extraocular yellow filters. Others, particularly those testing subjects with generally defined ocular disease (e.g., Zigman, 1990), photophobia (e.g., Gawande et al, 1992) and albanism (e.g., Provines et al, 1997), do not historically show the same benefit.

As mentioned previously, contrast is commonly defined as the difference in intensity between an object and its background (Schwartz, 1994)[2]. Contrast sensitivity testing often involves presentation of various sine wave gratings that vary in both frequency and in contrast, and determination of whether the subject in question can resolve the grating. High and extremely

[2] It should be noted that Schwartz's definition of contrast is specific to the spatial domain. Contrast is not, however,

low spatial frequencies are generally difficult to resolve, as are gratings presented in low contrast. Often, these gratings are achromatic. When considering the effects of extraocular yellow filters on contrast sensitivity, it is important to note that the influence of yellow filters is not the same for achromatic gratings as it is for chromatic gratings. For example, research by Wolffsohn et al (2000) suggests that extraocular yellow filters do not improve contrast sensitivity with achromatic (i.e., gray scale) gratings, but markedly improve contrast sensitivity when the grating in question is white on a short-wave background. These results compare well as a contrast sensitivity analog with those of Luria (1972), who found that detection thresholds for a brief flash of a yellow target on a short-wave background were reduced with addition of a yellow filter. Consequently, adding a yellow filter likely alters detection thresholds and contrast sensitivity by influencing chromatic channels, rather than luminance channels (e.g., the two stimuli used in Wolffsohn et al were, reportedly, isoluminant).

As stated previously, the multi-lamellar retina is anatomically configured in a way that allows for amplification of the appearance of edges in the visual field. Consequently, it is possible to think of the human vision system as a contrast engine of sorts, where edges detected, enhanced and, at times, created in the retina are amplified throughout the rest of the vision system. For example, C-type horizontal cells in the retina tend to be receptive to a relatively narrow waveband and tend to increase firing rates when the stimulus is of a specific, corresponding wavelength. Bipolar cells have concentric receptive fields and exhibit spatial antagonism, which causes these cells to increase firing rates only when light falls on the “ON” portion of the receptive field. Consequently, bipolar cells with the highest firing rates are those that detect a luminance gradient (i.e., an edge) *within* a single receptive field. Given the retina's response to contrast, any alteration of a visual scene that enhances contrast of a given target

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only relevant to the spatial domain.

relative to its surrounding spectral environment should improve detectability of that target. This phenomenon is known as contrast enhancement.

Human primates are equipped quite early in life with their own intraocular version of the extraocular chromatic filter in the form of MP. As mentioned previously, MP absorbs a relatively narrow band of short-wave light, peaking at about 460 nm (e.g., Landrum & Bone, 2001), which is consistent with extraocular yellow filters tested in previous experiments (e.g., Wolffsohn et al., 2000). Given the fact that contrast sensitivity is often improved in healthy observers by extraocular yellow filters, it is possible that higher levels of MP (i.e., a denser intraocular filter) are also capable of enhancing contrast. Consequently, the purpose of the current investigation is to provide a first test of the contrast enhancement prediction of the *visual function hypothesis.* In a natural scene, it is often the case that an object of interest (i.e., a target) must be viewed against a short-wave background (i.e., the sky). In an ecologically valid recreation of this scenario, it is hypothesized that MP will enhance the appearance of the edge between the target and the background by absorbing the short-wave portion of the visible spectrum of the background relative to the target (**See Figure 1**).

Methods.

Subjects.

A total of 23 subjects (n = 16 females, n = 7 males; n = 21 Caucasian, 1 Asian, 1 African American), aged 18-29 years (M = 22.74 years, SD = 3.63 years) were selected from the undergraduate research pool and the graduate student population from the University of Georgia Psychology Department. All subjects were non-smokers, presented with visual acuity correctable to 20:40 or better and had no presence of or history of ocular disease. Subjects were not currently

and had not been taking lutein supplements for at least six months prior to the experiment. Informed consent was obtained from each subject prior to testing, and the tenets of the Declaration of Helsinki were adhered to at all times. All subjects' data were analyzed using Microcal Origins, version 7.2.

Materials and Procedure.

Macular Pigment Assessment. MP was assessed psychophysically in Newtonian view using a desktop device (Macular Metrics, Rehoboth, MA) that utilized customized heterochromatic flicker photometry (cHFP). This device uses light-emitting diodes (20 nm half-bandpass) to generate a 460 nm background and test targets that can be varied in terms of their spatial dimensions and, consequently, the portion of the retina that they test. The test target is composed of a 570 nm reference light (not absorbed by the pigments) and a 460 nm test light, the intensity of which is varied by the subject (for a complete description, see Wooten et al, 1999). Essentially, the subject varies the intensity of the 460 nm test to match the luminance of the reference. The intensity required to make this match is a measure of the subject's MP optical density. Five measures are typically taken for a given spatial configuration (e.g., at 30 minutes of retinal eccentricity) and the average within each condition is calculated by a connected software system. These measures are compared with a peripheral reference point (at seven degrees of eccentricity) used to equate subjects for individual differences in retinal sensitivity and lenticular absorption. MP density peaks in the center of the fovea and decreases exponentially with eccentricity (e.g., Snodderly et al, 1984). By the seven-degree reference point, MP density is almost nonexistent. Consequently, MP density is computed by comparing all other points on the spatial profile to the seven-degree reference.

HFP is one of the most widely used and highly validated psychophysical techniques for assessing MP density (see Hammond et al, 2005). The denotation of "customized" HFP refers to an ability to optimize viewing conditions (e.g., low-frequency flicker rates) for each subject and has been validated in a wide variety of subjects. It is particularly ideal for an aging population, given the fact that it is not prone to confounding by various artifacts such as intraocular debris and increased lens density that tend to be prevalent in older adults (e.g., Hammond et al, 2005).

Contrast Enhancement. Two goals for measuring contrast enhancement were 1), to gauge whether the contrast enhancement effect related to MP, and 2), to disentangle the optical effect of contrast enhancement from non-optical improvements in visual function. Consequently, contrast enhancement was measured using careful psychophysical methodology with well-controlled, optically defined stimuli. The target and surround were presented in Maxwellian view using a three-channel optical system with a 1 kW xenon arc-lamp. A custom-fit dental impression apparatus was used to maintain head position and, consequently, light entry through the pupil. The target was composed of a high-contrast grating (100% contrast, 5 cycles/degree) superimposed with a 2.3 μW, 570 nm one-degree disk (approximately 40 nm half-bandpass). In the first measurement condition, the target was surrounded by a relatively bright (702 μW) 425 nm annulus, which enabled a sharp edge between the target and the surround. In the second condition, the same target was surrounded by a broadband, achromatic light source that enabled provision of the same edge. The rationale for both of these conditions was to provide an ecologically valid stimulus arrangement (high-contrast, mid-wave target with either a short-wave "blue sky" surround or a broadband surround) stimulus arrangement, whose surround was either highly absorbed by MP (short-wave surround) or moderately absorbed by MP (MP absorbs only the short-wave portion of the achromatic surround).

Subjects were aligned with the optical system in such a way that the image was in focus with the center of the pupil. Absolute thresholds were assessed via the ascending method of limits; the intensity of the short-wave annulus was incrementally increased by the experimenter, and subjects used a buzzer to indicate the point at which the target dimmed and was no longer resolvable. The phenomenon of brightness induction (e.g., Heinemann, 1955; Heinemann & Chase, 1995; Blakeslee & McCourt, 2008) predicts that the brightness of light in one test region (e.g., the target, positioned on the central retina) will change when other regions of the retina (e.g., those stimulated by the surround) are simultaneously illuminated. In other words, brightness induction is determined by a ratio of luminance of a given test region to luminance of an adjacent region (e.g., Hainemann & Chase, 1995) (**See Figure 2**). This ratio is, in general, relatively small (Heinemann, 1955). The functional implication of brightness induction where the present study is concerned is that the target's perceived brightness will become depressed once the ratio becomes sufficiently small. Given the aforementioned fact that the ratio is already relatively small, brightness depression is relatively easy to determine for most subjects.

An ascending and descending method of limits was used to determine the intensity of the surround needed to cause the yellow grating target to disappear. The experimenter began the trial at a point where either the yellow grating test was clearly visible (ascending trials) or clearly not visible (descending trials). The surround was then attenuated in small steps (equivalent to about 0.05 OD) until threshold was reached (either the target appeared or disappeared). An average of about 5 ascending and 5 descending trials was used to determine threshold. Based on brightness induction (typically contrast is about 1%), these thresholds tended to be quite sharp.

Results.

MP Density. The average MP density for the sample was 0.45 log-units (*SD* = 0.18 log-units) at 30-minutes of retinal eccentricity, which is higher than the average for the adult population in the United States (approximately 0.23 log units) at the same locus (e.g., Curran-Celentano et al, 2001; Hammond & Caruso-Avery, 2000).

Contrast Enhancement. MP density was significantly related to the intensity of the 425-nm surround ($r = 0.47$, $p < 0.01$) (**See Figure 3)**. The task required subjects to determine the point at which brightness induction was reached, or the point where an increase in the luminance of the surround causes a distinct dimming of the target. Consequently, the results suggest that subjects with higher MP density required a more intense surround to reach the brightness induction point than did subjects with lower MP density (i.e., MP was absorbing more of the surround). The same basic trend was seen with the broadband surround, although it was not statistically significant ($r = 0.26$, $p < 0.11$) (**See Figure 4**). This result was not surprising given the fact that MP absorbs less of the broadband surround.

Comment.

The two primary goals for the present experiment were to determine whether MP was capable of enhancing contrast, and whether contrast enhancement occurred as a function of MP's optical properties. These results support the hypothesis that MP is capable of enhancing contrast given the right wavelength conditions. In addition, it is likely, given the young, healthy population tested and careful psychophysical procedures, that MP enhances contrast in an analogous way to extraocular yellow filters used by Wolffsohn et al (2000) and others (e.g., Luria, 1972).

The relation between MP density and contrast enhancement was moderate, suggesting that MP accounted for approximately 22% of the variance in contrast enhancement. There are two major methodological factors that account for a moderate result of what was hypothesized to be a purely optical phenomenon. First, the two basic options for the stimulus configuration are a target-surround arrangement and a target-background arrangement. In the current study, a target-background arrangement could not be employed, as increasing the intensity of the background would also cause an increase in the intensity of the target, thus effectively eliminating the phenomenon of brightness induction. The center-surround stimulus arrangement was, therefore, necessary. As stated previously, the human vision system is extremely sensitive to the presence of edges, and any misalignment, even extremely slight misalignment, of the target relative to the surround and the subject relative to the target and surround will alter the appearance of the edge. Consequently, alignment imperfections undoubtedly accounted for some of the noise present in the line (**See Figures 3 and 4**). Such misalignments occur easily in Maxwellian-view (e.g., due to optical parallax).

The second methodological issue deals with the interference filter used to form the short-wave surround. The *ex vivo* absorption spectrum for MP suggests that MP tends to absorb light most effectively in the 400-500 nm region, with peak absorbance occurring at about 460 nm. In the present investigation, the surround peaked at about 425 nm. MP's absorption is only about 65% at 425 nm (**See Figure 5**). Given this fact, the slope of the least squares regression line in the present investigation (Y = 0.01-0.40X) is predicted almost perfectly. Use of a 460 nm surround would, consequently, be expected to yield a steeper slope.

CHAPTER THREE: NEURAL EFFICIENCY ARM

Overview.

