SIMULTANEOUS ERG AND VEP RESPONSES TO DIFFERENT INTEROCULAR CONTRAST RATIOS AS A FUNCTION OF SPATIAL AND TEMPORAL FREQUENCIES IN ANISOMETROPIC AMBLYOPES

A thesis presented to the graduate faculty of New England College of Optometry in partial fulfillment of the requirements for the degree of Master of Science

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PRELIMINARY STUDY ON SIMULTANEOUS ERG AND VEP RESPONSES TO DIFFERENT INTEROCULAR CONTRAST RATIOS AS A FUNCTION OF SPATIAL AND TEMPORAL FREQUENCIES IN ANISOMETROPIC AMBLYOPES

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Abstract

SIMULTANEOUS ERG AND VEP RESPONSES TO DIFFERENT INTEROCULAR CONTRAST RATIOS AS A FUNCTION OF SPATIAL AND TEMPORAL FREQUENCIES IN ANISOMETROPIC AMBLYOPES

John Marc Hamilton

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PURPOSE

The goal of this project was to investigate the relationship between retinal (ERGs), cortical (VEPs), and subjective responses (contrast balance point) in anisometropic amblyopes and normal age-matched controls to examine the role of the retina and cortex in amblyopia. This study was designed to examine the precise cause of vision loss in amblyopia by testing the hypothesis that the spatial and temporal frequency dependence of the contrast balance point is due to both retinal and cortical factors.

METHODS

Twenty participants were recruited (13 normals, 7 anisometropic amblyopes). All participants underwent pre-testing involving a review of systemic and ocular health, ETDRS best-corrected visual acuity, auto-refraction, axial length measurement, and pupil size measurement. This preliminary testing assured that subjects met their group's inclusion/exclusion criteria. Participants wore their best correction in a trial frame during all testing. Each participant's subjective contrast balance point was next obtained using band-pass filtered Sloan letters via an adaptive staircase procedure. Letter stimuli of 1 and 4 cycles per degree, with varying Michelson contrasts, were displayed on a uniform gray background (60 cd/m^2) on a 120 Hz luminance-calibrated monitor, while participants wore stereo-shutter gasses running at 60 Hz. The balance point was defined as the interocular contrast ratio yielding letter recognition in each eye with equal probability. Steady-state pattern ERGs and pattern VEPs were then recorded simultaneously using a five-channel electrophysiological amplifier and a

dichoptic viewing system with a 2-mirror Wheatstone stereoscope. Subjects fixated on a cross while dichoptically viewing two ~5x15 degree checkerboard stimuli with average luminance of 60cd/m^2. The stimuli reversed in time (4 Hz or 10 Hz) across a range of spatial frequencies (1 and 4 cycles per degree) and across a range of contrast ratios including the exact subjective contrast balance point of the subject. The ERGs and VEPs were recorded in accordance with ISCEV standard electrode placement. Recordings were fast fourier transformed to analyze amplitude and phase. Retinal and cortical electrophysiologic response dynamics of the amblyopic, fellow, and normal eyes were compared using ANOVA and regression analysis for each spatial and temporal frequency and for the whole range of luminance contrast tested.

RESULTS

Our balance point data is consistent with the literature. Our ERG and VEP results demonstrate variable agreement with past studies involving correlations with contrast, spatial and temporal properties of stimulation. Our ERG results suggest that in some cases at high contrast, the retina of amblyopic eyes is deficient to some extent. Similarly, our monocular VEP results also suggest some post-retinal deficiency in amblyopic eyes. From these results we can suggest that the retinal deficiency contributes to the post-retinal defect recorded at the cortex. However, as past studies have reported electrophysiogical deficiencies to be attributable to eye movements, once we accounted for eye movements, we observed no ERG, no monocular VEP, and no binocular beat VEP differences between normal versus amblyopic versus fellow eyes in any of our testing conditions. Our electrophysiological findings did not correlate to the subjective balance point measurements.

CONCLUSIONS

Our study does not suggest any significant retinal or post-retinal functional deficiencies in amblyopic subjects. This study provides a comprehensive framework and addresses important factors, limitations and future considerations for assessing retinal function, monocular visual pathway function, and binocular visual pathway function in amblyopes.

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List of Abbreviations

CRT: Cathode Ray Tube

DTL: Dawson-Trick-Litzkow

ETDRS: Early Treatment Diabetic Retinopathy Study

FDA: Food and Drug Administration

Hz: Hertz

ID: Identification

ISCEV: International Society for Clinical Electrophysiology of Vision

LCD: Liquid Crystal Display

NECO: New England College of Optometry

NEE: New England Eye Center

OD: Oculus Dexter, meaning Right Eye

OS: Oculus Sinister, meaning Left Eye

SD: Standard Deviation

SE: Standard Error

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1- Introduction

1.1- Overview

Amblyopia, colloquially referred to as “lazy eye”, is a serious visual disorder affecting between 2 to 4% of children under age six years, making it the number one cause of monocular visual impairment in this population[1]. Amblyopia is a unilateral or less frequently bilateral condition in which the best-corrected visual acuity is worse than 20/20 despite a normal structural eye examination due to the presence of at least one amblyopic risk factor early in life[2]. Visual acuity is the primary measure of amblyopia and is clinically diagnosed when there is at least a 2-line difference between eyes using the logMAR (log of minimum angle of resolution) scale, or in bilateral cases, when the best-corrected visual acuity is poorer than 20/40 in the better-seeing eye at age 4 years or older (or worse than 20/50 in patients under age 4 years)[3]. Visual acuity in amblyopia ranges from mildly reduced from normal (20/20) to functionally blind (20/200 or worse)[2]. It is caused by a disturbance of normal visual input during the critical period of visual development (widely accepted to span from birth to age 8 years)[4], and the risk of amblyopia increases with relevant family history, prematurity, developmental delay, and maternal smoking, use of drugs or alcohol. Amblyopia is most commonly associated with anisometropia (asymmetric refractive error as small as 0.50 diopters) and manifest strabismus, and less frequently with form deprivation (ie. caused by congenital cataract or ptosis) [2,3,5].

The visual processes that are defective in amblyopia manifest at threshold and suprathreshold levels, and include numerous deficits rather than simply reduced visual acuity[6], such as reduction in contrast sensitivity[6], reduced binocular vision, increased sensitivity to crowding[7], abnormal spatial distortions and uncertainty[8], unsteady and inaccurate monocular fixation[9], poor eye tracking[10], and errors in accommodation[11]. A major hypothesis for these deficits is an abnormal binocular interaction with the fellow eye exhibiting stronger inhibitory cortical input than the amblyopic eye resulting in interocular suppression[12].

For patients with deprivational amblyopia, treatment begins by correcting the structural abnormality interfering with clear retinal image formation. For all types of amblyopia, the first step in treatment involves appropriate refractive correction as indicated, which alone may improve vision or may require additional treatment such as patching or atropine penalization[2]. Once the underlying etiology is corrected, current standard of care revolves around impairing or occluding the fellow, better-seeing eye for 2-6 hours per day to interfere with its dominance of visual cortex while promoting development of visual pathways to the amblyopic eye. For centuries it has been accepted that occlusion of the fellow, sound eye may be achieved with an adhesive patch or by using blurring filters (Bangerter foils)[13]. In recent decades, atropine drops have been shown to be equally effective as patching. Atropine is a cycloplegic agent that blurs vision for more than 24 hours so can be a good option for uncooperative patients[14]. The earlier the treatment within the critical period provides the best visual outcome given the higher plasticity of the young brain[15]. Deprivation amblyopia is the most resistant to treatment while anisometropic amblyopia carries the best prognosis, likely a result of some preserved degree of binocularity in these patients. One study suggested that after successful treatment, amblyopia recurred in 25% of children within a year of stopping therapy, emphasizing the importance of slow tapering of treatment to prevent recurrence[2,3].

Rather than fully occluding or significantly blurring the fellow eye, which may further disrupt binocularity and be less than ideal for promoting binocular function, newer binocular approaches to treatment involve just enough blur or contrast reduction to the fellow eye, while simultaneously engaging the amblyopic eye[16]. It has been suggested that this binocular approach may improve not only visual acuity, but also binocular function, and can be achieved by wearing red-green, polarized, or liquid crystal display shutter glasses to provide different but effectively balanced inputs to the eyes. Perceptual learning, which has shown promising treatment results, incorporates these binocular strategies by having the patient perform repetitive discriminative tasks to improve perception[3,17].

Generally, it is accepted that amblyopia is a problem of cortical preference to the fellow (normal) eye while the amblyopic eye is suppressed[1]. It has been shown that suppression can be alleviated by balancing the input in the two eyes, i.e. using a stronger stimulus for the amblyopic eye (i.e. higher contrast) and a weaker stimulus (i.e. lower contrast) for the fellow eye. This is called the balance point, or the contrast ratio between the two eyes of an amblyope that results in binocularity[12], and it remains unclear whether the neural generators of the balance point originate in the retina or cortex.

Contrast processing involves two streams, namely the magnocellular (MC) and parvocellular (PC) pathways which are present at the level of the retina and lateral geniculate nucleus, with further processing of MC and PC signals in the cortex, namely the primary visual cortex[18]. The magnocellular pathway has high-contrast gain and is involved in the detection of brief, achromatic stimuli of low contrast, whereas the parvocellular stream primarily responds to high spatial frequency, high contrast, chromatic and achromatic stimuli[19]. Past studies have reported contrast sensitivity in amblyopia to be normal or near normal at low spatial frequencies but reduced at high spatial frequencies. Reduced contrast sensitivity at high spatial frequency, or visual acuity, would represent a selective parvocellular pathway deficit[20,21]. However more recent studies suggest that amblyopes have reduced contrast sensitivity at all spatial frequencies which would represent nonselective deficits in both the parvocellular and magnocellular pathways[22,23]. In anisometropic amblyopia, there are inconsistencies in the literature concerning selective (parvo or magno) high spatial frequency loss versus nonselective (parvo and magno) generalized loss across all spatial frequencies[18,22,23]. Past studies have not specifically isolated the parvo and magno pathways. The two streams overlap with the relative weight of their responses dependent upon the spatial, temporal and chromatic properties of the stimulus, and on the adaptation level[18]. Zele et al.[18] designed a stimulus to isolate magnocellular and parvocellular vision and showed through psychophysical testing that anisometropic amblyopes have reduced spatial contrast sensitivity across the range of spatial

frequencies tested (0.25-8.0 cycles per degree) as a result of dysfunction in both the magno and parvo streams. They reported that the fellow eyes were normal or near normal across those spatial frequencies tested. Furthermore, they discussed that although they were able to isolate a given retinal pathway (MC or PC), that the nature of their results represents more than just retinal ganglion cell function, given that their results did not show typical band-pass characteristics of retinal ganglion cells[24]. Therefore they suggested that postretinal processing has a significant role in contrast sensitivity, and possibly abnormal processing of MC or PC signals in the cortex of amblyopes.

There is no clear concensus in the literature regarding the neural basis of amblyopia. The precise cause of vision loss in amblyopia has been the subject of extensive research. Researchers have examined anatomical, physiological and many psychophysical aspects of amblyopia with variable and conflicting findings[25].

Brain imaging studies using functional Magnetic Resonance Imaging (fMRI) have shown changes in the lateral geniculate nucleus (LGN) corresponding to the amblyopic eye[26], which are consistent with morphological changes identified originally by Wiesel and Hubel[27]. Retinal imaging studies using Optical Coherence Tomography (OCT) provide mixed results as to whether there are structural differences between the amblyopic and the fellow eye's retinae. Most of the OCT data agree that amblyopes have an overall thicker macula and fovea for the amblyopic compared to the fellow eye[28]; however, retinal nerve fiber layer (RNFL) thickness differences have not been consistent among studies[29,30].

Amblyopia has been studied with Visual Evoked Potentials (VEPs), and the majority of studies have found decreased amplitude and increased latency of the amblyopic VEP response. Rather than any clinically observable primary deficit in the amblyopic pathway, the abnormal VEP response is thought to be induced by abnormal binocular interactions from the unbalanced images in each eye, or else, from the suppression of the amblyopic eye by the fellow eye. This suppression results in loss of binocularity; however, since binocular vision can be restored by

balancing the images each eye sees, it is suggested that the mechanism/neurons for binocular vision is intact but dysfunctional in amblyopes[31]. Most VEP studies, however, have used stimuli that are equal between the two eyes, therefore facilitating suppression of the amblyopic pathway.

While VEP studies all agree that the amblyopic eye exhibits an abnormal VEP signal, the results are much more equivocal for electroretinograms (ERGs). A number of functional studies[32–36] assessed the activity of retinal ganglion cells using the pattern ERG (PERG) with high contrast reversing luminance gratings or checkerboards of varying spatial frequency or check size, respectively. Most of the research groups[32–35] found that the amplitude and/or the latency of the PERG is affected in the amblyopic eye when compared to the PERG of the fellow eye. In contrast, Hess et al.[37] found no difference in the PERG responses of anisometropic and strabismic subjects, and they attributed their results to the fact that they corrected for the unstable fixation of amblyopic subjects. A common characteristic of all the aforementioned functional studies is that they used stimuli of high luminance contrast (>80%). This type of stimulation fails to fully characterize retinal function in amblyopes because high contrast stimuli do not assess the sensitivity of the retinal neurons under near-threshold conditions where it is known that contrast sensitivity (i.e. sensitivity at contrast threshold) is affected in amblyopes, while suprathreshold apparent contrast is not[38].

Kwon et al.[12] conducted an experiment with amblyopes where they measured the contrast balance point as a function of spatial frequency. They found that the balance point depends on the spatial frequency of the stimulus. In other words, the spatial frequency of the stimulus affects the degree of suppression on the amblyopic eye by the fellow eye, with more suppression occurring for higher spatial frequencies. Current unpublished data from Dr. Peter Bex's lab suggests that the balance point also changes as a function of temporal frequency, with higher temporal frequencies exhibiting lower balance point than lower temporal frequencies, when the two eyes are stimulated with the same temporal frequency. It is still

unclear, however, whether this spatial and temporal frequency dependence of the balance point is due to retinal or cortical factors.

The following section will review the basics and background of the tests used in this study.

1.2- Binocular Balance Point Experiment

Binocular vision - meaning both eyes working together - is important to depth perception and to other complex visual functions such as reading, object recognition and visual-motor coordination[39,40]. There is a risk of developing amblyopia anytime normal binocularity is disrupted by any condition causing unequal input between the eyes. Literature suggests that abnormal binocular interaction plays a significant role in the etiology of amblyopia[41]. Binocular vision in amblyopia involves deficits in the excitatory connections important for binocular summation and stereopsis[42], and increased inhibitory connections facilitating interocular suppression[43]. Studies have found that suppression can be alleviated and binocularity can be restored in some amblyopes by using binocularly balanced contrast stimuli, meaning equating the effective contrast of the two eyes. They suggest that the balanced stimuli promote binocularity possibly through strengthening of the excitatory binocular connections responsible for binocular summation[41,44–46]. Amblyopia treatments using binocularly balanced stimuli have been shown to improve visual acuity and stereoacuity[47].

Many studies have demonstrated that abnormal binocular interaction shows spatial-frequency dependency[21,42,48], and Kwon et al.[12] designed the first clinical modality to evaluate binocular imbalance as a function of spatial frequency. They used an innovative computer-based approach to calculate the balance point (BP) between the two eyes, defined as the interocular contrast-ratio required to report a letter of a specific spatial frequency in each eye with equal probability (50%). Therefore a BP of 0.50 suggests that the input signals from both eyes are equal (perfect binocular balance), whereas a value different from 0.50 indicates binocular imbalance. They tested 12 amblyopes (10 anisometropic amblyopes, 1 strabismic

amblyope, and 1 mixed anisometropic and strabismic amblyope) and 12 normals and showed that the balance points of amblyopes increased with increasing spatial frequency. For instance, the BP of the amblyopic eye increased from 0.63 at 0.5 cycles per degree (c/deg), to 0.90 at 5c/deg, meaning at low 0.5c/deg spatial frequency, 63% contrast was needed in the amblyopic eye to balance 37% contrast in the fellow eye, while for high 5c/deg spatial frequency, 90% contrast was required in the amblyopic eye to balance 10% contrast in the fellow eye. Across all spatial frequencies tested, the balance points of the amblyopic group (0.75 ± 0.13 SE) were higher than in the normal control group (0.53 ± 0.05 SE). The higher BP in amblyopia suggests that the input from the amblyopic eye is suppressed by the fellow eye, even more so at higher spatial frequencies. The BP of the normal group was always close to 0.50, indicating that the inputs from both eyes are treated approximately equally, with only slight eye dominance, independent of spatial frequency[12]. They reported reasonably good test-retest reliability with their methods, with repeated measures all within less than a mean difference of 0.09 (< ~9%).

1.3- Pattern ERG

Electroretinography (ERG) is a reliable, objective test of retinal function, which records the electrical signals of the retina in response to a stimulus. ERG records retinal activity independent of the visual pathways and cortex. The electrical responses are recorded using a combination of electrodes on or around the eye, namely active, reference and ground electrodes[49].

Pattern ERG (PERG) is a local retinal response largely covering the macular area and representing ganglion cell function, driven by photoreceptors and other retinal cells. PERG is a sensitive measure of the integrity of the optics and retinal cells within the retinal area subtended by the stimulus. PERG can be used to identify central retinal dysfunction or to monitor retinal ganglion cell dysfunction in diseases such as glaucoma[49].

The pattern ERG signal is evoked by a contrast-reversing pattern of constant mean luminance, commonly a white-and-black checkerboard presented on a monitor. The

checkerboard tiles continually reverse at the programmed temporal frequency. The resulting waveform morphology is dependent on the temporal frequency used ie. transient versus steady-state. At low temporal frequencies <3Hz whereby the retina has time to complete its response prior to the next contrast reversal, transient signals are obtained. The components of the transient ERG are measured by amplitude and latency. At higher temporal frequencies, the retina does not have time to fully generate its response prior to the subsequent contrast reversal and so the waveforms overlap. Steady-state ERG signals are "frequency-following", meaning the resulting waveform represents the temporal frequency of the stimulus used. Steady-state ERGs be analyzed by amplitude and phase using Fourier analysis[49]. Amplitude represents the magnitude of the electrophysiological response which is correlated to the number and vitality of retinal neurons contributing to the recorded signal. Phase is used in the frequency domain to signify rate of propagation of the recorded signal, which is analogous to latency in the time domain. Phase is also an indication of vitality of activated neurons, which may or may not be associated with changes in amplitude. There are two different sign conventions for phase. In this thesis, positive or increased phase change means increasing delay, increasing phase lag, or slower signal (latency increased), while negative or decreased phase means decreasing delay, phase advancement, or faster propagation of signal (latency shortened). Other studies have assigned the opposite sign convention[50]. Reduced or delayed signals may indicate neuronal dysfunction either by diminished number of neurons or by abnormal connectivity, or both[51].

Most previous studies done using transient PERG showed reduced amplitude and increased latency in the amblyopic eye compared to the healthy control eye[32,52]. Other studies showed no difference in either amplitude or latency between control and amblyopic eyes[53,54].

Like transient PERG, the literature is not clear when it comes to the use of steady-state PERG (SSPERG) in amblyopes. Some studies found decreased amplitude in amblyopic eyes

compared to normal controls[35], whereas other studies did not observe any significant differences between amblyopic eyes and controls[34].

Several studies have investigated the effects of stimulus contrast, spatial and temporal frequency on the amplitude and phase of steady-state PERG in normal subjects. Porciatti et al.[51] showed that amplitude progressively increased with increasing contrast in an approximate linear fashion to reach a value about 82% larger between 12% and 99% contrast. They demonstrated that phase also increased with increasing stimulus contrast between 12% and 99% contrast. They suggested that reduced stimulus contrast at constant mean luminance leads to phase advancement (latency shortening) because there are two major classes of retinal ganglion cells that generate the PERG response: M-cells and P-cells. M-cells are much more sensitive to luminance contrast than P-cells and generate a faster response[55,56], therefore low contrast stimuli elicit greater contribution from M-cells compared to high contrast stimuli, producing PERG phase advancement[57]. It has been shown that phase increases and amplitude decreases with increasing stimulus spatial frequency[58,59], and that phase increases linearly with increasing temporal frequency[58]. With regards to the effect of temporal frequency on amplitude, there is an interaction between temporal and spatial frequency so the form of the temporal function depends on the spatial frequency used. For example, at 0.4c/deg there is a maximum in the response at an intermediate temporal frequency around 8-10 Hz and attenuation for both higher and lower temporal frequencies. At 4c/deg, the largest amplitude occurs at the lowest temporal frequency[58].

1.4- Pattern VEP

Visual evoked potential (VEP) is a recording of the electrical activity of the visual cortex in response to a stimulus. VEPs represent the functional integrity of the central vision of the entire visual pathway, originating at the photoreceptors all the way to the visual cortex. Active electrodes are placed over the occipital cortex to measure the signal triggered by visual stimulation. VEP is used clinically to objectively measure visual acuity and to aid in the

diagnosis and monitoring of a variety of ocular conditions[60]. In unilateral conditions such as optic neuritis and amblyopia, the amplitude and latency of the affected eye is significantly different from the fellow eye[60,61].

There are different types of VEP - pattern-reversal, pattern onset/offset, and flash - which differ by their stimulation used. Like pattern-reversal ERG, the pattern-reversal VEP signal is generated by a contrast-reversing checkerboard pattern of constant mean luminance, presented on a monitor. The size of the checkerboard tiles is specified by their size in degrees, and the tiles continually reverse back-and-forth at the programmed temporal frequency[60]. Pattern VEP produces very small amplitude signals which are sensitive to eye movement so they are measured by averaging many trials[60,62]. Similar to ERGs discussed earlier, the main determinant of the VEP waveform morphology is the temporal frequency of the stimulus. At higher temporal frequencies where the visual system never returns to its resting state throughout the duration of recording, the waveform becomes an approximately sinusoidal frequency-following response, termed a steady-state VEP (SSVEP). VEPs recorded at lower temporal frequencies are called transient VEPs[60]. The primary source of the SSVEP response is the striate cortex (V1), but there are other sources as well[63,64].

VEP changes in amblyopia have been an area of interest since the 1970's. The earliest studies of pattern VEPs in amblyopia showed markedly reduced amplitude of the amblyopic eye compared to the fellow eye when each eye was tested separately, which correlated to visual acuity. These studies showed increased signal latency[65,66]. Other studies have also demonstrated abnormal VEP amplitude and latency in the amblyopic eye[67–71]. Later studies reported on average a 9.0% decrease in VEP amplitude of the amblyopic eye relative to the fellow eye in anisometropic amblyopia[72]. Some other studies have compared the amplitude of steady-state VEPs in normal versus amblyopic eyes[73,74]. While all types of amblyopia have demonstrated decreased VEP amplitude, findings vary depending on their subject selection particularly the depth of amblyopia, and the type of VEP stimulation used. Studies to date have

commonly measured VEP responses at a single contrast level which fails to fully characterize the amblyopic deficiency for contrast processing. Responses should be recorded across a range of contrasts for each eye to obtain a more complete picture of the amblyopic deficiency[75].

As previously discussed with SSPERG, amplitude and phase of the steady-state VEP also depend on contrast, spatial and temporal properties of the stimulation. These relationships have been extensively studied. Several studies have reported higher SSVEP amplitude with increasing stimulus contrast in controls and amblyopes[75–78]. Strasburger et al.[79] conducted an investigation in normal human subjects concerning the effects of stimulus spatial frequency and contrast on SSVEP amplitude using checkerboard stimulation. Their study showed that amplitude generally increases with increasing contrast and with increasing spatial frequency; however they noted a characteristic double-peaked pattern to their amplitude versus spatial frequency response function which increased at lower and higher spatial frequencies but decayed at intermediate frequencies around 3c/deg. This plot had a low frequency peak at 1.6c/deg (standard error of 0.18c/deg), a trough at 3.2c/deg (standard error of 0.3c/deg), and a high frequency peak at 7.2c/deg (standard error of 0.75c/deg). This general shape can be described by gain reduction at intermediate spatial frequencies and gain increase at low and high spatial frequencies[79]. They suggested that amplitude did not correlate to contrast sensitivity or perception which could be attributable to an interaction or cancellation of electrical signals being measured at the electrode site but originating from different areas. Norcia et al.[50] studied a wider range of spatial frequencies and demonstrated that amplitude generally increases with increasing spatial frequency up to about 8-11c/deg at which point amplitude then decreases with increasing spatial frequency. These findings were in agreement with Strasburger et al.[79] who, along with Norcia et al.[50], further showed that SSVEP phase increases with increasing stimulus spatial frequency, having a similar double-peaked response function to the aforementioned amplitude plot with its trough located in the same spatial frequency range. These results were consistent with findings of a study performed in macaque monkeys by

Nakayama et al.[80] who also reported that phase increases while amplitude decreases with increasing temporal frequency. Other studies have demonstrated that higher temporal frequencies and higher contrast conditions show faster propagation speeds[78,79,81].

1.4.1- Frequency-tagging VEP: Working with Simultaneous Stimuli

VEPs can be recorded monocularly or binocularly[60]. As discussed previously, many studies have employed monocular recordings, however comparing monocular and binocular results gives information regarding binocular interactions. Depending on the VEP protocol, binocular stimulation can show suppression, summation, or binocular facilitation[31,61,82–90]. In suppression, the binocular signal is less than the average monocular signal. Summation produces a binocular signal that is greater than the average monocular signal, up to the sum of the two monocular responses. In binocular facilitation, the binocular signal is greater than twice the average monocular response. Pattern-reversal SSVEP studies have demonstrated binocular summation in normal human subjects[61,91]. In subjects with abnormal binocular vision which was defined by decreased stereoacuity, binocular summation may be reduced or absent. A pattern-reversal VEP study resulted in mixed findings in these binocular deficient subjects by showing that some participants had normal binocular summation while others did not[90].

