Understanding the Mechanisms Underlying Brain Plasticity in Adult Humans

by

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Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

Purpose

The human brain changes significantly with age. The plasticity of the visual cortex is thought to decrease into adulthood while childrens' brains are highly plastic. This change in plasticity is thought to be due, in part, to an inhibitory neurotransmitter known as gamma-aminobutyric acid (GABA). Recent research has established a general increase in GABA levels from childhood into adulthood, thought to be associated, in part, with the decrease in plasticity. It is unclear, however, whether GABA levels affect the changes in plasticity that occur from young adulthood into older age. In older age, a further decrease in GABA levels has been suggested. The purpose of this thesis is threefold: (1) To implement a strategy for inducing long-term potentiation (LTP), (2) to understand the associations between psychophysical and physiological measures of neuroplasticity within the primary visual cortex, and (3) to assess the effect of age on both measures. We hypothesize that as plasticity continues to decline into older age, GABA levels will continue to increase.

Methods

Binocular rivalry alternation rates (ARs) were used as a behavioural measure of cortical GABA levels. A dichoptic presentation using red/green glasses was displayed on an Asus 3D Vision Ready monitor. Young (18-40 years) and older (60-80 years) participants wearing red/green glasses indicated whether they perceived the red grating, green grating, or a mix of the two–referred to as piecemeal–using 3 keys. Visually-evoked potentials (VEPs) were then used as a measure of the change in plasticity following a rapid onset/offset

checkerboard stimulus thought to induce LTP within the primary visual cortex. VEPs before and after the inducing stimulus were recorded and compared.

Results

ARs were significantly slower in older adults compared to the young adults. Pre to post waveform amplitudes had relatively lower LTP in the young adults compared to the older adults; however neither group showed significant LTP ($p>0.05$ for main effect of pre versus post VEP amplitude).

Conclusions

No correlation between AR and LTP was observed. AR was slower in older adults than in young adults. LTP was relatively greater in older adults compared with the young adult group. A decrease in GABA levels with older age, as most studies have found, alludes to an increase in ARs; however this was not the case in the present results. In contrast, while slower ARs suggests an increase in GABA levels, LTP was relatively greater in older adults suggesting a decrease in GABA levels. These data indicate that either AR or LTP, or both AR and LTP, are inadequate measures of GABA concentration or inaccurate measures of plasticity, or that GABA and LTP may not be directly related in the tested sample. Changes in neurotransmitter concentrations with age may lead to neural adaptations that alter the response to both rivalry and LTP in unexpected ways.

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Lastly but certainly not least, thank you to my loving family for their everlasting support and encouragement.

Dedication

I lovingly dedicate this thesis to the one who made me who I am today.

Thank you for always believing in me.

To my late uncle, you are my motivation. May this and all that follows make you proud.

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Chapter 1

Introduction

Human characteristics change remarkably over a lifetime. Our daily experiences shape and alter our behaviour, thoughts and overall personality. Contrary to previous findings, however, there is minimal change in the number or density of neurons within most areas of the brain following early childhood development; rather the changes in thoughts, behaviours and memories are more largely dependent on the reformation of neural branching and the brain's ability to alter its neural connections[1]. With time and experience, the branching of neurons is either strengthened or weakened depending on the use of the neurons involved in a particular pathway. The malleability of the neuronal circuits of the brain is referred to as neural plasticity[2]. The primary visual cortex, like other sensory areas of the brain, relies largely on early childhood experiences for proper structural and functional development. Abnormalities in development, such as minimal or lack of visual input, can result in impaired or loss of function of essential synaptic circuitry. Additionally, changes in neural function involving behaviour, memory and movement can occur due to disease

or brain injury over a lifetime. Such injuries to the brain currently have limited treatment opportunities with the belief that the adult brain is unable to change significantly.

As in many areas of the brain, the visual cortex experiences a phase of increased plasticity throughout early childhood development known as the critical period. While in some cortical areas, such as the motor cortex, there is evidence for substantial reorganization following damage to the brain from disorders such as stroke[3,4], it is more difficult for such changes to occur within the primary visual cortex[5–7]. This is associated with reduced plasticity following the closure of the critical period, which is thought to be more clearly defined than in other areas of the brain[5,8]. Insufficient visual imput to one eye throughout the critical period results in a shift in ocular dominance to the unaffected eye, causing reduced vision, or amblyopia, in one eye due to abnormal cortical processing. Research advances in understanding the mechanisms that underlie visual cortex plasticity demonstrate that changes to the brain can theoretically be enhanced in adults, providing new directions in possible treatments and neurorehabilitation[9–12].

Hebbian plasticity, named after the psychologist Donald Hebb, is a theory describing a mechanism of synaptic plasticity that reflects changes in synaptic strength. The changes result from specific patterns of neural activity and are believed to play a key role in the process of learning[2,13,14]. The theory states that repetition of a neural function or correlated input to a particular synapse will strengthen the synapses and increase communication between the neurons involved in that particular circuit–an idea often summarized with the phrase "neurons that fire together, wire together"[2,13]. For instance, correlated neural activity will strengthen the synapse between those two neurons[2]. Conversely, decorrelated activity can, in turn, weaken synapses[2].

One fundamental synaptic strengthening mechanism is known as long-term potentiation, or LTP. LTP is a phenomenon that alters the strength of synaptic connections and is thought to underlie the processes of learning and memory formation and can be induced through visual stimulation[15,16]. As with learning and memory formation, the ability to make changes in synaptic strength between neurons varies with age[2,5]. As the brain matures, it becomes increasingly more difficult for existing connections to strengthen or for new connections to form, particularly in the primary visual cortex–a result thought to be caused, in part, by the increase of the neurotransmitter known as gamma-aminobutyric acid (GABA)[2,5,17,18]. Consequently, we aim to understand the correlation between LTP induction and GABA levels in humans to provide a foundation for understanding the mechanisms that underlie brain plasticity in adults.

1.1 Overview of the Visual Pathway

The development of the visual system, as well as other neural functions, is dependent on early experience and input from the surrounding environment. Frequent and diverse visual stimuli are essential to ensure proper functionality of visual areas within the brain. The eye is the light-sensitive organ that converts the energy of a photon into an electrical signal and transmits the signal through the optic nerve to the primary visual cortex and associated areas. The process of transforming, interpreting and understanding a visual input from the environment is known as perception.

A visual stimulus must first be focused on the posterior layer of the eye–known as the retina–before it can be processed in the brain. The photoreceptors of the retina capture

the light entering the eye while the retinal ganglion cells (RGCs) encode features of the visual stimulus such as colour and contrast[19]. The majority of this information (from >90% of ganglion cells) is then conducted towards the brain in three primary pathways: the parvocellular (P) pathway, the magnocellular (M) pathway and the koniocellular (K) pathway[20–23]. Each pathway is responsible for encoding specific features of the stimulus, which are processed first at the lateral geniculate nucleus (LGN) of the thalamus, then further in the primary visual cortex and other associated areas[19].

Information from the P, M and K pathways remain separated in the LGN. The P pathway carries information about colour and is sensitive to high spatial frequencies and low temporal frequencies[22–24]. Conversely, the M pathway carries information regarding luminance and contrast, and is sensitive to low spatial frequencies, high temporal frequencies, as well as motion[21–23]. The K pathway seems to be more heterogeneous than the other two and is not fully understood[22–24]. All three pathways form synapses in the LGN onto distinct groups of neurons that continue to the primary visual cortex.

The organization of visual input continues into the brain, forming not only distinct pathways of information but also separating input from each from each eye[22–24]. The representations of each eye within the visual cortex are known as ocular dominance columns and are thought to play a role in binocular vision[22–24]. Early studies in cortical plasticity found that monocular deprivation during development, simulating amblyopia–a neurodevelopmental disorder in which early visual deprivation in one of both eyes results in reduced visual function due to abnormal visual cortex development–resulted in the degradation of this pattern of organization. This causes a shift in visual input preference from one eye to the other, known as ocular dominance plasticity[25]. The development of the visual cortex

and normal binocular vision is therefore dependent largely on equal visual input to both eyes.

1.1.1 Processing of the Extrastriate Cortex

The primary visual cortex contributes to two functionally distinct pathways known as the dorsal stream and the ventral stream. The P and M pathways provide input to each of the major streams within the primary visual cortex. The dorsal stream processes spatial information and motion perception and receives information primarily from the M pathway[22,26,27]. The ventral stream, which includes area V2 and V4 of the extrastriate cortex, is responsible for identifying information relating to form perception and receives information from the P pathway[22,26,27]. The separate streams of information are often referred to as the "where" and the "what" pathways, respectively[27].

The development of these two visual streams has been studied extensively to understand the functional and structural consequences of atypical development of the visual system. Research on the rate of development of each system presents contradicting results with some studies, demonstrating later ventral visual stream development and others indicating the opposite[27]. Nonetheless, stimulation of the visual system in the early stages of life is essential to ensure proper functionality in later years and throughout adulthood.