A well-functioning, efficient vision system must be able to detect changes in the sensory environment, however minute, and to minimize neural response to irrelevant stimuli. Furthermore, an efficient vision system must be able to perform these tasks as rapidly as possible without a significant increase in noise. The various factors that contribute to this process are not fully known. What is known, however, is that the vision system exhibits altered function at both retinal and cortical levels depending on nutritional status. In animal models, for example, early malnutrition has been related to reduced nitric oxide synthase activity in the rat visual cortex (e.g., Borba et al, 2000) and increased norepinepherine release (e.g., Soto-Moyano et al, 1998), which leads to reduced neural plasticity, decreased synaptic pruning in early development and interhemispheric asymmetry of visual evoked potentials. Supplementation with taurine has been related to modulated signal transmission in both retina and visual cortex in rat (e.g., Mi et al, 2000). Zinc deficiency is related to changes in the development of the non-human primate visual cortex (e.g., Dyck et al, 2003). In humans, vitamin A deficiency is a well-known cause of night blindness (e.g., Sklan, 1987) and corneal xerosis (e.g., Menon & Vijayaraghavan, 1980). Both of these conditions are reversible in many cases after improving vitamin A status. In addition, docosahexaenoic acid (DHA) supplementation, even above that usually received in breast milk, is related to accelerated maturation of the visual cortex and retina (e.g., Uauy et al, 1992; Hoffman et al, 2004), and to improved visual function in early life that seem to be maintained throughout the lifespan (for review, see Uauy & Dangour, 2006).

L and Z are the only carotenoids found in high concentration in the primate retina.

Furthermore, they are also found in high concentrations in the frontal and occipital cortices, where they form approximately 66-77% of total carotenoid concentration (Craft et al, 2004). Given the fact that nutritional factors can influence neuronal function, it is important to understand how L and Z, which are located in functionally important regions of the CNS, are related to neural function. The bulk of past research on L and Z has focused on their ability to protect the neural retina from diseases such as AMD. L and Z have multiple properties, however, that suggest an ability to influence neural function independently of an ability to prevent damage.

In order to influence neural function, L and Z need to be located in proximity to (or, ideally, embedded in) neurons. Looking first at the retina, L and Z are located in highest concentration in the in the inner and outer plexiform layers of the fovea (Snodderly, et al, 1984), which is the most metabolically and neurologically active site in the retina. L and Z are located in neural cell membranes (e.g., Sujak et al, 1999) and axon projections (e.g., Crabtree et al, 2001), where they bind with the paclitaxil site on β-tubulin proteins (Bernstein et al, 1997; Crabtree et al, 2001). L and Z's specific arrangement within the cortex is not yet known. It is likely, however, that L and Z will bind similarly in the occipital cortex, which has a high concentration of α- and β-tubulin (e.g., Cronly-Dillon & Perry, 1979).

It has been established that L and Z are found in neural tissue, and that, at least in retinal tissue, the nature of how L and Z are embedded may give some clue as to their function. L and Z are, as mentioned above, located in neural cell membranes. Here, L, for example, is positioned both orthogonal to and flush with the lipid bilayer (e.g., Sujak et al, 1999). This dual-orientation makes L (in its orthogonal configuration) capable of serving as structural support for a membrane that is PUFA-rich and, therefore, quite fluid. In its parallel state, L is able to influence the formation of gap junctions and second messenger systems, which enable interneuronal and

neural-glial communication (e.g., Vaney et al, 1998; Stahl & Seis, 2001). It must be reiterated, however, that the bulk of this research has been focused on retinal L and Z; although it is likely that L and Z serve the same function in neural tissue regardless of its locus, this fact remains unknown.

In addition to support and communication functions, L and Z are also capable of augmenting neuron metabolic functions in the retina. As stated previously, for example, research by Aleman et al (2001) and Liew et al (2006) suggest that MP density is related to retinal thickness, which influences oxygen tension. L and Z are also thought to regulate blood flow via anti-inflammatory (e.g., Izumi-Nagai et al, 2007) and anti-atherosclerotic (e.g., Dwyer et al, 2001) mechanisms. The functional consequences of L and Z's influence on retinal metabolism are unknown. When blood flow is impeded to neural tissue in general, however, it is known that cell loss, angiogenesis and apoptosis tend to occur with increased frequency. Interestingly, L and Z are known to prevent apoptosis (e.g., Chucair et al, 2007).

Without further behavioral data, the sum of these facts is simply the suggestion of a relation between L, Z and neural function. In order to investigate the hypothesis further in human subjects, it is first necessary to determine quantities of L and Z in tissues of interest, and to then relate these quantities to some biomarker of function. Two of the most commonly used methods of L and Z assessment in human subjects research are dietary assessment via self-report and serum analysis via high-performance liquid chromatography (HPLC). Both of these methods have been useful in determining relations between xanthophyll status and disease state, but both methods are problematic in important ways. For example, common dietary assessment methods, such as the 24-hour food recall and the Food Frequency Questionnaire are commonly known to be subject to similar biases, such as recall ability of the participant, self-report biases and

seasonal variation in dietary habits. Serum xanthophyll analyses, while overcoming self-report biases, are generally only a sound indicator of *recent* dietary or supplemental intake (e.g., Landrum et al, 1997).

In addition to these facts, it is necessary to note that the question of *function* is usually quite complex, especially for a system such as the CNS that tends to compensate for acquired cell loss (i.e., maintain function despite loss) over a period of decades. Consequently, one-time measures of xanthophyll status, especially those that only reflect recent dietary behavior, are often insufficient to predict the impact of xanthophylls on the lifespan development of the function in question. While the above-mentioned problems are important considerations for assessing L and Z status, perhaps the most important limitation of common assessment methods is that neither serum nor dietary intake assessment is able to accurately give information about how well L and Z are absorbed and concentrated into tissues of interest. In the context of the present investigation, for example, neither serum xanthophyll status nor dietary intake data are able to highly predict xanthophyll concentration in the CNS. In the neural retina, for example, relations between serum xanthophylls and MP density and between dietary intake of xanthophylls and MP density are relatively weak (e.g., $r = 0.21$ and 0.25, respectively in a large ($n = 280$) Midwestern population from Curran-Celentano et al, 2001). Similar data are, unfortunately, not available for other regions of the CNS. Given the relatively weak associations between diet, serum and tissue concentrations (i.e., MP density), it is necessary to find an index of L and Z status that reflects longer-term dietary intakes, that is proximal to the tissue of interest and that can be assessed *in vivo*.

The purpose of the present investigation was, therefore, to undertake an initial exploration of the neural efficiency hypothesis using established psychophysical methodology to

both test neural efficiency and to provide a measure of MP, which will serve as a biomarker for L and Z *embedded in* central nervous tissue. The rationale for using MP in this way is based on the fact that MP density is a collection of L and Z that have crossed the blood-retina barrier and have been intercalated into retinal tissue. L and Z are, as also mentioned previously, the dominant carotenoids in the occipital cortex. Given that they are not endogenous to the human body (e.g., Mares-Perlman et al, 2002), it follows that carotenoids must also cross the blood-brain barrier. Given these similarities and the fact that carotenoid density in the brain also varies across individuals like MP density (Craft et al, 2004), it is likely that MP density is a biomarker for L and Z embedded in occipital cortex. That is, individuals with higher MP density also likely have higher cortical levels of L and Z. As stated previously, this assumption has not yet been tested empirically. It is hypothesized that, as in previous studies, the TMTF will decrease with age. With regard to MP density, it is hypothesized that higher levels of MP will relate to increased sensitivity to flicker.

Methods.

Subjects. A total of 57 subjects, ranging in age from 15-84 years (M = 40.13 years, SD = 19.77 years) (**See Figure 6**) were recruited from the University of Georgia and from the Athens-Clarke Co. community. Approximately 61% of the sample was female, and the majority of subjects (91.2%) were Caucasian. The remaining 8% were of Asian descent. All subjects were free of ocular disease, had visual acuity correctable to 20:40 (Snellen notation) or better and were not currently and had not been taking L or Z supplements for at least 6 months prior to participation. With one exception, all subjects were capable of giving informed consent. In the

exception (a 15-year old female), parental consent was issued, and verbal agreement was received from the subject. The tenets of the Declaration of Helsinki were adhered to at all times.

Materials and Procedure.

MP Density. MP density was assessed using the same desktop device described previously (Macular Metrics, Rehoboth, MA). The customized HFP technique described previously was also employed, with two exceptions. The first exception is that MP density was assessed along the entire spatial profile, as opposed to only a central locus. Consequently, MP density was assessed at 7.5 minutes, 30 minutes, 1-degree and 1.75-degrees of retinal eccentricity. In order to compare the spatial profile between individuals, area under the curve was computed, using Microcal Origin, version 7.2. The second exception is the fact that MP density was carried out in a wide age-range of individuals. Consequently, the CAREDS protocol, which has been validated in older subjects, was followed during the measurement process (Snodderly et al, 2004).

Neural Efficiency. In order to measure neural efficiency by the criteria defined above (i.e., the ability to detect and communicate the presence of rapid changes in the environment without an increase in noise), a dynamic measure of visual processing speed was necessary. Many of the most significant changes in the aging or deteriorating neural system include changes in processing speed. In healthy older adults, for example, decreased processing abilities and speed are seen in both simple (e.g., contrast sensitivity; Anstey et al, 2006) and complex representations (e.g., similar faces in a facial recognition task; Habak, et al., 2008). Furthermore, changes in visual processing are often related to changes in memory in healthy adults (e.g., Kemps & Newson, 2006; Anstey et al, 2006; Anstey et al, 2001). It should be noted that similar changes in other sensory modalities (e.g., audition) do not consistently show relations with other

cognitive processes (e.g., Anstey et al, 2001). These changes occur in older age independently of disease, but are often accelerated in the disease state and can, therefore, be confounded with presence of disease (overt or covert). One possible explanation for reduced processing speed in even healthy older adults is the fact that anatomical changes, such as gradual demyelination of neurons including the optic nerve, are pervasive in older age (Peters, 2002).

One factor that needs to be considered when selecting a dynamic measure of visual processing is the measure's ability to tax the vision system, and to express function over a wide range of points. The above-mentioned studies, for example, tended to test either simple or complex visual representations without including a range of representations within the same sample. In addition, assessments of complex visual stimuli make it difficult to determine whether function loss is due to retinal cell loss or loss along other regions of the visual stream. Given these issues, the human temporal modulation transfer function (TMTF) was chosen as the primary dependent measure of neural efficiency of the vision system. The TMTF is the temporal analog of the contrast sensitivity function, characterizing responses to changes in temporal vision much as the contrast sensitivity function characterizes responses to changes in spatial vision. The TMTF is generally assessed by presenting a simple stimulus (e.g., a solid disk of a particular wavelength and luminance) that varies in both frequency and in depth of modulation. Subjects view the stimulus over a wide range of frequencies and temporal contrast levels and determine whether or not the appearance of flicker is visible. Much of the past research on temporal visual function that has investigated the TMTF has only addressed the high frequency end. At 100% modulation, the critical flicker fusion frequency (CFF) can be assessed. Past research suggests that CFF is decreased in a variety of circumstances, including older age (e.g., Tyler, 1989; Haegerstrom-Portnoy et al, 1999) and in the presence of retinal degeneration (e.g., Phipps et al,

2004). Recent research by Hammond & Wooten (2005) suggests that CFF is also related to MP density. Consequently, TMTFs are particularly sensitive indices of visual function, of neurological decline and of retinal health.

The human TMTF is dependent on several factors, such as pupil size, adaptation state of the subject, luminance of the stimulus test field, stimulus size and duration (Hart, 1993). The present investigation focused on the relation between MP density (as a biomarker of retinal and cortical L and Z) and the shape and absolute sensitivity of the TMTF. To control for factors known to influence the TMTF and to enable analysis of both shape and sensitivity of the function, a novel apparatus was engineered (Macular Metrics; Rehoboth, MA). The apparatus utilizes a 660 nm, 20nm half-bandpass LED light source (Nichia Corp., Mountville, PA) to present a one-degree target on a 10-degree background. The choice of 660 nm was made to maximize L-cone activity, but to not allow absorption of the stimulus by MP or the crystalline lens. An occluder was positioned in front of the background to enable presentation of a four-minute black space between the target and its background, effectively making a center-surround orientation (**See Figure 7**). This space between target and surround was maintained to reduce small errors of fixation and optical blur. Presence of a fixation point located 7-degress in the periphery enabled measurement of the complete TMTF in both the fovea (with central fixation) and in the peripheral retina (using the fixation point at 7-degrees), where MP density is, effectively, zero. As mentioned previously, luminance influences the TMTF (the exact relation is specified by the Ferry-Porter law, e.g., Schwartz, 1994). Given the fact that pupil size influences retinal illumination, subjects viewed the 25 cd/m^2 stimulus through an eyepiece containing a 3 mm artificial pupil. Ambient light in the test room was kept below 1 foot-candle during the testing procedure.