When using a non-stereoscopic stimulus in subjects with normal binocular vision, binocular stimulation consistently produces increased VEP amplitude – relative to the average monocular signal – due to binocular summation[61,83,84,86,91]. However, when a stereoscopic stimulus is presented, meaning having non-zero binocular disparity, the binocular VEP amplitude is reduced to less than the average monocular amplitude[31,82,83,86,88]. The binocular amplitude reduction with stereoscopic stimuli is a result of fusional suppression, which is normal interocular suppression[82,92]. During stereoscopic binocular vision, monocular position information from each eye is used to calculate the cyclopean position, as well as to produce stereopsis[85,93–95]. It has been proposed that once the cycloplean position has been established, the monocular position information must be suppressed to eliminate competing position

information from the right eye, left eye, and the calculated cyclopean position. This suppression of monocular information, referred to as fusional suppression, plays an essential role in fusion during stereopsis[85]. Fusional suppression noted in binocular VEPs is a normal process in stereopsis which only occurs with non-zero binocular disparity[85,96].

SSVEP studies have been conducted using multifrequency stimulation with a single active electrode over Oz to gather the multiple frequency stimulations[97,98]. Hebert-Lalonde et al.[97] used a frequency-tagging electrophysiological method to identify central and peripheral visual field defects, extracting two cycle frequencies presented at the same time in twenty-two normal and 5 visually impaired participants. Hohberger et al.[98] suggest that like multifocal electroretinography (mfERG) can reduce recording time without a loss in signal-to-noise ratio, multifrequency stimulation is an efficient method for recording pattern-reversal SSVEPs.

Dichoptic multifrequency stimulation is important for the assessment of binocular interaction in this study. The SSVEPs in this study were recorded binocularly, however the resultant VEP signal could be "frequency-tagged" with the temporal frequencies of the two stimuli used, allowing each eye to be analyzed independently. For example, a 4Hz component corresponding to one eye, and a 10Hz component corresponding to the other eye, could be extracted and analyzed from the single waveform that was binocularly generated. The difference between the two temporal frequencies used in the dichoptic stimulation, 6Hz in this example, is termed the "beat frequency". The beat frequency is an index of binocularity, as it is not present within the stimulus, it is thought to be generated by the confluence of monocular signals in the binocular neural pathways. Baitch and Levi have demonstrated that a beat response is present in stereonormal adults, and that it is reduced or absent in subjects with poor stereoacuity[99]. Stevens et al.[100] later performed a pediatric study, comparing beat VEPs in strabismic and/or amblyopic children with those of stereonormal children, and reported that dichoptic amplitudes were significantly lower for the stereodeficient group.

1.5- Clinical Relevance of Research Goals

Amblyopia is a serious visual disorder associated with visual acuity loss, decreased stereopsis, contrast sensitivity and other visual deficits. Visual loss is permanent unless treated before age six years, within the critical period while the visual pathway exhibits pronounced neural plasticity[2]. The prevalence and profound effects of amblyopia makes it very important for the optometric community to detect and treat it. Current traditional treatment options include full optical correction, penalization and occlusion of the normal eye. Other treatments have been proposed such as perceptual learning[101], electrical or magnetic brain stimulation[102–104], monocular game-based methods[105,106], and dichoptic game-based designs that provide a balanced binocular input[107]. Treatment efficacy has commonly been evaluated using psychophysical tasks which may improve with practice and be subject to response bias and motivational factors. Objective measures of visual improvement are important to more effectively evaluate treatment regimens[75]. Functional magnetic resonance imaging has previously been used in studies to objectively study amblyopic deficits[108] however MRI equipment is costly and not readily available to clinicians outside of major academic centers. ERG and VEP provide objective measures that are cheaper and more clinically straightforward[75].

Despite various treatment protocols, the underlying cause of the deficit in amblyopia remains a topic of extensive research. It is unclear whether the source of change is of retinal or cortical origin, even though conventional wisdom suggests that the amblyopic deficits are of cortical and/or thalamic origin. In this study, steady-state pattern ERG (SSPERG) and pattern VEP (SSPVEP) responses were recorded that were not only suprathreshold but also close to threshold so as to estimate the dynamic range of the retinal and cortical neurons, and these responses were correlated with the results of the psychophysical balance point testing. Currently there is no single clinical factor that can be used to predict the presence of, or development of amblyopia in very young children. New physiologic and anatomic markers could

provide valuable insight into the pathophysiology of amblyopia, which if incorporated into a screening or diagnostic system could lead to earlier detection and treatment for future generations.

1.6- Specific Experimental Issue or Question

The goal of this project was to simultaneously record steady-state PERGs and PVEPs with different contrast ratios between the two eyes of anisometropic amblyopes to examine the relationship between the retinal (ERGs) and cortical (VEPs) responses. This study was designed to test the hypothesis that the spatial and temporal frequency dependence of the balance point is due to both retinal and cortical factors.

2- Materials and Methods

2.1- Subjects

The study was approved by the New England College of Optometry Institutional Review Board and complies with all institutional requirements regarding the use of human subjects. Twenty participants were recruited (13 normal subjects, 7 anisometropic amblyopes). Normals were recruited from the NECO student population. Normals who responded to the email invitation were screened for normal ocular health and no history of amblyopia. Amblyopes were recruited from the NECO Center for Eye Care and the NECO student population. Amblyopes were identified by ICD-10 code and they were contacted if their records satisfied the study criteria. NECO Center for Eye Care participants had authorized the release of their personal information for "Engaging in research that has been approved by the Institutional Review Board in compliance with NECO/NEE policy." Those who responded to the phone or email invitation were invited for a screening. After participants expressed an interest in being enrolled, the study was explained and the consent form was reviewed with them in detail. Informed consent was obtained in accordance with the Declaration of Helsinki and the NECO Institutional Review Board.

All participants underwent pre-testing involving a review of systemic and ocular health, ETDRS best-corrected visual acuity, auto-refraction, axial length measurement, and pupil size measurement. Auto-refraction was obtained using Nidek ARK-1 equipment (NIDEK, Japan). Axial length measurement was achieved using a Lenstar biometry device (Haag-Streit, Switzerland). Pupil size was manually measured using a Rosenbaum Pocket Vision Screener (Merck Frosst, Canada). This preliminary testing assured that subjects met their group's inclusion/exclusion criteria. Inclusion and exclusion criteria are outlined in Tables 1 and 2.

All participants were between 18 and 30 years of age at time of enrollment into the study, had no history of systemic or neurological disease affecting vision, and no history of epilepsy or other seizures. The control group was age-matched with the amblyopia group.

Normal controls had a maximum spherical equivalent of -6.00 diopters in either eye. All normals had best-corrected visual acuity of 20/20 or better in both eyes. Amblyopes had no co-existing ocular condition, other than amblyopia, that may affect vision. Amblyopes had a maximum spherical equivalent refractive error of -6.00 diopters in their amblyopic eye, an inter-ocular best-corrected acuity difference of 2 or more ETDRS lines, anisometropia of at least 0.50 diopters spherical equivalent and/or a minimum 1.50 diopters difference in astigmatism between their eyes. Participants wore their best correction in a trial frame during all testing. Participant characteristics are summarized in Tables 3 and 4.

Inclusion Criteria
▪ Between 18 and 30 years of age at the date of enrollment into the study ▪ BCVA of 0.0 logMAR (20/20 Snellen equivalent) or better in both eyes
Exclusion Criteria
▪ History of systemic disease that may affect vision ▪ Presence of ocular disease that may affect vision ▪ Any neurological disease that may affect vision ▪ History of Epilepsy or other seizures ▪ Myopia greater than a spherical equivalent of -6.00 D in any eye

Table 1: Inclusion and exclusion criteria for normal control participants

Inclusion Criteria
▪ Between 18 and 30 years of age at the date of enrollment into the study ▪ Interocular acuity difference of 2 or more ETDRS lines, anisometropia of ≥0.50 diopters (D) of spherical equivalent and/or ≥1.50 D difference in astigmatism between the eyes
Exclusion Criteria
▪ History of systemic disease that may affect vision ▪ Presence of co-existing ocular disease, other than Amblyopia, that may affect vision ▪ Any neurological disease that may affect vision ▪ History of Epilepsy or other seizures ▪ Myopia greater than a spherical equivalent of -6.00 D in the amblyopic eye

Table 2: Inclusion and exclusion criteria for amblyopic participants

Subject ID	Gender	Age (Yrs)	Best Corrected ETDRS Snellen Acuity		Auto-Refraction (Diopters)		Spherical Equivalent Refraction (Diopters)		Anisometropia [SE, (Diopters)]	Axial Length (mm)		Pupils[2]	
			OD[1]	OS[1]	OD	OS	OD	OS		OD	OS	Dark (OD,OS)	Light (OD,OS)
N1	Female	30	20/20	20/20	+0.75-0.75x088	+0.50-0.50x102	+0.50	+0.25	0.25	23.78	23.75	7,7	6,6
N2	Male	28	20/20	20/20	-0.25-0.25x178	pl-0.25x020	-0.25	plano	0.25	25.58	25.42	6,6	4,4
N3	Male	25	20/20	20/20	+0.75-0.50x134	+0.50-0.50x045	+0.50	+0.25	0.25	23.30	23.39	7,6	6,5
N4	Male	24	20/20	20/20	-4.00sph	-4.00sph	-4.00	-4.00	0.00	24.36	24.29	7,7	6,6
N5	Male	24	20/20	20/20	-5.25-0.25x110	-4.75-0.75x025	-5.25	-5.00	0.25	26.03	25.65	6,6	5,5
N6	Female	24	20/20	20/20	+0.25-0.75x170	+0.75-0.75x076	plano	+0.50	0.50	23.38	23.54	7,7	6,6
N7	Male	23	20/20	20/20	pl-0.25x005	pl-0.50x081	plano	-0.25	0.25	24.21	24.28	7,7	6,6
N8	Male	25	20/20	20/20	-3.25sph	-3.50sph	-3.25	-3.50	0.25	24.32	24.34	7,7	6,6
N9	Male	26	20/20	20/20	-4.75-2.25x003	-4.75-1.50x179	-5.75	-5.50	0.25	24.75	24.52	6,6	4,4
N10	Female	24	20/20	20/20	-0.75-1.50x006	-1.75-1.75x172	-1.50	-2.50	0.50	24.68	25.03	6,6	5,5
N11	Female	23	20/20	20/20	+0.25-1.00x120	-1.75-0.50x045	-0.25	-2.00	1.75	24.08	24.87	7,7	5,5
N12	Female	22	20/20	20/20	-2.25-0.25x060	-2.25-0.50x147	-2.25	-2.50	0.25	24.12	24.12	7,7	5,5
N13	Male	29	20/20	20/20	+0.25sph	+0.75-0.25x146	+0.25	+0.75	0.50	22.71	22.79	6,6	4,4

Table 3: Summary of control participant characteristics. 1OD=right eye, OS=left eye throughout all figures and tables. 2All participants in this study had equal, round, normally reactive pupils without relative afferent pupillary defect

Subject ID	Gender	Age (Yrs)	Best Corrected ETDRS Snellen Acuity		Auto-Refraction (Diopters)		Spherical Equivalent Refraction (Diopters)		Anisometropia [SE, (Diopters)]/ Difference in Cylinder (Diopters)	Axial Length (mm)		Pupils	
			OD	OS	OD	OS	OD	OS		OD	OS	Dark (OD,OS)	Light (OD,OS)
A1	Male	23	20/32	20/16	+3.50	+2.25	+3.50	+2.25	1.25/0.00	22.72	23.01	8,8	6,6
A2	Female	23	20/20	20/50	+3.75-0.50x105	+5.00-0.25x077	+3.50	+5.00	1.50/0.25	22.43	21.87	6,6	4,4
A3	Male	30	20/20	20/40	+0.75-1.50x084	+3.00-5.25x160	plano	+0.50	0.50/3.75	23.20	22.86	7,7	5,5
A4	Female	24	20/32	20/16	-2.75-0.50x092	-1.25-1.50x110	-3.00	-2.00	1.00/1.00	25.48	24.79	7,7	5,5
A5	Male	20	20/16	20/40	+0.25-0.75x002	+3.00-1.25x025	plano	+2.50	2.50/0.50	25.12	24.10	6,6	4,4
A6	Female	21	20/16	20/32	+3.75-1.50x180	+6.50-1.50x006	+3.00	+5.75	2.75/0.00	22.84	21.75	7,7	5,5
A7	Male	23	20/40	20/20	+0.50-0.25x062	+1.50sph	+0.50	+1.50	1.00/0.00	23.00	22.83	7,7	6,6

Table 4: Summary of amblyopic participant characteristics.

2.2- Balance Point

Band-pass filtered Sloan letters were used for measuring the contrast balance point. The spatial frequency of the test letters were 1 and 4 cycles/degree at a viewing distance of 65 centimeters. Test letters were displayed on a uniform gray background (60 cd/m^2) with varying Michelson contrasts. The stimuli were generated and controlled using MATLAB with Psychophysics Toolbox extensions for Windows. Stimuli were presented on a 120 Hz luminance-calibrated LCD monitor (BenQ XL2720). Participants wore stereo-shutter glasses running at 60 Hz (nVidia Corp., Santa Clara, CA).

The balance point stimuli, equipment and procedure of this study were adapted from methods used by Kwon and colleagues (Kwon et al.[12]). A different letter chart was presented to the two eyes of a subject via the stereo-shutter glasses (Figure 1). At each position, the identity and interocular contrast ratio of the letter on each chart differed while the spatial frequency of the letter remained the same. The interocular contrast ratio was randomized across 5 letter slots in each row to avoid any ascending or descending pattern of contrast arrangement, which might otherwise bias participants' responses. Participants viewed the chart and were instructed to read the chart-letters aloud, from left-to-right, and from top line to bottom line, as quickly and accurately as possible. If participants experienced binocular rivalry, which may occur when the interocular contrast ratio reaches a subject's contrast balance point, they were instructed to report the more dominant percept (whichever came first or whichever appeared to be the stronger percept).

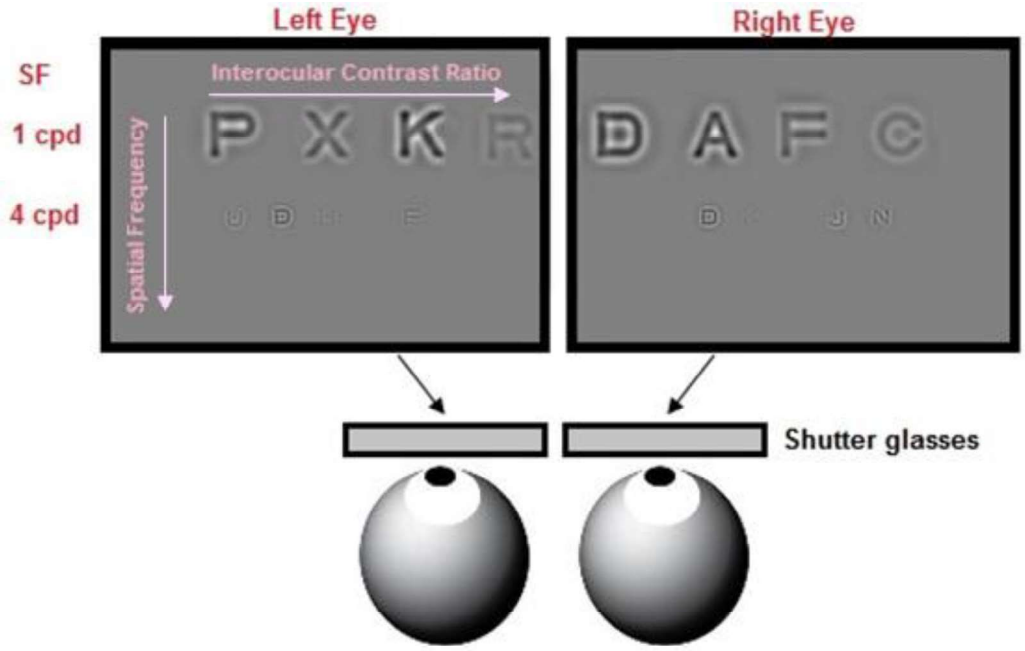


Figure 1: Binocular balance experiment - different letter charts being separately presented to the two eyes via shutter glasses. Schematic adapted from Kwon et al.[12]

The relative contrast of the letter in each eye was determined via an adaptive staircase procedure. The balance point was defined as the interocular contrast ratio yielding letter recognition in each eye with equal probability (50%). The experiment outputted 2 balance point contrast ratios, one ratio for each spatial frequency tested, 1 and 4 cycles per degree (c/deg), as shown in Figure 2. The output was defaulted as the left eye in all cases. In the example of Figure 2, the left eye balance point contrast was 50% at 1c/deg and 51% at 4c/deg, meaning the right eye contrast was 50% and 49% respectively, giving a balance point contrast ratio of 0.50/0.50 at 1c/deg, and 0.51/0.49 at 4c/deg. Each contrast ratio calculated here was then used to define the balance point ratio used in the electrophysiological recordings of that spatial frequency.

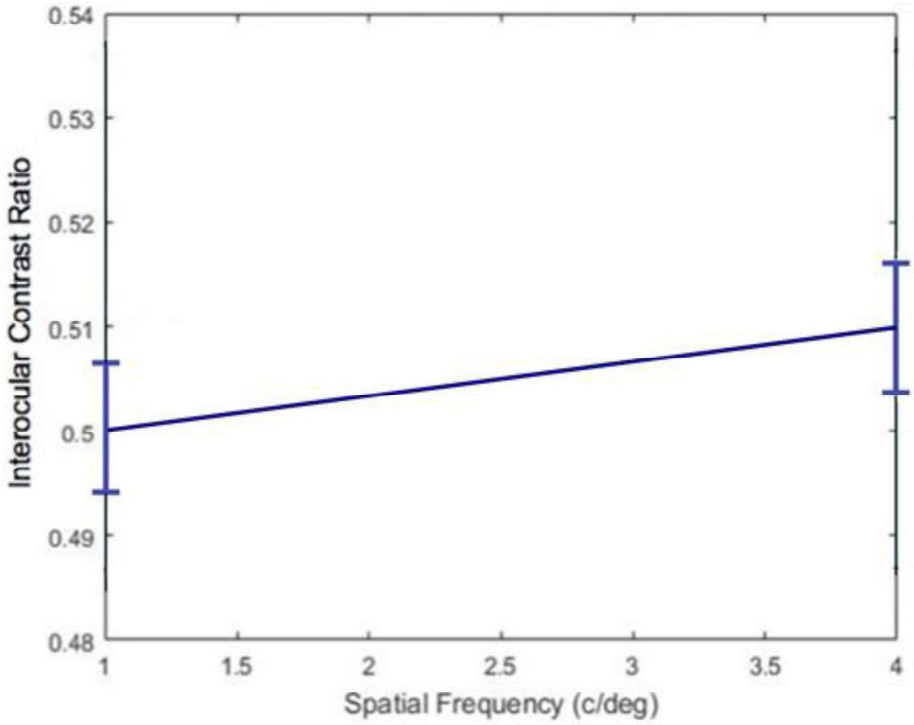


Figure 2: Balance point experiment MATLAB output of interocular contrast ratio needed for the subject to report letter shown to either eye with equal probability, at 1 and 4 c/deg.

2.3- Electrophysiological Recordings

The electrophysiological experiments were recorded using an FDA-approved clinical system (Espion, Diagnosys LLC, Lowell, MA). This recording system was connected to a computer running Visual Psychophysics Engine (VPE) software (Cambridge Research Systems, UK) controlling a customized stimulus on a 12-inch by 16-inch CRT monitor (SONY Trinitron) with a 120Hz refresh rate. During electrophysiological testing, pattern ERGs and pattern VEPs were recorded simultaneously using a five-channel electrophysiological amplifier, and a dichoptic viewing system with a 2-mirror Wheatstone stereoscope. Two stimuli were presented on a calibrated CRT screen so as to be viewed dichoptically (i.e. right eye views the right stimulus and left eye views the left stimulus). The stimuli consisted of a ~5x15 degree checkerboard pattern that reversed in time (4 Hz or 10 Hz) across a range of spatial frequencies (1 and 4 cycles per degree) at a viewing distance of 107 centimeters with average luminance of 60cd/m^2. A check size of 0.5 degrees was used for the 1c/deg stimulus and a check size of

0.125 degrees was used for the 4c/deg stimulus. It should be noted, however, that a checkerboard is a complex stimulus containing a wide range of spatial frequencies. A fixation mark (red cross) was present in the center of each stimulus. The contrast ratio of the two stimuli ranged between 100%:0% (right:left contrast) and up to 0%:100% in steps of 25% contrast (i.e. 100%:0%, 75%:25%, etc) including the exact contrast balance point of the subject (i.e. 6 total contrast levels). This contrast range was repeated for each spatial frequency and each temporal frequency, through protocols 1-4, as outlined in Table 5 below. The order of the presentation was randomized from subject to subject using an online list randomizer (RANDOM.ORG).

Protocol	Step	Channel 1 ERG OD % Contrast	Channel 2 ERG OS % Contrast	Channel 3 VEP % Contrast OD/OS
1 c/deg OU 10 Hz OD 4 Hz OS	1	100	100	100/100
	2	100	0	100/0
	3	75	25	75/25
	4	Subjective Balance Point (BP)	Subjective Balance Point (BP)	BP/BP
	5	25	75	25/75
	6	0	100	0/100
1 c/deg OU 4 Hz OD 10 Hz OS	1	100	100	100/100
	2	100	0	100/0
	3	75	25	75/25
	4	Subjective Balance Point (BP)	Subjective Balance Point (BP)	BP/BP
	5	25	75	25/75
	6	0	100	0/100
4 c/deg OU 10 Hz OD 4 Hz OS	1	100	100	100/100
	2	100	0	100/0
	3	75	25	75/25
	4	Subjective Balance Point (BP)	Subjective Balance Point (BP)	BP/BP
	5	25	75	25/75
	6	0	100	0/100
4 c/deg OU 4 Hz OD 10 Hz OS	1	100	100	100/100
	2	100	0	100/0
	3	75	25	75/25
	4	Subjective Balance Point (BP)	Subjective Balance Point (BP)	BP/BP
	5	25	75	25/75
	6	0	100	0/100

Table 5: Recording protocols for electrophysiological experiments

The ERGs and VEPs were recorded under dim room illumination. Impedance was always kept below 5 KΩ. Each resulting waveform was an average of 250 sweeps recorded over 2 minutes. Participants were instructed to fixate the marking in the center of the screen. They were allowed to blink as needed however were advised to minimize blinking. Participants were given breaks between recordings. The ERGs were recorded from each eye using DTL active electrodes and 9mm gold-cup reference and ground electrodes. Microconductive DTL thread electrodes (Diagnosys, LLC, USA) were draped on the conjunctiva at the inferior limbus. A gold-cup skin electrode (Diagnosys LLC, USA) was placed in the center of each subject's forehead. A reference gold-cup skin electrode was clipped onto each earlobe (Diagnosys LLC, USA). The VEPs were recorded using standard 9mm gold-cup electrodes (Diagnosys LLC, USA) placed on the scalp according to the ISCEV 10/20 system, as illustrated in Figure 3. Nuprep Skin Prep Gel (Weaver and Company, USA) was used to prepare the areas of skin coming into contact with a gold-cup electrode. Ten20 Conductive Paste (Weaver and Company, USA) was used for gold-cup conductivity and adhesiveness. A headband was additionally used to secure the VEP electrode setup. ERG and VEP electrode placement was done in accordance with ISCEV (International Society for Clinical Electrophysiology of Vision) guidelines[49,60]. The subject's head was stabilized with a customized, sterile bite bar made with standard dental impression material (Kerr Extrude XP silicone impression technique). The bite bar was positioned for proper alignment through the stereoscope which was attached above. This setup (Figure 4) ensured continuous alignment between the eyes and the stimulus. Repeated checks were made to assure good comfort and that participants were achieving clear and single vision through the dichoptic setup.

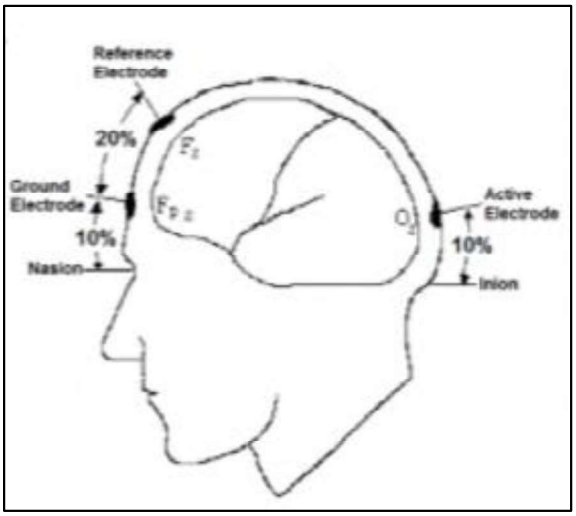


Figure 3: Placement of active, reference and ground VEP electrodes on the head in accordance with ISCEV 10/20 standards[60].

The VEPs had frequency following responses meaning their signals consisted of a 4 Hz component that corresponded to the 4 Hz stimulus of one eye, and a 10 Hz component that corresponded to the 10 Hz stimulus of the other eye. The VEPs were recorded binocularly however frequency tagging made it possible to analyze each eye independently. For example, with 4 Hz presented to the left eye and 10 Hz presented to the right eye, the left function was tagged by the 4 Hz component of the signal, and the right was represented by its 10 Hz component. The recorded binocular beat VEP signal, an index of binocularity, had a temporal frequency equaling the difference of the temporal frequencies used. For example, with 4 Hz and 10 Hz frequencies used (one on each eye), the recorded signal had a frequency equal to the difference which in this case is 6 Hz.