1.2 Neuroplasticity and the Critical Period

The ease and extent of changes within the brain are more pronounced throughout early childhood development[8,28]. Increases in neuroplasticity known as critical periods occur in many regions of the brain and at various times throughout early childhood development[29,30]. Sensory stimulation during these relatively short periods is essential to the proper development of the neuronal connections of a particular function within the brain[5,30]. Studies with rats and kittens, as well as monkeys, show shifts in ocular dominance following periods of monocular deprivation during the early stages of development[31–33]. Deprivation outside the critical period, however, has little to no effect on ocular dominance cortical processing[31,32]. Although neural changes occur following the closure of the critical period, the primary visual cortex becomes less plastic. Therefore, the development and functionality of the ocular dominance columns within the brain is experience-dependent.

The critical period for visual acuity and ocular dominance in humans is conventionally thought to close around the age of 7 years[34], when the plasticity of the primary and extrastriate visual cortex steadily declines[35,36]. In other words, the brain's potential to restructure its connections in the visual cortex becomes increasingly more difficult throughout childhood and later years[1]. In the past, developmental and neurological vision disorders, such as amblyopia, were deemed permanent if not treated during the critical period of brain development, due to the presumed lack of plasticity in the adult brain[1]. However, recent findings demonstrate that while the opening of the critical period is relatively sudden, the closure is rather gradual and incomplete, indicating that changes can in fact be made

within the adult brain[1].

1.2.1 Mechanisms of Neuroplasticity

1.2.1.1 Visually-Evoked Potentials (VEPs)

Visually evoked potentials (VEPs) are an event-related potential induced by a visual stimulus and recorded from an electro-encephalogram (EEG)[37]. VEPs are a common technique used to measure visual cortex activity in humans[37]. Recent studies have used changes in VEP waveform amplitudes to assess changes in primary visual cortex plasticity[16,38].

A brief appearance of a high contrast image–most commonly a checkerboard–produces a specific pattern of negative and positive waveform components. Pattern reversal waveforms, where the black and white checks reverse positions, and pattern onset waveforms, where a static checkerboard is presented on and off periodically, are two common methods of presenting a pattern stimulus due to having more inter-subject reliability than other methods[37].

1.2.1.1.1 Typical VEP Waveforms

Different recording methods and stimulus properties result in different VEP waveforms. Additionally, waveforms can vary within and between populations. Typical pattern onset waveforms in response to a pattern flicker separated by a luminance-matched blank screen look like figure 1.1–a primary positive component approximately 90ms after the stimulus appears (C1), a primary negative component at approximately 120ms (C2) and a second

positive component at approximately 180ms (C3) (figure 1.1)[37]. Pattern reversal waveforms look similar to figure 1.2–a primary negative component approximately 80ms after the stimulus appears (N80), a primary positive component at approximately 100ms (P100) and a second negative component at approximately 135ms (N135) (figure 1.2)[37]. These expected waveforms provide a measure of abnormalities within the visual pathway and can be used to diagnose and assess retinal and neural pathologies[37].

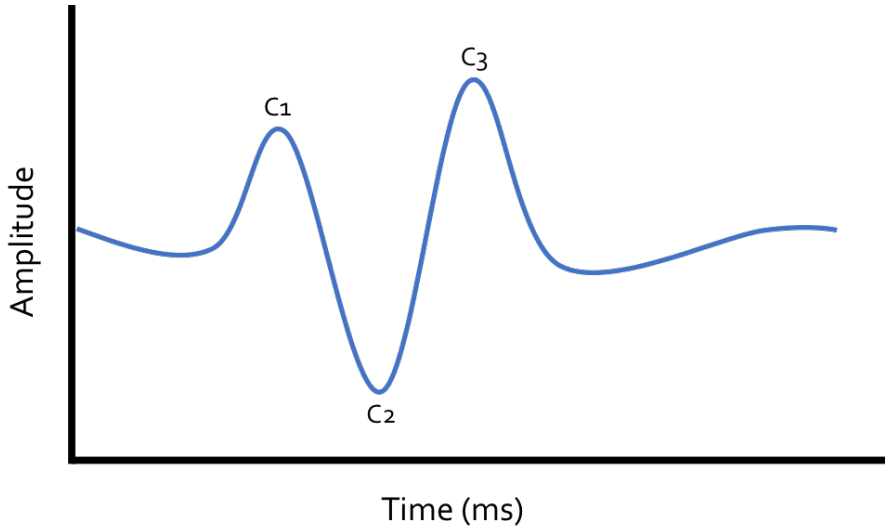

Figure 1.1: A schematic representation of a waveform in response to a pattern onset checkerboard stimulus.

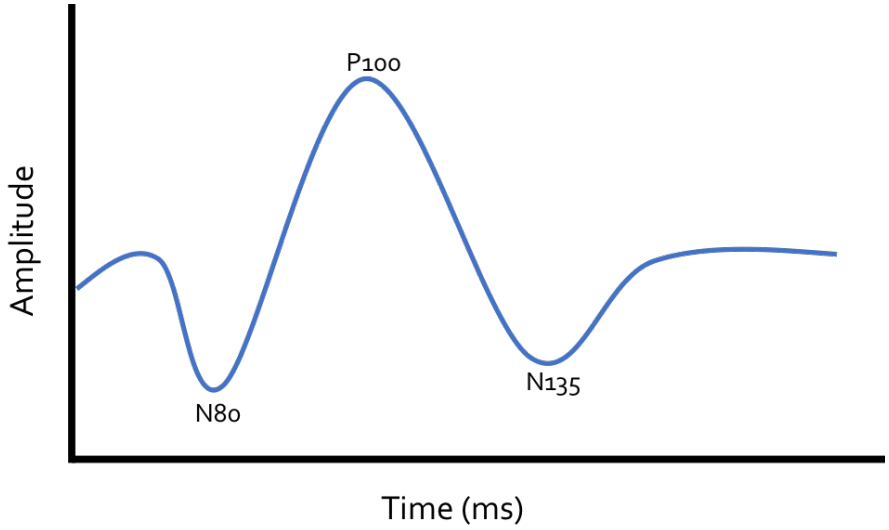

Figure 1.2: A schematic representation of a waveform in response to a pattern reversal checkerboard stimulus.

Factors such as biological sex and age may affect VEP waveforms. Studies have demonstrated that female waveforms are more variable, possibly due to hormonal changes[39], while others have demonstrated changes in component latency with age[37]. Theoretically, repetition of a high contrast stimulus may affect the amplitudes of some VEP components indicating an increase or decrease in plasticity compared to baseline measures[16,40]. This technique is based on the phenomenon known as long-term potentiation.

1.2.1.2 Long Term Potentiation and Plasticity

Long-term potentiation (LTP) is the activity-dependent process of strengthening and increasing communication between synapses[16,41–43]. This phenomenon is central to the process of learning and memory formation and is primarily associated with the excitatory neurotransmitter glutamate as well as the -amino-3-hydroxy-5-methyl-4-isoxazole propionate

(AMPA) and the N-methyl D-aspartate (NMDA) glutamate receptors[16,44]. An increase of glutamate in the synapse after repeated stimulation of a neuron will result in a cascade of events, gradually leading to an increase of AMPA receptors as well as additional synapses between the two neurons[45]. Therefore, LTP strengthens the communication between the neurons and alters the processing of information within the brain. In the past, LTP responses have been observed in animal models[16,46,47] and isolated human cortical tissue by electrically stimulating the tissue and recording evoked potentials and intracellular excitatory post synaptic potentials (EPSPs)[48,49]. Additionally, noninvasive techniques such as transcranial magnetic stimulation (TMS) have been used to induce LTP in humans[15,50,51]. More recently, visual or auditory stimuli have been found to induce an LTP-like response in humans as measured by changes in evoked potential amplitude as well as by activity increases shown using brain imaging techniques[16,41,50]. The complex relationship between LTP and adaptation is still being investigated, however it is believed that adaptation stabilizes neuronal circuits rather than counteract the effects of LTP induction[52]. For example, viewing rapidly contrast reversing visual stimuli increases the magnitude of VEPs extracted from EEG[38,51,53]. This effect has been linked to the induction of long-term potentiation as effects are stimulus specific[16,38,41,54,55]. For instance, LTP of a horizontally-oriented stimulus will show effects for that same orientation and no effects for vertical stimuli[55,56]. The change in VEP amplitude provides an electrophysiological index of visual cortex plasticity[38,51].

1.2.1.2.1 VEP Stimuli Parameters

Since high contrast patterned visual stimuli provide information about visual resolution and elicit more consistent waveforms across individuals compared to unpatterened (flash) stimuli, a checkerboard is the most commonly used stimulus to produce reliable and consistent results[57]. Checkerboards have been used in most studies that test the ability of a visual stimulus to produce an LTP-like response[16,38,42,51,58–61]. To elicit LTP, the onset/offset or contrast reversal rate is either set to a high frequency for a short period of time[16] or a constant slow frequency for a longer period of time[38]. Regardless of the method used to measure the LTP-like response, each demonstrated VEP potentiation following the induction of LTP as compared to the baseline recordings–typically taken at a frequency of 1Hz[16,42,51,55,59–61]. The two primary papers for this approach vary slightly in method and results[16,38].