Subjects were equipped with a dial that changed the percent of modulation. The following logged frequencies (listed as log Hz) were tested in both the center and the periphery for each subject: 1.5, 1.4, 1.3, 1.2, 1.1, 1, 0.8, 0.6, and 0.4 log Hz, which correspond to frequencies ranging from about 32 Hz to approximately 2.5 Hz. This range was selected to encompass the entire range of frequencies commonly tested. Frequencies were presented to subjects in a random order, and subjects were required to turn the knob from 0% modulation (a stable stimulus) until the stimulus reached a just-noticeable state of flicker. Five trials were assessed for each subject on each frequency in both center and periphery. With regard to analysis of the TMTF data, Jarvis et al (2003) compared various modeling and analysis strategies for assessing TMTF data across species. This comparison suggested use of integrated area under the TMTF curve for the provision of one, single value that can represent the curve for its comparison against other variables (e.g., MP density) (Jarvis et al, 2003). Consequently, this method was used, and all analyses were performed using Microcal Origin (version 7.2).

Results.

MP Density. The full spatial profile for all subjects tested is presented in **Figure 8**. MP density in the present study was relatively high (approx. 0.48 log units at 30 minutes of eccentricity) compared with United States norms (e.g., Curran-Celentano et al, 2001). Anatomical studies suggest that the spatial distribution for MP shows peak MP density in the central fovea with exponential decline in MP density with increasing eccentricity. In order to assess the validity of MP measurements, the data were fit with a first-order exponential decay to determine goodness of fit to an exponential function. The data fit well ($r = 0.999$), which suggests a high degree of validity in the measurement. In order to determine whether MP density

would co-vary with age (a known covariate of the TMTF), the relation between MP density and age was assessed (**See Figure 9**). MP density was not determined to be related to age (r = 0.11, $p > 0.05$), which compares well with past investigations.

TMTF. The present investigation is the first of its kind to measure the TMTF via a novel apparatus that is optically pristine and capable of controlling confounds (e.g., pupil size, luminance) that influence the TMTF, both in the center and in the periphery. The general shape of the TMTF is presented in **Figure 10**. The shape is, generally, a band-pass shape with a peak in the mid-frequency range (approximately 0.8 log-Hz or 6.31 Hz in the center and 1.0 log-Hz or 10 Hz in the periphery), which is expected based on original measurements from Kelly (1961).

As expected, a slight age-decline in temporal visual function (r = -0.31, $p < 0.015$) was seen when collapsing across frequencies (**See Figure 11**). It should be noted that individuals who were three or more standard deviations away from the mean and those who were unable to resolve all of the temporal frequencies (generally older adults) were removed from this analysis (n = 8). Estimation of the unresolved frequencies (usually in the high frequency range) would be expected to improve this result. The age-decline is stronger in the high-frequencies (r = -0.48, $p < 0.0001$) (**See Figure 12**) than in the low-frequency conditions (r = -0.25, $p < 0.03$) (**see Figure 13**). The above-listed age-declines were significant in the central TMTF but were not present in the peripheral measure (**See Figure 14**).

Relations between MP density and the TMTF. The primary research question was whether MP density related to the TMTF in younger, healthy subjects. Given the fact that MP density was not related to age in this sample, we were able to increase statistical power by collapsing across age (i.e., it was not necessary to control for age as a co-variate). The relation between MP and area under the TMTF was moderate in our sample in both the center (r = 0.26,

$p < 0.035$) (**See Figure 15**) and the periphery ($r = 0.31$, $p < 0.015$) (**See Figure 16**). Moreover, an assessment of the difference between central TMTF (where MP is densest) and peripheral TMTF (where there is, effectively, no MP) shows no difference between the two measures in their association with MP density (**See Figure 17**).

Comment.

This study was the first of its kind to measure both the central and peripheral TMTF in a wide range of subjects. As expected, the TMTF decreases with age across a wide range of frequencies (i.e., from approximately 2-32 Hz). This result is consistent with those of others who have measured the entire TMTF (e.g., Mayer et al, 1988). On average, the TMTF tended to decline about three times more steeply at high frequencies as opposed to lower frequencies. This result may occur as a result of functional declines more centrally located (i.e., extra-retinally), given the fact that limits in the ability to perceive flicker at higher frequencies are generally thought to be a cortical phenomenon, while limits in the ability to perceive lower frequencies are thought to be due to limitations in retinal inhibitory mechanisms. Given the fact that the older adults in the current investigation, although relatively small in number, were generally healthy and had no presence of CNS or ocular disease, the age-decline in TMTF may be thought of as a feature of normal aging, rather than as an indicator of disease. Additional investigations with a larger sample of older adults would be beneficial, as would further investigation in a well-segmented (i.e., young, middle-aged, young-old and old-old) sample.

The relation between TMTF and MP density was moderate, which is not unexpected given the number of factors that contribute to visual processing. One issue that undoubtedly tempers this result, however, is the fact that the bulk of the subjects were well-educated

individuals from the University of Georgia community. Although a wide range of MP densities was present in the sample (MP varied by about a factor of 10 in this sample as it does in the population), the bulk of participants were higher than the national average. Increased variability in MP density (i.e., more subjects with low levels) would likely yield a stronger result. For example, an analysis of our own archival data of 355 relatively young subjects (M = 28.7 years, SD = 17 years) suggests that individuals with low MP density (ranging from near zero to about 0.2 log units) had average CFF values of approximately 3 Hz lower than individuals with high MP density (0.41-0.81 log units) (unpublished). Recall that CFF is the limit case for temporal vision (i.e., 100% temporal modulation). This result is also consistent with results from Wooten & Hammond (2005). These results would be expected to increase in magnitude with reduced temporal contrast. Another limitation, of course, is simply based on the assumption that MP predicts the amount of L and Z post-receptorally. Although this is likely, the strength of this correlation is unknown and could also be quite moderate.

Perhaps the most interesting result is the fact that the relation between MP density and the TMTF was the same in both the central TMTF and the peripheral TMTF. Given the fact that MP density is highest in the central one-degree of the retina (where the central TMTF was assessed) and is almost non-existent after about 7 degrees (where the peripheral TMTF was assessed), any influence L and Z may have on the TMTF is likely post-receptoral in nature. Otherwise, the TMTF would be expected to be improved in the central condition relative to the peripheral condition. Consequently, the idea that MP density may serve as a biomarker of cortical L and Z warrants further investigation.

The MP-TMTF relation can be interpreted in at least two ways. The first is consistent with the predictions of the neural efficiency hypothesis. L, for instance, has been shown to

improve gap junction communication, which could improve intercellular communication within the nervous system (Stahl & Seis, 2001). Consequently, higher MP density (as a biomarker for higher levels of L and Z in other regions of the CNS) could theoretically improve signaling efficiency throughout the visual system. The second interpretation is that the type of loss being measured by the TMTF shows up relatively early and that this loss progresses as a linear function of time. In that case, the protective effects of MP (*the protective hypothesis*) might also influence temporal visual function and might reduce losses as a constant function of time. Recent papers arguing that protection of the retina is quite significant even in infancy (e.g., see Zimmer and Hammond, 2007) are consistent with this interpretation. Unfortunately, the results of the present investigation are not capable of solving this issue. One could argue, however, that protective effects of L and Z would be more pronounced in the center of the retina. The fact that the MP-TMTF relation was essentially the same in both the center and periphery argues for the neural efficiency hypothesis.

CHAPTER FOUR: GENERAL DISCUSSION

The general conclusion of these studies is that MP is related to both temporal vision and optical improvements, which supports the idea that MP density acutely improves visual function. Furthermore, these improvements seem to occur via a variety of mechanisms. The well-established *protective hypothesis* suggests that MP density preserves visual function by protecting the retina from degeneration and disease, thus preserving the number of functional cells available over the lifespan. The *visual function hypothesis* suggests that MP density is able to directly improve specific aspects of visual function (e.g., performance under glare conditions and contrast enhancement) via a purely optical (i.e., filtration) mechanism. The *neural efficiency hypothesis* predicts that L and Z improve neural processing throughout the visual system. The current study provides the first test of, and support for, this hypothesis. The fact that relatively moderate, but significant, results were seen in some of the studies is not surprising. As mentioned previously, methodological issues in the contrast enhancement arm and sampling issues in the neural efficiency arm may have attenuated the relations that we found. In the neural efficiency arm, however, one additional factor is simply that visual function is a complex process capable of being influenced by many external factors. L is likely just one of those factors.

There are multiple implications for these results. With regard to the contrast enhancement result, perhaps the most important point to note is the fact that this result supports the idea that MP is capable of directly altering visual function. In this particular instance, MP seems to improve contrast enhancement via the same mechanism as extraocular filtered lenses. From a practical standpoint, given the fact that tasks that involve distance viewing of a target against a short-wave background (e.g., air traffic control, sharp shooting) are facilitated by the addition of

yellow extraocular filters (for review, see Wooten & Hammond, 2002), it follows that increased MP density may also facilitate such tasks. These examples are relatively specific; this result was, however, obtained via assessment with an ecologically valid stimulus configuration and should generalize to any task in which perception of an edge between a target and its short-wave background will improve ability to detect the target, whether it is an aircraft on the horizon or oncoming traffic in the distance.

With regard to the neural efficiency arm, several implications should be mentioned. The first point of interest is the fact that this study provides the first example of the ability to measure the full TMTF in both the central retina and in the periphery in a portable device. Given the fact that the TMTF is a sound measure of visual processing ability, the clinical applications of a novel measurement strategy warrant further exploration. The second point that should be addressed is the fact that measures of visual processing have been correlated with a variety of measures of cognitive function. For example, the CFF has been linked to MP density (Wooten & Hammond, 2005) and to general intelligence in adults (e.g., Zlody, 1965) and in older children (e.g., Cross, 1963). CFF is also subject to attention and learning, as CFF decreases as perceptual load increases (Carmel et al, 2007) and can be improved with increased visual experience (e.g., Seitz et al, 2005). Given the fact that the CFF is considered to be a sound measure of visual processing speed and is related to other indices of cognitive function (recall that L and Z are also the dominant carotenoids in the frontal lobe; Craft et al, 2004), the relation between MP density (again, as a biomarker of cortical L and Z) should be investigated. Preliminary work in this area (e.g., Renzi et al, in preparation) suggests that MP density is related to a variety of cognitive indices (e.g., the Salthouse battery, including portions of the Mini-Mental State Examination, the Wechsler Adult Intelligence Scale and basic reaction time indices) in healthy older adults.

One result that is reasonably clear from the neural efficiency study is the fact that relations between MP density and neural function need further investigation. Of particular interest is the fact that increasing CNS L and Z may have some impact on visual and, possibly, cognitive function. Prior to examining these relations (and, inevitably, confounding neural efficiency effects of MP with protective effects of MP), however, it is necessary to relate MP density and the TMTF to other validated measures of visual function, such as steady-state visual evoked response potentials. An investigation is currently underway to test these relations using stimuli that mirror those used in the TMTF portion of the current study. In addition, it will be necessary in the future to relate MP density to cortical levels of L and Z, although such an undertaking will be difficult, given the variable nature of carotenoid density in CNS tissue.

One additional point that should be mentioned is that both the contrast enhancement results and the neural efficiency results are amenable to experimental investigation using L and Z supplementation. With regard to contrast enhancement, past research by Stringham & Hammond (2008) suggests that a dose of approximately 10-12 mg of L+Z for approximately 6 months is sufficient to raise MP density and improve function under disability glare conditions in young, healthy subjects. Given the magnitude of results anticipated with further contrast enhancement testing, such an intervention would be expected to improve contrast enhancement as well. With regard to neural efficiency, supplementation with L and Z is, unfortunately, not quite as straightforward. The magnitude of the effect in the present investigation was relatively low to moderate. If this low magnitude of effect is due merely to the complex nature of visual function and the probability that L and Z play only a small part of it, then immediate effects of supplementation might not be obvious. If the effect is due to a sampling bias (as was undoubtedly at least partly the case in the present investigation), then supplementation in a more

heterogeneous population may yield more dramatic results. Regardless of the reason for relatively low magnitude correlations, one thing to consider is the fact that whatever MP does for neural tissue, it probably does it over the entire lifespan. Consequently, a relatively small-scale effect at one time-point may actually be important over the lifespan.