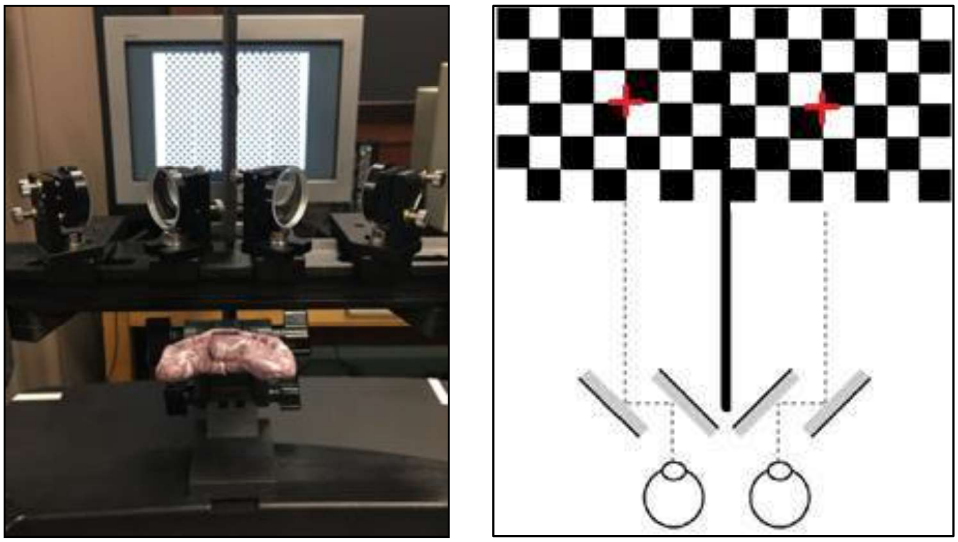


Figure 4: Photo of electrophysiological setup with bite bar and 2-mirror Wheatstone stereoscope for dichoptic stimulation (left). Overhead illustration of electrophysiological setup without bite bar (right, not drawn in scale)

Figure 5 below illustrates dichoptic ERG responses from the right and left eyes, using the same spatial frequency checkerboard (1 c/deg) in both eyes, with the right stimulus reversing at 10 Hz, and the left stimulus reversing at 4 Hz.

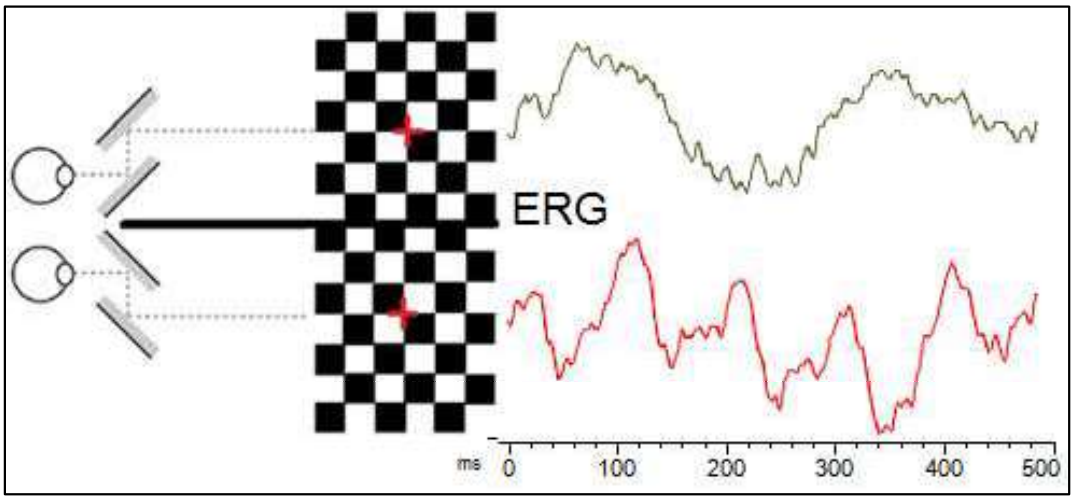


Figure 5: Dichoptic ERG responses. The subject viewed a checkerboard of the same spatial frequency in both eyes, which reversed at 10Hz in the right eye (red) and 4Hz in the left eye (green).

Figure 6 below illustrates a dichoptic VEP recording, using the same spatial frequency checkerboard (1 c/deg) in both eyes, with the right stimulus reversing at 10 Hz, and the left stimulus reversing at 4 Hz. The waveform on top is the binocularly evoked VEP signal, which is fast fourier transformed on bottom showing the separate eye-tagging frequencies (10Hz from the right eye, 4Hz from the left eye, 6Hz binocular beat frequency).

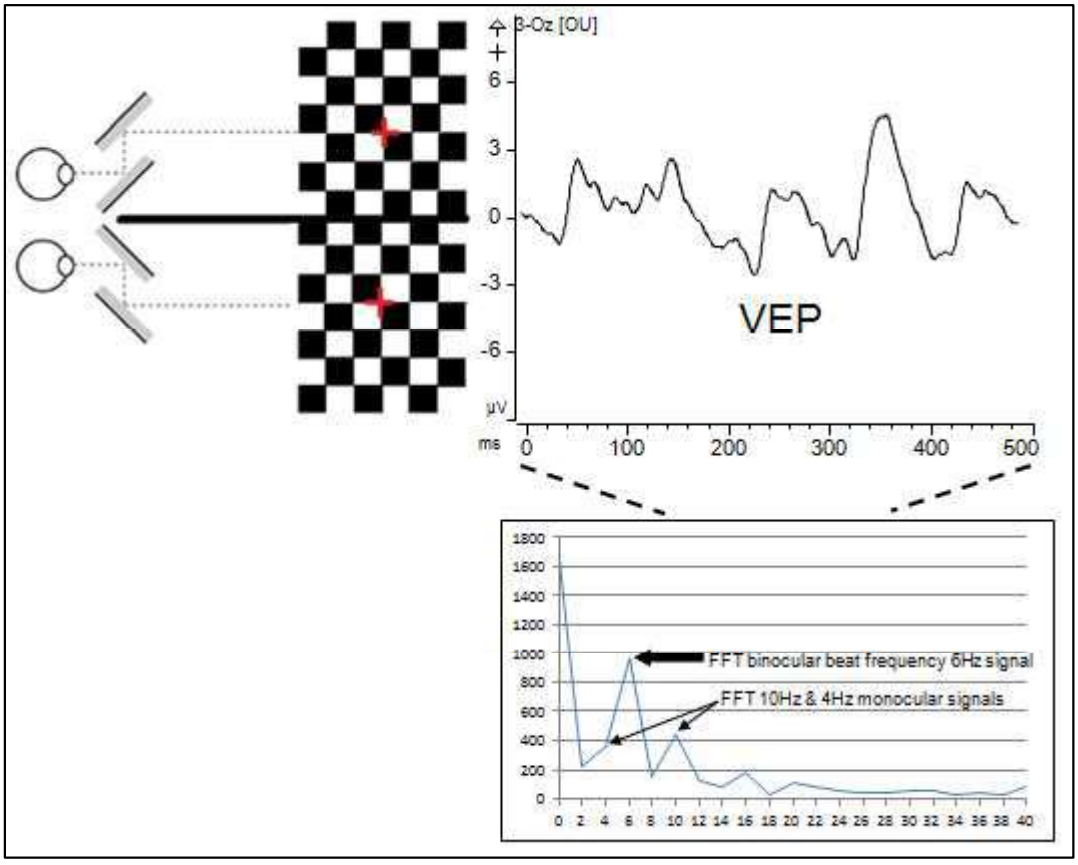


Figure 6: Dichoptic VEP frequency tagged responses. The subject viewed a checkerboard of the same spatial frequency in both eyes, which reversed at 10Hz in the right eye and 4Hz in the left eye. The binocularly evoked waveform is shown in the top plot, while the frequency tagged responses are illustrated in the FFT below (10Hz, 4Hz, 6Hz).

2.4- Data Processing

Raw ERG and VEP waveforms were exported from the Espion system and processed by MatLab using a Fast Fourier Transform (FFT) script to calculate the power spectrum. Sample MatLab code is included in the appendix (Figure A1).

The phase was calculated by transforming the radians acquired by the FFT, into x, y cartesian vectors by multiplying the amplitude by the sine (y) and cosine (x) of the radians. The x and y vectors were then averaged and transformed into degrees.

2.5- Statistics

Multiple-way Analysis of variance (ANOVA) and appropriate statistical corrections (i.e. Bonferroni for multiple corrections) were applied to investigate differences between different stimulating conditions (i.e. spatial and temporal frequency and contrast ratio). Retinal response dynamics of the amblyopic eye were compared with those of the fellow eye of the amblyopes for each spatial and temporal frequency and for the whole range of luminance contrast. The retinal responses of the fellow eyes were also compared with the retinal responses of the non-amblyopic controls. The monocular cortical responses of the amblyopic eye were compared with the monocular cortical responses of the fellow eye. The monocular cortical responses of the fellow eye were also compared to the normal cortical responses of the control eyes. Since the experiments were also recorded binocularly, the amblyopes' binocular beat cortical responses were compared with the normals' responses for each spatial and temporal frequency and for the whole range of luminance contrast.

3- Results

3.1- Demographics

Twenty participants – 7 amblyopes and 13 normals - were enrolled in this study. All participants met their respective inclusion/exclusion criteria.

The amblyopes included 3 females and 4 males. The mean age of the amblyopes was 23.4 ± 3.2 (1 SD) years (range 20-30 years). The mean ETDRS Snellen equivalent best-corrected visual acuity of the amblyopic eyes was approximately 20/40 [mean logMAR 0.27 ± 0.07 (1 SD)]. The mean amblyopic spherical equivalent refractive error was +2.11 ± 3.03 (1 SD) diopters (range -3.00 to +5.75 D). The amblyopes had a mean spherical equivalent anisometropia of 1.50 ± 0.77 (1 SD) diopters (range 0.50 to 2.75 D), and mean difference in cylinder of 0.79 ± 1.36 (1 SD) diopters (range 0.00 to 3.75 D). The mean axial length of the amblyopic eyes was 23.11 ± 1.30 (1 SD) millimeters (range 21.75 to 25.48 mm). The amblyopic mean pupil size under dark illumination measured 6.86 ± 0.69 (1 SD) millimeters, while their mean pupil size in light illumination measured 5.00 ± 0.82 (1 SD) millimeters.

The mean ETDRS Snellen equivalent best-corrected visual acuity of the fellow eyes was approximately 20/20 [mean logMAR -0.06 ± 0.05 (1 SD)]. Their mean spherical equivalent refractive error was +1.65 ± 2.18 (1 SD) diopters (range -2.00 to +3.50 D). The mean axial length of fellow eyes was 23.46 ± 1.05 (1 SD) millimeters (range 22.43 to 25.12 mm). The fellow eyes' mean normal pupil size under dark illumination measured 6.86 ± 0.69 (1 SD) millimeters, and their mean normal pupil size in light illumination measured 5.00 ± 0.82 (1 SD) millimeters.

The normal control group consisted of 26 eyes of 5 females and 8 males with mean age 25.2 ± 2.4 (1 SD) years (range 22-30 years). The mean ETDRS Snellen equivalent best-corrected visual acuity of the normal eyes was 20/20 [mean logMAR 0.00 ± 0.00 (1 SD)]. The mean spherical equivalent refractive error of all normal eyes was -1.72 ± 2.18 (1 SD) diopters (range -5.75 to +0.75 D). The mean spherical equivalent refractive error of the normal right eyes was -1.63 ± 2.24 (1 SD) diopters (range -5.75 to +0.50 D). The mean spherical equivalent

refractive error of the normal left eyes was -1.81 ± 2.21 (1 SD) diopters (range -5.50 to +0.75 D). The mean spherical equivalent anisometropia was 0.40 ± 0.43 (1 SD) diopters (range 0.00 to 1.75 D). The mean axial length of all normal eyes was 24.28 ± 0.84 (1 SD) millimeters (range 22.71 to 26.03 mm). The mean axial length of the normal right eyes was 24.25 ± 0.90 (1 SD) millimeters (range 22.71 to 26.03 mm). The mean axial length of the normal left eyes was 24.31 ± 0.82 (1 SD) millimeters (range 22.79 to 25.65 mm). The mean normal pupil size under dark illumination measured 6.58 ± 0.50 (1 SD) millimeters (OD 6.62 ± 0.51, OS 6.54 ± 0.52). The mean normal pupil size in light illumination measured 5.19 ± 0.80 (1 SD) millimeters (OD 5.23 ± 0.83, OS 5.15 ± 0.80). In terms of normal right eyes compared to normal left eyes, there were no statistically significant differences in visual acuity, spherical equivalent refractive error, axial length, or pupil size under dark or light illumination.

There was no statistically significant difference in age between amblyopic and normal subjects ($p>0.05$). There were no significant differences in axial length ($p>0.05$) or pupil size under dark or light illumination ($p>0.05$) between amblyopic, fellow and normal eyes.

There were statistically significant differences in logMAR visual acuity between amblyopic and normal eyes ($p = 0.000$), and amblyopic and fellow eyes ($p = 0.000$), but no significant difference was found between normal and fellow eyes ($p>0.05$). There was a significant difference in spherical equivalent anisometropia between amblyopic and normal subjects ($p = 0.013$).

3.2- Binocular Balance Point

The binocular balance point (BP) was obtained from each subject using the experiment adapted from Kwon et al.[12] Figure 7 shows the mean balance points as a function of spatial frequency (SF) for the amblyopic and normal control subjects. At 1c/deg, the BP for the amblyopic group was 76.43 ± 2.13 (mean ± SE), and 50.46 ± 1.03 (mean ± SE) for the normal group. At 4c/deg, the BP for the amblyopic group was 81.43 ± 2.96 (mean ± SE), and 47.23 ± 1.34 (mean ± SE) in the normals. Across both spatial frequencies, the balance points for the amblyopes were higher than in the normals. The BP of the amblyopic group changed with SF, while it remained constant in the normals across the two different spatial frequencies.

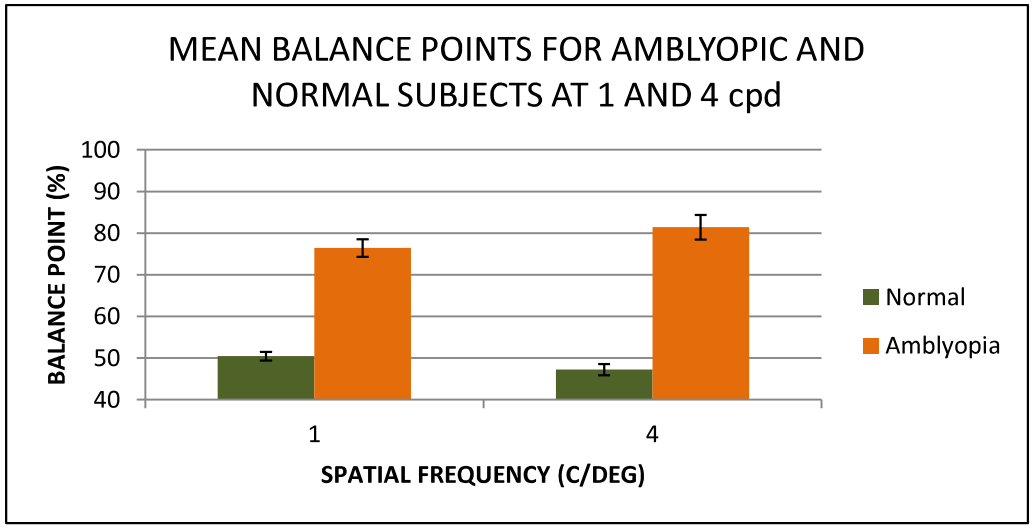


Figure 7: Mean balance points for amblyopic and normal control groups at two spatial frequencies. Error bars represent ± 1 Standard Error of the Mean.

3.3- Electrophysiological Recordings

Dichoptic steady-state PERG and PVEP data were successfully recorded from all subjects. Figures 8, 10 and 12 show examples of raw data exported directly from the Espion system with the amplitudes in microvolts on the y-axis, and timing in milliseconds along the x-axis. Below each raw data sample, figures 9, 11 and 13 show the fast fourier transform (FFT) analysis of the temporal signals inherent in the recordings. The FFT analysis was done in 2 Hz intervals as depicted on the x-axis in the figures, while the y-axis shows amplitude in nanovolts.

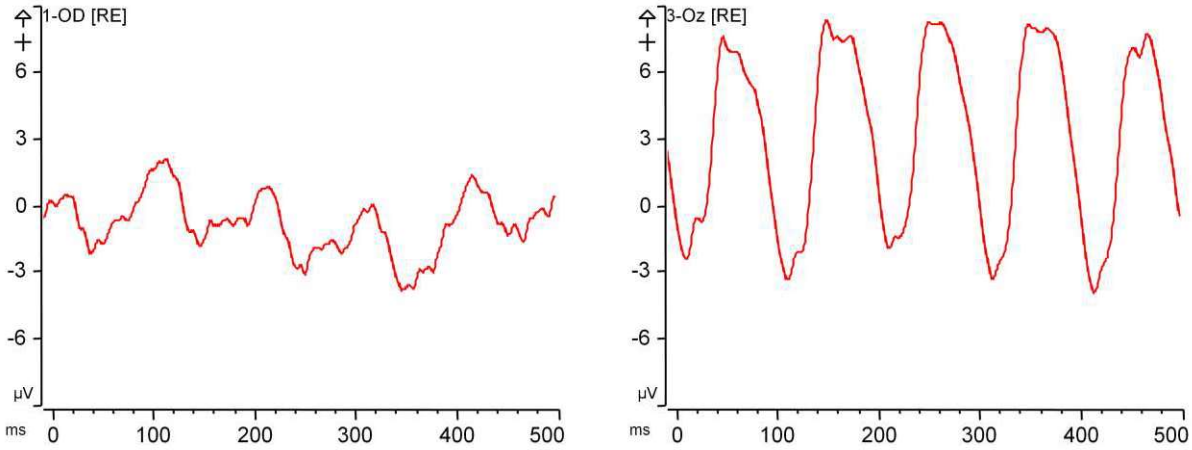


Figure 8: Simultaneous PERG (left), PVEP (right) recorded from the right eye of a normal subject, using a 10Hz reversing checkerboard, 1 c/deg, 100% contrast. X-axis: latency in milliseconds. Y-axis: amplitude in microvolts.

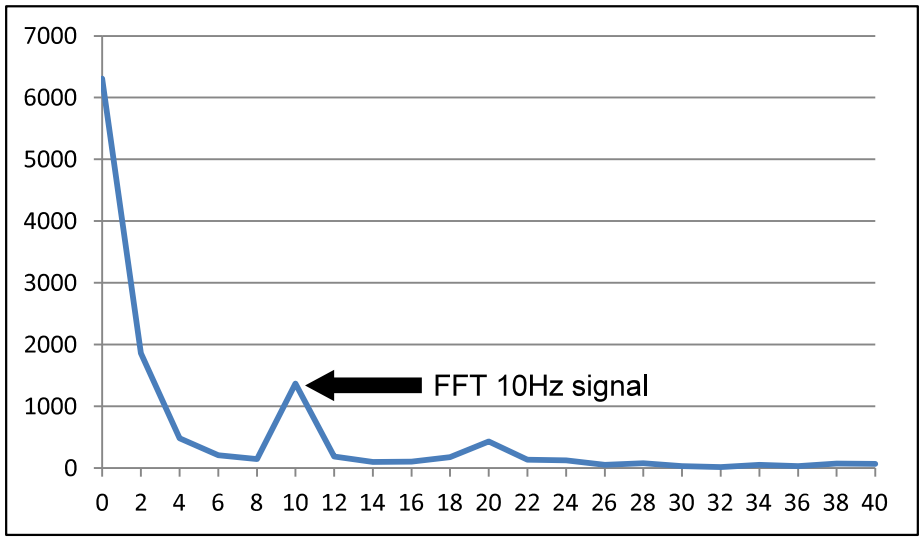


Figure 9: Fast fourier transform (FFT) analysis of PERG in figure 8, showing 10Hz signal. X-axis shows temporal frequency of the signal, y-axis shows amplitude of the signal in nanovolts.

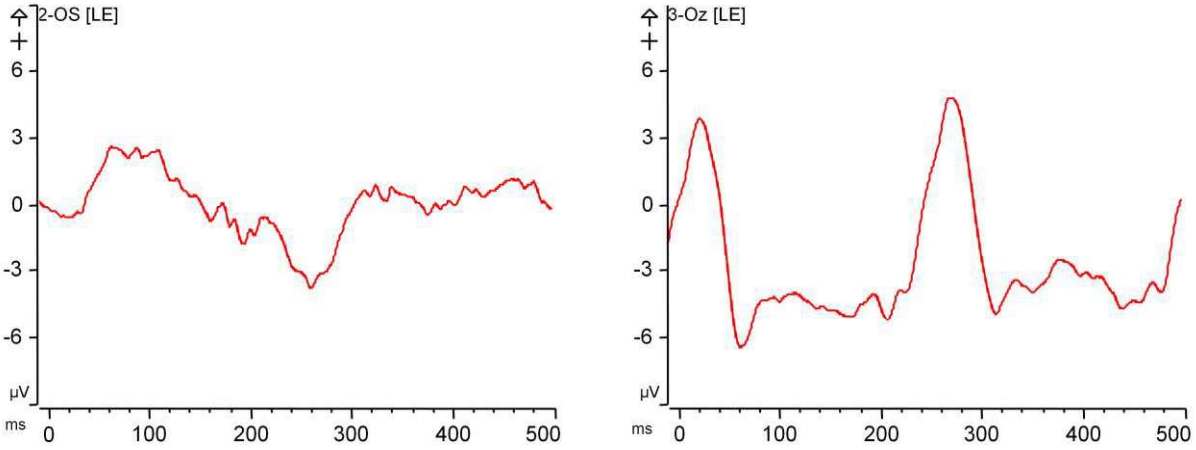


Figure 10: Simultaneous PERG (left), PVEP (right) recorded from the left eye of a normal subject, using a 4Hz reversing checkerboard, 1 c/deg, 100% contrast. X-axis: latency in milliseconds. Y-axis: amplitude in microvolts.

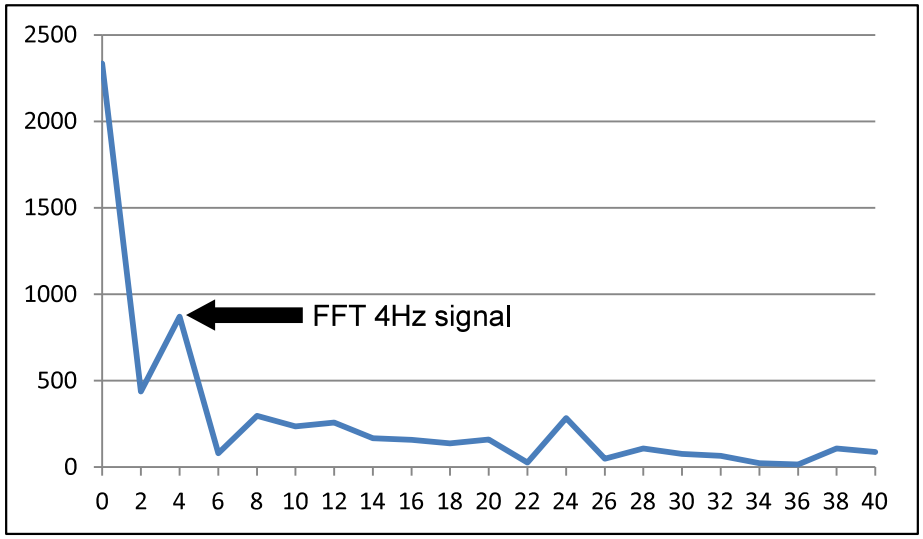


Figure 11: Fast fourier transform (FFT) analysis of PERG in figure 10, showing 4Hz signal. X-axis shows temporal frequency of the signal, y-axis shows amplitude of the signal in nanovolts.

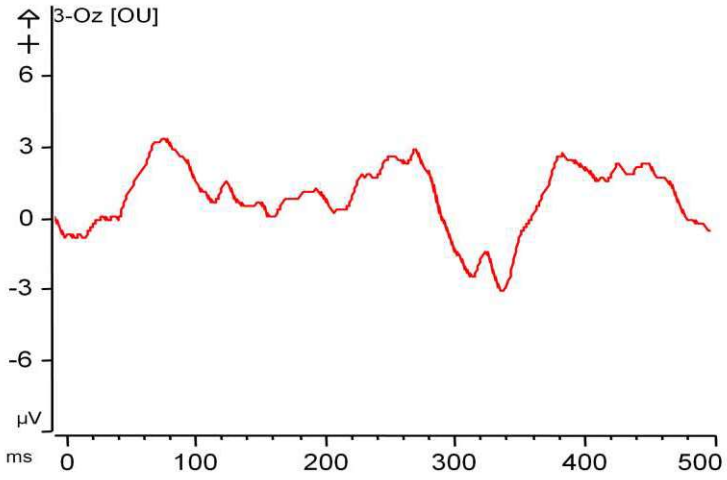


Figure 12: Binocular beat PVEP recorded from a normal subject, using a 10 Hz OD, 4 Hz OS (interocular difference of 6 Hz) reversing checkerboard, 1 c/deg at 100% contrast in both eyes. X-axis: latency in milliseconds. Y-axis: amplitude in microvolts.

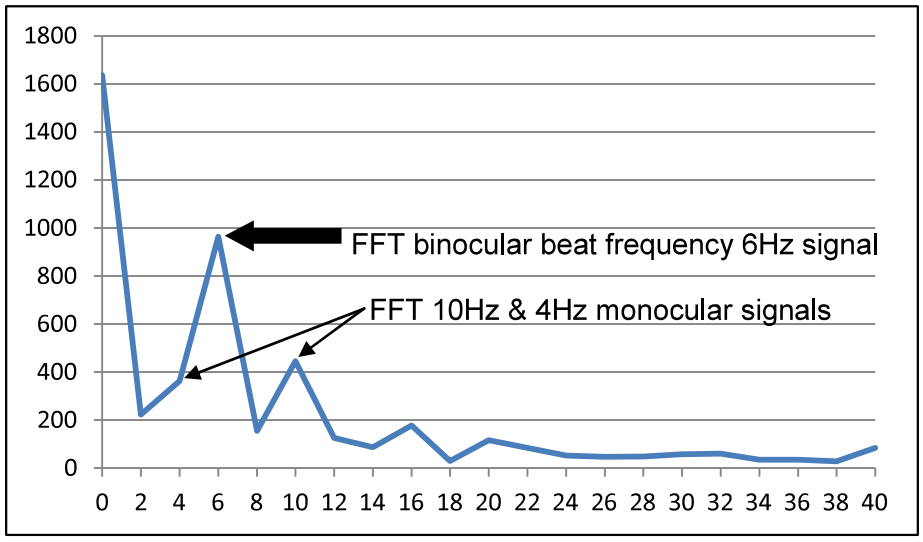


Figure 13: Fast fourier transform (FFT) analysis of binocular VEP in figure 12, showing a 6Hz beat signal equal to the difference between the dichoptic temporal frequencies (10Hz and 4z) which are also present in the power spectrum. X-axis shows temporal frequency of the signal, y-axis shows amplitude of the signal in nanovolts.