1.2.1.2.2 The LTP of VEPs by Teyler et. al.[16]

Teyler and colleagues were the first to demonstrate that rapid stimulation by a visual stimulus–what they called photic tetanus–resulted in an increase in VEP amplitude[16]. A checkerboard (0.3 degree checksize, 4 degrees of visual angle) was presented to one half of the visual field, either right or left. This hemifield pattern allowed for one hemisphere to act as a control in comparison to the condition following the photic tetanus. A pattern onset flicker of the hemifield checkerboard was presented at a frequency of 1Hz for 7 minutes. The right and left hemifield checkerboards were randomly and equally presented throughout the pre and post measure. The photic tetanus condition was randomly chosen to be a left or

right hemifield checkerboard flickering at a frequency of 9Hz for 2 minutes. The other hemisphere was used as a control. Participants were asked to close their eyes for 2 minutes immediately following the photic tetanus and before the post measure.

A 128-electrode cap was used to measure waveforms. A dipole of 7 channels centered around parietal electrodes P7 and P8–positions used for the 10-20 system for electrode placement–provided the largest signal. Significant increases in amplitude in the post-tetanus measure were found in the N2 component of the hemisphere ipsilateral to the checkerboard hemifield as compared to the control hemisphere.

1.2.1.2.3 The LTP of VEPS by Normann et. al.[38]

As compared with the above protocol, Normann et. al. used a full-field pattern reversal checkerboard stimulus (0.3 degree check size) reversing at a frequency of 2 reversals per second (rps) (1Hz) for 20 seconds as a pre and post measure. There were two conditions. In the first condition, the checkerboard reversed at 19rps (9.5Hz) for 10 minutes. In the control condition, the checkerboard reversed at 2rps, just as in the pre and post measures for 10 minutes. Unexpectedly, the control condition showed a more pronounced potentiation effect than did 10 minutes at 19rps. Between stimulations, participants were asked to read out numbers presented on a grey screen for 2 minutes. The centre electrode Oz was analyzed. Significant potentiation in the N135 component and a possible depotentiation in the P100 component was found.

1.2.1.2.4 Factors Influencing LTP

The nature and permanence of LTP has been investigated extensively in recent years. It was found that this LTP-like effect does not diminish over time in the absence of visual stimulation, but only when it is depotentiated with the baseline frequency[16,59,62]. For example, the LTP-like effect after the photic tetanus will depotentiate only after the participant repeatedly views the baseline stimulus at 1Hz[16,60]. In other words, a slow baseline following the photic tetanus will depotentiate the response. Although the experiment lasted only a few hours, theoretically, the changes that occur therefore remain unless otherwise transformed. This demonstrates a possibility for long-lasting changes in the adult brain.

In the following years, sinusoidal gratings, both vertical and horizontal, were used instead of checkerboards to illustrate the specificity of the LTP-like response. Vertical gratings flickering for two minutes resulted in a potentiated response to vertical gratings, but no effect was seen for horizontal gratings signifying orientation specificity[53,55]. Although it requires further investigation, the technique of inducing LTP through visual stimulation provides an objective measure of visual cortex plasticity.

Most studies used a 64 channel EEG system and analysed LTP by reducing the surface amplitude data to equivalent dipoles. However, clinical VEP systems typically have only 1 to 4 channels and the possibility of using clinical systems to evaluate LTP and techniques to optimise LTP for use in clinical settings are currently unknown. Optimizing the methodology and protocol for practical and clinical use is necessary for further implications in future studies. Teyler and Normann's protocols were replicated and modified by several other labs which reported similar results as well as expanded on the effects and implica-

tions of inducing LTP using visual stimuli[15,51,55,56,58]. Although it is difficult to compare between the two studies as they differ significantly, optimizing the method for clinical use is yet to be done. A quick, reliable and objective measure using VEPs as a measure of plasticity is critical if it is to be used on clinical patients or as treatment in the future.

Recent research shows that the closure of critical periods, from infancy into childhood, is, in part, due to an increase in inhibition mediated by the inhibitory neurotransmitter gamma-aminobutyric acid, or GABA[8,28]. This change in GABA levels has been seen in many areas of the brain including the somatic sensory, motor, auditory and visual cortex[63]. GABAergic neurons are the major supply of inhibitory neurotransmitters in the brain and play a primary role in the organization of the cerebral cortex. The initial development of GABA within the brain is also an essential component for the beginning of the critical period[64]. In other words, a gradual onset of GABA levels is thought to open the critical periods while further increases with time will close them; however the relative levels of GABA as compared to adults remain low[8,11]. Consequently, animal studies have shown that inhibiting GABA in young adults is crucial for enhancing developmental plasticity and controlling the critical period of brain development[8,11]. One study found that reduction of GABA via pharmacological intracortical infusion of 3-mercaptopropionic acid (MPA), a drug known to reduce GABA concentration within the brain, reactivates and induces visual cortex plasticity in young adult rats[11]. Although invasive, this provides insight into the role that GABA has in neuroplasticity of the primary visual cortex following the closure of the critical period. Similarly, inhibiting GABA production through gene-targeted disruption was found to prevent the atypical development of the visual cortex that occurs following brief deprivation in a mouse model[64].

Generally, GABA levels are thought to increase from early childhood development into young adulthood. In contrast, GABA levels into older adulthood seemingly decline in the primary visual cortex as well as other areas and are thought to be a cause for the visual degradation that may occur with age[65,66]. The effect that this natural decline in GABA has on visual cortex plasticity is currently unknown. Previous psychophysical studies with humans have reported a decrease in alternation rate as well as stronger suppression with age[40,67]. These differences that occur with age were potentially explained by a decrease in inhibition with age, allowing for stronger and longer percept durations during rivalry[40]. It is understood that GABA levels increase from early childhood development into young adulthood. However, a decline in GABA levels from young adulthood into older age is thought to be associated with the cognitive decline that occurs in older adulthood. This seemingly contradicting trend is a motivating factor for understanding the effect of age on GABA levels. Evidently, measuring and assessing intracortical GABA levels as well as understanding its effect on the critical period and plasticity has been a focus of recent research as results may lead to promoting the recovery of abnormal visual development as well as the effects of brain damage.

1.2.1.2.5 Assessing GABA Levels

1.2.1.2.5.1 Animals Measuring GABA levels in vivo has been achieved with various techniques within animal models. For instance, one group established that high-performance liquid chromatography (HPLC) is able to measure very low levels of GABA allowing for detection of small changes in a rat's cerebral cortex[68]. Another technique, although low in spatial resolution, is single-photon emission computed tomography (SPECT)[69].

While a number of invasive techniques exist to quantify and inhibit GABA with animal models, none are applicable to humans and therefore other methods are required.

1.2.1.2.5.2 Humans GABA levels in humans have typically been quantified using imaging techniques such as magnetic resonance spectroscopy (MRS)[18,70–72]. MRS is a technique used to assess the biochemical composition of the brain as well as other organs using signals from hydrogen molecules and protons[73]. The concentration of specific chemicals within the brain can be used to evaluate physiological changes related to disorders such as stroke, Alzheimer's disease and Parkinson's disease[73]. To measure the effect of GABA concentration on brain structure and function, studies have used specific drugs that alter GABA levels. For instance, maintaining or increasing GABA levels has effects on some epileptic disorders[74], while inhibiting GABA has shown increases in neuroplasticity[6,64]. Although using MRS to determine the concentration of GABA levels in vivo is less invasive than those techniques used on animal models, it requires expensive scanners and specialist expertise. Other indirect measures are also possible (see below).

1.2.1.2.6 GABA and Age

GABA inhibition within the primary visual cortex is thought to play a role in the closure of the critical period, reducing plasticity as the brain matures. Conversely, studies have shown that GABA levels within some regions of the brain in fact decline with age[65,75,76]. One study used MRS to measure intracortical GABA levels specifically the frontal and parietal regions of tha brain in adults between the ages of 20 and 76. Results showed a significant decrease in GABA levels with age, consistent with previous animal studies[75].

Several studies have also shown that the decrease in GABA levels may underly some age-related cognitive decline[77–79]. It is still unclear, however, what the effect of age is on GABA levels within the primary visual cortex and the impact it has on visual processing and neural plasticity.

1.2.1.2.7 Binocular Rivalry as a Behavioural Measure of GABA

Lower levels of GABA, measured using MRS, have recently been shown to correlate with a faster alternation rate for a phenomenon known as binocular rivalry (Rho = 0.506)[18]. Consequently, binocular rivalry, specifically alternation rates, may provide a behavioural measure of cortical GABA levels. Binocular rivalry is a phenomenon that occurs when two different images are presented to each eye simultaneously. The images are alternately suppressed as one image dominates at a time[80]. The frequency of switching from one image to the other is known as the alternation rate. This is thought to be a result of a balance of excitation and inhibition in the visual cortex. The mechanism of binocular rivalry is not yet understood; however it is thought to encompass higher and lower level areas of the visual pathway[81]. The fluctuations that occur throughout the visual pathway result in a bistable perception of the rivalrous images.