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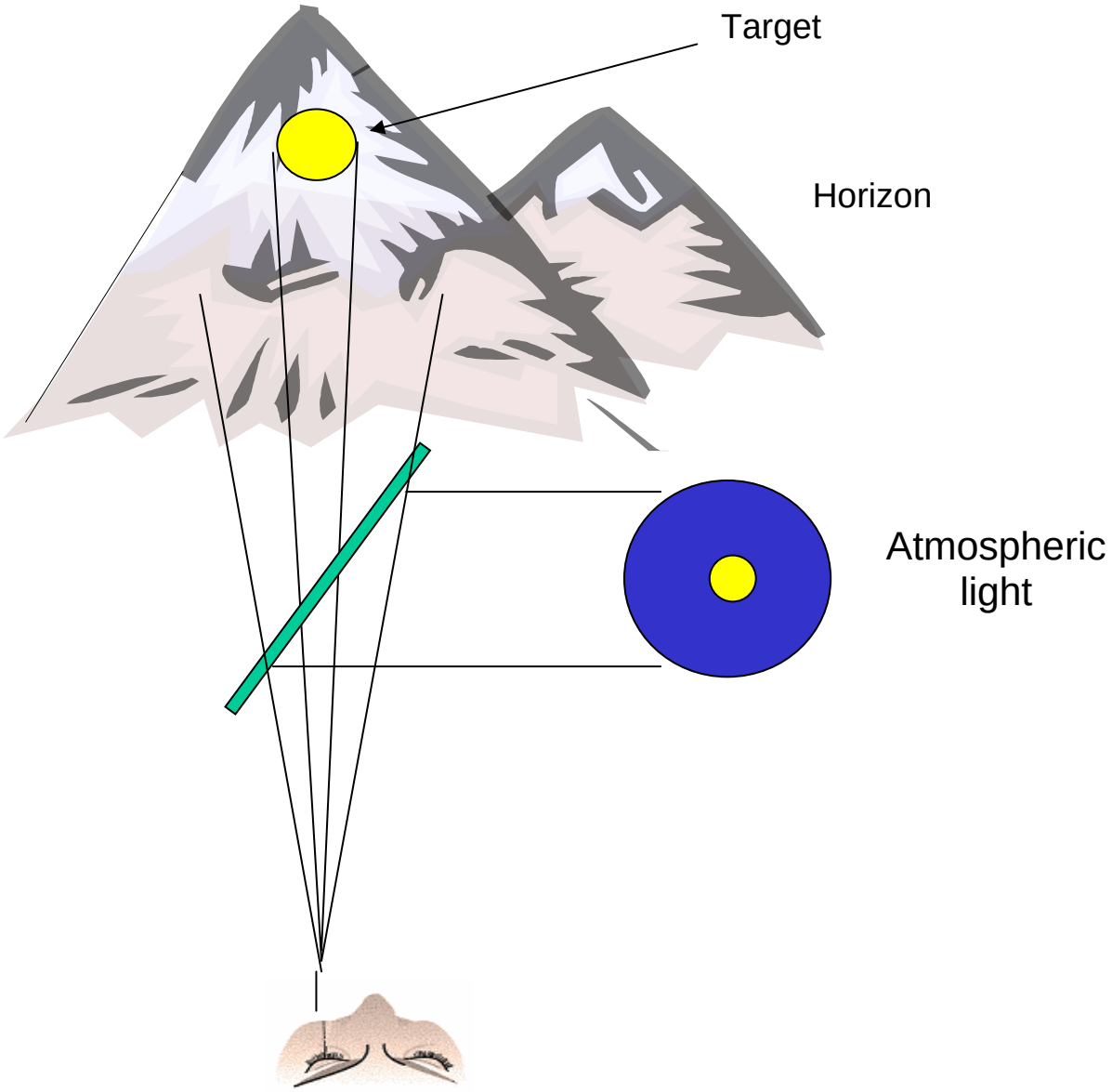

Figure 1. Schematic of the relation between naturally viewed stimuli relate to laboratory stimuli.

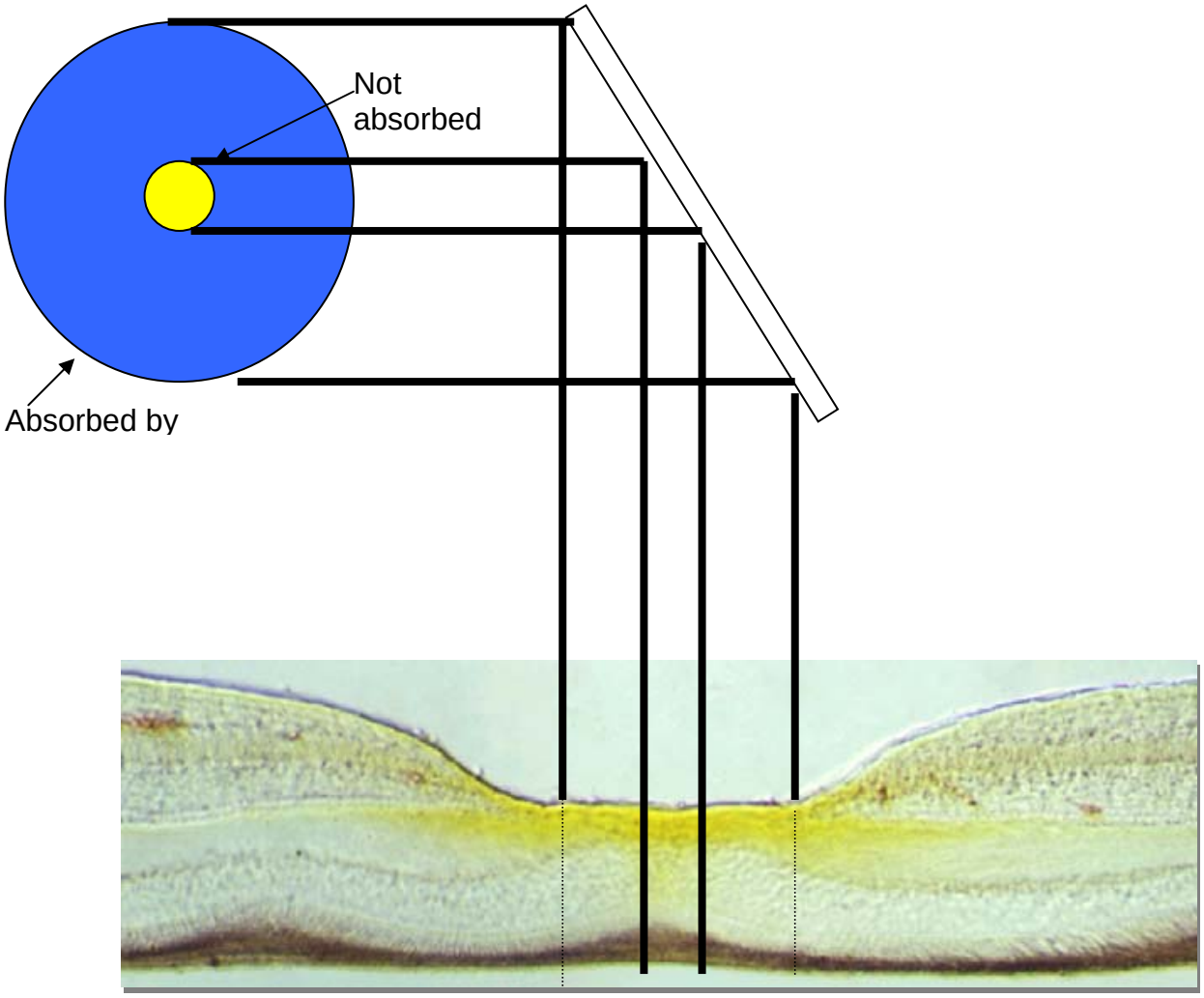

Figure 2. Differential retinal illuminance in the fovea and parafovea in the contrast enhancement task.

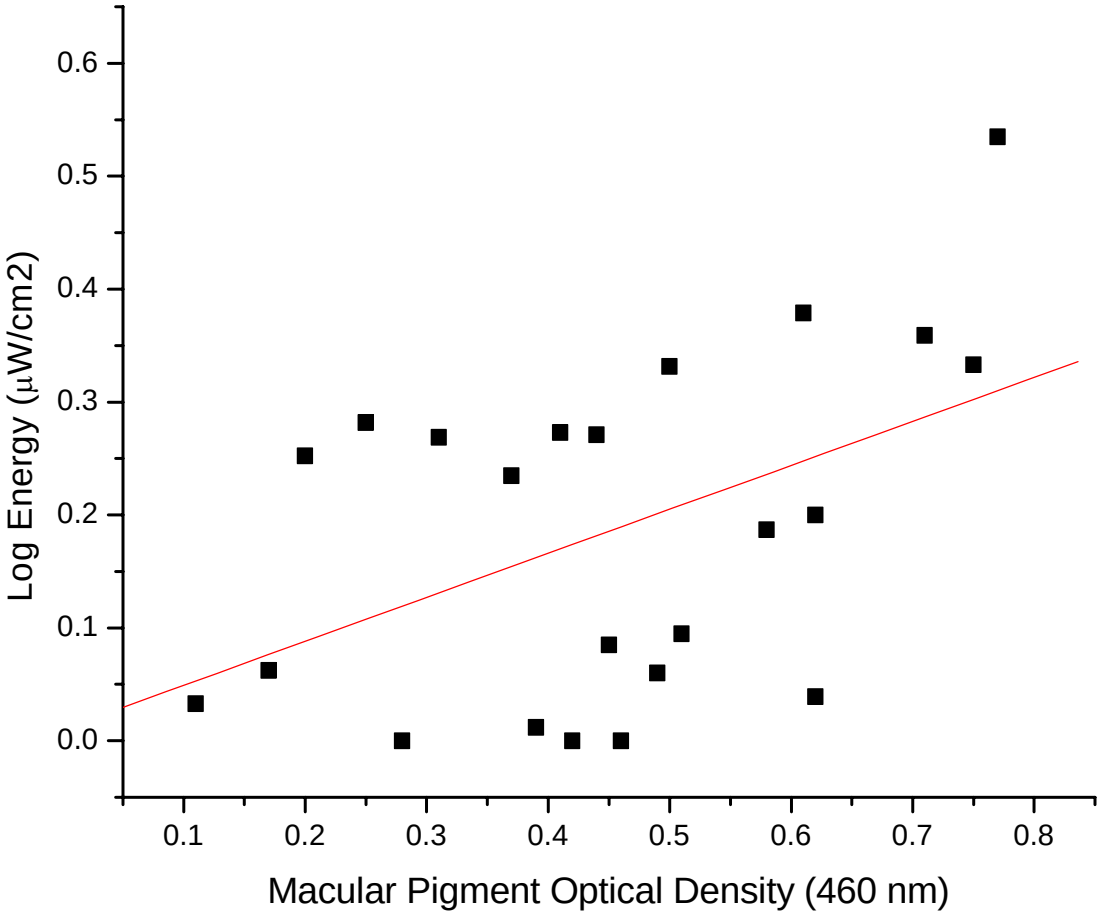

Figure 3. Log radiance of the 425 nm surround required to veil a 570 nm target, presented as a function of MP density. MP density was tested at 30 minutes of retinal eccentricity. The line indicates the least squares regression fit to the data, $Y = 0.01 - 0.40X$; $r = 0.47$, $p < 0.01$.