3.3.1- Electroretinogram Responses

Figures 14 through 25 illustrate the mean SSPERG amplitude and phase results as a function of contrast for each spatial/temporal condition.

3.3.1.2- Dichoptic ERG Normative Data

In figure 14 below, the dichoptic ERG amplitude in normals, at 1 cpd (cycles per degree) in both eyes, progressively increases in an approximate linear fashion between 25% and 100% contrast at 4Hz ($t = 2.998$, $p = 0.005$) and at 10Hz ($t = 5.431$, $p = 0.000$). There was no statistically significant amplitude difference between 4Hz and 10Hz stimulation at any contrast level ($p>0.05$).

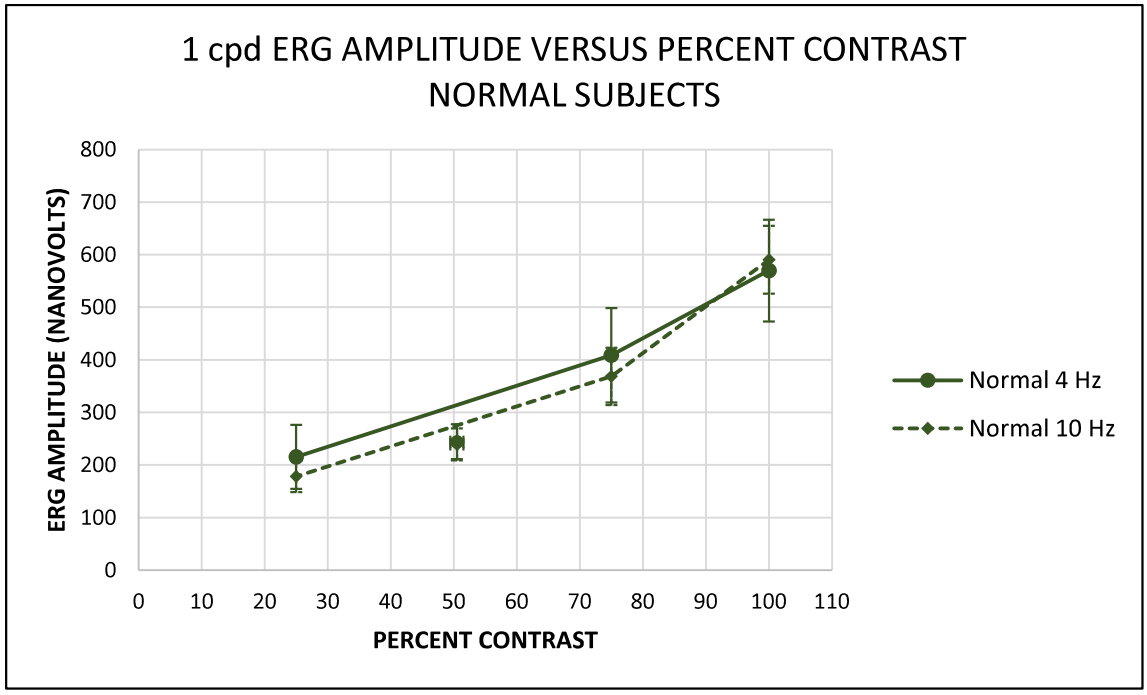


Figure 14: PERG mean amplitude (± 1 SE) as a function of contrast for two different values of temporal frequency recorded dichoptically, at 1 cpd both eyes, in normals

In figure 15, the dichoptic ERG phase in normals, at 1cpd in both eyes, progressively increases with increasing stimulus contrast at 10Hz ($t = 2.830$, $p = 0.007$), but there was no significant correlation between phase and contrast at 4Hz ($t = -0.678$, $p = 0.502$). There was a significant difference in phase between 4Hz and 10Hz at 100% ($p = 0.002$), 75% ($p = 0.000$) and subjective balance point ($p = 0.045$) contrast levels, but no significant difference at 25% ($p>0.05$).

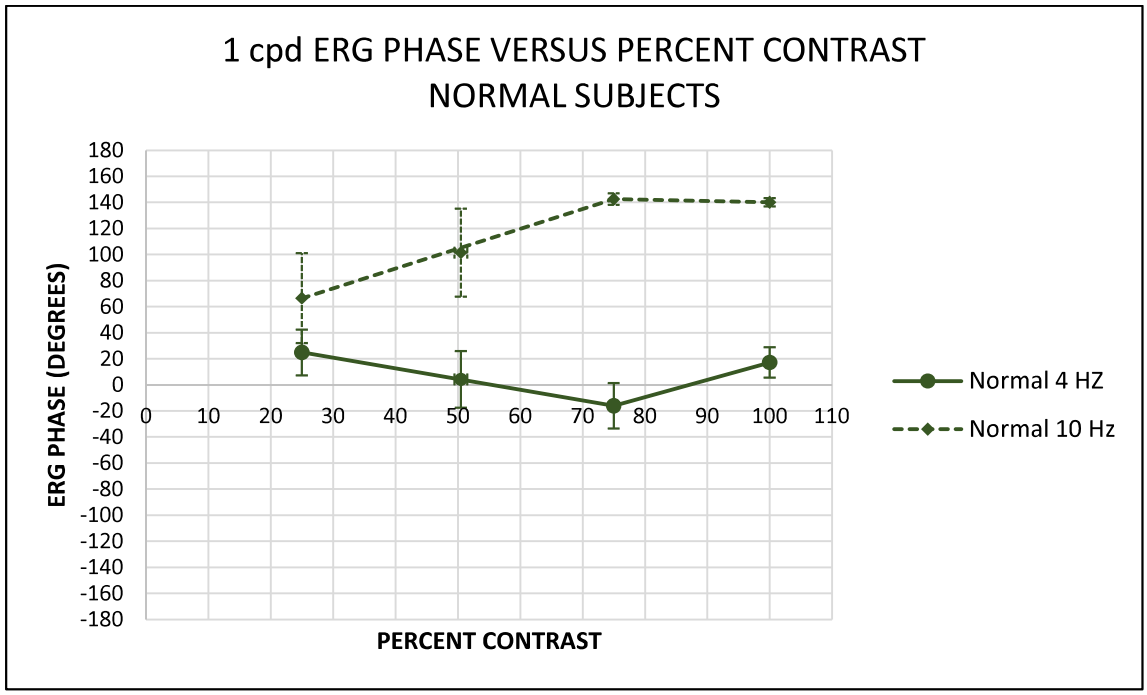


Figure 15: PERG mean phase (± 1 SE) as a function of contrast for two different values of temporal frequency recorded dichoptically, at 1 c/deg both eyes, in normals

In figure 16, the dichoptic ERG amplitude in normals, at 4 cpd in both eyes, increases with increasing contrast at 10Hz (t = 4.459, p = 0.000), but there was no correlation between amplitude and contrast at 4Hz (t = 1.397, p = 0.171). There was no statistically significant amplitude difference between 4Hz and 10Hz stimulation at any contrast level (p>0.05).

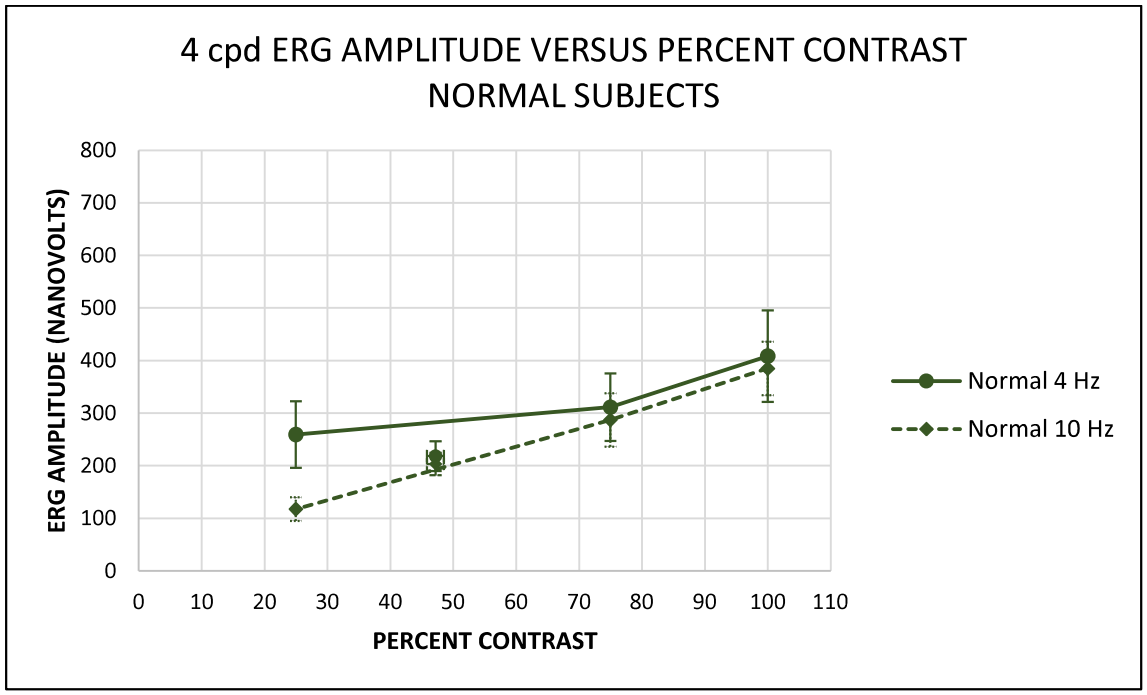


Figure 16: PERG mean amplitude (± 1 SE) as a function of contrast for two different values of temporal frequency recorded dichoptically, at 4 cpd both eyes, in normals

In figure 17, the dichoptic ERG phase in normals, at 4cpd in both eyes, progressively increases with increasing stimulus contrast at 4Hz ($t = 3.003$, $p = 0.005$) and 10Hz ($t = 2.337$, $p = 0.025$). There was no statistically significant difference in phase between 10Hz versus 4Hz at any contrast level ($p>0.05$).

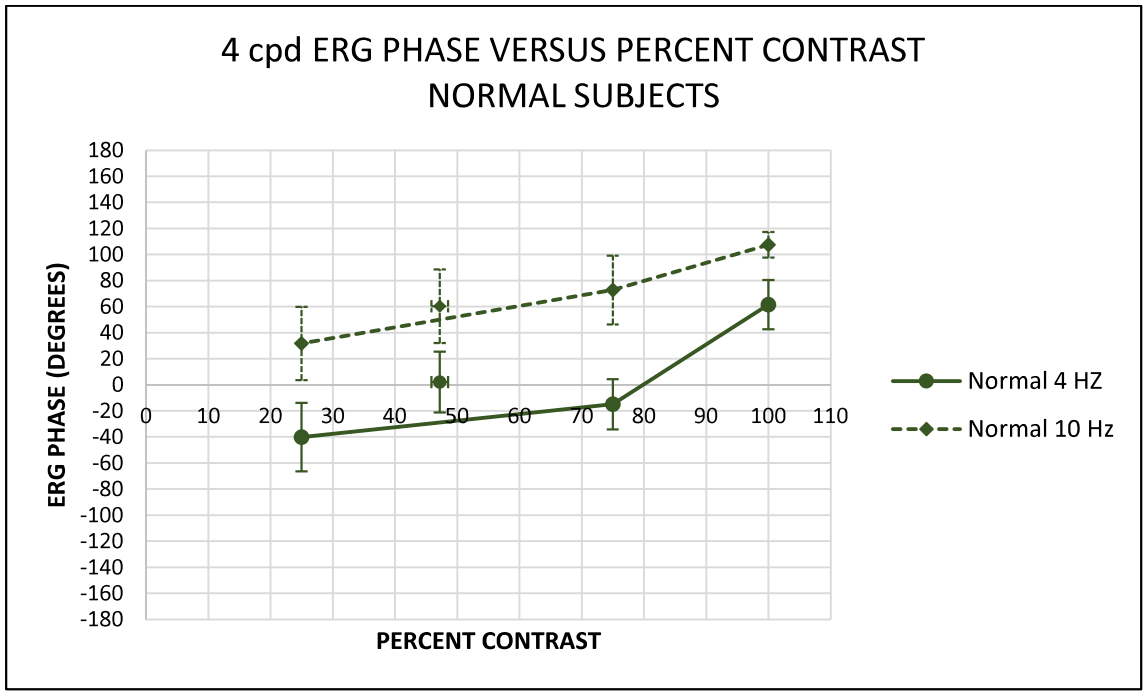


Figure 17: PERG mean phase (± 1 SE) as a function of contrast for two different values of temporal frequency recorded dichoptically, at 4 c/deg both eyes, in normals

When analyzing normal eyes stimulated at 4Hz, there was no significant difference in ERG amplitude nor in ERG phase between 1 c/deg and 4 c/deg at any contrast level ($p>0.05$). At 10Hz, there was a significant difference in amplitude between 1 c/deg and 4 c/deg at 100% contrast ($p = 0.032$), but no other effects of spatial frequency on normal ERG amplitude at any other contrast level ($p>0.05$). There was no spatial frequency dependence of ERG phase in normals at 10Hz at any contrast level ($p>0.05$).

3.3.1.3- ERG Analysis Between Groups

Figures 18 through 25 show the mean SSPERG amplitude and phase results as a function of contrast for each spatial/temporal condition, in normal versus amblyopic versus fellow eyes. The mean data at balance point contrast are shown as single data points throughout the figures.

Regression analysis was performed to investigate any significant correlation between contrast and amplitude or phase for each spatial/temporal condition in normal, amblyopic and fellow eyes. ANOVA and Bonferroni post-hoc analysis was used to investigate differences between normal versus amblyopic versus fellow eyes for each combination of spatial and temporal frequency across the range of luminance contrast.

In figure 18 below, showing all groups stimulated at 1cpd, 4Hz, there were no statistically significant differences in ERG amplitude between any groups at any contrast level ($p>0.05$). ERG amplitude significantly increases with increasing contrast in normal eyes ($t = 2.998$, $p = 0.005$), but there was no significant correlation between amplitude and contrast in amblyopic eyes ($t = 0.710$, $p = 0.486$) or fellow eyes ($t = 1.689$, $p = 0.108$).

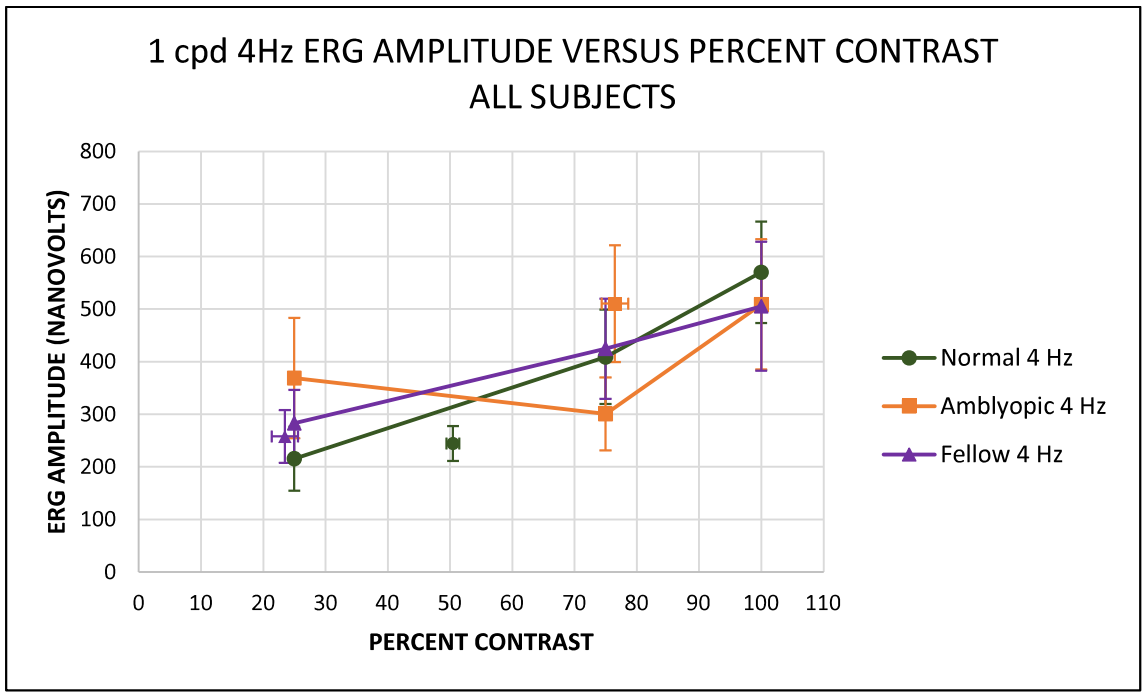


Figure 18: PERG mean amplitude (± 1 SE) as a function of contrast, at 1c/deg spatial frequency, 4Hz temporal frequency, in normal control vs amblyopic vs fellow eyes

In figure 19 below, showing all groups stimulated at 1cpd, 4Hz, there were no statistically significant differences in ERG phase between any groups at any contrast level ($p>0.05$). There was no significant correlation between ERG phase and contrast in normal eyes ($t = -0.678$, $p = 0.502$), amblyopic eyes ($t = -0.907$, $p = 0.376$), or fellow eyes ($t = 1.704$, $p = 0.105$).

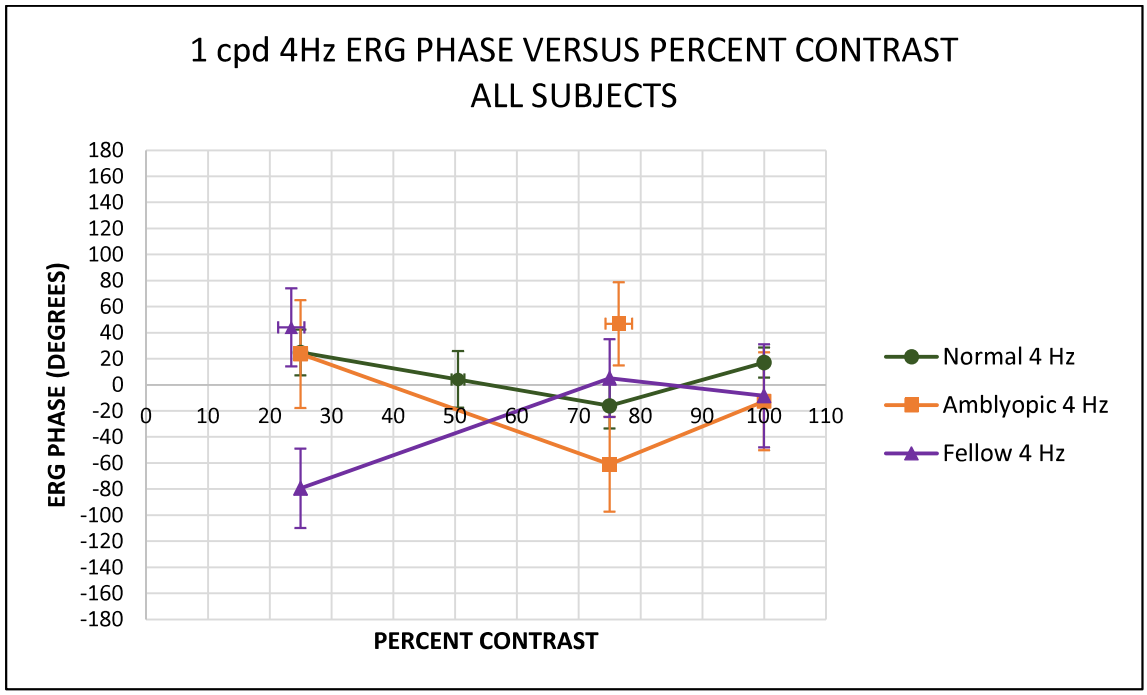


Figure 19: PERG mean phase (± 1 SE) as a function of contrast, at 1c/deg spatial frequency, 4Hz temporal frequency, in normal control vs amblyopic vs fellow eyes

In figure 20 below, showing all groups stimulated at 1cpd, 10Hz, there were no statistically significant differences in ERG amplitude between any groups at 25%, 75% or subjective balance point contrast ($p>0.05$). At 100% contrast, there was a statistically significant difference in amplitude between normal and fellow eyes ($p = 0.012$), but no significant difference between normal and amblyopic eyes, or amblyopic and fellow eyes ($p>0.05$). ERG amplitude significantly increases with increasing contrast in normal eyes ($t = 5.431$, $p = 0.000$) and amblyopic eyes ($t = 3.774$, $p = 0.001$), but there was no significant correlation in fellow eyes ($t = 1.851$, $p = 0.080$).

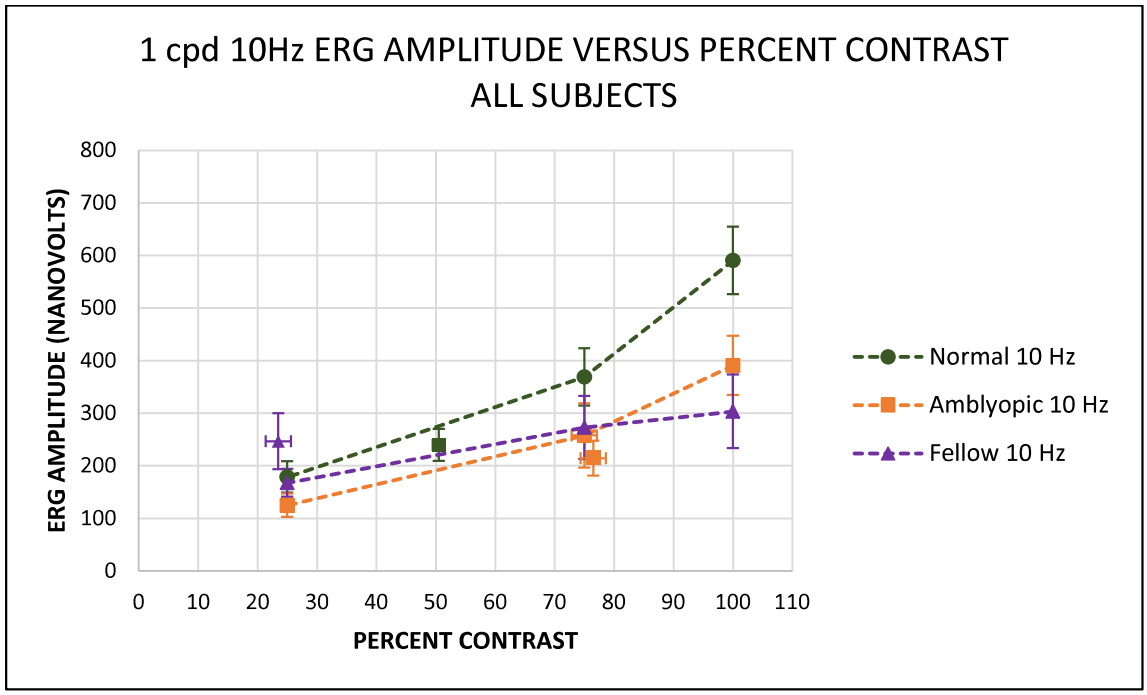


Figure 20: PERG mean amplitude (± 1 SE) as a function of contrast, at 1c/deg spatial frequency, 10Hz temporal frequency, in normal control vs amblyopic vs fellow eyes

In figure 21 below, showing all groups stimulated at 1cpd, 10Hz, there were no statistically significant differences in ERG phase between any groups at any contrast level ($p>0.05$). ERG phase significantly increases with increasing contrast in normal eyes ($t = 2.830$, $p = 0.007$) and amblyopic eyes ($t = 2.155$, $p = 0.044$), but there was no correlation observed in fellow eyes ($t = 1.204$, $p = 0.243$).

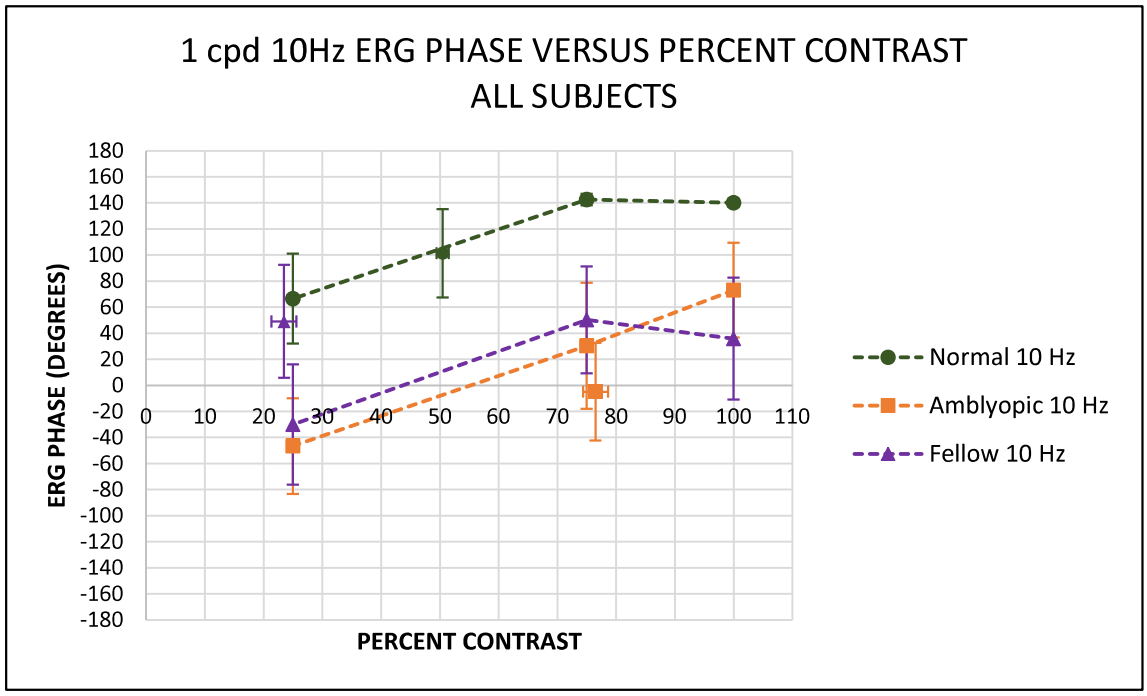


Figure 21: PERG mean phase (± 1 SE) as a function of contrast, at 1c/deg spatial frequency, 10Hz temporal frequency, in normal control vs amblyopic vs fellow eyes

In figure 22 below, showing all groups stimulated at 4cpd, 4Hz, there were no statistically significant differences in ERG amplitude between any groups at any contrast level ($p>0.05$). There was no significant correlation between ERG amplitude and contrast in normal eyes ($t = 1.397$, $p = 0.171$), amblyopic eyes ($t = 1.464$, $p = 0.160$), or fellow eyes ($t = 1.421$, $p = 0.172$).