The concentration of GABA in children is expected to be relatively lower as compared to adults, thought to be correlated with higher plasticity[18]. Consistent with the theory, children have faster alternation rates than do adults[82–84]. Following the critical period, GABA levels seemingly increase slightly resulting in relatively reduced plasticity as compared to the critical period. However, interestingly enough, a reduction in GABA levels

with older age has been found to correlate with perceptual changes[40,75]. For instance, a decrease in GABA levels have been correlated to an age-related decline in 3D shape discrimination[40]. The effect of naturally reducing GABA levels with age on visual cortex plasticity is unknown. This raises the intriguing possibility that older adults may show enhanced LTP compared to younger adults. Alternation rates provide an indirect measure to assess the neural changes that occur with age.

1.3 Summary

The development of the visual system is a delicate and time sensitive occurrence. If the brain is not stimulated properly throughout early childhood development, the structure and functionality of the brain will be affected. Neurodevelopmental disorders become increasingly difficult to treat in adulthood due to the decline in neuroplasticity with age. However, investigating the mechanisms underlying plasticity in the adult brain can provide insight into more effective treatments for neurorehabilitation in the future. By bringing together two different techniques, namely binocular rivalry and LTP, the question of whether GABA levels influence visual cortex plasticity in adult humans can be addressed. GABA and measures of plasticity can therefore be used to understand the changes that occur from early life to adulthood.

Chapter 2

Rationale and Objectives

Throughout early childhood development, the brain is highly plastic and develops rapidly[8,28]. While the rate of neuronal change within specific regions of the brain is particularly high during the critical period, the plasticity of the brain steadily declines into adulthood. Although it was once thought that neuroplasticity is minimal after the closure of the critical period, recent research has proved that the brain does in fact have the potential to change, strengthen connections as well as form new ones well into adulthood, albeit at a slower rate[1]. These findings have changed the perception that developmental and neurological disorders are permanent if not treated during the critical period of brain development. Understanding the mechanisms involved in the high rates of plasticity in children as opposed to the slower changes in the adult brain allows for the possibility of treatments for adult patients with disorders such as amblyopia. Recent findings demonstrate that neuroplasticity can in fact be assessed within adults by eliciting the synaptic mechanism of long-term potentiation. Long-term potentiation (LTP), the process

of strengthening synapses by persistent repetition of a particular activity, is fundamental to the process of learning and memory formation[16].Additionally, significant efforts have been made to understand the role of neurochemicals in the varying rates of plasticity in the human brain. For instance, the age-related decline in plasticity has been attributed, in part, to an increase in inhibition mediated by the inhibitory neurotransmitter GABA[8,28]. Studies have shown that inhibiting the effects of GABA is essential for inducing developmental plasticity and controlling the critical period of brain development[8]. In other words, the reduction of GABA may stimulate plasticity in the adult brain.

The objectives of this thesis are:

1. To identify the best method of LTP induction using visually-evoked potentials (VEPs) in humans.

2. To investigate whether or not a relationship exists between GABA levels, assessed indirectly using binocular rivalry alternation rates, and the extent of LTP induction in the human visual cortex.

3. To study the effect that age (young adulthood vs. older adulthood) may have on alternation rates and LTP.

Chapter 3

General Methods

3.1 Experimental Design

The study is divided into pilot studies and a main study. In the pilot studies, stimulus paradigms were investigated. The most optimal protocol was chosen for the main study–a cross sectional comparison between two distinct age groups. In this chapter, the strategies, instruments and techniques used are explained. The following chapters provide more details that are specific to each experiment.

3.2 Psychophysical Measure of Alternation Rates with Binocular Rivalry

3.2.1 Equipment

Binocular rivalry was induced by presenting red and green orthogonally oriented sinusoidal gratings separately to each eye. Gratings had spatial frequencies of 0.5, 1, 1.5 or 2 cycles per degree (cpd). The stimulus–programmed using Matlab–were round, subtending 6.1 degrees of the visual angle on a black background. The viewing distance was 60cm. The red and green colour of the stimulus was calibrated based on the red/green gel of the glasses being used. A black fixation cross was in the centre of each grating.

3.2.2 Protocol

The psychophysical measure comprised of 6 to 10 trials. Each trial consisted of the stimulus being presented for 60 seconds. Participants, while wearing the red/green glasses, were asked to indicate whether they were seeing the red grating, the green grating, or a mix of the two (known as piecemeal) using the three indicated keyboard presses and to press the key for the whole duration that they saw the percept. Piecemeal was defined as seeing equal perepts at the same time, or seeing a mixture of red and green (yellow). Participants were given an optional break between each trial.

3.3 Electrophysiological Measures

3.3.1 Equipment

Visually evoked potentials (VEPs) were measured using a clinical-grade EEG system (Espion E2 electrophysiology testing system version 5.2) available within the Optometry Clinic at the School of Optometry and Vision Science. Five Ag/AgCl or silver cup electrodes were placed on a participant using the 10/20 system–an international system of scalp electrode placement for electroencephalogram (EEG) experiments[85]. Three electrodes were placed at the back of the head, specifically at Oz–10% above the inion, measured based on the distance from the inion to the nasion–and either 10% (PO7 and PO8) or 15% (P7 and P8) lateral to Oz on either side, measured based on the circumference of the head. A reference electrode was placed 30% above the nasion at Fz (figure 3.1). A ground electrode was placed on the right ear. The scalp and ear areas where electrodes were to be placed were cleaned using NuPrep Skin Prep Gel to reduce impedance and improve conductivity. Electrodes were placed on the scalp using Ten20 Conductive Paste. Scalp areas were cleaned after the experiment using alcohol swabs.

Three active channels were monitored on the system. Recording was synchronised to the pattern change (reversal or onset). Records of individual presentations (sweeps) were averaged. Sweeps with artifacts such as blinks or other high voltage signals were rejected from the average. Averaged waveforms are called steps' and 3 steps were averaged for an overall pre and post measure separately. Waveforms were sampled at a frequency of 1000Hz with band pass filters of 0.3 to 100 Hz as set in the clinical system.

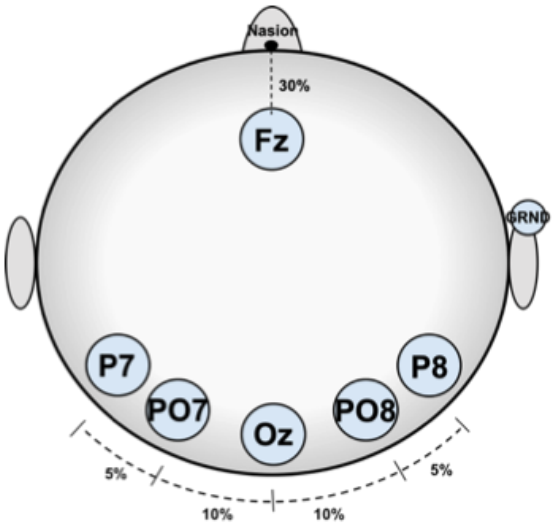

Figure 3.1: Electrode placement for electrophysiological measure.

3.3.2 Stimuli

High contrast black and white patterns were presented on a screen with a visual angle of 7.7 degrees. Patterns were checkerboards that contained equal areas of black and white and were either phase reversed or presented as onset/offset from a luminance-matched grey background so that pattern changes did not result in change of the overall luminance of the screen. There is a range of used stimuli such as vertical and horizontal gratings; however, checkerboards are most commonly used. The stimulus was presented on a 60Hz 20" CRT screen at a 1.5-meter viewing distance. Participants were asked to fixate on a red cross in the centre of the screen with correction for distance as needed. The experiment was performed with no room lighting. The Espion E2 monitor was the control monitor and was turned away from the participant to eliminate any light distractions.

3.3.3 Protocol

All VEP testing consisted of an initial stimulus as a pre measure, a tetanizing stimulus– a stimulus that induces LTP–followed by a repeat of the initial stimulus as a post measure. After the tetanizing stimulus, participants were asked to close their eyes for 2 minutes before the post measure was recorded. The sweeps for the pre and post measures were averaged separately. The tetanizing waveforms were not analyzed.

3.3.4 Summary

Methods and protocols were chosen based on recent literature regarding GABA levels and sensory-induced LTP[16,38]. Pilot data was first collected in order to determine the most optimal stimulus paradigms and protocol for the main study.