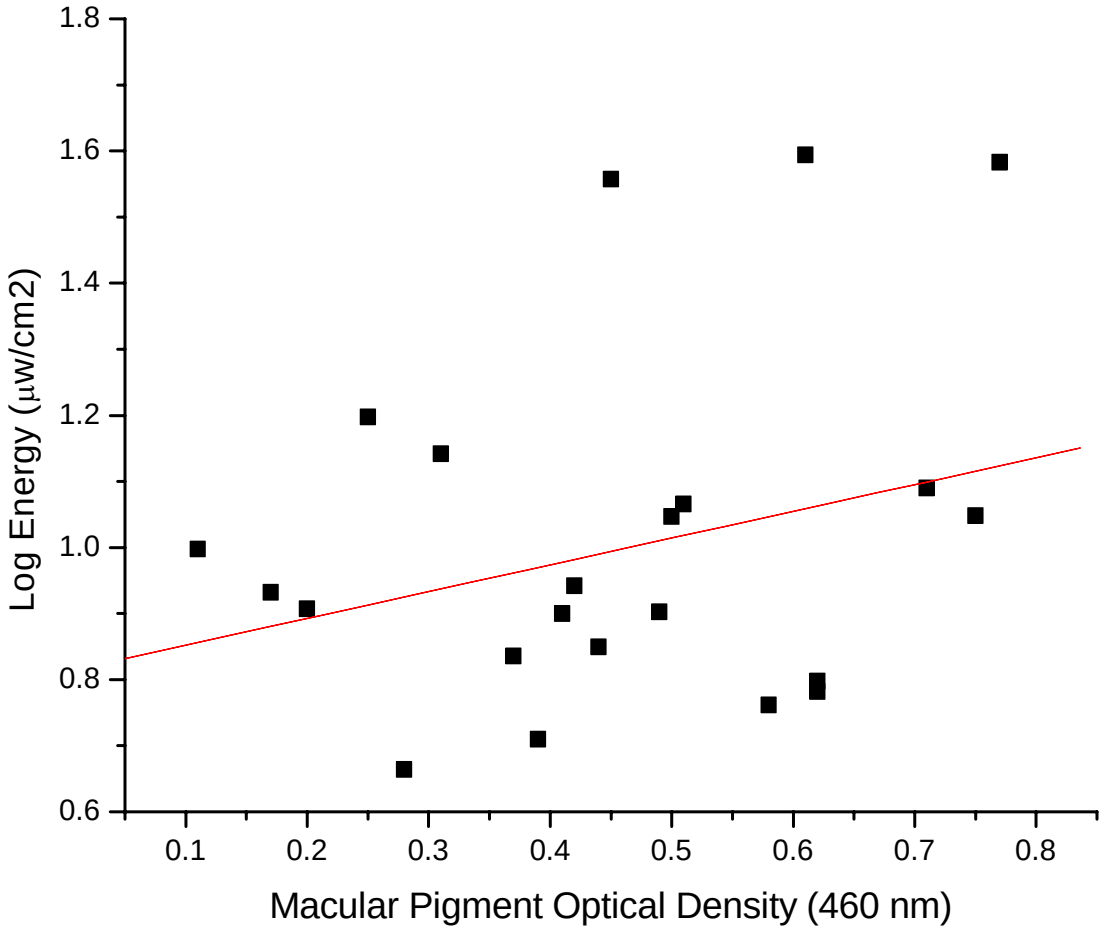

Figure 4. Log radiance of the broadband surround required to veil a 570 nm target, presented as a function of MP density. MP density was tested at 30-minutes of retinal eccentricity. The line indicates the least squares regression fit to the data Y = 0.81 – 0.40X, $r = 0.26$, $p < 0.11$.

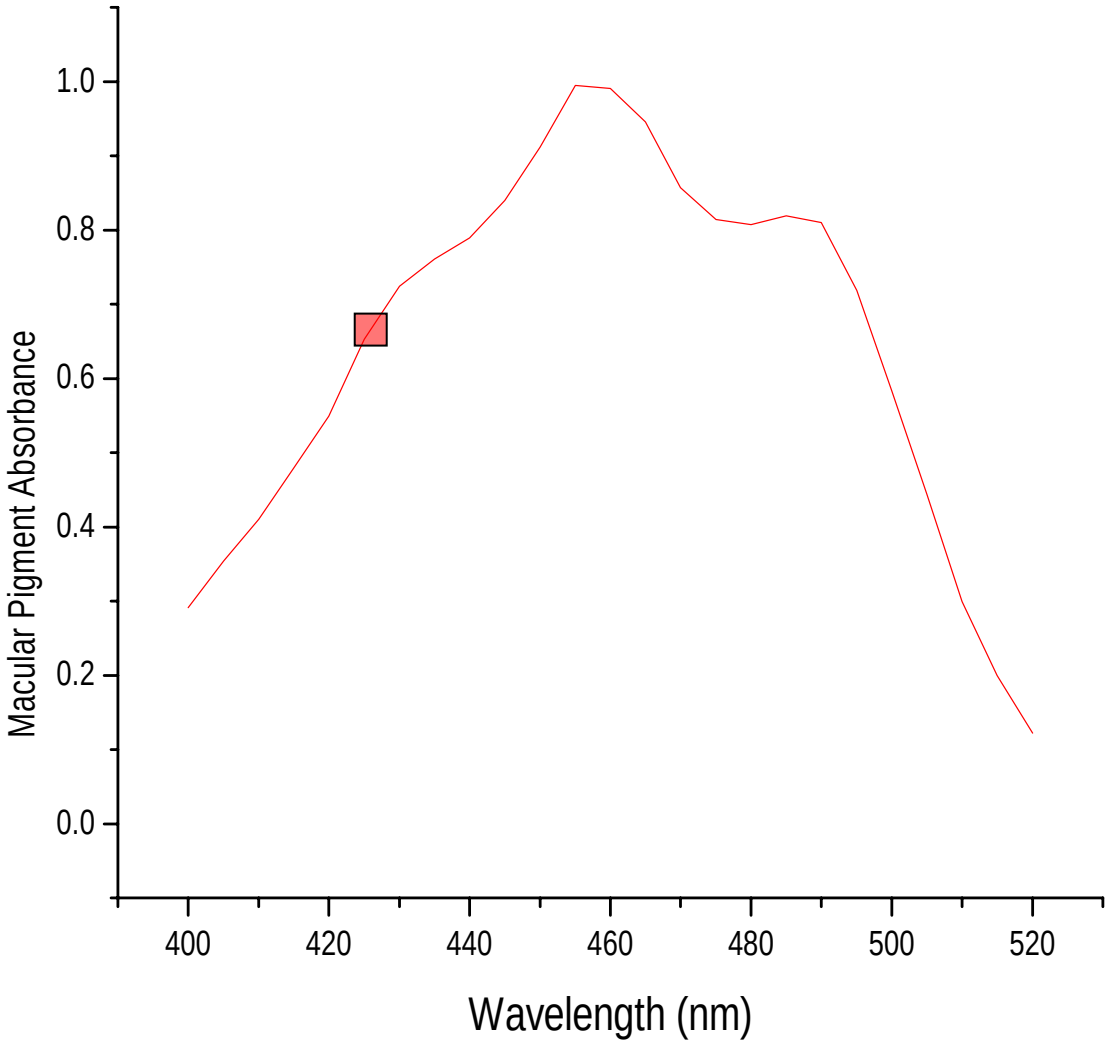

Figure 5. *Ex vivo* absorption spectrum for MP (derived from Hammond et al, 2005). Square indicates MP's absorbance (approx. 65%) of the 425 nm short-wave surround.

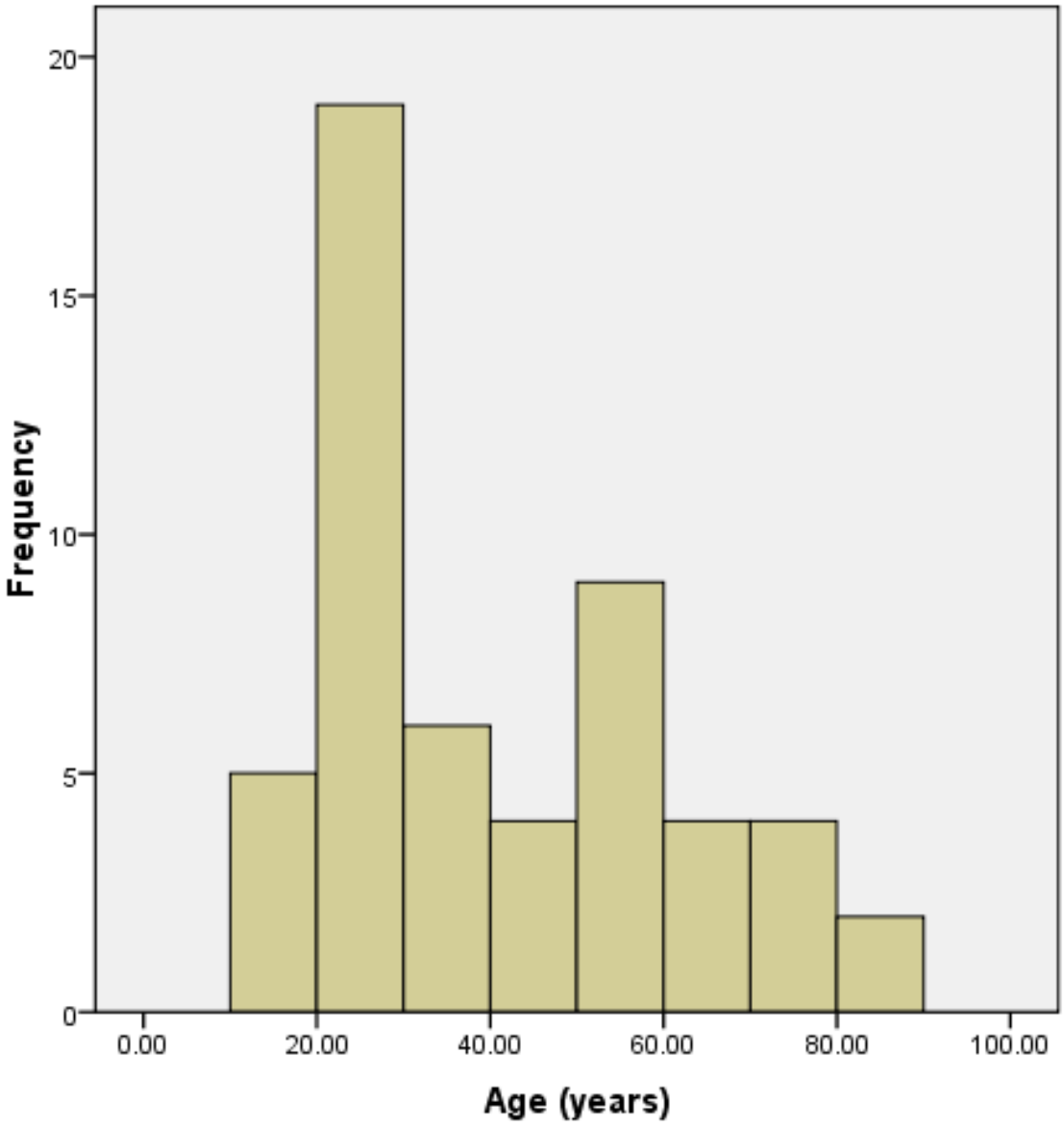

Figure 6. Frequency distribution of subjects sampled in the neural efficiency arm.

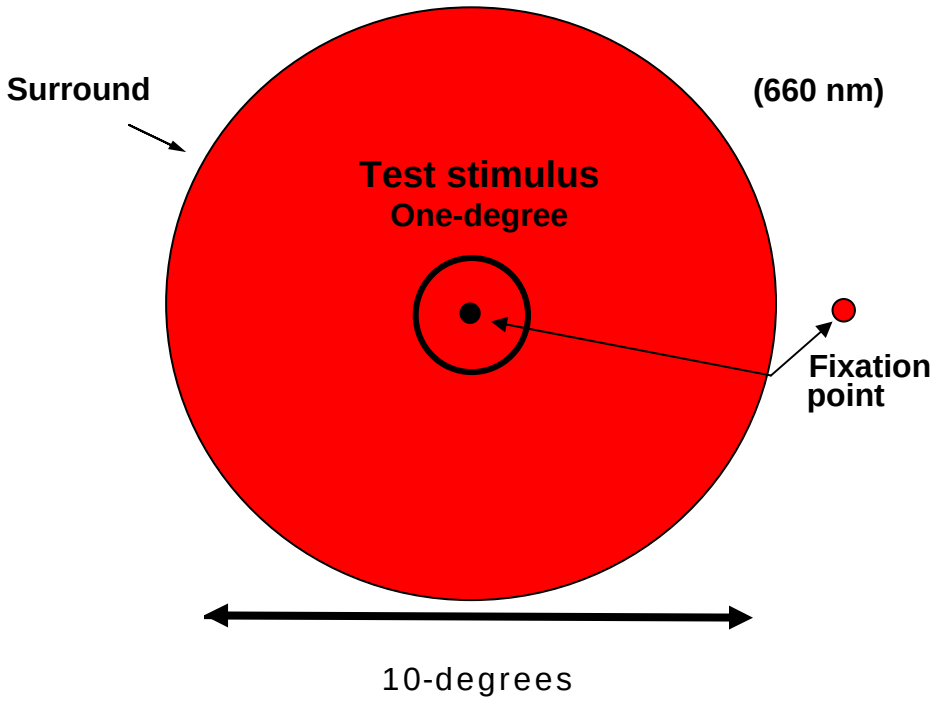

Figure 7. Stimulus presentation for TMTF measurement.