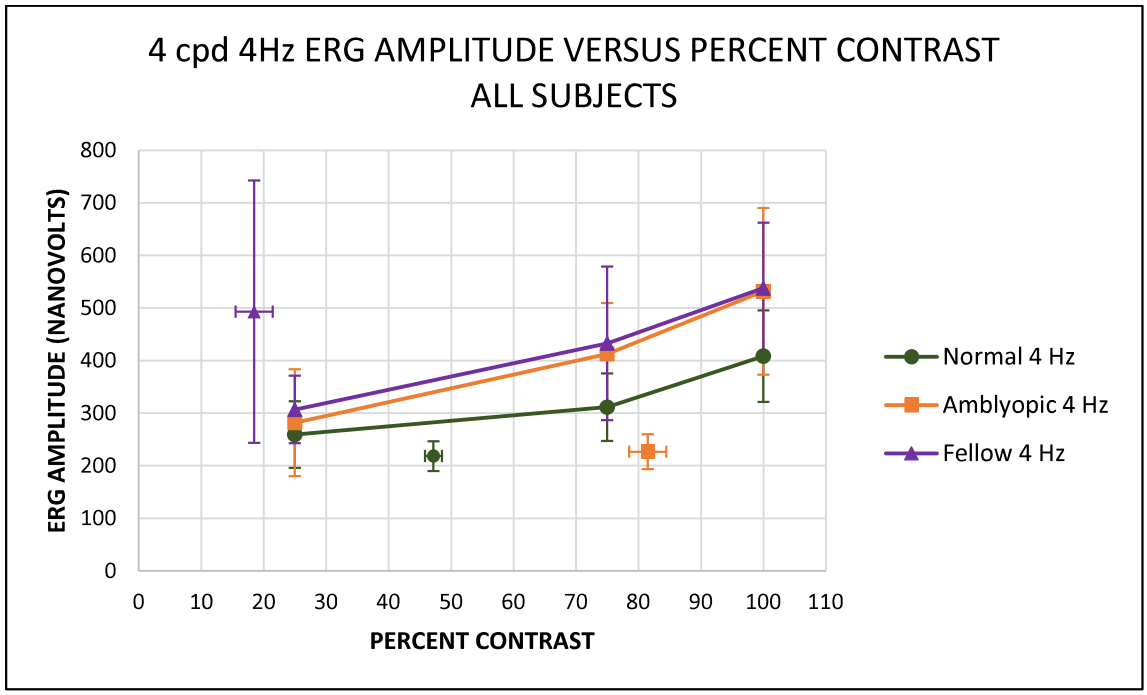


Figure 22: PERG mean amplitude (± 1 SE) as a function of contrast, at 4c/deg spatial frequency, 4Hz temporal frequency, in normal control vs amblyopic vs fellow eyes

In figure 23 below, showing all groups stimulated at 4cpd, 4Hz, there were no statistically significant differences in ERG phase between any groups at any contrast level ($p>0.05$). ERG phase significantly increases with increasing contrast in normal eyes ($t = 3.003$, $p = 0.005$), but there was no significant relationship between phase and contrast in amblyopic eyes ($t = -0.739$, $p = 0.469$) or fellow eyes ($t = 1.148$, $p = 0.265$).

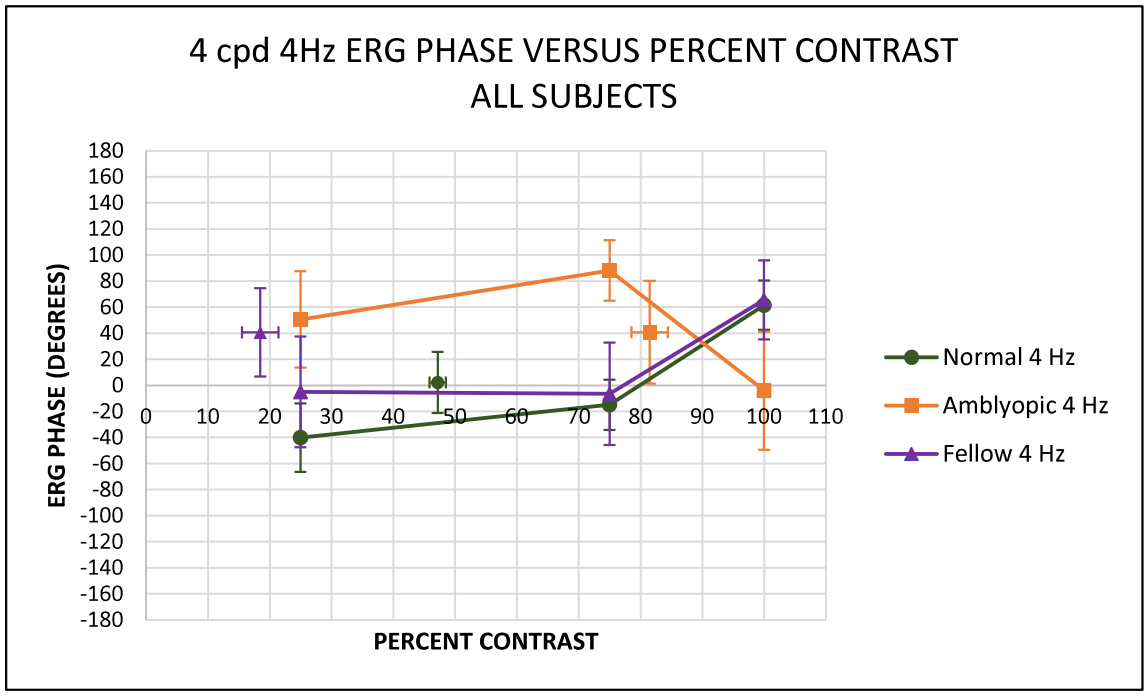


Figure 23: PERG mean phase (± 1 SE) as a function of contrast, at 4c/deg spatial frequency, 4Hz temporal frequency, in normal control vs amblyopic vs fellow eyes

In figure 24 below, showing all groups stimulated at 4cpd, 10Hz, there were no statistically significant differences in ERG amplitude between any groups at 25%, subjective balance point, or 75% contrast ($p>0.05$). At 100% contrast, there was a statistical difference between normal and amblyopic eyes ($p = 0.000$), normal and fellow eyes ($p = 0.003$), but no statistical difference between amblyopic and fellow eyes ($p>0.05$). There was a significant positive correlation between ERG amplitude and contrast in normal eyes ($t = 4.459$, $p = 0.000$), but no correlation in amblyopic eyes ($t = -0.566$, $p = 0.578$) or fellow eyes ($t = -0.746$, $p = 0.465$).

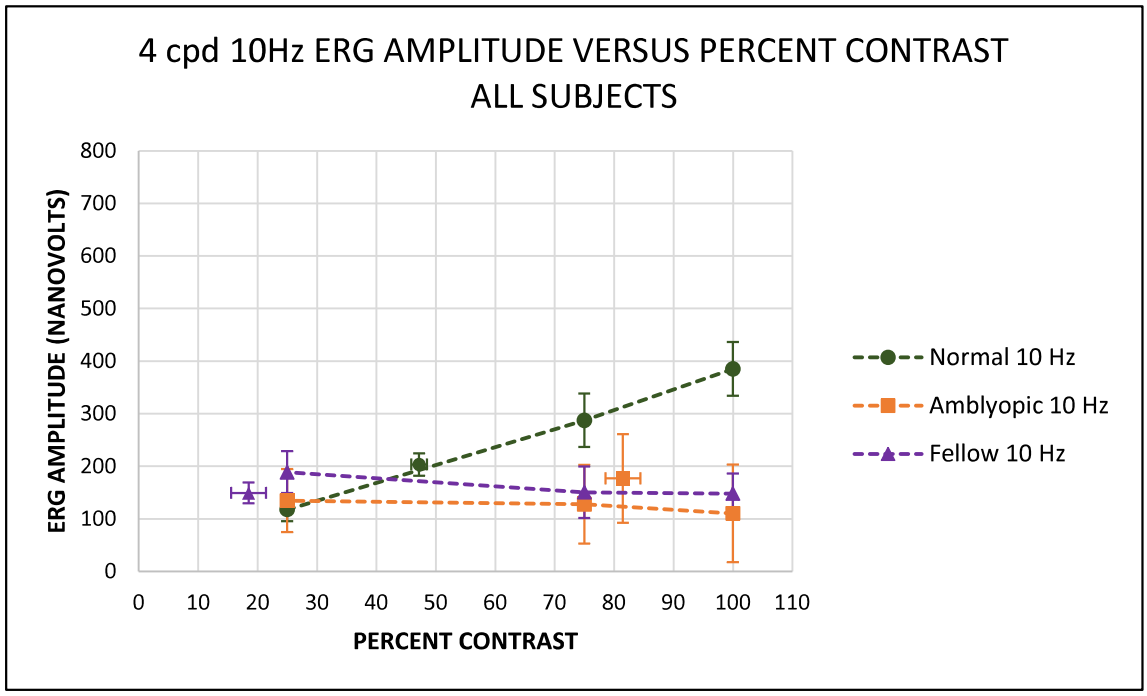


Figure 24: PERG mean amplitude (± 1 SE) as a function of contrast, at 4c/deg spatial frequency, 10Hz temporal frequency, in normal control vs amblyopic vs fellow eyes

In figure 25 below, showing all groups stimulated at 4cpd, 10Hz, there were no statistically significant differences in ERG phase between any groups at any contrast level ($p>0.05$). ERG phase significantly increases with increasing contrast in normal eyes ($t = 2.337$, $p = 0.025$), but there was no significant correlation between phase and contrast in amblyopic eyes ($t = 0.328$, $p = 0.747$) or fellow eyes ($t = 0.419$, $p = 0.680$).

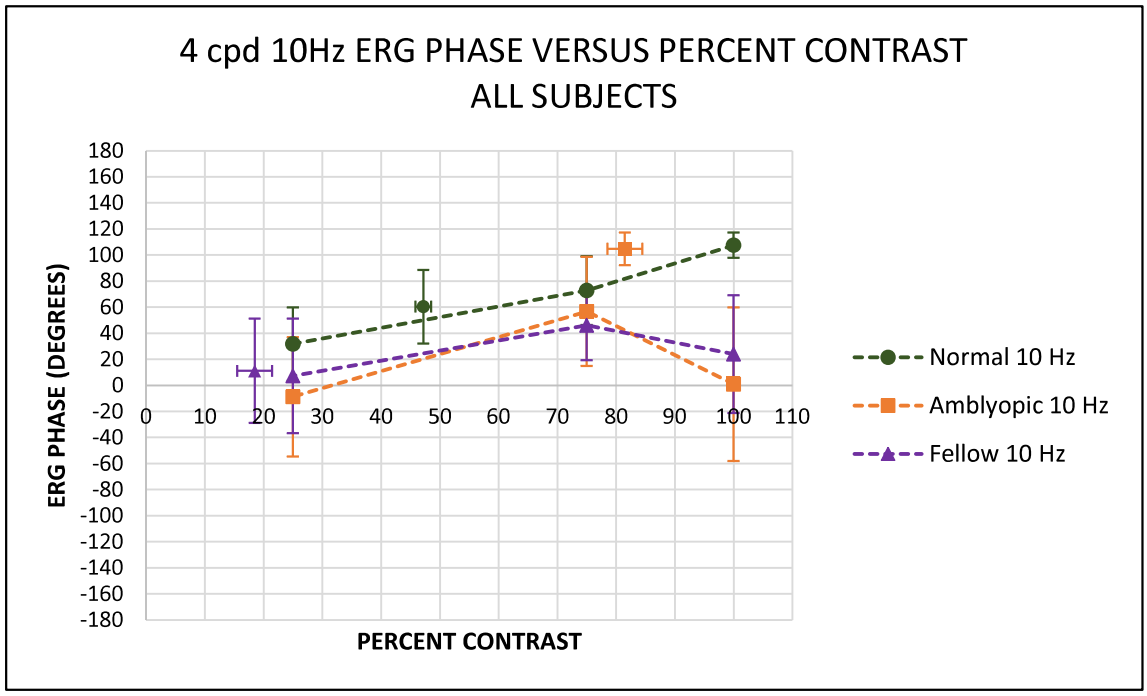


Figure 25: PERG mean phase (± 1 SE) as a function of contrast, at 4c/deg spatial frequency, 10Hz temporal frequency, in normal control vs amblyopic vs fellow eyes

3.3.1.4- Temporal and Spatial Frequency Dependence of ERG in Amblyopic Subjects

With spatial frequency kept constant at 1c/deg, there was no effect of temporal frequency on ERG amplitude or phase at any contrast level ($p>0.05$) in amblyopic or fellow eyes. With spatial frequency held constant at 4c/deg, there was a significant difference in amplitude between 4Hz and 10Hz at 100% contrast ($p = 0.011$) in amblyopic eyes, but no other effects of temporal frequency on ERG amplitude at any other contrast level in amblyopic or fellow eyes ($p>0.05$). There was no effect of temporal frequency on ERG phase at 4c/deg at any contrast in amblyopic or fellow eyes ($p>0.05$).

With temporal frequency held constant at 4Hz, there was no spatial frequency dependence of ERG amplitude or phase at any contrast level ($p>0.05$) in amblyopic or fellow eyes. At 10Hz, there was a significant difference in amplitude between 1c/deg and 4c/deg at 100% contrast ($p = 0.000$) in amblyopic eyes, but no other effects of spatial frequency on ERG amplitude at any other contrast level ($p>0.05$) in amblyopic or fellow eyes. There was no effect of spatial frequency on ERG phase at 10Hz at any contrast in amblyopic or fellow eyes ($p>0.05$).

3.3.1.5- ERG Comparisons of 100% Contrast Bilaterally Versus 100% Contrast Unilaterally

Given that our dichoptic ERG findings involve independent analysis of each eye, this analysis was performed to confirm that our monocular frequency-tagged data is in fact representative of monocular signal and not affected by simultaneous recording of the other eye. The following tables 6 and 7 summarize ANOVA statistics of ERG amplitude and phase, respectively, when comparing the binocular 100%-100% contrast condition (step 1: 100% contrast bilaterally) to the monocular 100% contrast condition (100% contrast unilaterally, other eye 0%) within each group (in normal, amblyopic, or fellow eyes) across all of the testing protocols. There were no significant differences in ERG amplitude or phase between these contrast conditions within any of the groups under any protocol ($p>0.05$).

Protocol	Group	Contrast Condition	Group	Contrast Condition	Sig.
1 cpd 4 Hz	Normal	100% bilaterally	Normal	100% unilaterally	1
	Amblyopic	100% bilaterally	Amblyopic	100% unilaterally	1
	Fellow	100% bilaterally	Fellow	100% unilaterally	1
1 cpd 10 Hz	Normal	100% bilaterally	Normal	100% unilaterally	0.244
	Amblyopic	100% bilaterally	Amblyopic	100% unilaterally	1
	Fellow	100% bilaterally	Fellow	100% unilaterally	1
4 cpd 4 Hz	Normal	100% bilaterally	Normal	100% unilaterally	1
	Amblyopic	100% bilaterally	Amblyopic	100% unilaterally	1
	Fellow	100% bilaterally	Fellow	100% unilaterally	1
4 cpd 10 Hz	Normal	100% bilaterally	Normal	100% unilaterally	0.323
	Amblyopic	100% bilaterally	Amblyopic	100% unilaterally	1
	Fellow	100% bilaterally	Fellow	100% unilaterally	1

Table 6: ANOVA analysis of ERG amplitude between 100% contrast bilaterally versus 100% contrast unilaterally in normal, amblyopic and fellow eyes

Protocol	Group	Contrast Condition	Group	Contrast Condition	Sig.
1 cpd 4 Hz	Normal	100% bilaterally	Normal	100% unilaterally	1
	Amblyopic	100% bilaterally	Amblyopic	100% unilaterally	1
	Fellow	100% bilaterally	Fellow	100% unilaterally	1
1 cpd 10 Hz	Normal	100% bilaterally	Normal	100% unilaterally	1
	Amblyopic	100% bilaterally	Amblyopic	100% unilaterally	1
	Fellow	100% bilaterally	Fellow	100% unilaterally	1
4 cpd 4 Hz	Normal	100% bilaterally	Normal	100% unilaterally	1
	Amblyopic	100% bilaterally	Amblyopic	100% unilaterally	1
	Fellow	100% bilaterally	Fellow	100% unilaterally	1
4 cpd 10 Hz	Normal	100% bilaterally	Normal	100% unilaterally	1
	Amblyopic	100% bilaterally	Amblyopic	100% unilaterally	1
	Fellow	100% bilaterally	Fellow	100% unilaterally	1

Table 7: ANOVA analysis of ERG phase between 100% contrast bilaterally versus 100% contrast unilaterally in normal, amblyopic and fellow eyes

3.3.2- VEP Responses

Figures 26 through 37 illustrate the mean SSPVEP amplitude and phase results as a function of contrast for each spatial/temporal condition.

3.3.2.2- Dichoptic VEP Normative Data

In figure 26 below, the dichoptic VEP amplitude in normals, at 1 cpd in both eyes, increases with increasing contrast between 25% and 100% contrast at both 4Hz ($t = 3.948$, $p = 0.000$) and at 10Hz ($t = 3.403$, $p = 0.002$). There was a statistically significant amplitude difference between 4Hz and 10Hz stimulation at 100% contrast ($p = 0.011$), but otherwise no statistical differences at any other contrast level ($p>0.05$).

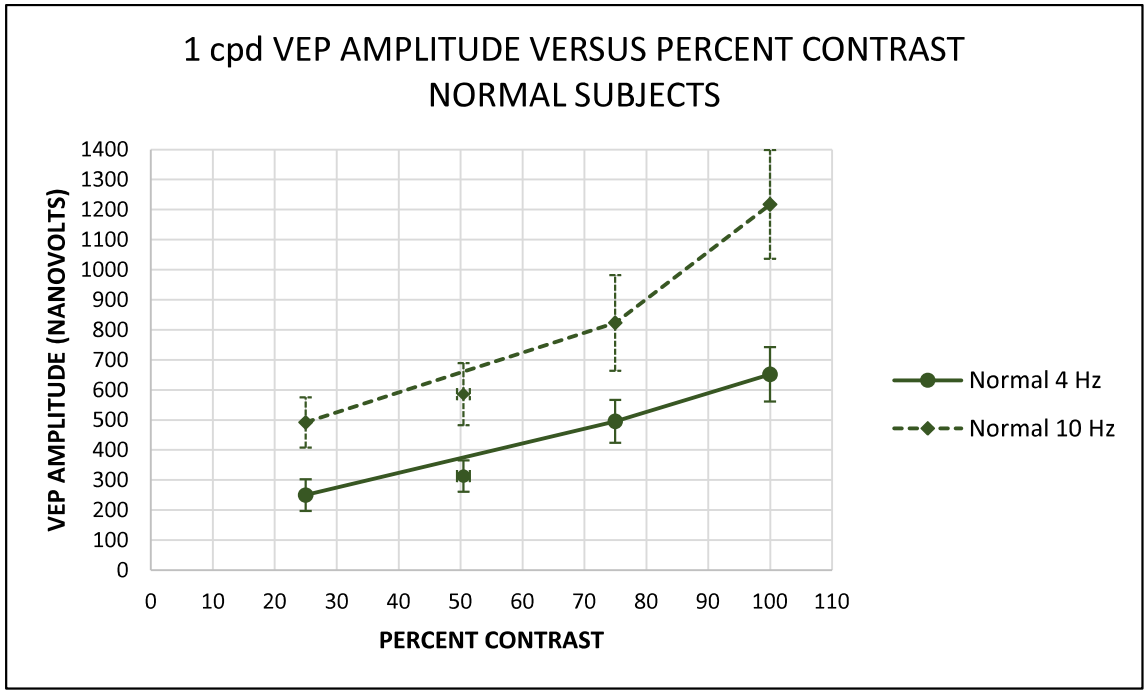


Figure 26: PVEP mean amplitude (± 1 SE) as a function of contrast for two different values of temporal frequency recorded dichoptically, at 1 c/deg both eyes, in normals

In figure 27, the dichoptic VEP phase in normals, at 1cpd in both eyes, has no significant correlation with contrast at 4Hz (t = -0.385, p = 0.702) or 10 Hz (t = 0.966, p = 0.340). There were no statistically significant differences in VEP phase between any groups at any contrast level (p>0.05).

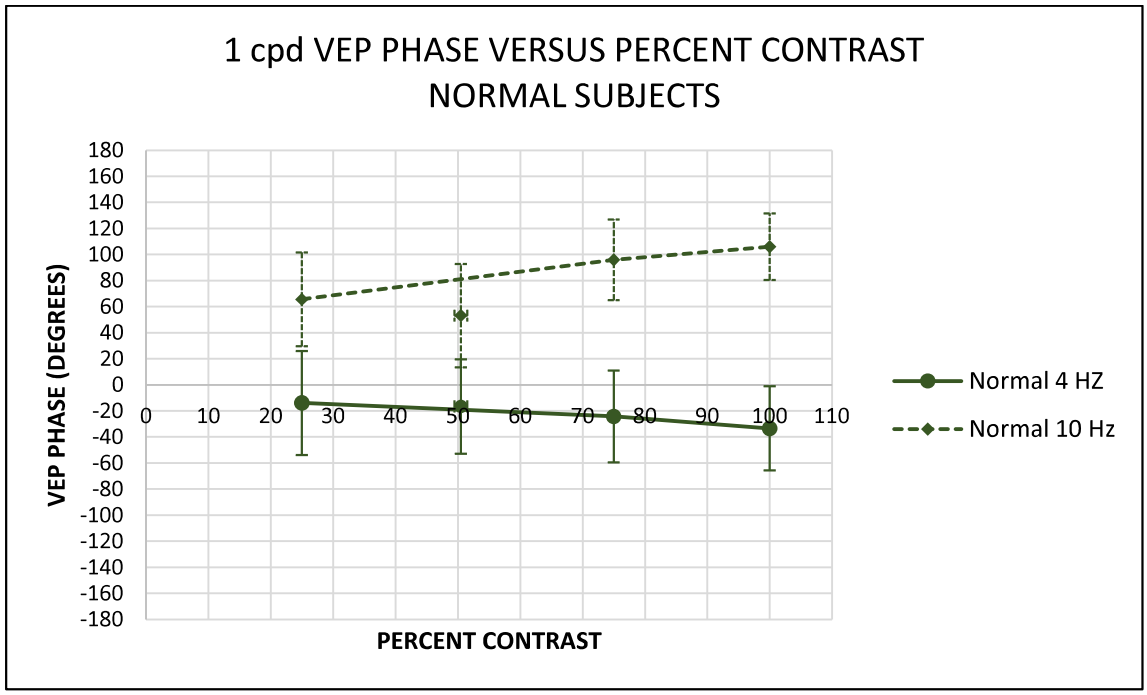


Figure 27: PVEP mean phase (± 1 SE) as a function of contrast for two different values of temporal frequency recorded dichoptically, at 1 c/deg both eyes, in normals

In figure 28, the dichoptic VEP amplitude in normals, at 4 cpd in both eyes, increases with increasing contrast at 10Hz (t = 2.723, p = 0.010) and at 4Hz (t = 3.301, p = 0.002). There was no statistically significant amplitude difference between 4Hz and 10Hz stimulation at any contrast level (p>0.05).