Chapter 4

Pilot Studies

4.1 Studies of Binocular Rivalry

Data were collected within the lab to determine behavioural differences in alternation rate based on the spatial frequency and orientation of the stimulus. Four spatial frequencies (0.5, 1.0, 1.5 and 2.0 cpd) as well as two orthogonal grating orientations (45/135 and 90/180 degrees) were used. Combinations of the two variables were randomized during 40 60-second trials on 9 participants. Average alternation rates were calculated for each individual trial using the following formula then averaged across all 9 participants.

$$alternation\ rate\ of\ trial = \frac{number\ of\ alternations}{time\ of\ last\ button\ press - time\ of\ first\ button\ press}$$

The results indicated that the largest spatial frequency of 0.5cpd resulted in the least time spent in piecemeal (figure 4.1). The alternation rates for all spatial frequencies were

not normally distributed. As a result, a Wilcoxon test indicated significant differences between all pairs of spatial frequencies except 0.5 and 1 cpd, and 1 and 1.5 cpd (see Appendix A for p values). No significant difference was found between the two orientations (Z=-1.381, p=0.167) (figure 4.2). As a result, further data were collected using the 0.5 cpd gratings and the oblique orientation (45/135 degrees).

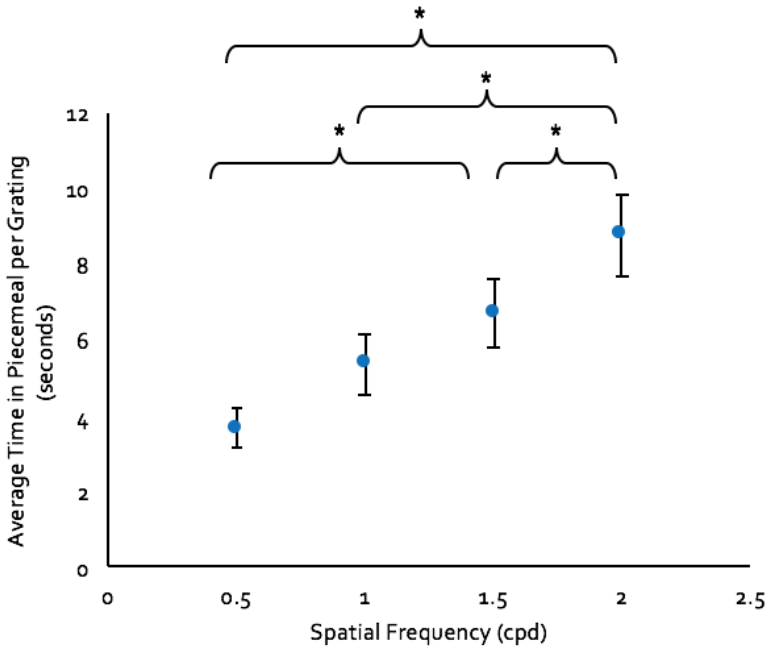

Figure 4.1: Average time spent in piecemeal for 9 observers plotted against spatial frequency. Asterisks indicate significant differences in alternation rates between indicated spatial frequencies. Error bars = SEM.

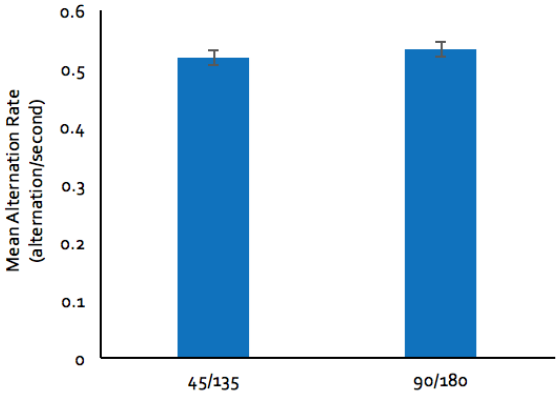

Figure 4.2: Average alternation rates of 9 participants for two grating orientations.

Blinking may have an effect on binocular rivalry measures, although studies have shown that the effect does not significantly change the alternation rates[86,87]. Nevertheless, pilot data were collected with the purpose of assessing the effect of blinking on alternation rates. Blinks were manually counted and averaged across 10 60-second trials. A Spearman correlation on data for 5 participants showed no significant association between blinking and alternation rates (R^2=0.16, p = 0.792). Lastly, a small study was done wherein the red/green glasses were reversed for half the trials to calculate eye dominance. This information ensured that participants were relatively equally dominant so as to provide an accurate measure of alternation rates. One spatial frequency of 0.5cpd and one orientation (45/135) was used for this study. After 3 trials, the glasses were reversed for a total of 6 10-second trials for 8 participants. Average alternation rates of the first and last three trials for 7 of 8 participants were not significantly different (t=-0.475, p=0.647). Reversing the glasses allowed for eye dominance interpretation. All 8 participants spent on average an equal amount of time seeing red when the red lens was on either eye, indicating

relatively equal dominance (Mean time (seconds) red:green = 21.9:22.9). The average time spent in red for the first and second three trials were not significantly different (t=-0.665, p=0.510). This study protocol of 8 participants was chosen for the psychophysical thesis data collection.

4.2 VEP Protocol Selection

4.2.1 Protocol 1

Data were collected on 10 participants based on a published on/off VEP protocol[16]. While Teyler and colleagues used a hemifield checkerboard to provide a control cerebral hemisphere for each participant, software limitations only allowed for a full field checkerboard in our study. Pre and post baseline measurements consisted of 3 minutes (30 sweeps/result, 2 results/step, 3 steps) of on/off checkerboard presented at a frequency of 1Hz. The stimulus was on for 35ms and off for 965ms; however no stimulus onset asynchrony between flashes was possible as was done with Teyler and colleagues. There were two conditions that each participant completed at least 24 hours apart. The first condition was the photic tetanus, where the full-field checkerboard flickered on and off at a frequency of 9Hz for 2 minutes. The control condition was 2 minutes at a frequency of 1Hz. The control condition was always second to the 9Hz flicker. Participants were asked to fixate on a centre red cross throughout the protocol. Following the 2-minute photic tetanus or control condition, participants were asked to close their eyes for 2 minutes before post measures were recorded. Electrodes were placed at Oz, PO7 and PO8 on the occipital

lobe.

4.2.2 Results

Based on the average waveforms of 6 male participants, Teyler and colleagues found significant differences pre to post in one component which they referred to as N1b at approximately 176ms (figure 4.3)[16]. No N1b component was present in the average results for all participants in our data. The waveforms presented with a double-peaked positive component and, qualitatively, no increase in amplitude in the post waveform was seen (figure 4.4).

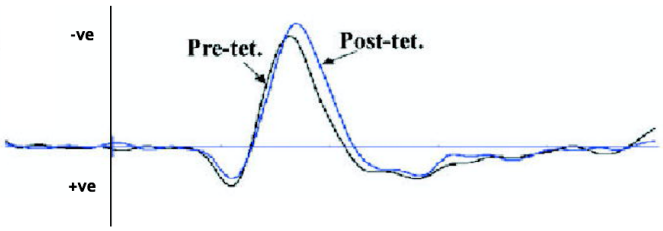

Figure 4.3: Waveform schematic representing the potentiation of the N1b complex reported by Teyler and colleagues. Positive is up on the y-axis. Modified from Teyler et. al.[16].

In comparing the data with that of the article's, these results may have been due to the placement of the electrodes. While a 128-electrode net was used for data collection in the original paper, only 3 channels were available in the clinical system we used. Therefore, we hypothesized that the effects may be better replicated if the lateral electrodes were placed 15% out from Oz, on P7 and P8 instead because potentiation includes parietal components in the original study. Three participants were tested with the single difference of placing the electrodes further out from the centre. Still no difference pre and post was found in

the nature of the waveforms and no potentiation was evident.

As the stimulus was presented for only 35ms, it was possible that the unexpected waveform was a combination of the on and off VEP response. Consequently, three participants were tested with the photic tetanus protocol with the stimulus remaining on for 490ms and off for 510ms (figure 4.5). Results varied across participants however waveforms were generally more consistent with previous studies than the 35ms protocol.

4.2.3 Protocol 2

Some studies in later years modified Teyler's protocol to induce LTP and found similar results. Normann and colleagues used a full-field reversal checkerboard protocol[38]. Their pre and post measures consisted of two 20-second recordings for a reversal rate of 2 reversals per second (rps), which is equivalent to 1Hz. They had two conditions: the photic tetanus condition of 19rps and the control condition of 2rps, each lasting for 10 minutes followed by two minutes of reading numbers on a screen. Interestingly, their control condition showed increases in VEP amplitude–similar to Teyler's results–while the 19rps, equivalent to approximately 9Hz, showed no potentiation. Protocol 2 matched Norman's control condition and was performed on 3 participants with one modification: participants closed their eyes for 2 minutes instead of reading numbers or a grey screen. Channel electrodes were placed at Oz, PO7 and PO8.