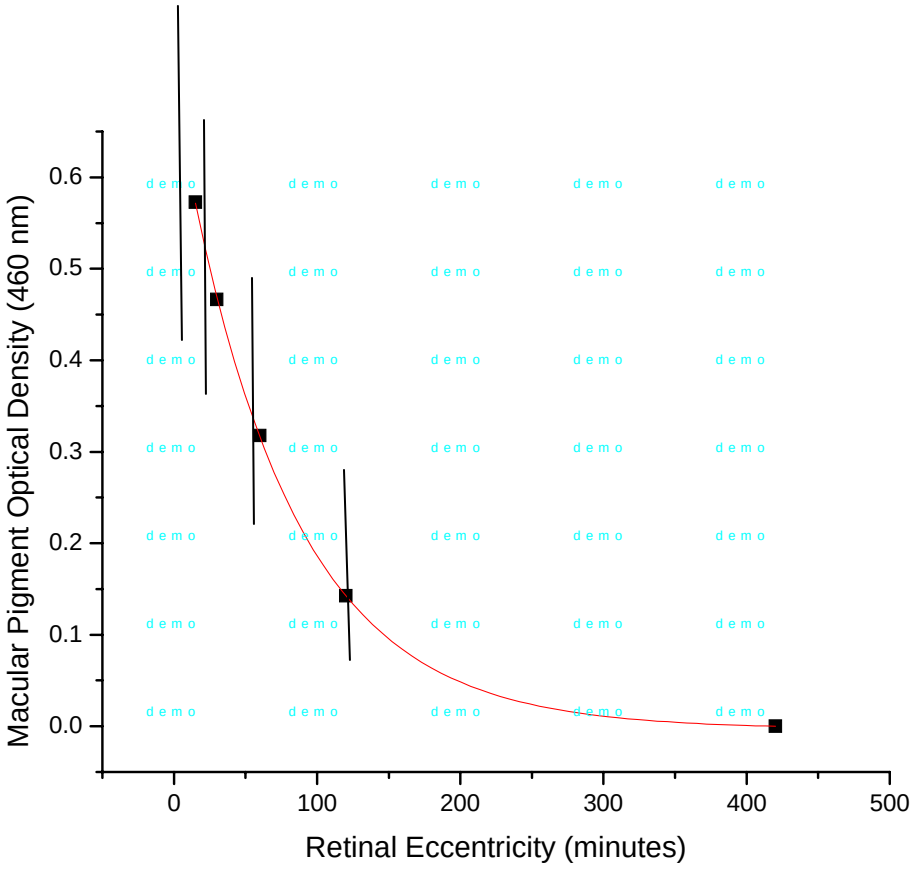

Figure 8. Spatial profile for MP density in the entire neural efficiency sample (N = 57). The points were fit by a first-order exponential decay (r = 0.999). Error bars depict standard deviation, as standard error of the mean was too small at each point to depict on this figure.

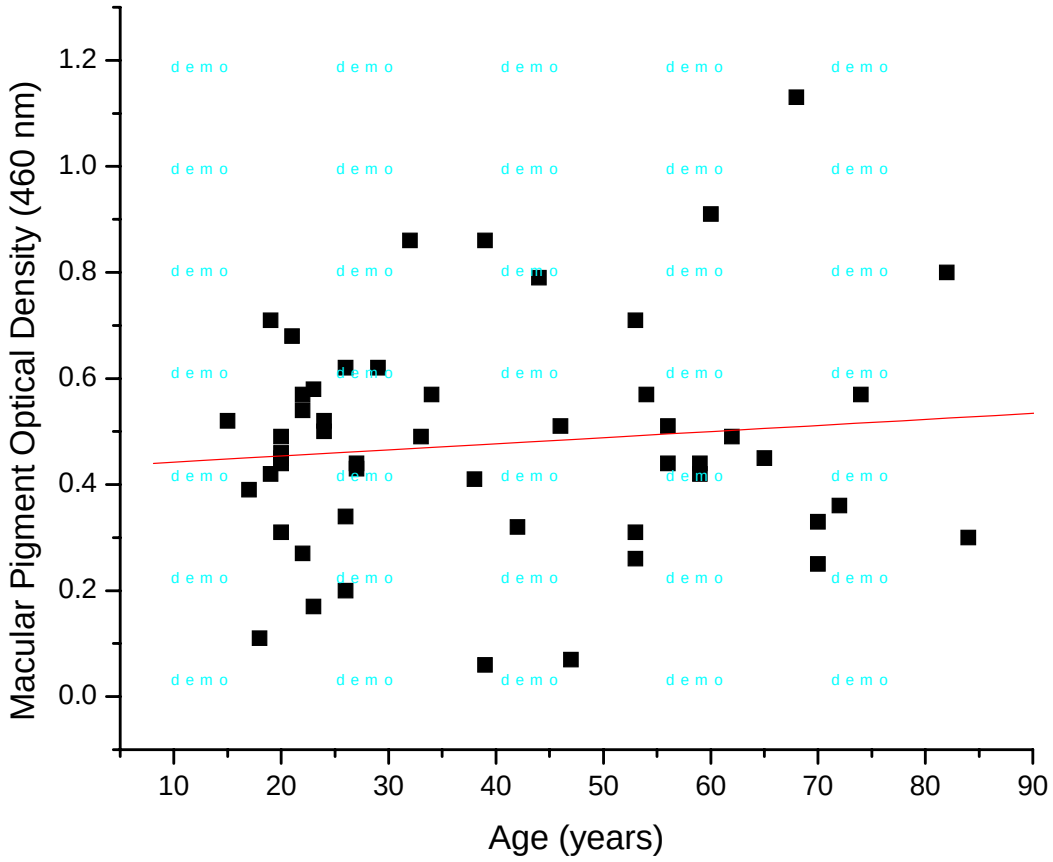

Figure 9. The relation between MP density and age in the study sample ($r = 0.11$, $p > 0.05$).

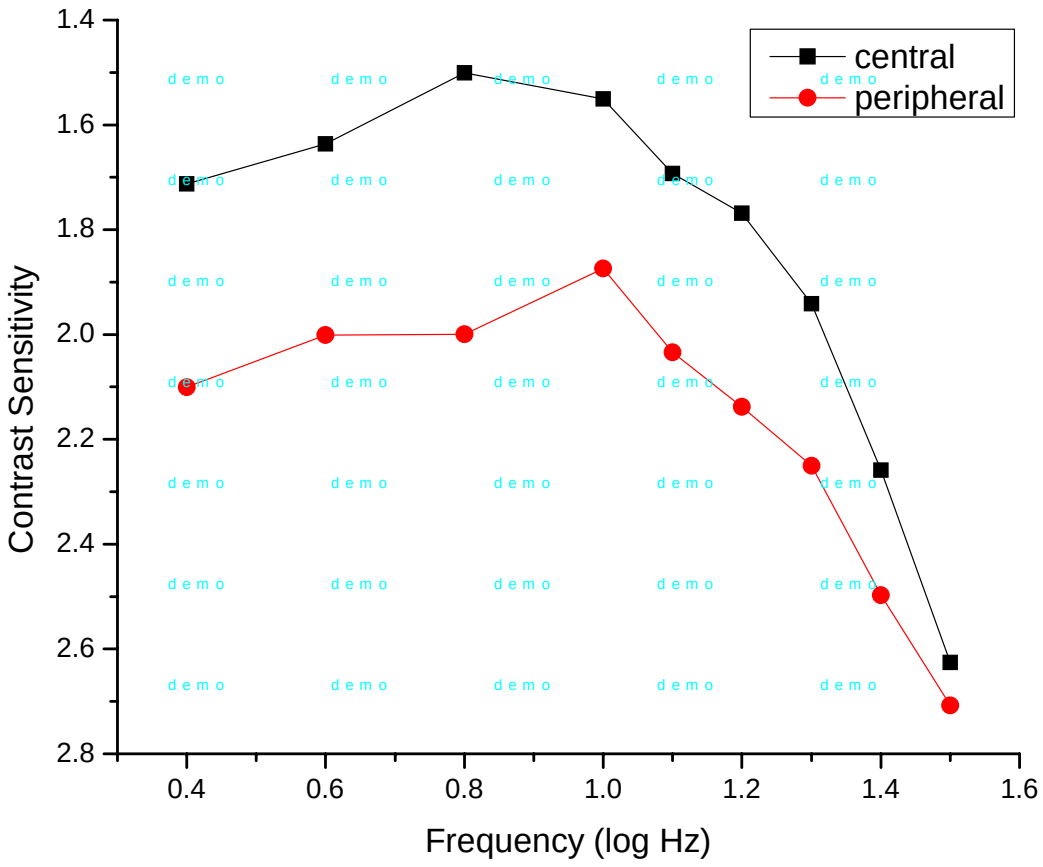

Figure 10. Mean TMTF collapsed across age, assessed in the center and in the periphery.

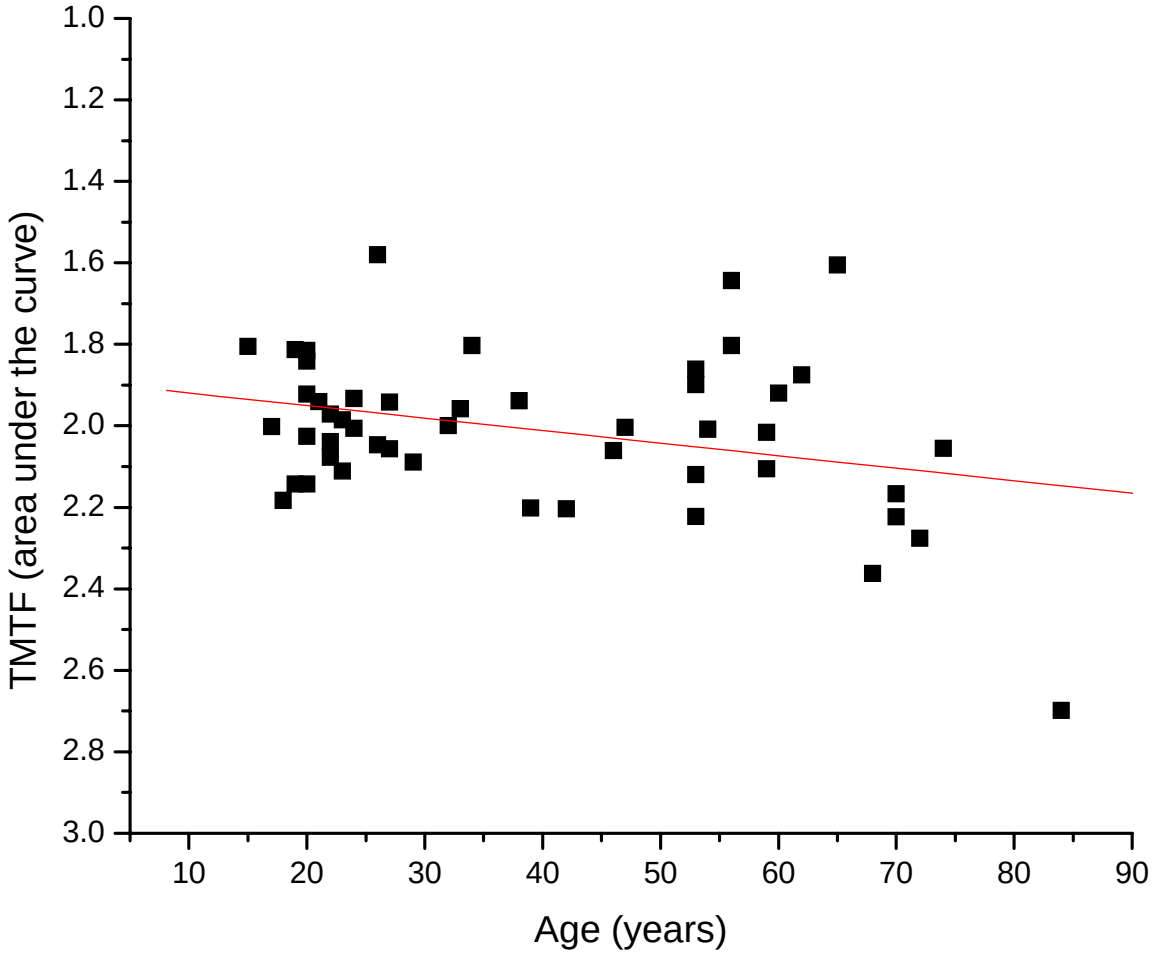

Figure 11. The relation between TMTF and age, collapsed across all frequencies tested.