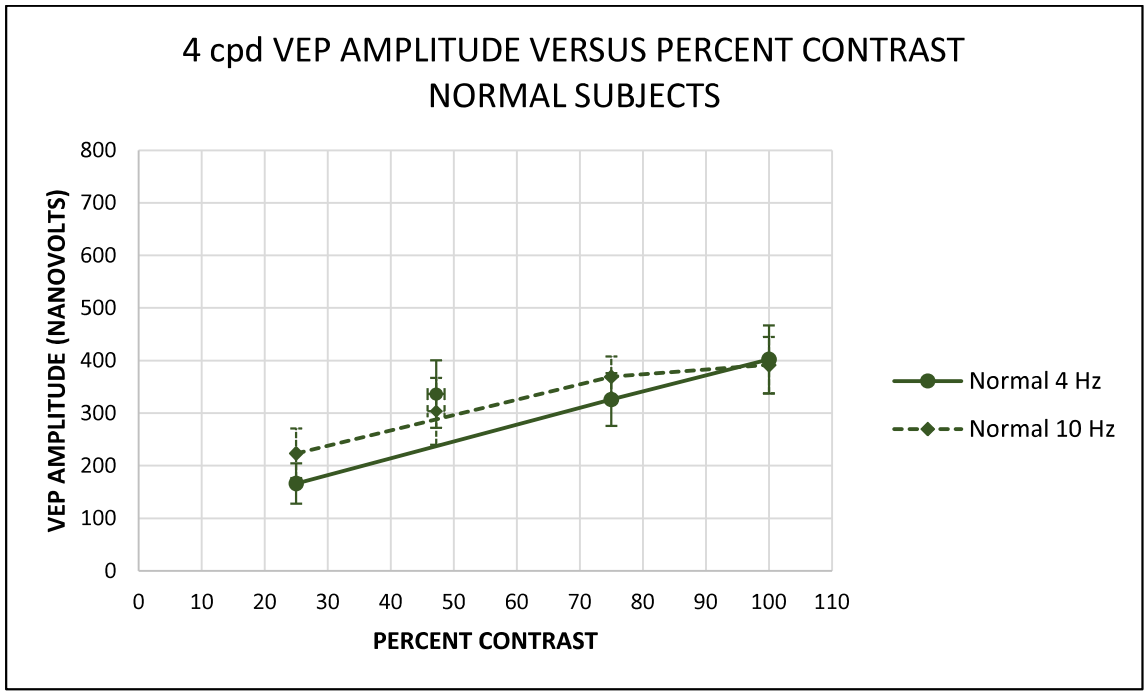


Figure 28: PVEP mean amplitude (± 1 SE) as a function of contrast for two different values of temporal frequency recorded dichoptically, at 4 c/deg both eyes, in normals

In figure 29, the dichoptic VEP phase in normals, at 4cpd in both eyes, does not show any significant regression at 4 Hz (t = 1.015, p = 0.317) or 10 Hz (t = 0.990, p = 0.328). There were no statistically significant differences in VEP phase between any groups at any contrast level (p>0.05).

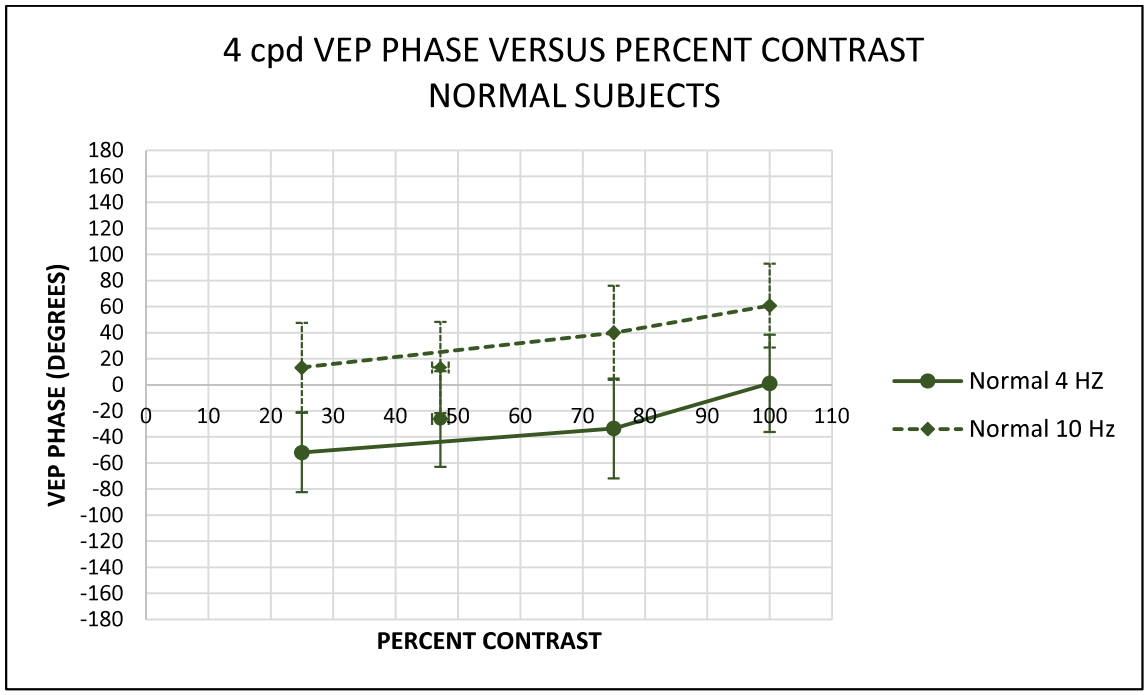


Figure 29: PVEP mean phase (± 1 SE) as a function of contrast for two different values of temporal frequency recorded dichoptically, at 4 c/deg both eyes, in normals

When analyzing normal eyes stimulated at 4Hz, there was no significant difference in VEP amplitude nor in VEP phase between 1 c/deg and 4 c/deg at any contrast level (p>0.05). At 10Hz, there was a significant difference in amplitude between 1 c/deg and 4 c/deg at 100% contrast (p = 0.000), but no other effects of spatial frequency on normal VEP amplitude at any other contrast level (p>0.05). There was no spatial frequency dependence of VEP phase in normals at 10Hz at any contrast level (p>0.05).

3.3.2.3- VEP Analysis Between Groups

Figures 30 through 37 show the mean SSPVEP amplitude and phase results as a function of contrast for each spatial/temporal condition, in normal versus amblyopic versus fellow eyes. The mean data at balance point contrast are shown as single data points throughout the figures.

Regression analysis was performed to investigate any significant correlation between contrast and amplitude or phase for each spatial/temporal condition in normal, amblyopic and fellow eyes. ANOVA and Bonferroni post-hoc analysis was used to investigate differences between normal versus amblyopic versus fellow eyes for each combination of spatial and temporal frequency across the range of luminance contrast.

In figure 30 below, showing all groups stimulated at 1cpd, 4Hz, there were no statistically significant differences in VEP amplitude between any groups at any contrast level ($p>0.05$). VEP amplitude significantly increases with increasing contrast in normal eyes ($t = 3.948$, $p = 0.000$), but contrast is not a significant predictor of amplitude in amblyopic eyes ($t = 1.119$, $p = 0.277$) or fellow eyes ($t = 1.518$, $p = 0.145$).

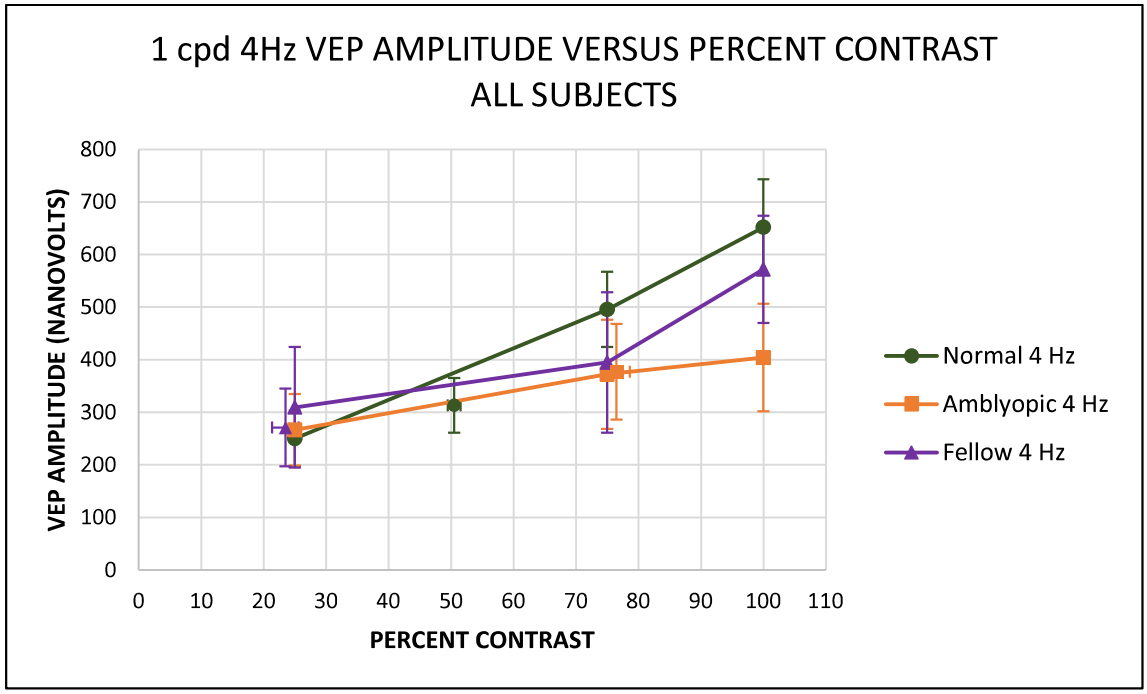


Figure 30: PVEP mean amplitude (± 1 SE) as a function of contrast, at 1c/deg spatial frequency, 4Hz temporal frequency, in normal control vs amblyopic vs fellow eyes

In figure 31 below, showing all groups stimulated at 1cpd, 4Hz, there were no statistically significant differences in VEP phase between any groups at any contrast level ($p>0.05$). VEP phase showed no significant correlation with contrast in normal eyes ($t = -0.385$, $p = 0.702$), amblyopic eyes ($t = 0.263$, $p = 0.795$) or fellow eyes ($t = 0.314$, $p = 0.757$).

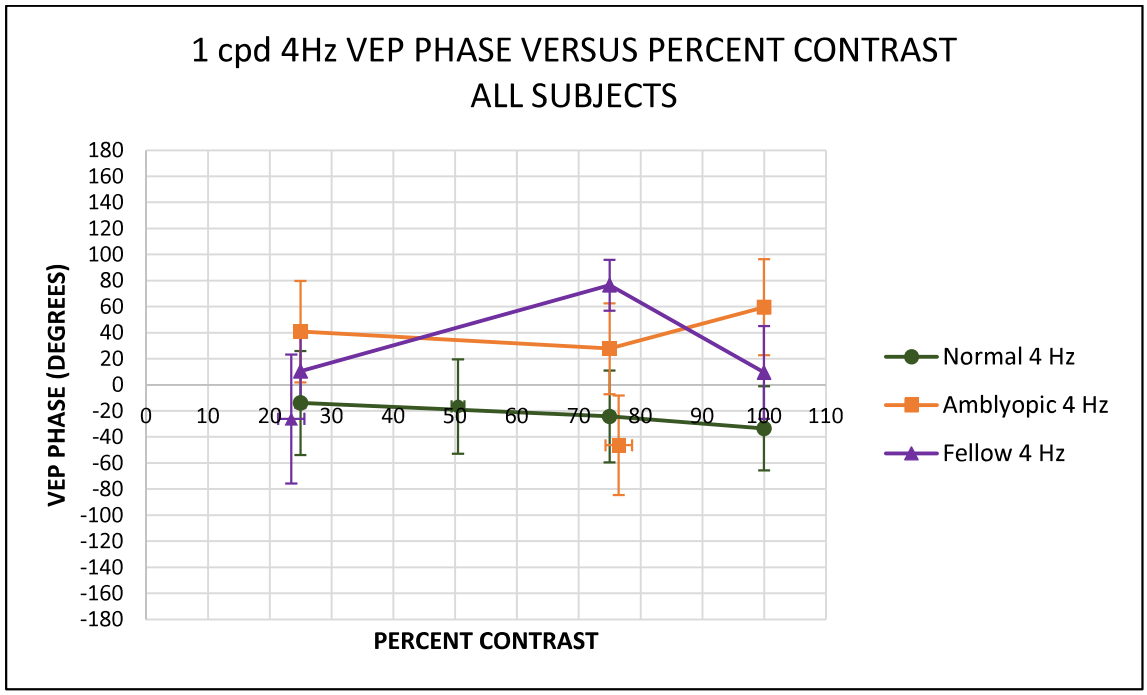


Figure 31: PVEP mean phase (± 1 SE) as a function of contrast, at 1c/deg spatial frequency, 4Hz temporal frequency, in normal control vs amblyopic vs fellow eyes

In figure 32 below, showing all groups stimulated at 1cpd, 10Hz, there was a significant VEP amplitude difference at 100% contrast between normal and amblyopic eyes ($p = 0.004$) and normal and fellow eyes ($p = 0.014$), but otherwise no other statistically significant differences in amplitude between groups at any other contrast level ($p>0.05$). VEP amplitude significantly increases with increasing contrast in normal eyes ($t = 3.403$, $p = 0.002$), but there was no significant correlation in amblyopic eyes ($t = 0.190$, $p = 0.851$) or fellow eyes ($t = -0.099$, $p = 0.922$).

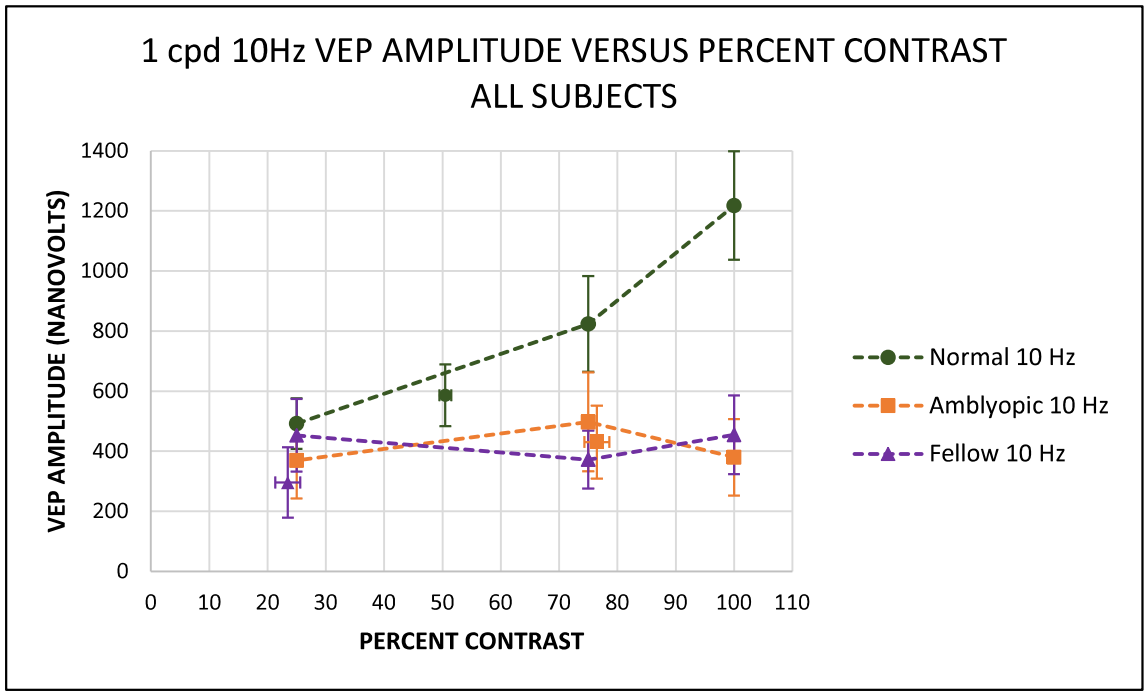


Figure 32: PVEP mean amplitude (± 1 SE) as a function of contrast, at 1c/deg spatial frequency, 10Hz temporal frequency, in normal control vs amblyopic vs fellow eyes

In figure 33 below, showing all groups stimulated at 1cpd, 10Hz, there were no statistically significant differences in VEP phase between any groups at any contrast level ($p>0.05$). There was no significant correlation between VEP phase and contrast in normal eyes ($t = 0.966$, $p = 0.340$), amblyopic eyes ($t = -1.941$, $p = 0.067$), or fellow eyes ($t = -0.204$, $p = 0.840$).

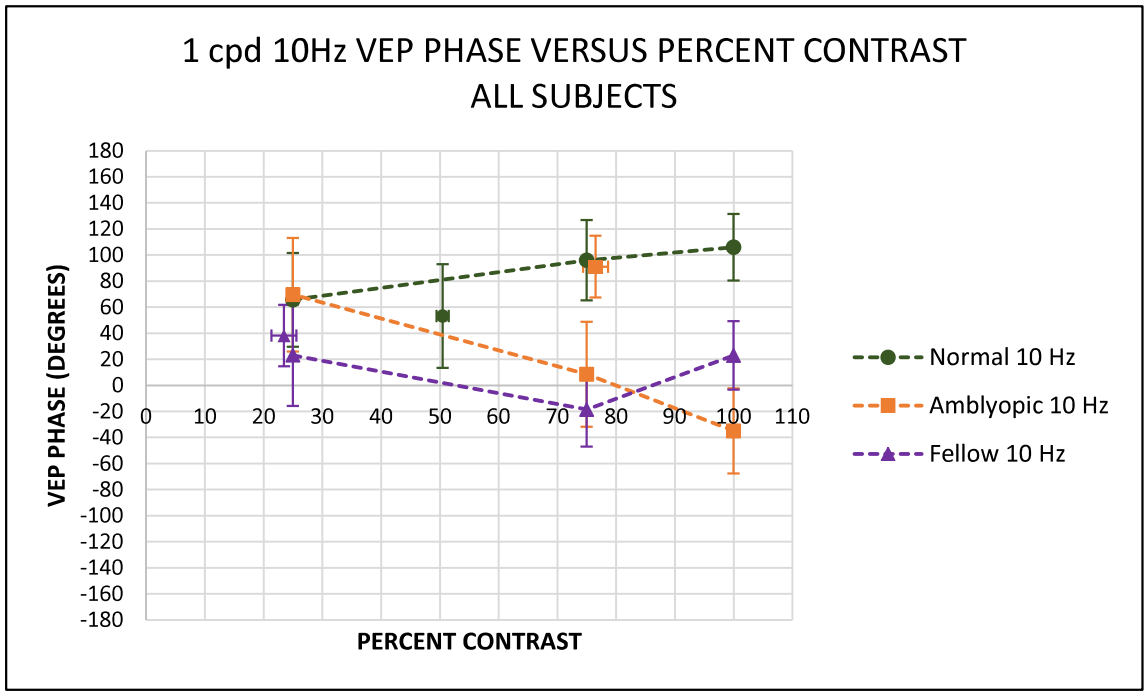


Figure 33: PVEP mean phase (± 1 SE) as a function of contrast, at 1c/deg spatial frequency, 10Hz temporal frequency, in normal control vs amblyopic vs fellow eyes

In figure 34 below, showing all groups stimulated at 4cpd, 4Hz, there were no statistically significant differences in VEP amplitude between any groups at any contrast level ($p>0.05$). VEP amplitude significantly increases with increasing contrast in normal eyes ($t = 3.301$, $p = 0.002$), but there was no significant relationship in amblyopic eyes ($t = 1.510$, $p = 0.148$) or fellow eyes ($t = 0.510$, $p = 0.616$).

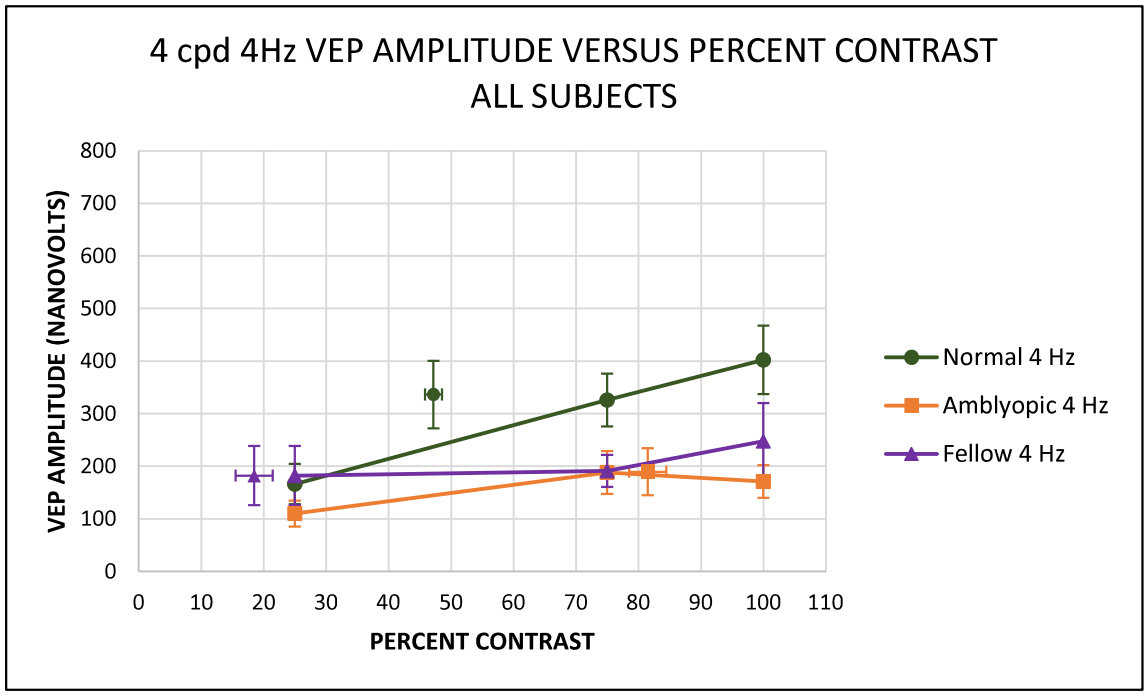


Figure 34: PVEP mean amplitude (± 1 SE) as a function of contrast, at 4c/deg spatial frequency, 4Hz temporal frequency, in normal control vs amblyopic vs fellow eyes

In figure 35 below, showing all groups stimulated at 4cpd, 4Hz, there were no statistically significant differences in VEP phase between any groups at any contrast level ($p>0.05$). There was no significant regression between VEP phase and contrast in normal eyes ($t = 1.015$, $p = 0.317$), amblyopic eyes ($t = 0.626$, $p = 0.539$), or fellow eyes ($t = 0.151$, $p = 0.882$).

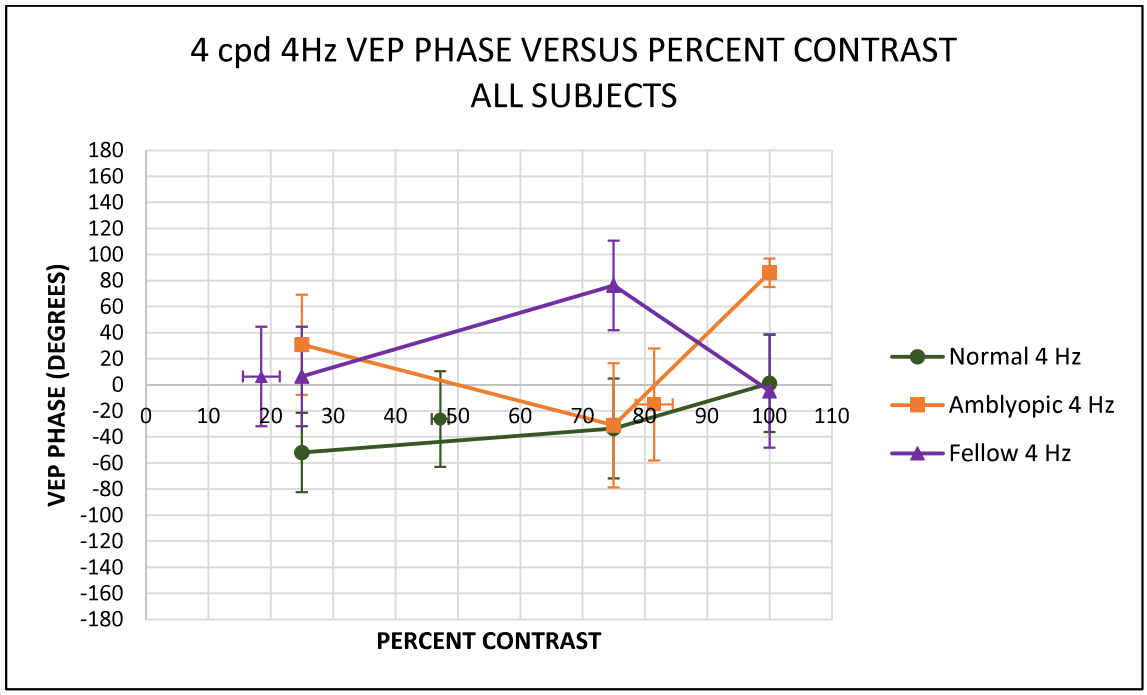


Figure 35: PVEP mean phase (± 1 SE) as a function of contrast, at 4c/deg spatial frequency, 4Hz temporal frequency, in normal control vs amblyopic vs fellow eyes

In figure 36 below, showing all groups stimulated at 4cpd, 10Hz, there were no statistically significant differences in VEP amplitude between any groups at any contrast level ($p>0.05$). VEP amplitude significantly increases with increasing contrast in normal eyes ($t = 2.723$, $p = 0.010$), but there was no significant correlation in amblyopic eyes ($t = 1.505$, $p = 0.149$) or fellow eyes ($t = 1.180$, $p = 0.252$).