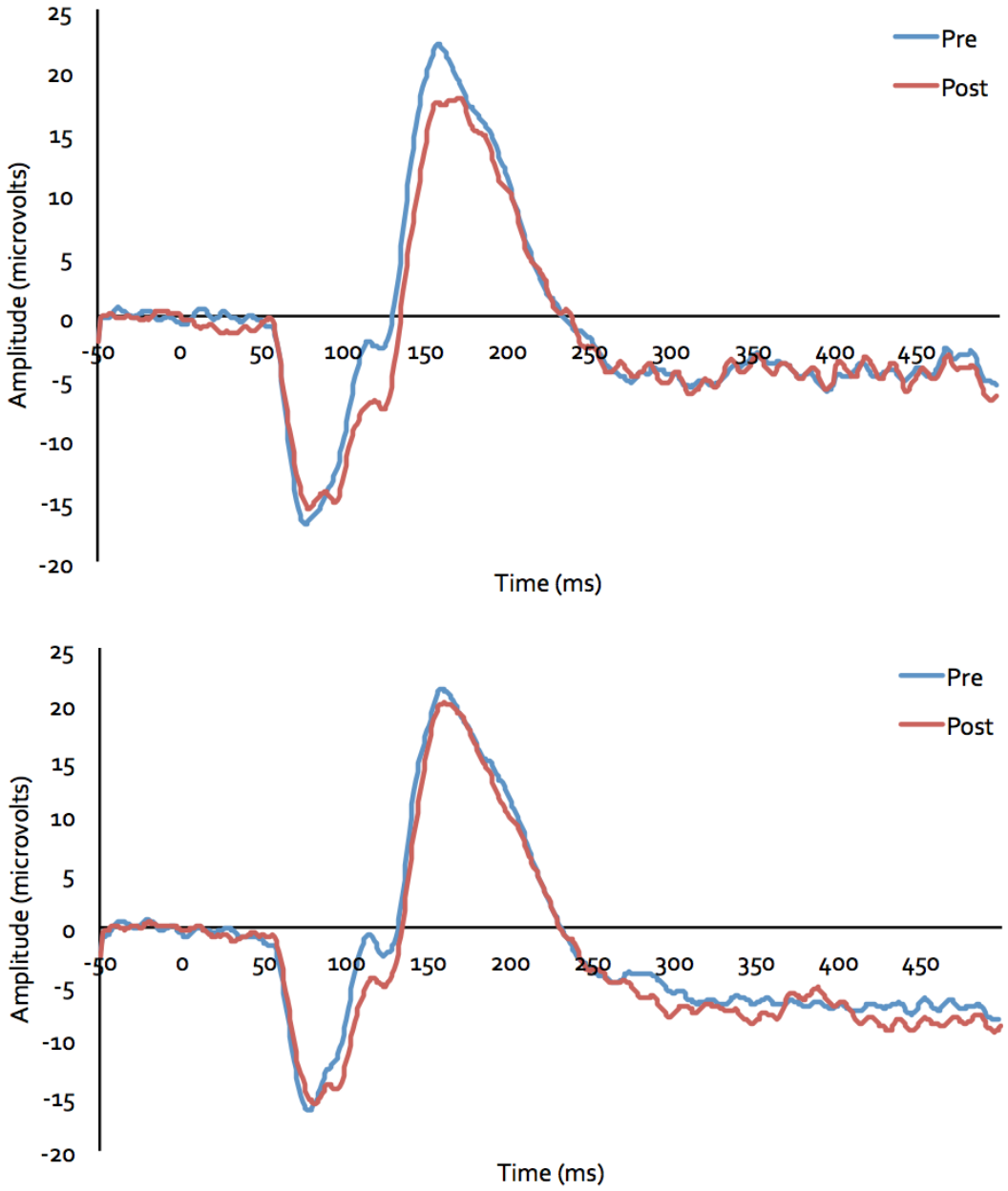

Figure 4.4: Group average of 10 participants for the 9Hz condition (top) and the control condition (bottom) for channel 1.

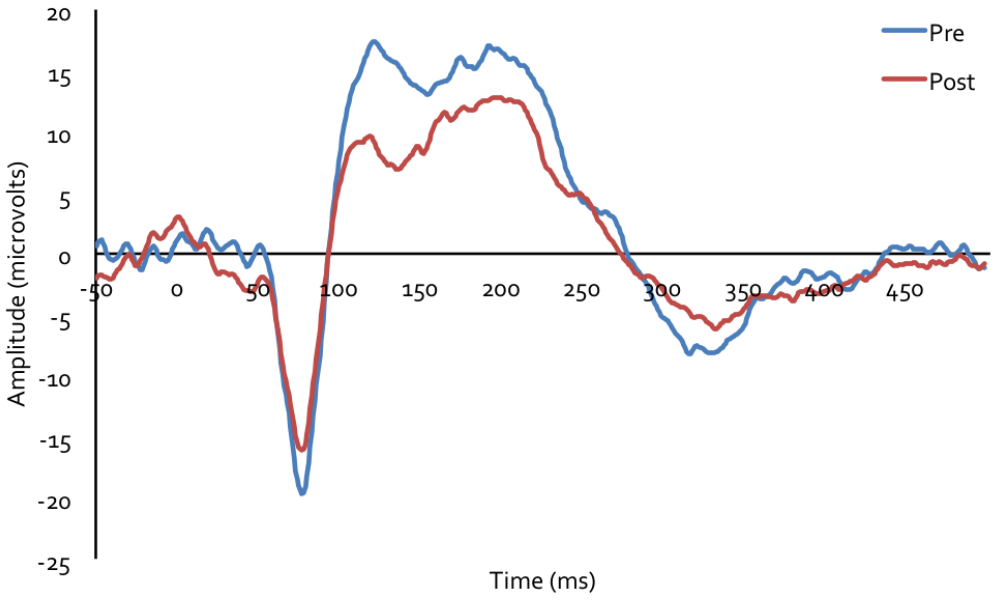

Figure 4.5: Average waveform of 3 participants for prolonged stimulus onset (490ms on, 510ms off) for channel 1.

4.2.3.1 Results

Once again, waveforms for all three participants did not appear as reported in the literature (figure 4.6). A prominent N80 component was present, followed by the P100 component, however a shallow N135, if any, followed. We observed a depotentiaion of the VEP after tetanization, however, the short pre- and post- recording times resulted in low signal to noise ratios.

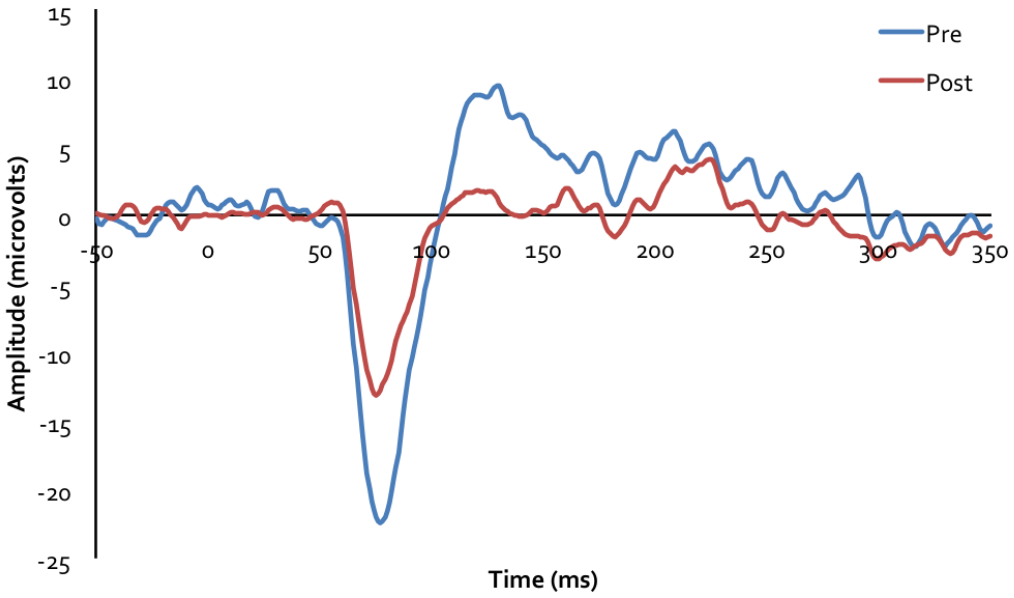

Figure 4.6: Average waveform of 3 participants for replication of the pattern reversal protocol for channel 1[88].

4.3 Discussion

Protocol 1, a modified version of Teyler and colleague's original methods[16], showed no qualitative difference in pre and post waveforms as anticipated. The average waveforms presented a broad, double positive peak, possibly masking the second negative component that was previously found to change significantly[16]. Extending the duration of the stimulus, however, did not unveil the negative component. Nonetheless, a longer stimulus duration showed waveforms that were more consistent with previous studies. The pattern-reversal protocol matched to that of Normann et al made it more difficult to increase the signal to noise ratio due to the short pre and post measures as opposed to the pattern onset

protocol.

Since the original protocol used for this method involved a pattern onset stimulus, this was the chosen protocol used for the study, applied to a clinical system. As a result, the modified prolonged stimulus onset of 490ms was chosen for the main study over the original 35ms onset time.

Chapter 5

Main Study Methods

5.1 Introduction

Following the pilot studies, a main study was conducted to compare LTP and rivalry alternation rates between younger and older adults.

5.1.1 Recruitment

The younger adult population (18-40 years) was recruited from throughout the University of Waterloo through poster advertisements and emails. The older adult population (60-80 years) was recruited using the Waterloo Research in Aging Participant (WRAP) pool. Participants were required to have good vision with or without glasses or contact lenses, and to be in good health.