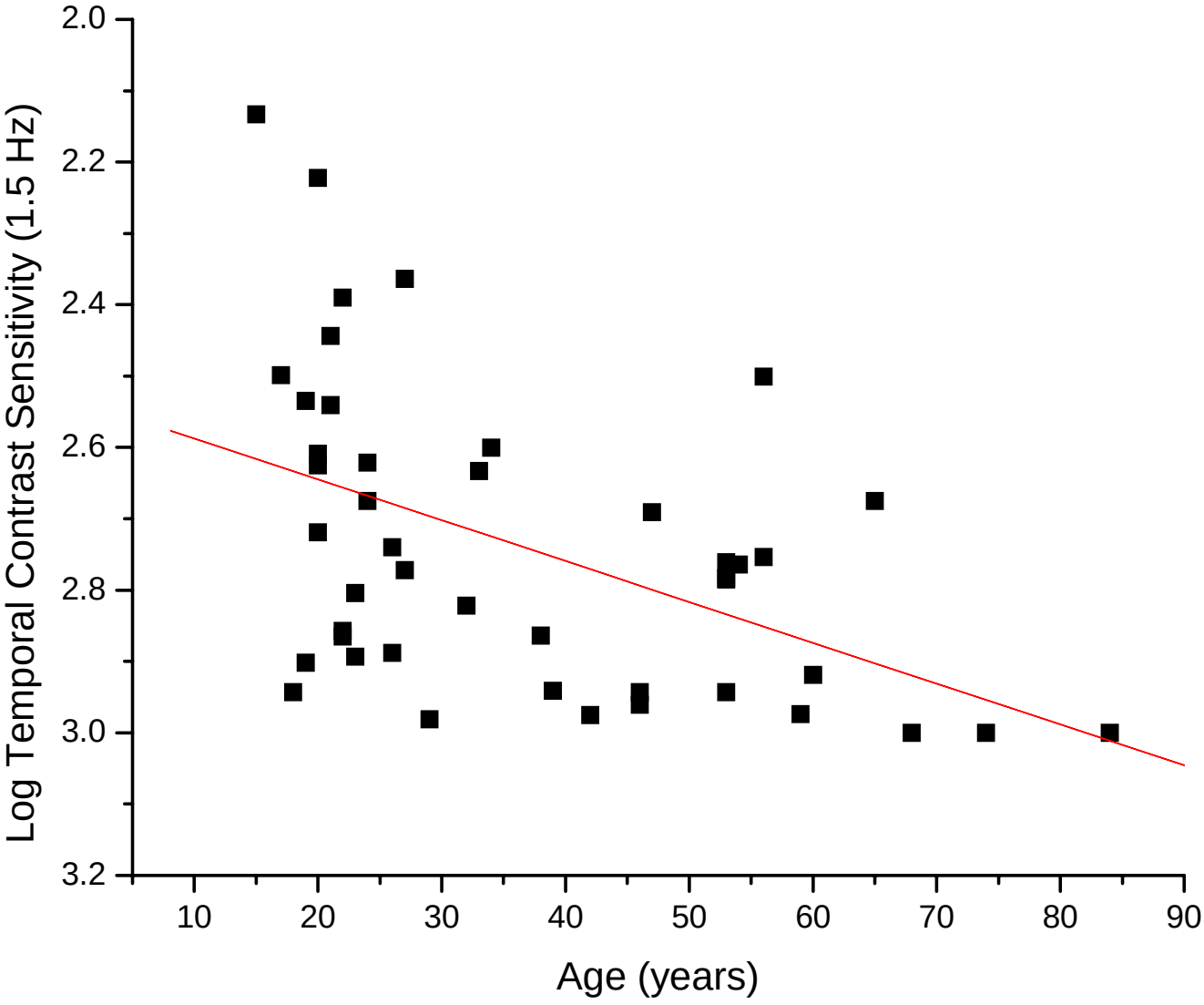

Figure 12. The relation between TMTF and age in the high frequency (approx 32 Hz) region of the function (r = -0.48, p < 0.0001).

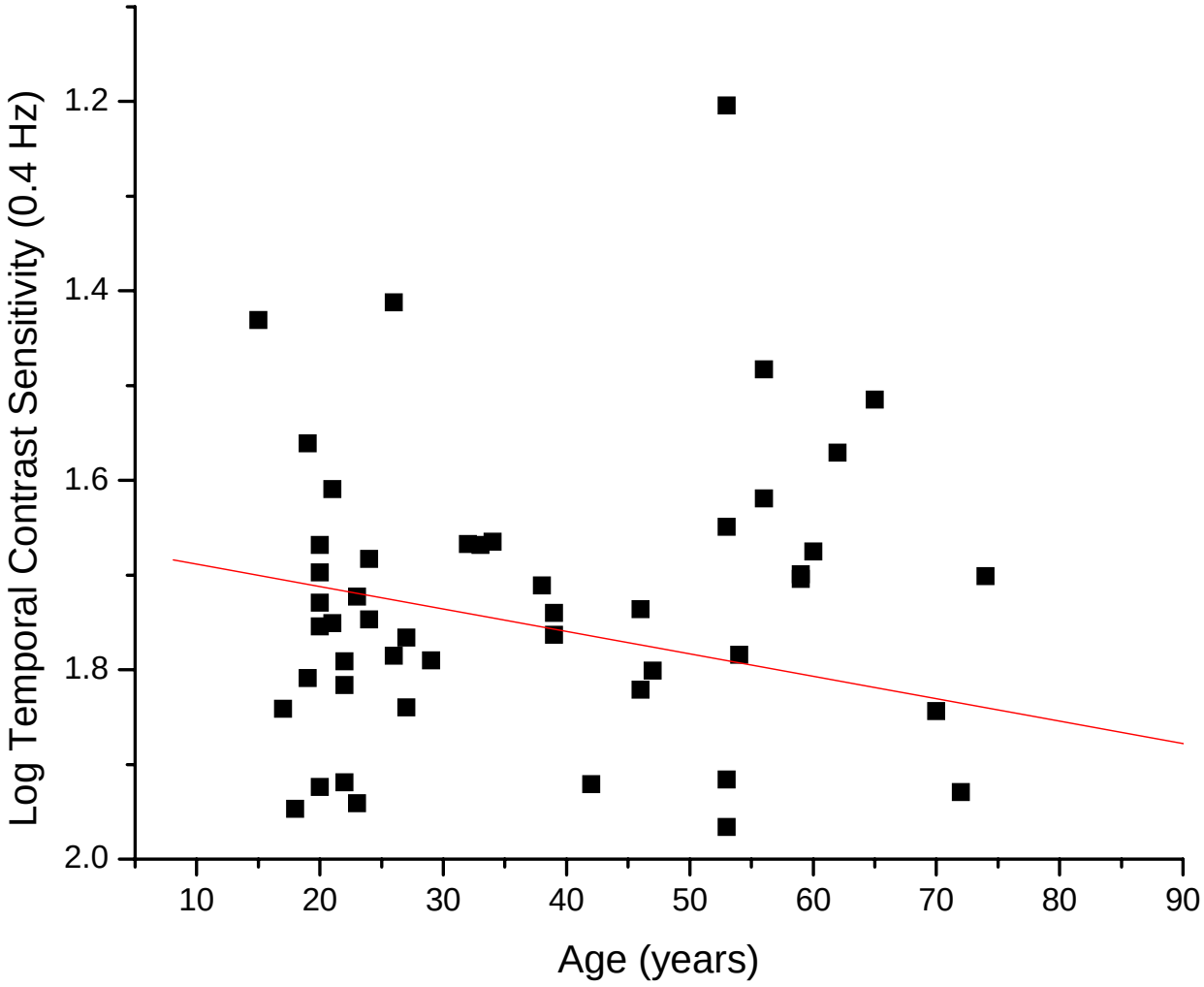

Figure 13. The relation between TMTF and age in the low frequency (approx 2.5 Hz) region of the function (r = -0.25, p < 0.03).

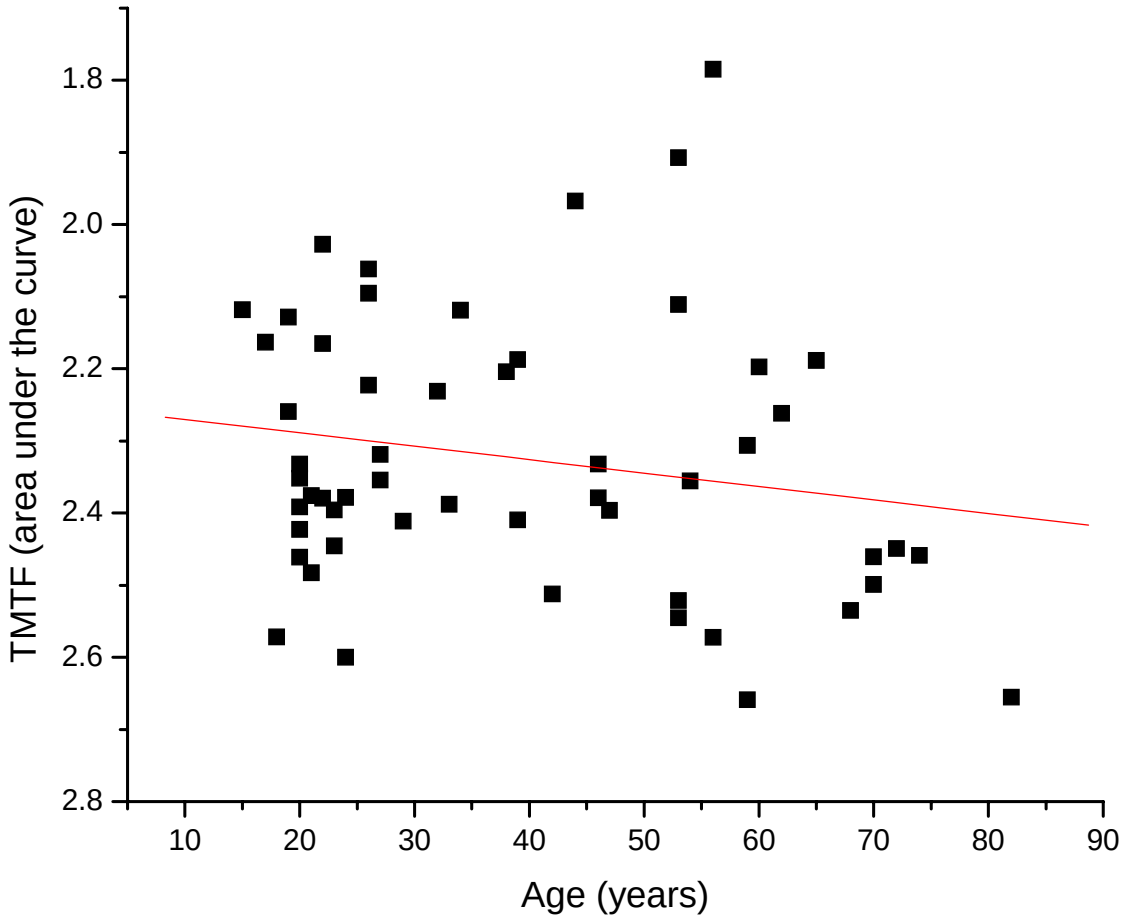

Figure 14. The relation between peripheral TMTF and age ($p > 0.09$) collapsed across frequencies.