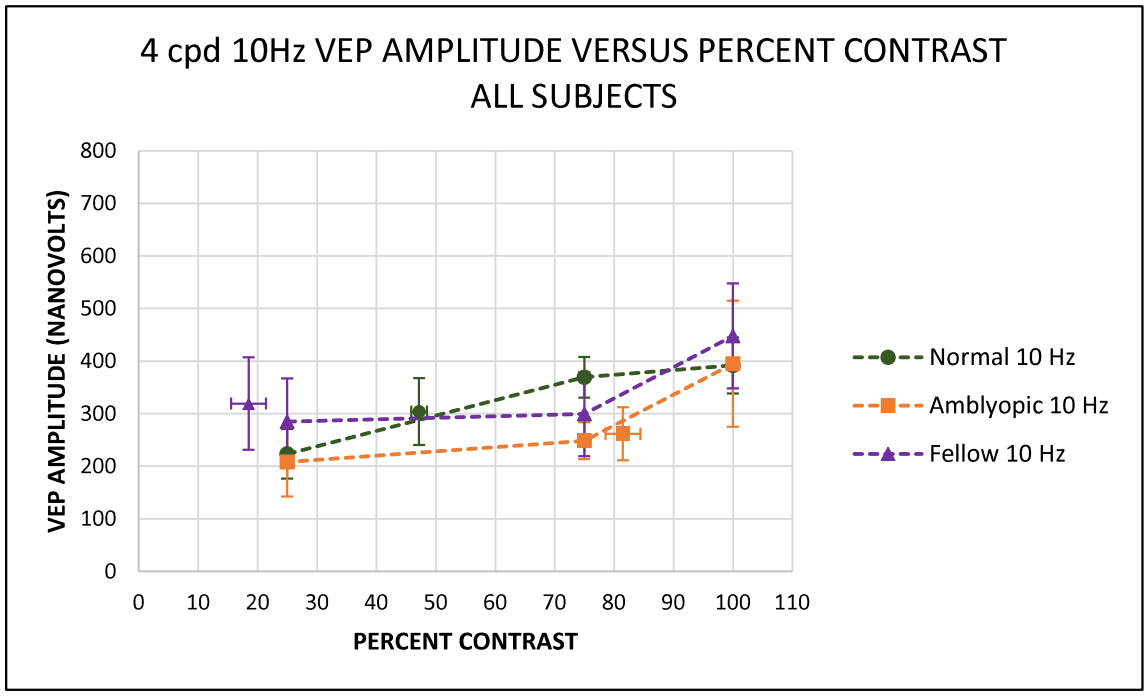


Figure 36: PVEP mean amplitude (± 1 SE) as a function of contrast, at 4c/deg spatial frequency, 10Hz temporal frequency, in normal control vs amblyopic vs fellow eyes

In figure 37 below, showing all groups stimulated at 4cpd, 10Hz, there were no statistically significant differences in VEP phase between any groups at any contrast level ($p>0.05$). There was no significant correlation between VEP phase and contrast in normal eyes ($t = 0.990$, $p = 0.328$), amblyopic eyes ($t = -0.976$, $p = 0.341$), or fellow eyes ($t = 0.654$, $p = 0.521$).

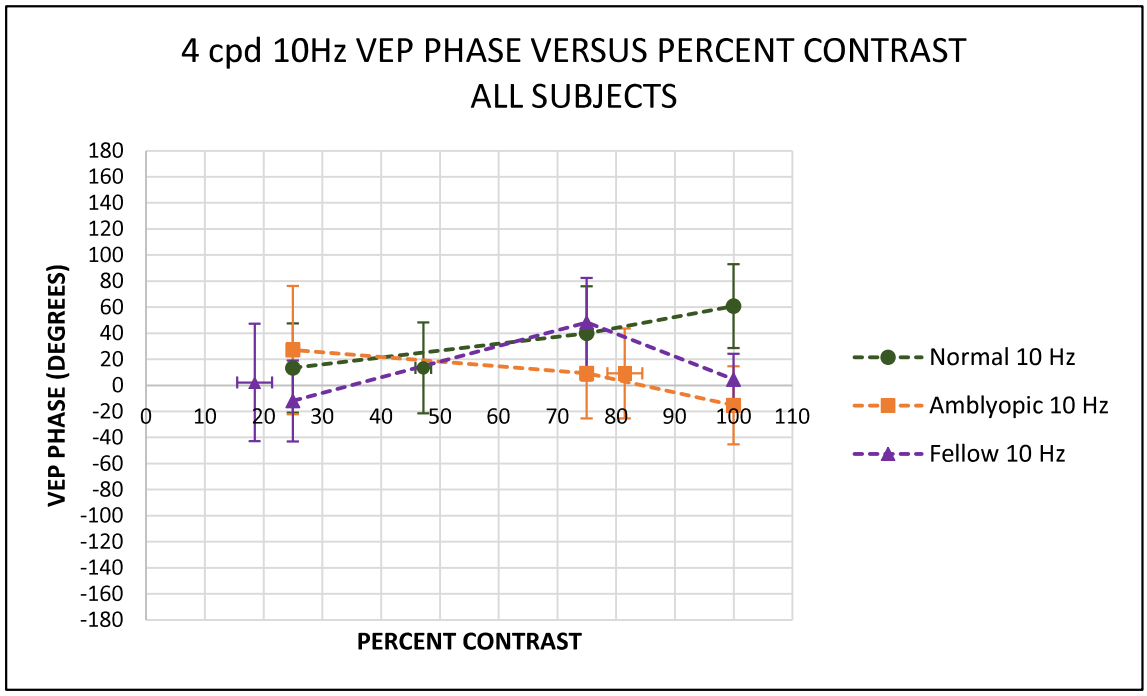


Figure 37: PVEP mean phase (± 1 SE) as a function of contrast, at 4c/deg spatial frequency, 10Hz temporal frequency, in normal control vs amblyopic vs fellow eyes

3.3.2.4- Temporal and Spatial Frequency Dependence of VEP in Amblyopic Subjects

With spatial frequency kept constant at 1c/deg, there was no significant effect of temporal frequency on VEP amplitude or phase at any contrast level ($p>0.05$) in amblyopic or fellow eyes. With spatial frequency held constant at 4c/deg, there was no significant effect of temporal frequency on VEP amplitude or phase at any contrast level in amblyopic or fellow eyes ($p>0.05$).

With temporal frequency held constant at 4Hz, there was no spatial frequency dependence of VEP amplitude or phase at any contrast level ($p>0.05$) in amblyopic or fellow eyes. At 10Hz, there was no significant effect of spatial frequency on VEP amplitude or phase at any contrast in amblyopic or fellow eyes ($p>0.05$).

3.3.2.5- VEP Comparisons of 100% Contrast Bilaterally Versus 100% Contrast Unilaterally

Since our dichoptic VEP findings include separate analysis of each eye, this analysis was done to show that our monocular frequency-tagged data is in fact representative of monocular signal and not affected by simultaneous recording of the other eye. The following tables 8 and 9 summarize ANOVA statistics of VEP amplitude and phase, respectively, when comparing the binocular 100%-100% contrast condition (step 1: 100% contrast bilaterally) to the monocular 100% contrast condition (100% contrast unilaterally, other eye 0%) within each group (in normal, amblyopic, or fellow eyes) across all of the testing protocols. There was a statistically significant difference in VEP amplitude between these contrast conditions in normal eyes at 1cpd 4Hz ($p = 0.044$, power 44%), otherwise there were no other significant differences in VEP amplitude or phase found ($p>0.05$).

Protocol	Group	Contrast Condition	Group	Contrast Condition	Sig.
1 cpd 4 Hz	Normal	100% bilaterally	Normal	100% unilaterally	0.044
	Amblyopic	100% bilaterally	Amblyopic	100% unilaterally	1
	Fellow	100% bilaterally	Fellow	100% unilaterally	1
1 cpd 10 Hz	Normal	100% bilaterally	Normal	100% unilaterally	0.066
	Amblyopic	100% bilaterally	Amblyopic	100% unilaterally	1
	Fellow	100% bilaterally	Fellow	100% unilaterally	1
4 cpd 4 Hz	Normal	100% bilaterally	Normal	100% unilaterally	1
	Amblyopic	100% bilaterally	Amblyopic	100% unilaterally	1
	Fellow	100% bilaterally	Fellow	100% unilaterally	1
4 cpd 10 Hz	Normal	100% bilaterally	Normal	100% unilaterally	1
	Amblyopic	100% bilaterally	Amblyopic	100% unilaterally	1
	Fellow	100% bilaterally	Fellow	100% unilaterally	1

Table 8: ANOVA analysis of VEP amplitude between 100% contrast bilaterally versus 100% contrast unilaterally in normal, amblyopic and fellow eyes

Protocol	Group	Contrast Condition	Group	Contrast Condition	Sig.
1 cpd 4 Hz	Normal	100% bilaterally	Normal	100% unilaterally	1
	Amblyopic	100% bilaterally	Amblyopic	100% unilaterally	1
	Fellow	100% bilaterally	Fellow	100% unilaterally	1
1 cpd 10 Hz	Normal	100% bilaterally	Normal	100% unilaterally	1
	Amblyopic	100% bilaterally	Amblyopic	100% unilaterally	1
	Fellow	100% bilaterally	Fellow	100% unilaterally	1
4 cpd 4 Hz	Normal	100% bilaterally	Normal	100% unilaterally	1
	Amblyopic	100% bilaterally	Amblyopic	100% unilaterally	1
	Fellow	100% bilaterally	Fellow	100% unilaterally	1
4 cpd 10 Hz	Normal	100% bilaterally	Normal	100% unilaterally	1
	Amblyopic	100% bilaterally	Amblyopic	100% unilaterally	1
	Fellow	100% bilaterally	Fellow	100% unilaterally	1

Table 9: ANOVA analysis of VEP phase between 100% contrast bilaterally versus 100% contrast unilaterally in normal, amblyopic and fellow eyes

3.3.3- Beat VEP Analysis Between Groups

Figures 38 through 39 show the mean SSPVEP beat amplitude results as a function of contrast for each spatial/temporal condition, in normal versus amblyopic subjects. The mean data at 100% contrast bilaterally are shown as single data points throughout the figures.

In figures 38 and 39 below, at 1cpd and 4cpd respectively, there were no statistically significant differences in beat (6 Hz signal) VEP amplitude between any groups at any contrast level ($p>0.05$). There were also no significant differences in beat VEP amplitude between any contrast conditions within any of the groups ($p>0.05$).

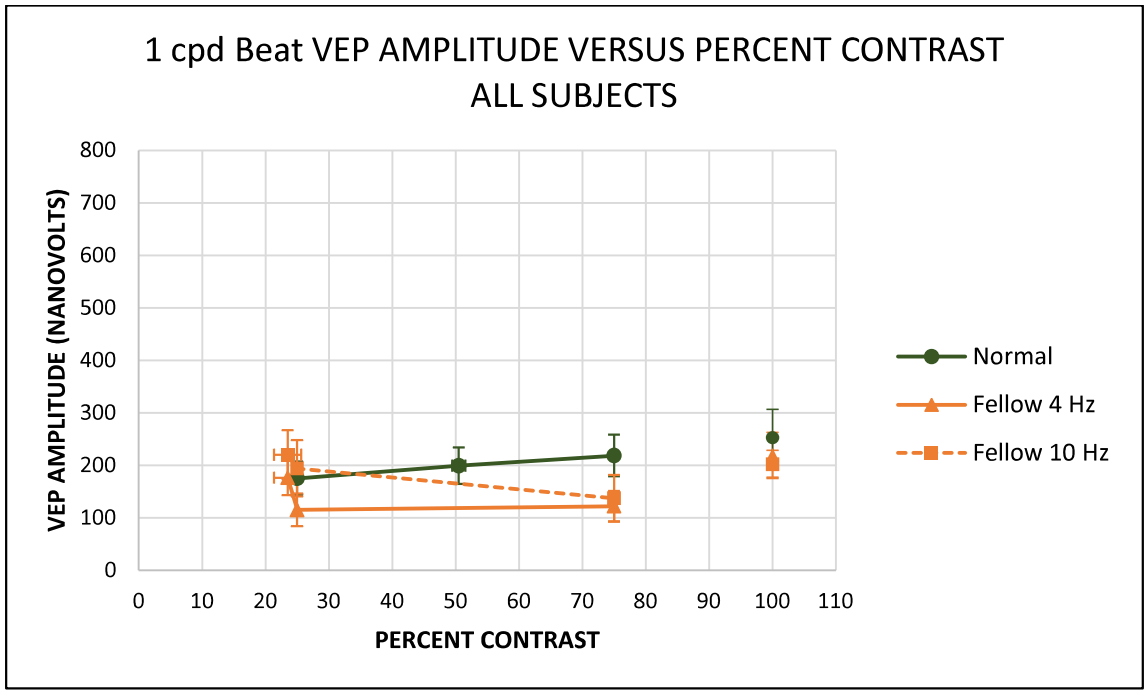


Figure 38: PVEP mean beat (6 Hz) amplitude (± 1 SE) as a function of contrast, at 1c/deg spatial frequency, in normal control vs amblyopic subjects with fellow eye 4Hz stimulation vs amblyopic subjects with fellow eye 10Hz stimulation

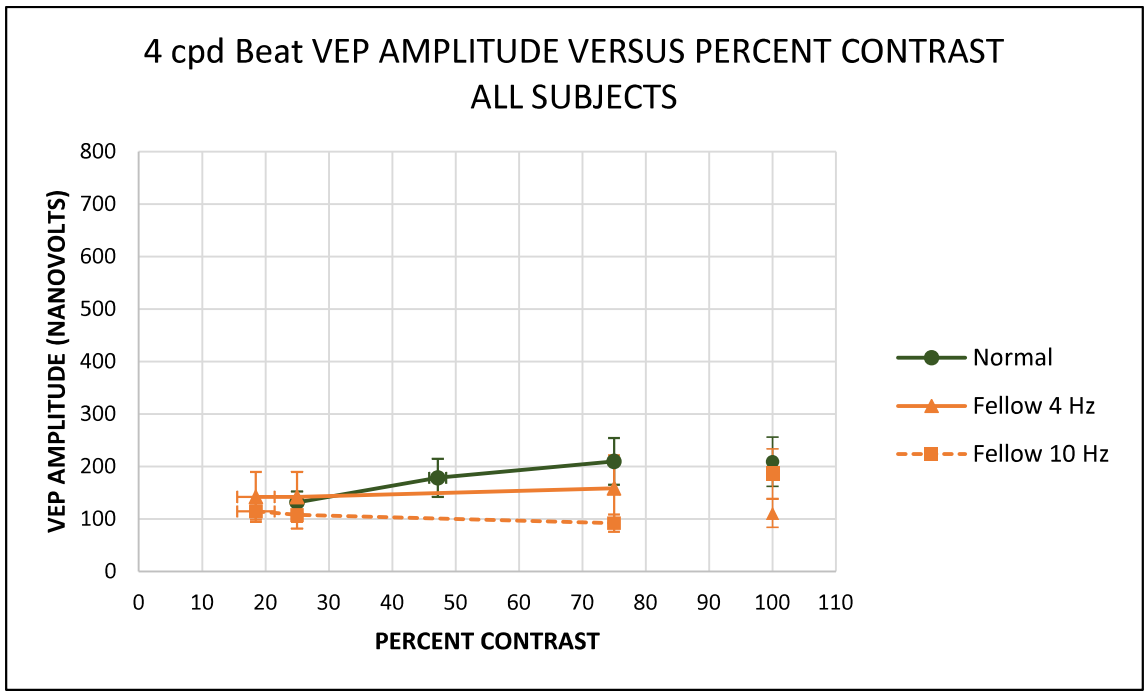


Figure 39: PVEP mean beat (6 Hz) amplitude (± 1 SE) as a function of contrast, at 4c/deg spatial frequency, in normal control vs amblyopic subjects with fellow eye 4Hz stimulation vs amblyopic subjects with fellow eye 10Hz stimulation

4- Discussion

The purpose of this project was to investigate the relationship between retinal (ERGs), cortical (VEPs), and subjective responses (contrast balance point) in anisometropic amblyopes and normal age-matched controls to examine the role of the retina and cortex in amblyopia. Retinal and cortical electrophysiologic response dynamics of the amblyopic, fellow, and normal eyes were compared for each spatial and temporal frequency and for the whole range of luminance contrast tested, inclusive of the subjective contrast balance point. This study was designed to examine the precise cause of vision loss in amblyopia by testing the hypothesis that the spatial and temporal frequency dependence of the contrast balance point is due to both retinal and cortical factors. This would suggest that the neural generators of the balance point originate in both the retina and cortex, and ultimately, that both the retina and cortex contribute to the pathophysiology of anisometropic amblyopia.

Our amblyopic and normal subjects were age and sex-matched to account for well documented changes in PERG and PVEP data with aging and between sexes[109,110]. Furthermore, there were no significant differences in axial length or pupil size between amblyopic, fellow and normal eyes, which assured that these factors did not confound our electrophysiology results[111,112].

The average balance points (BP) measured in this study [1c/deg: 76.43% ± 2.13% (mean ± SE) for the amblyopic group and 50.46% ± 1.03% for the normal group; 4c/deg: 81.43% ± 2.96% for the amblyopic group and 47.23% ± 1.34% for the normal group] were within range of results reported by Kwon et al.[12] In their 12 amblyopic observers, Kwon et al. found that the BP of the weak amblyopic eye increased from 63% at 0.5c/deg to 90% at 5c/deg. This indicates that binocular imbalance is stronger at higher spatial frequencies compared to lower spatial frequencies, meaning that with increasing spatial frequency, the contrast in the weak eye has to be further increased while the contrast in the strong eye must be accordingly further reduced to achieve binocularly balanced vision. In their normal participants, they consistently

found BP values close to 50%, independent of spatial frequency. They further reported that for a given spatial frequency, the amblyopic eye needs a higher contrast to balance the fellow eye (75% for the amblyopic eye versus 53% for the non-dominant eye of normal controls). The results of our study are in good agreement with the findings of Kwon et al, as expected, given that our balance point experiment was modeled after theirs. The balance points we measured for the amblyopes were higher than in the normals across both spatial frequencies tested, the binocular imbalance of the amblyopic group increased with increasing spatial frequency, while the BP remained essentially constant in the normals independent of spatial frequency. Any minor differences between the results of our study and others could be explained by differences in the classes or in the depths of amblyopia tested.

With regards to our electroretinogram results in our normal group compared to published literature in normals, we demonstrated that amplitude progressively increases in an approximate linear fashion between 25% and 100% contrast at 4Hz and at 10Hz, which agrees with trends observed by Porciati et al.[51] They also found that phase increased with increasing stimulus contrast between 12% and 99% contrast due to relative response weightings of M and P cells at different contrasts, and we also demonstrated this trend in most of our normative data. At 10Hz temporal frequency and 100% contrast, we observed significantly greater amplitude at 1c/deg compared to 4c/deg, which is consistent with past work demonstrating that amplitude decreases with increasing spatial frequency[59]. However we did not find any other spatial frequency dependence of ERG amplitude in any of our other contrast levels or testing parameters. We did not demonstrate any effect of spatial frequency on ERG phase in our study, despite past studies[58] reporting that phase increases with increasing spatial frequency. Our study did not show any significant temporal frequency dependence of ERG amplitude in any of our testing conditions, despite past studies having observed an effect of temporal frequency on amplitude with spatial-temporal interactions[58]. We consistently showed increased phase at 10Hz

compared to 4Hz, which is in agreement with previously published findings[58], however our differences were only significant at higher contrasts at 1c/deg.

When it comes to our electroretinogram findings in our amblyopic group compared to our normal group, we showed that at 10Hz, at 100% contrast only, at both spatial frequencies tested, amplitude was significantly greater in normal versus amblyopic eyes and in normal versus fellow eyes, but there was no significant difference between amblyopic and fellow eyes. Although normal amplitudes generally tended to be greater than the amplitudes of amblyopic and fellow eyes, particularly at higher-end contrasts, none of these differences in amplitude were significant between normal versus amblyopic versus fellow eyes in any of our other testing conditions. There were essentially no significant relationships between ERG amplitude and contrast in our amblyopic or fellow eyes. As previously discussed, we demonstrated that ERG phase significantly increases with increasing contrast in normal eyes, but we did not observe any significant correlation between phase and contrast in amblyopic eyes or fellow eyes. Our findings showed neither trends nor significant differences in ERG phase between any of the groups in any of our tests. At 4c/deg 100% contrast, we observed an effect of temporal frequency on ERG amplitude in amblyopic eyes – 4Hz produces greater amplitude than 10Hz – but otherwise did not find any other temporal frequency dependence of ERG amplitude elsewhere in our study. Moreover, we did not demonstrate any effects of temporal frequency on ERG phase in any of our results. At 10Hz 100% contrast, we found greater amplitude at 1c/deg compared to 4c/deg in amblyopic eyes but otherwise did not observe any other spatial frequency dependence of ERG amplitude in amblyopic or fellow eyes under any other testing parameters. There was no effect of spatial frequency on ERG phase in amblyopic or fellow eyes under any condition.

Furthermore, given that an electroretinogram is a monocular recording acquired by an electrode specific to each eye, we would not expect any spill-over or effects from the other eye during binocular dichoptic recording, and our results support that. A comparison of the bilateral

100%-100% contrast condition to the unilateral 100% condition demonstrated no significant differences in ERG amplitude or phase in any groups under any of the testing conditions, thus implying reliable, independent analysis of the dichoptic ERG data specific to each eye.

Our ERG results strongly suggest that there is no difference in macular retinal function between normal versus amblyopic versus fellow eyes, which is in good agreement with past studies[34,37]. Under a few 100% contrast conditions our results showed significantly reduced ERG signals in amblyopic eyes compared to normal eyes, also supported by a past study[35], which implies an anomaly at the level of the retina; however it has been widely suggested that these reduced ERG responses were a result of unstable fixation in amblyopes, and once eye movements are controlled for, the responses equalize[34,37]. As expected, in our study we did not note amblyopic retinal function to be reduced at subthreshold contrasts[6]. To our knowledge, no other study has comprehensively studied ERG responses in amblyopes across such a wide range of contrasts as done in this project, but the variability of our results and lack of significance in our correlations between amplitude, phase and contrast in amblyopic and fellow eyes may be a result of our small sample size, especially given the inherent variability of ERG responses[37]. We did notice that our amblyopic participants had some difficulty maintaining fixation, while our normal participants did not. Steady fixation is important for obtaining reliable electrophysiological responses[34,37] and so as a result, our amblyopic recordings were significantly noisier due to eye movement artifacts, which will be further addressed and accounted for below in our discussion of bin analysis.

With regards to our visual evoked potentials in our normal group, we demonstrated that SSVEP amplitude significantly increases with increasing stimulus contrast, which is in good agreement with past studies[75–78]. We observed that phase also generally increases with increasing contrast however this relationship was not significant and is not consistent with previous findings[78,79,81]. Current literature suggests that both amplitude and phase increase with increasing stimulus spatial frequency[50,79], however our results, granted essentially without

significance, show that both amplitude and phase generally decrease at 4c/deg compared to 1c/deg. At 10Hz 100% contrast, there was significantly greater amplitude at 1c/deg versus 4c/deg, but otherwise our results did not reveal any other effects of spatial frequency on VEP amplitude. We did not observe any spatial frequency dependence of VEP phase in any of our normative testing. We found that 10Hz stimulation generally produced greater amplitude and phase compared to 4Hz, but these differences were not significant under any condition and therefore we cannot suggest any temporal frequency dependence of VEP amplitude nor phase based on our findings.

Our visual evoked potentials in our amblyopic group demonstrated through all testing that stimulus contrast was not a significant predictor of VEP amplitude in amblyopic eyes or fellow eyes – although a very questionable trend of increasing amplitude with increasing contrast may be appreciated, this was not significant. We demonstrated that at 1cpd, 10Hz, 100% contrast, there was a significant VEP amplitude difference between normal and amblyopic eyes, and between normal and fellow eyes, but otherwise no other significant differences between groups under any condition. VEP amplitude in normals generally tended to be greater than amblyopic and fellow eyes throughout the range of testing however these differences were not significant. We did not observe any significant correlation between VEP phase and contrast in amblyopic eyes or fellow eyes. Our VEP phase data was quite scattered and variable, and to that effect, we did not identify any trends nor find any significant differences in VEP phase between any groups in any of our results. We did not demonstrate any spatial or temporal frequency dependence of VEP amplitude or phase in amblyopic or fellow eyes throughout any of our testing.

Considering that our VEP signals were binocularly acquired from a single electrode, the VEP waveform comprises numerous temporal frequencies inclusive of those present in our dichoptic stimulus. Since our findings include separate analysis of each eye, it is important to show that our monocular frequency-tagged data is in fact representative of monocular signal

and not affected by simultaneous recording of the other eye. To do so, we compared the binocular 100%-100% contrast condition (100% contrast bilaterally) to the monocular 100% contrast condition (100% contrast unilaterally, other eye 0%) and essentially found no differences in VEP amplitude or phase between these two contrast conditions, aside from one significant difference at 1cpd 4Hz in normal eyes only, with low 44% power. Therefore we suggest that our method of frequency-tagging to extract monocular function from a binocular dichoptic VEP response is a reliable means of independently analyzing the right and left visual pathways.

Our monocular VEP results generally suggest that there is no significant difference in the functional integrity of the visual pathway between normal versus amblyopic versus fellow eyes. Our results do not agree with those of past studies that have widely reported reduced amplitude and increased latency of the amblyopic eye compared to the fellow eye when each eye was tested separately[65,66]. While previous studies have measured VEP responses at a single high contrast level, to our knowledge, we are the first study to record VEPs across a range of contrasts in amblyopia. Only under one condition, at 1c/deg 10Hz 100% contrast, did we observe reduced VEP amplitude in amblyopic compared to normal eyes, and as previously discussed with ERGs, we suspected that the anomaly was caused by noise contamination from eye movements.

When assessing the power spectrums of our binocular beat VEP data, we noticed that not all of our amblyopic subjects showed the expected 6Hz beat response. Often times the beat response was shifted to an adjacent frequency such as 5Hz or 7Hz, and we attributed this spillover of signal to eye movement artifacts. In both normals and amblyopes, we expected a peak amplitude response at the balance point secondary to peak binocular summation. Our normative data did not show any difference in beat amplitude across the range of contrast ratios. At 1c/deg, our amblyopic data showed increased amplitude at the balance point contrast ratio (23.5% contrast presented to the fellow eye, 76.5% contrast presented to the amblyopic

eye) compared to the reversed contrast condition (75% contrast presented to the fellow eye, 25% contrast presented to the amblyopic eye), however this difference was not significant. Interestingly, we did not find any significant difference in the binocular beat response measured at balance point compared to the response measured with 100% contrast presented bilaterally where we expected suppression and thus a reduced signal. Again, as previously described with our ERGs and VEPs, we supposed that our expected signals may have been contaminated by eye movements causing leakage of signal into surrounding frequency bins.

Our original ERG results suggest that in some cases, the retina of amblyopic eyes is deficient to some extent. Our original VEP results also suggest some post retinal deficiency in amblyopic eyes. From these results we can suggest that the retinal deficiency contributes to the post-retinal defect recorded at the cortex. Differences between our results and those of past studies may be due to differences in the spatial and temporal parameters of the stimulus, electrode types and placement, participant demographics and above all, the depth of amblyopia studied. It is also important to note that some past studies defined their normal control as the fellow "normal" eye[32], while others collected their normative data on a separate normal healthy population[37]. Plus it has been widely suggested that accurate refractive correction and fixation stability must be controlled for in amblyopic subjects to ensure that these factors are not responsible for any reduced responses[37].