5.1.2 Participant Screening

All screening measures (inclusion criteria) (Table 5.1) as well as the psychophysical task were performed in the same room under consistent room light conditions. The electrophysiological task was performed in a clinical testing room with minimal light. All measurements were taken continuously on the same day.

Table 5.1: Summary of inclusion and exclusion criteria

	Young	Older
Inclusion Criteria		
Cover Test	No strabismus, ≤6 exophoria	No strabismus, ≤6 exophoria
Worth 4 Dot	Normal binocular fusion	Normal binocular fusion
Stereoacuity	≤40”	≤200”
VA (OU)	≤0.3 logMAR	≤0.3 logMAR
Exclusion Criteria	Amblyopia; medication for depression/anxiety; glaucoma/cataracts; diabetic retinopathy; age-related macular degeneration; prone to seizures/epilepsy	

Those with amblyopia or disrupted binocular vision were excluded as the measure of alternation rates demands equal perception with both eyes. Additionally, certain medications for disorders such as anxiety, depression, psychosis and seizures alter the levels of GABA in the brain. Exclusion criteria was determined using a questionnaire (Appendix B). Participants who self-reported currently taking those medications or similar were excluded. Participants were given $20 in appreciation of their time, and parking costs (if applicable) were reimbursed.

This project was reviewed by, and received ethics clearance through a University of Waterloo Research Ethics Committee. All participants were informed of the nature of the study before participation and gave consent before any measures were taken (Appendix C). Our recruitment target was 30 participants in each group. In total, 30 young adults (19 female) and 14 older adults (10 female) were recruited. Fewer older adults were recruited than planned due to difficulties in identifying participants who fulfilled the study inclusion criteria. Electrophysiological data were not collected for one younger participant. Electrophysiological data for one older participant were excluded from analysis as she could not tolerate the tetanization stimulus.

5.1.2.1 Cover Test

A cover test is typically used to assess ocular alignment and consists of two measures: the unilateral or cover/uncover test as well as the alternating test assessing tropia and phoria respectively[89]. The cover test was performed while the participant was looking at either a near (40cm) and distant (6m) object. All participants showed no manifest deviations and phorias were within normal range based on Morgan's norms[90].

5.1.2.2 Worth 4 Dot Test

The Worth 4 dot test assesses binocular fusion and can reveal suppression at both distance and near[89]. Although this is considered a coarse assessment of binocular vision, it was a beneficial for our study purposes and reflected similar conditions to that of our psychophysical measure. Participants wore red/green glasses in the standard format to

disassociate their eyes as the test was performed at near (40cm) and distance (6m). All participants reported seeing 4 dots, indicating normal binocular vision.

5.1.2.3 Stereoacuity

The perception of depth is referred to as stereoacuity and is a central characteristic of binocular vision[89]. Both eyes receive slightly different images resulting in disparity which is required to perceive depth[89]. While there are several different methods to measure stereoacuity, the Stereo Fly test was used for this study. The Stereo Fly test uses crossed polaroid filters to present slightly different images to each eye[89]. Participants were asked to indicate which of four circles was protruding from the rest–known as the Wirt test[89]–while wearing the polarized glasses. The younger age group were required to see a minimum of 40 seconds of arc to be eligible for the study while the older age group population were required to see a minimum of 200 seconds of arc.

5.1.2.4 Visual Acuity

Visual acuity was assessed using the automated Freiburg Visual Acuity Test. The program uses the best parameter estimation by sequential testing (best PEST) to estimate the visual acuity threshold[91]. Landolt crowded Cs were presented on a 24" Asus monitor 6m away from the participant. Thresholds were recorded for the right eye and left eye as well as both eyes together. All measurements were recorded in logMAR units. Participants were excluded if their VA for both eyes was >0.3 logMAR.

5.1.3 Psychophysical Measurement of Alternation Rate

Binocular rivalry alternation rates were recorded as participants viewed a 0.5 cpd dichoptic grating that subtended 6.1 degrees of visual angle while wearing red/green glasses. Red and green gratings were orthogonally oriented and oblique presented at 45 and 135 degrees respectively. A chin rest was placed 57cm away from the monitor. A practice trial before the study allowed for participants to familiarize themselves with the keyboard presses. The study consisted of 6 trials, each lasting for 60 seconds. Participants took breaks for up to one minute between each trial. During the first three trials, the participants wore the red/green glasses so that the red lens was over the right eye. For the last three trials, the glasses were reversed so that the green lens was over the right eye. This was done to assess whether any participants who are highly dominant in one eye as this might have affected the rate of alternations per second. Blinks were counted manually, and later recorded for some participants using an IR video camera placed above the monitor.

5.1.4 Electrophysiological Measure

A modified version of Teyler and colleagues's protocol was chosen (see Chapter 4). A full-field checkerboard stimulus (0.3 degree check size, 7.7 degrees of the visual angle) was presented on a 60Hz 20" CRT screen 1.5m away from the participant (figure 6.1). With dimmed light conditions, the checkerboard was presented as 490ms onset and 510ms offset at a frequency of 1Hz for 3 minutes for both the pre and post measures. Immediately following the pre measure, the checkerboard was presented at frequency of 9Hz for two

minutes. Following tetanization, participants closed their eyes for 2 minutes. The 3-minute post measure was then recorded. Electrodes were placed at Oz, PO7 and PO8.

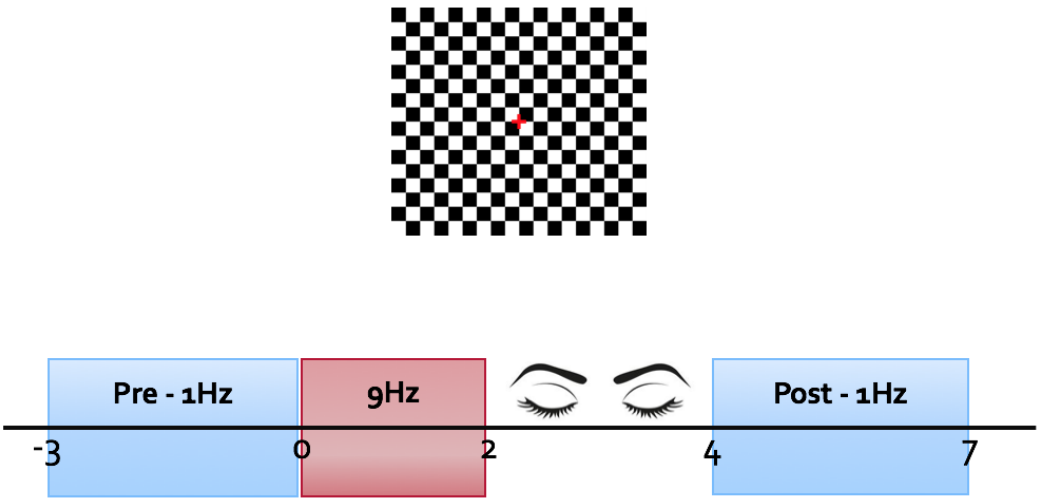

Figure 5.1: Electrophysiological stimulus used (top) and protocol schematic in minutes (bottom).

5.1.5 Statistical Analysis

The statistical software SPSS was used for analysis. The psychophysical and electrophysiological measures were analyzed both separately and together as described below.

5.1.5.1 Alternation Rates

Alternation rates were analyzed within and between groups. The effect of number of blinks on alternation rates, as well as differences between the age groups in alternation

rate were investigated using a dependent and independent t-test respectively.

5.1.5.2 VEP Analysis

Since the protocol chosen included a full-field, pattern-onset checkerboard, the VEP waveforms may be inverted for some channels due to the anatomy of the visual cortex in an individual's brain. Waveforms were exported from the Espion E2 system as Excel files and analyzed manually. The distance from the first prominent deflection from baseline, whether positive or negative, to the following peak or trough was calculated as the absolute difference between the two peak values in microvolts. This was labelled as Amplitude 1. Amplitude 2 was calculated in the same manner from the second peak or trough to the following peak or trough (figure 5.2). Peaks were identified based only on the pre waveform. The amplitudes were then measured at the same time points in the post waveform as the peaks identified in the pre waveform. Measurements were taken for all channels individually.

As expected, amplitudes 1 and 2 values varied considerably between participants depending on the signal-to-noise ratio as well as the maximum potentials of each individual participant. After analysis of the peak-to-peak amplitudes, the percent change between the pre and post waveforms was calculated.