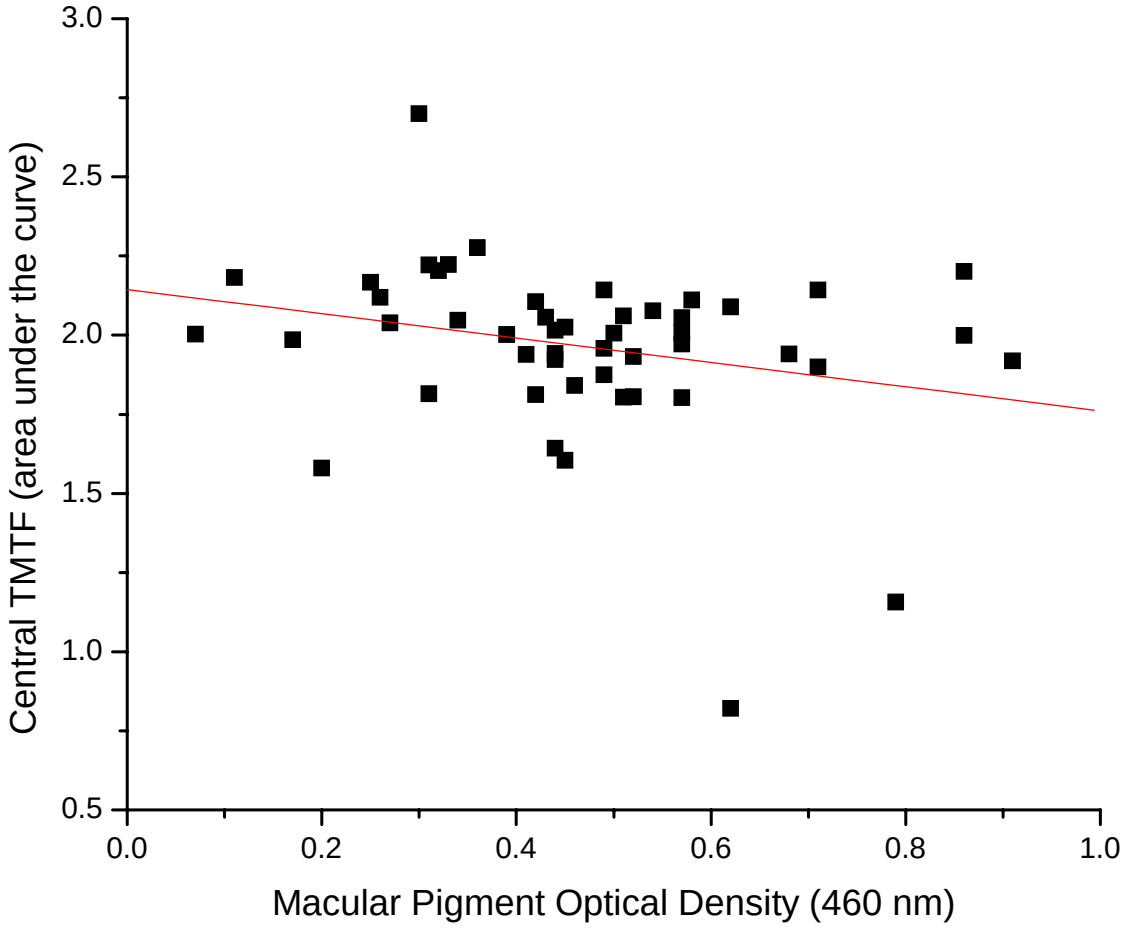

Figure 15. The relation between central TMTF and MP density (assessed at 30-minutes of eccentricity). Note the inverted y-axis, which is typical for this measure. Consequently, the negative slope actually indicates a positive association.

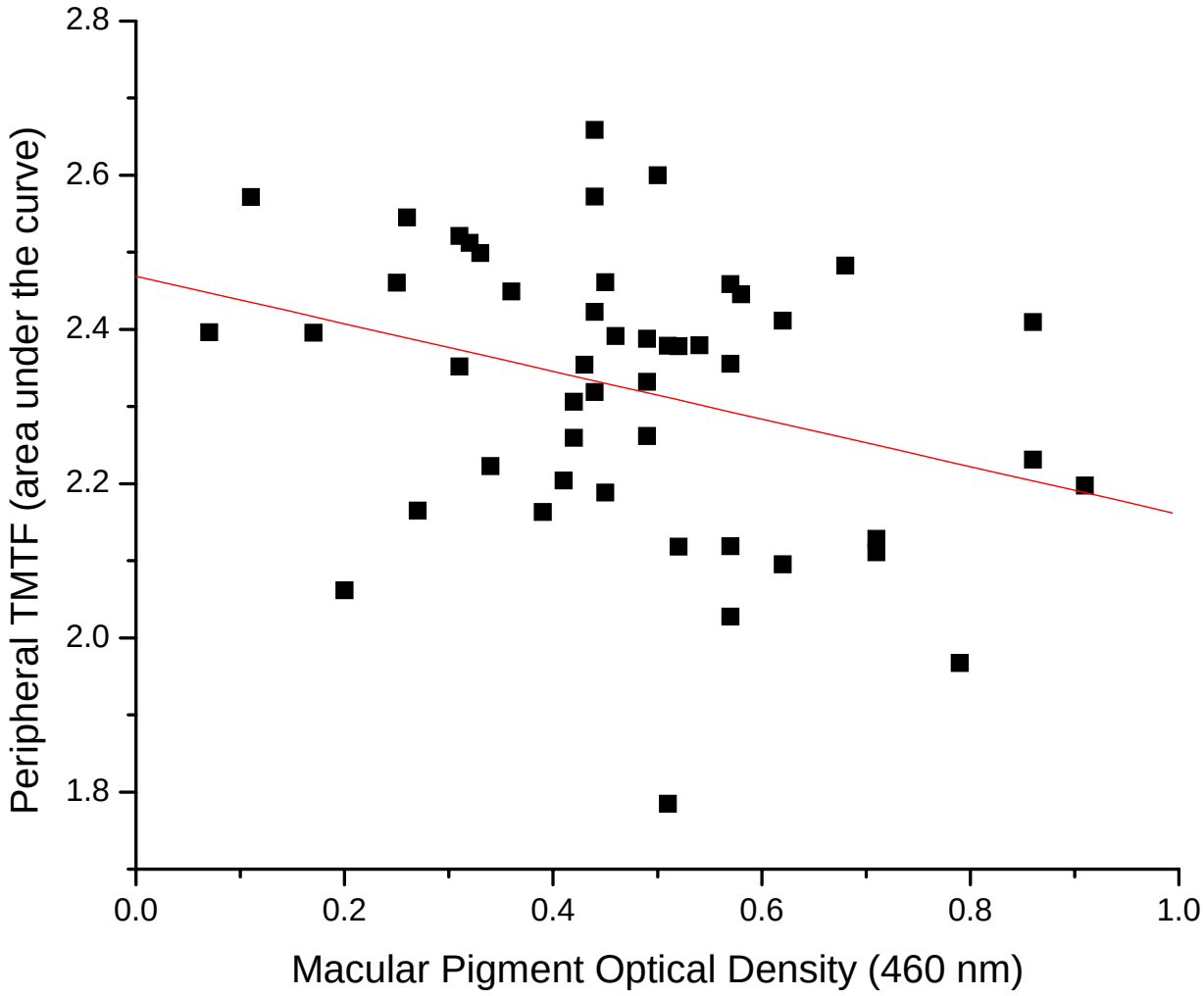

Figure 16. The relation between peripheral TMTF and MP density (assessed at 30-minutes of eccentricity). Note the inverted y-axis, which is typical for this measure. Consequently, the negative slope actually indicates a positive association.

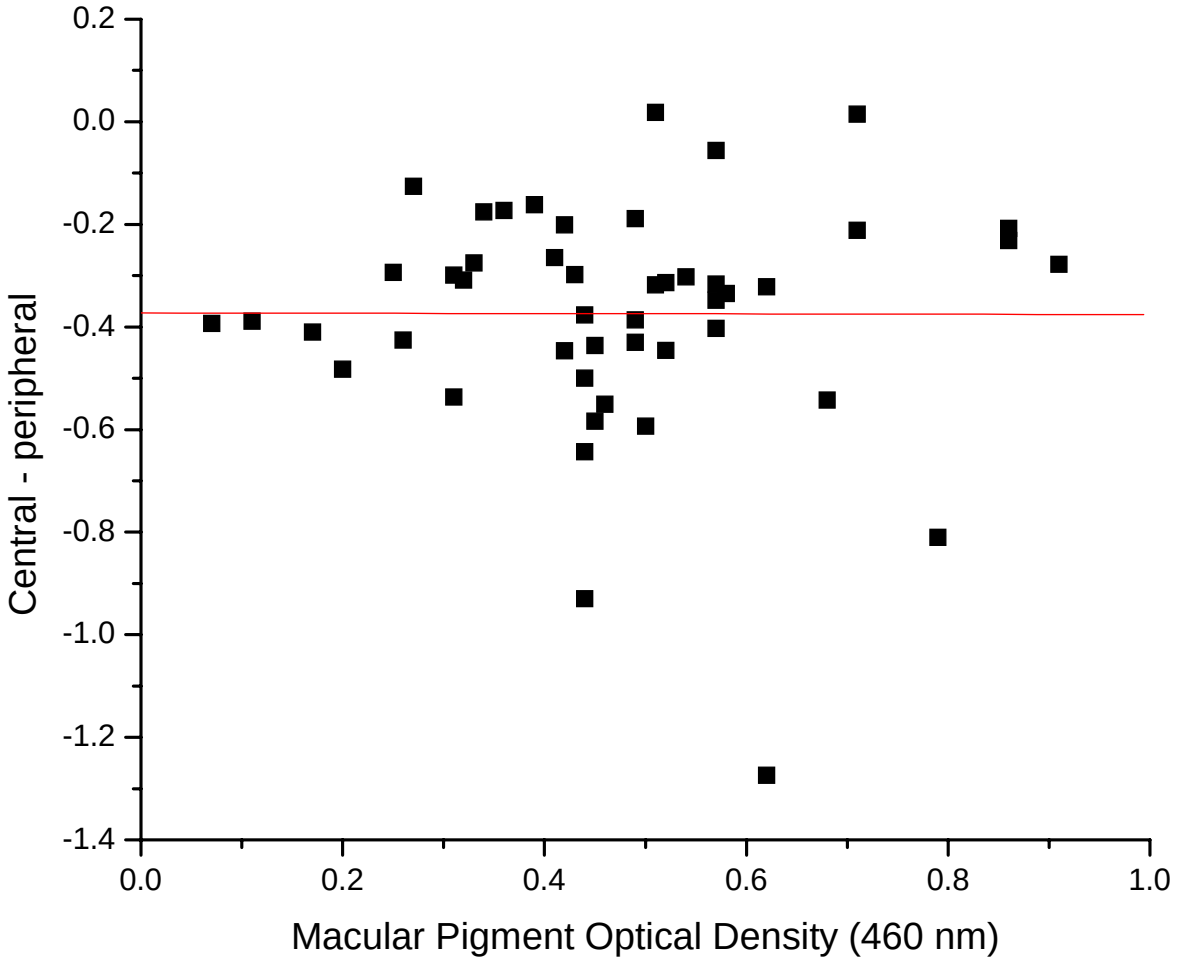

Figure 17. An assessment of the difference between central and peripheral TMTF area under the curve and its relation to MP density essentially yields a flat line (i.e., no difference)