It is well reported that electrophysiological recordings commonly use stimuli in the range of 3-20Hz and as high as 100Hz, and that the responses may be contaminated by measurement noise from potential sources such as eye or head movement[50]. A previous study described the signal of interest as being narrowband, while noise sources are broadband generally in the range of 8-12Hz[50]. The broadband noise causes lower signal-to-noise and thus a reduced amplitude measurement of the signal of interest[50]. While we took careful measures to diminish noise by averaging 250 sweeps per result and by ensuring good fixation and repeatable electrode positioning, our ERG and VEP signals may have been contaminated in

certain cases, more so in our amblyopic group due to excessive eye movement artifacts in these participants. Our amblyopic subjects took considerably longer to perform testing due to fixation instabilities and blinking likely associated with increased effort to fuse the stimulus. Given our small sample size, compounded by inherent variability in electrophysiological testing, we propose that added noise caused considerable spillover of our temporal frequencies of interest. For example, a 10Hz stimulus produces a 10Hz narrowband response when free of noise, however when contaminated by significant noise, it can result in a loss of amplitude at 10Hz due to spillover of that signal into surrounding temporal frequency bins. To differentiate it from the noise and ultimately determine if our frequency of interest was present, we performed analysis of adjacent bin frequencies. We calculated signal of a specific frequency by analyzing the power spectrum and calculating the area under the curve by integrating across a range of temporal frequencies – with the two adjacent frequencies. For example, when performing bin analysis on 10Hz signal, we integrated the area under the curve between 9Hz and 11Hz. Given the resolution of our FFT analysis was 2Hz, 9Hz and 11Hz needed to be extrapolated.

We performed this bin analysis in all of our ERG and VEP conditions where we observed any significant differences between groups, specifically at 1cpd 10Hz ERG amplitude at 100% contrast, 4cpd 10Hz ERG amplitude at 100% contrast, and 1cpd 10 Hz VEP amplitude at 100% contrast. There were no significant differences in amplitude between groups at any other contrast level so bin analysis was not applicable under any conditions other than those above.

Figure 40 below shows mean ERG amplitude for all groups stimulated at 1cpd 10Hz, and at 100% contrast, our original data showed a statistically significant difference in amplitude between normal and fellow eyes ($p = 0.012$), but no significant difference between normal and amblyopic eyes, or amblyopic and fellow eyes ($p>0.05$). The original data points at 100% contrast (prior to data processing to account for eye movements) for the amblyopic and fellow eyes are represented in grey. As can be visualized, once we ran our bin analysis on these two points, we observed increased signal amplitude for both points to an extent that there were no significant differences between any of the groups at 100% contrast ($p>0.05$). Our bin analysis suggests that there is no difference in retinal function between normal, amblyopic, and fellow eyes after compensating for signal spillover due to eye movements.

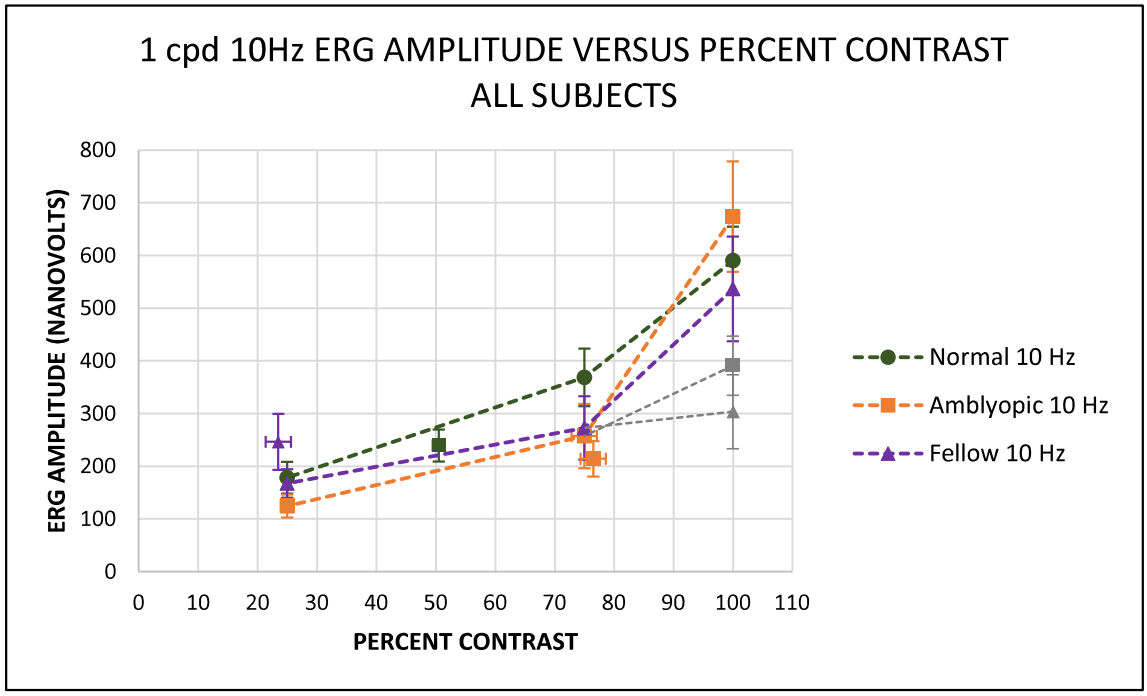


Figure 40: PERG mean amplitude (± 1 SE) as a function of contrast, at 1c/deg spatial frequency, 10Hz temporal frequency, in normal control vs amblyopic vs fellow eyes. The mean data at balance point contrast are shown as single data points. The original data points at 100% contrast - prior to accounting for eye movements - are plotted in grey.

Figure 41 below shows mean ERG amplitude for all groups stimulated at 4cpd 10Hz, and at 100% contrast, our original data demonstrated a statistically significant difference between normal and amblyopic eyes (p = 0.000), normal and fellow eyes (p = 0.003), but no statistical difference between amblyopic and fellow eyes (p>0.05). The original data points at 100% contrast for the amblyopic and fellow eyes are plotted in grey, and then following bin analysis on these two points to account for any signal leakage due to eye movements, we observed increased signal amplitude for both points to an extent that there were no significant differences between any of the groups at 100% contrast (p>0.05). Similar to the above condition, after performing bin analysis to compensate for signal distortion due to eye movements, our data here also suggests that there is no difference in retinal function between normal, amblyopic, and fellow eyes at any contrast level.

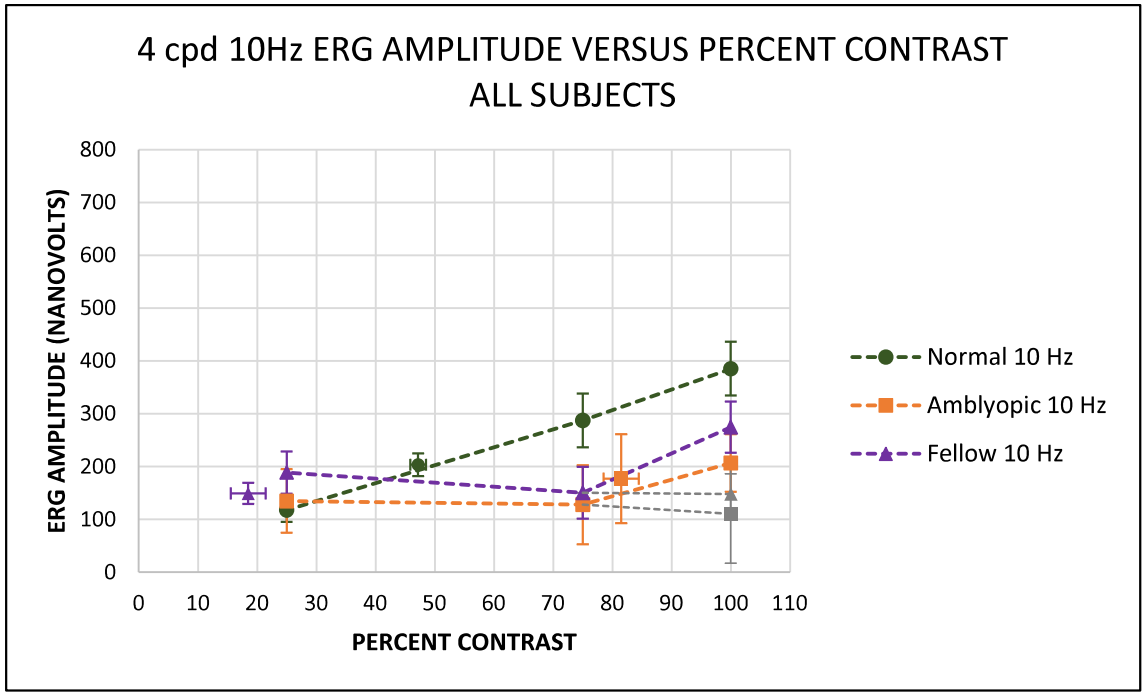


Figure 41: PERG mean amplitude (± 1 SE) as a function of contrast, at 4c/deg spatial frequency, 10Hz temporal frequency, in normal control vs amblyopic vs fellow eyes. The mean data at balance point contrast are shown as single data points. The original data points at 100% contrast - prior to accounting for eye movements - are plotted in grey.

Figure 42 below shows mean VEP amplitude for all groups stimulated at 1cpd 10Hz, and at 100% contrast, our original data revealed a statistically significant difference between normal and amblyopic eyes ($p = 0.004$) and normal and fellow eyes ($p = 0.014$), but otherwise no other statistically significant differences in amplitude between groups at any other contrast level ($p>0.05$). The original data points at 100% contrast for the amblyopic and fellow eyes are plotted in grey, and then following bin analysis on these two points, we observed increased signal amplitude for both points to an extent that there were no significant differences between any of the groups at 100% contrast ($p>0.05$). After performing bin analysis to compensate for signal spillover due to eye movements, our data here suggests that there is no difference in visual pathway function between normal, amblyopic, and fellow eyes at any contrast level.

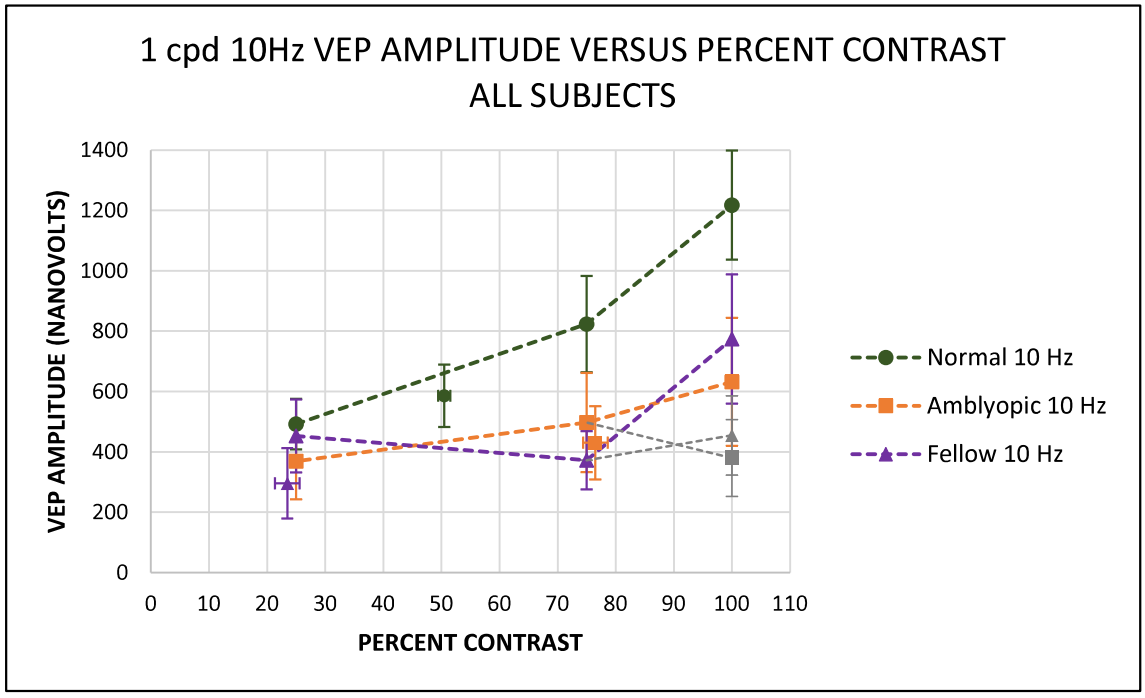


Figure 42: PVEP mean amplitude (± 1 SE) as a function of contrast, at 1c/deg spatial frequency, 10Hz temporal frequency, in normal control vs amblyopic vs fellow eyes. The mean data at balance point contrast are shown as single data points. The original data points at 100% contrast - prior to accounting for eye movements - are plotted in grey.

Figures 43 and 44 below show mean binocular beat VEP amplitude for all groups stimulated at 1cpd and 4cpd, respectively. The mean data at 100% contrast bilaterally are shown as single data points throughout the figures. The original data points (prior to data processing to account for eye movements) for the amblyopes are represented in grey. In figure 43, our amblyopic data (at both fellow 4Hz and fellow 10Hz) showed increased amplitude at the balance point contrast ratio (23.5% contrast presented to the fellow eye, 76.5% contrast presented to the amblyopic eye) compared to the reversed contrast condition (75% contrast presented to the fellow eye, 25% contrast presented to the amblyopic eye), however these differences were not significant. We did not find any significant difference in the binocular beat response measured at balance point compared to the response measured with 100% contrast presented bilaterally where we expected suppression and thus a reduced signal. We suspected that our expected 6Hz signals may have been contaminated by eye movements but after bin analysis across the entire range of contrasts, none of our results changed aside from increased magnitude of our signals.

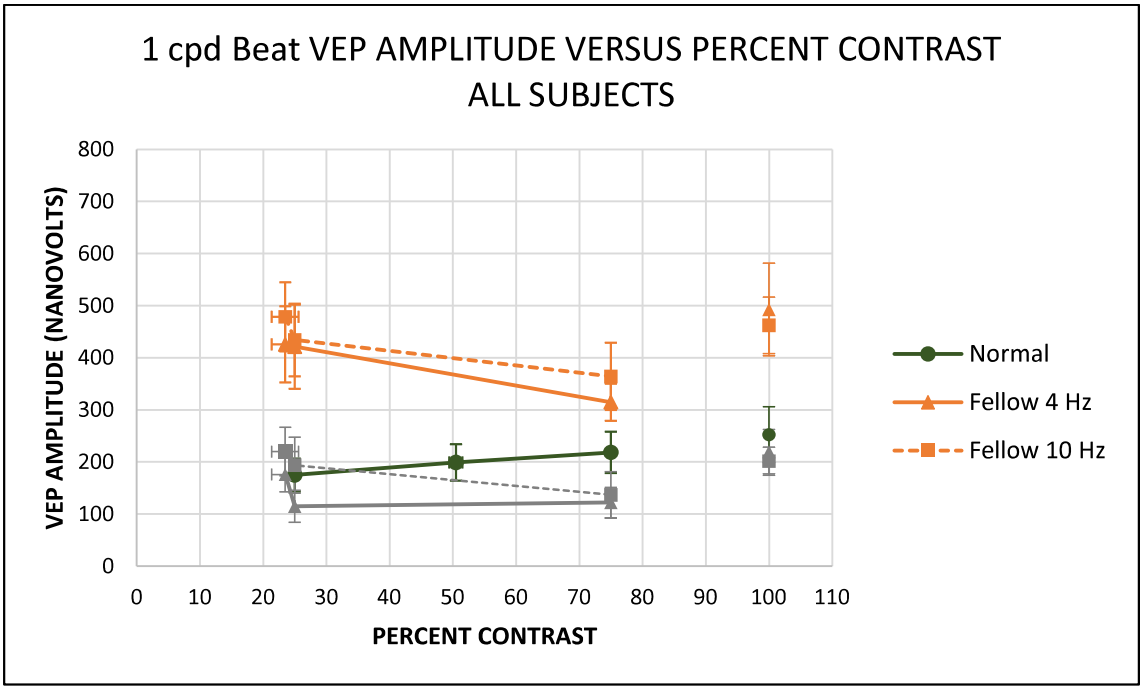


Figure 43: PVEP mean beat (6 Hz) amplitude (± 1 SE) as a function of contrast, at 1c/deg spatial frequency, in normal control vs amblyopic subjects with fellow eye 4Hz stimulation vs amblyopic subjects with fellow eye 10Hz stimulation. The mean data at 100% contrast bilaterally are shown as single data points. The original data points - prior to accounting for eye movements - are plotted in grey.

In figure 44 below, at 4cpd, our original data did not show any increased beat amplitude at balance point contrast. After bin analysis, the balance point contrast showed increased amplitude for 'fellow 10Hz' but it was not significant. Aside from generally increased magnitude of signals, there remained no significant differences in beat VEP amplitude between any contrast conditions within any of the groups ($p>0.05$).

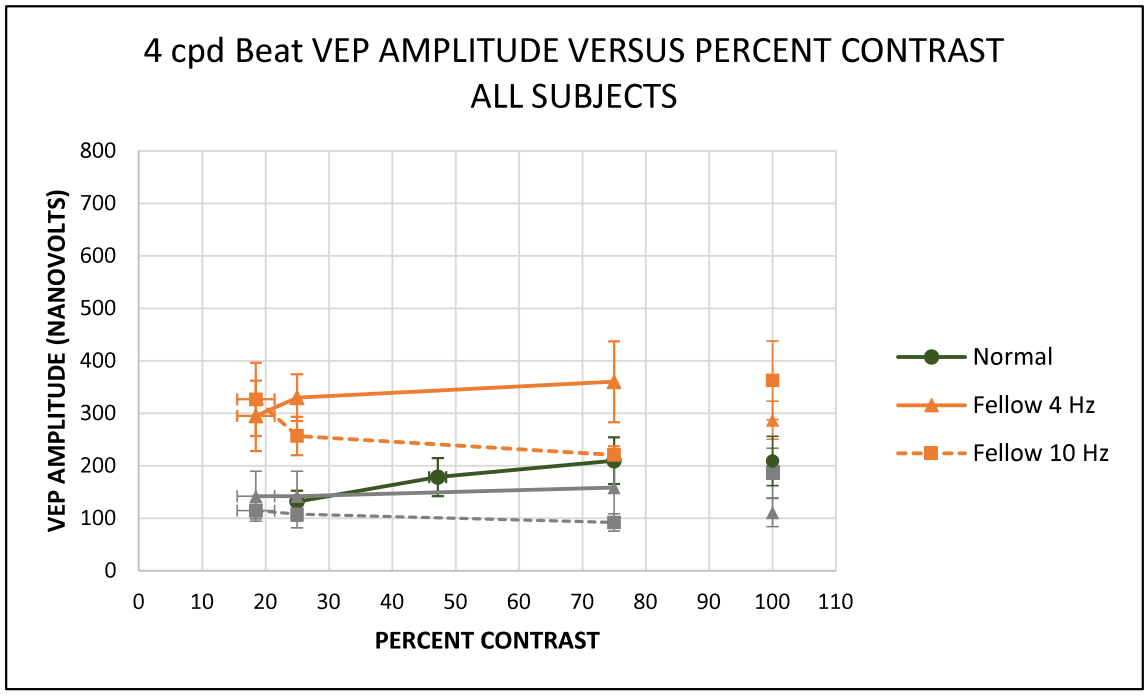


Figure 44: PVEP mean beat (6 Hz) amplitude (± 1 SE) as a function of contrast, at 4c/deg spatial frequency, in normal control vs amblyopic subjects with fellow eye 4Hz stimulation vs amblyopic subjects with fellow eye 10Hz stimulation. The mean data at 100% contrast bilaterally are shown as single data points. The original data points - prior to accounting for eye movements - are plotted in grey.

Once we accounted for eye movements with bin analysis, we observed no ERG, no monocular VEP, and no binocular beat VEP differences between normal versus amblyopic versus fellow eyes in any of our testing conditions. As previously discussed, amblyopic ERG deficiencies reported in the past and in our study can be attributed to unstable fixation, as once eye movements are accounted for, the ERGs normalized. Past VEP studies in amblyopia are less ambiguous, generally suggesting reduced function of the amblyopic visual pathway. While stable fixation is important for all electrophysiogical studies, the literature does not place as

much emphasis on the confounding effect of eye movements on VEPs in amblyopia. The results of our bin analysis suggest normal retinal function and normal visual pathway function in both eyes of the amblyopes recruited to our study. We were expecting to find higher amblyopic deficits at higher spatial frequencies and higher temporal frequencies, however this was not the case. We encountered more difficulty discerning 4Hz signals compared to 10Hz signals, likely because 4Hz is near the low frequency noise level so in some cases might have been washed out by noise lending to its higher variability.

This study provided a framework for assessing retinal function, monocular visual pathway function, and binocular visual pathway function in amblyopes. We did not show any significant retinal or post-retinal functional deficiencies in amblyopic subjects using our methods. A major reason for a lack of difference between our normals and amblyopes may be a result of the depth of amblyopia studied - with 20/40 as the mean amblyopic ETDRS Snellen equivalent best-corrected visual acuity. We did not perform any tests to detect presence of any suppression which would have been useful data to reference when interpreting our binocular beat VEP responses. Our ERG and VEP methods may not have shown adequate sensitivity to detect electrophysiological deficiencies in such mild cases of amblyopia, and our stimulus size may have extended beyond the area of central retina affected. Other studies that have demonstrated such deficiencies tested severe amblyopes in the range of 20/100 or worse visual acuity. With the screening and treatment strategies available today, it was already difficult to find potential research subjects in our population, let alone recruit anisometropic amblyopes with severe visual impairment into our study. With such mild visual impairment, our beat VEP protocol was not able to distinguish any significant differences in binocular cortical activity across the range of contrasts, inclusive of 100% contrast bilaterally, suggesting little to no suppression in our subjects. For this reason, the binocular beat VEP responses did not correlate to the subjective balance point measurements. The contrast ratio that yielded equal perception

between the two eyes of amblyopes did not produce a significantly peaked binocular beat response.

Taking these factors into account, including our limited number of participants, the mild depth of amblyopia studied, the spatial and temporal frequency parameters of our assessments, and the effects of eye movements, we can suggest that the amblyopes recruited to our study demonstrated normal retinal and post-retinal function. Future considerations would be to perform a monoptic (ie. 4Hz and 10Hz stimuli in the same eye) versus dichoptic (as done in this study) VEP recording so as to be able to compare monocular versus binocular cortical function using the same units of measurement, and thus be able to extrapolate binocular summation or suppression in that fashion. We could consider using a different set of spatial or temporal frequencies to increase the sensitivity or temporal resolution of our electrophysiological methods, to stay away from temporal frequencies in the range of low frequency noise, and to avoid having two temporal frequencies so close together (ie. 6Hz and 4Hz) which could contaminate each other. We used a 500ms sweep which resulted in 2Hz step resolution in FFT analysis, and because our study used 4Hz and 6Hz, those frequencies ended up being 1 step away from each other so could share common power. Ideally we could have avoided this and achieved 1Hz step FFT resolution by increasing the sweep time to 1 second and halving the number of sweeps (from 250 to 125) to maintain the same testing time. Increasing the sweep time to 1 second while maintaining the same number of 250 sweeps would have doubled the testing time from 2 to 4 hours which would have proven impractical for the participants. 1Hz step resolution would have also prevented the need to extrapolate the adjacent frequencies when performing our bin analysis. In adult amblyopes, the relationship between loss of acuity, loss of contrast sensitivity, and the reduction in electrophysiology is complex[35]. Increasing the depth of amblyopia would help to identify differences between groups, while increasing sample size would decrease variability seen in the study.

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Appendix

STIMULUS Settings	
Type	Checkerboard
Size	5 degrees width, 15 degrees height
Mode	Reversal
Average luminance	60cd/m^2
Fixation target	Centered red cross
Viewing distance	107 cm
Spatial frequency	1 c/deg, 4 c/deg
Temporal frequency	4 Hz, 10 Hz
Contrast	Variable
Contrast unit	Percent

ACQUISITION Settings	
Sample frequency	1000 Hz
Sweep pre-trigger time	10 ms
Sweep post-trigger time	500 ms
Sweeps per result	250
First sweep delay	0 ms
Inter sweep delay	0 ms
Trigger delay	0 ms
Glitch removal	0 ms
Drift removal	Disabled
Manual rejection of sweeps	Disabled
Reject first sweep always	Enabled
Pause after each sweep	Disabled
Baseline subtract (Auto-zero)	Enabled
Baseline pre-trigger	10 ms
Baseline range	10 ms

CHANNELS Settings	
Filter low frequency cutoff	0.312 Hz
Filter high frequency cutoff	50 Hz
Gain	8
Positive is up on axis	Enabled
Automatic rejection	Enabled: Maximum signal 300uV; Sweep range 100%

Table A1: System settings for electrophysiological recordings

```
dAmp = []; % holds amp for each trialFFTamp
dPhz = []; % holds phz for each trial
dInterAmp = [];
%ctr = 1;
x = (0:499)';
for col = 1 : 1 : columns

y = data(:, col);
%hold on;

[xData, yData] = prepareCurveData(x, y);
% Set up fittype and options.
ft = 'linearinterp';
% Fit model to data.'
[fitresult, gof] = fit( xData, yData, ft );

InterAmp = fitresult(0:0.9765625:499);

Fs = 1024;                    % Sampling frequency
T = 1/Fs;                     % Sample time
L = length(InterAmp);         % Length of signal
t = (0:L-1)*T;                % Time vector
NFFT = 2^nextpow2(L);       % Next power of 2 from length of y
Y = fft(y,NFFT)/L;
FFTfreq = transpose(Fs/2*linspace(0,1,NFFT/2+1));

FFTamp = 2*abs(Y(1:NFFT/2+1));
FFTphase = angle(Y(1:NFFT/2+1));
```

Figure A1: Sample MATLAB code of FFT transform to access amplitude and phase