A repeated measures ANOVA was used for each amplitude as the statistical method of choice. Three factors were considered: time = pre vs. post, electrodes = Oz vs. right vs. left channels and group = young vs. older. Potentiation was defined as an increase in the absolute value of either of the measured VEP amplitudes in the post waveform. A

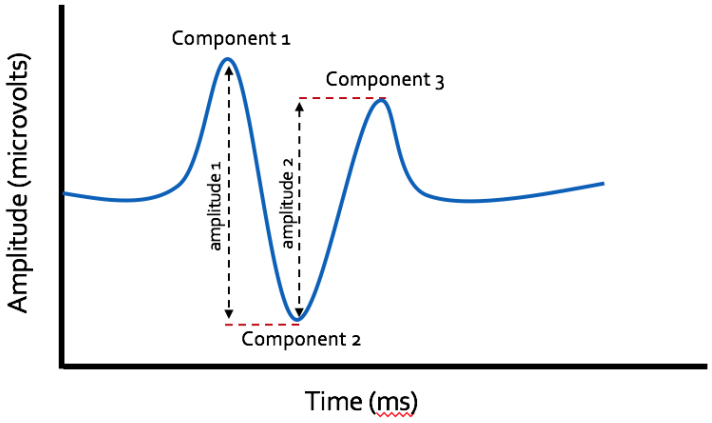

Figure 5.2: Schematic representation of a waveform and the measurements taken for amplitude 1 and amplitude 2.

decrease in post amplitude compared to the pre amplitude was defined as depotentiation. A regression analysis was then used to investigate the correlation between potentiation and alternation rate when controlling for electrodes as well as age group.

Chapter 6

Main Study Results

6.1 Population Demographics

A total of 30 young (mean age = 26 ± 3.9, 19 female) and 14 older (mean age = 68 ± 5.0, 4 male) participants were included in the study. Participants had no ocular deviations, normal binocular vision and met all inclusion and exclusion criteria. Stereoacuity and visual acuity (VA) measures were both within an acceptable range (Table 6.1).

Table 6.1: Mean demographics for each age group with standard deviations

	Mean Age	Median Stereoacuity	Mean VA (OU)
Young (n=30)	26 ± 3.9	20" (≤ 40")	-0.05logMAR ± 0.13
Older (n=14)	68 ± 5.0	63" (≤ 200")	-0.02 logMAR ± 0.09

6.2 Binocular Rivalry Alternation Rates

6.2.1 Effects of Blinks on Alternation Rates

The average number of blinks per minute for the young and older age group were 13 ± 10 and 10 ± 7 respectively (Table 6.2). The Shapiro-Wilk test showed that the blink rates were not normally distributed for either group. A Wilocoxon test showed no significant difference between the blink means (Z=-1.758, p=0.079).

Table 6.2: Means with standard deviations and medians for blinks and alternation rates

	Blinks		AR	
	Mean	Median	Mean	Median
Young	13 ± 10	9	0.59 ± 0.15	0.59
Older	10 ± 7	8	0.37 ± 0.14	0.36

Additionally, no significant correlation was found between the average number of blinks per minute and the alternation rates for both groups together (R^2 = 0.03, p=0.225). Analyzed separately, no significant correlation was found for the young population (R^2 = 0.05, p=0.229) (figure 6.1). The older population also showed no correlation between blinks per minute and alternation rate although approaching significance (R^2 = 0.25, p=0.063) (figure 6.2). However, the correlation was driven by one participant with an extreme blink rate. Without this participant, the correlation fell to an R^2 of 0 (R^2=0, p=0.953) (figure 6.3).

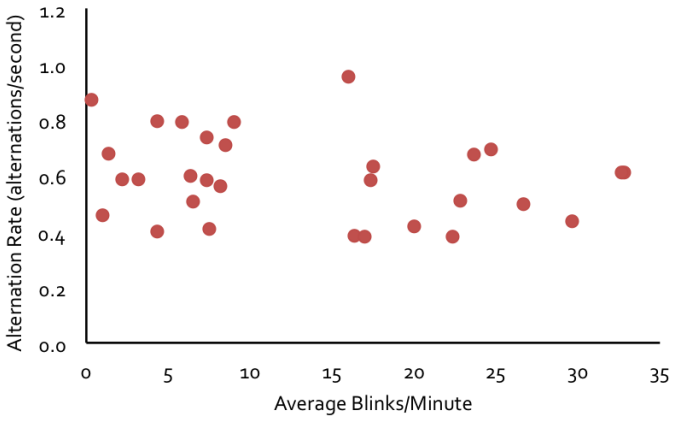

Figure 6.1: Scattergram of alternation rate against blink rate for the younger group. Each point represents the average data for one participant.

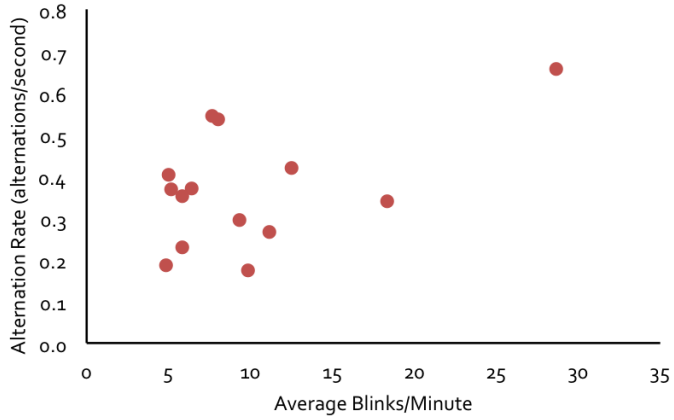

Figure 6.2: Scattergram of alternation rate against blink rate for the older group. Each point represents the average data for one participant.

6.2.2 Alternation Rate and Age

The Shapiro-Wilk test showed that the alternation rates were normally distributed for both groups individually and there were no outliers. Levene's statistic showed that the

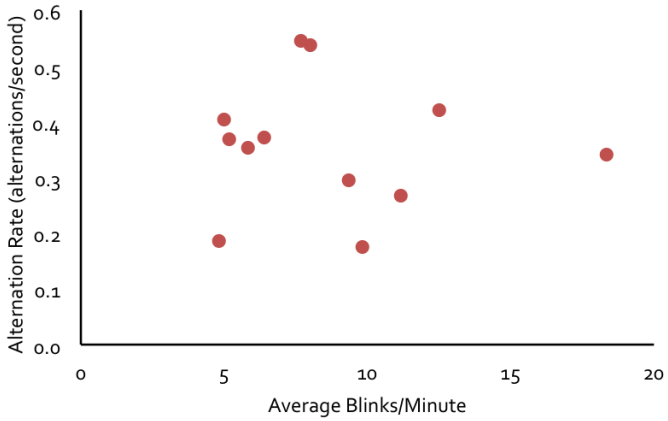

Figure 6.3: Scattergram of alternation rate against blink rate for the older group excluding one outlier with a high blink rate.

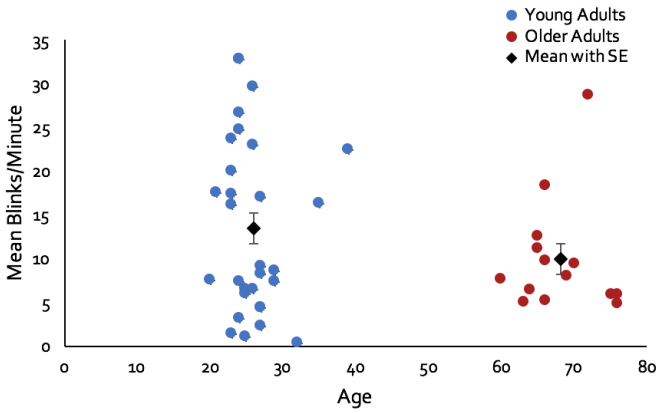

Figure 6.4: Scattergram of blink rates for both age groups. Blue represents the young population. Red represented the older population. The mean with standard error is plotted for each age group.

values had equal variances between the two groups. An independent t-test indicated that the alternation rate was significantly lower in the older group than in the young group (t_{42} = 4.667, p<0.001) (figure 6.5).

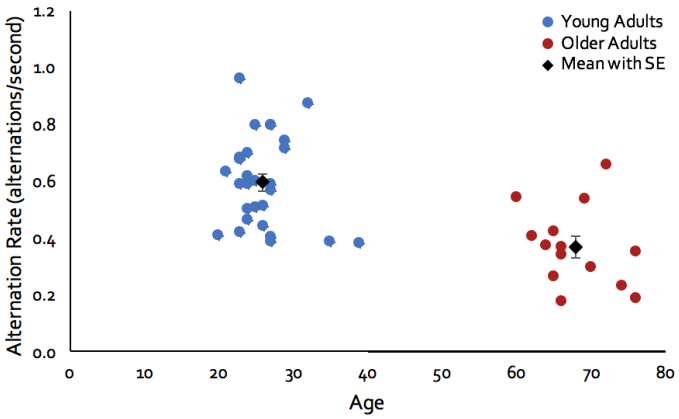

Figure 6.5: Individual alternation rate data for each age group. Blue represents the young population. Red represents the older population. The mean with standard error is plotted for each age group.

6.3 Electrophysiological Measure of Plasticity

One participant in the young population did not have a recorded VEP measure due to technical difficulties. VEP analyses for the young population were therefore performed on 29 participants (figure 6.6). Two participants in the older population were excluded from analysis. One participant was unable to tolerate the 9Hz flicker during the VEP recording while another had a very low signal-to-noise ratio. Therefore, VEP analysis for the older population was performed on 12 participants (figure 6.6).

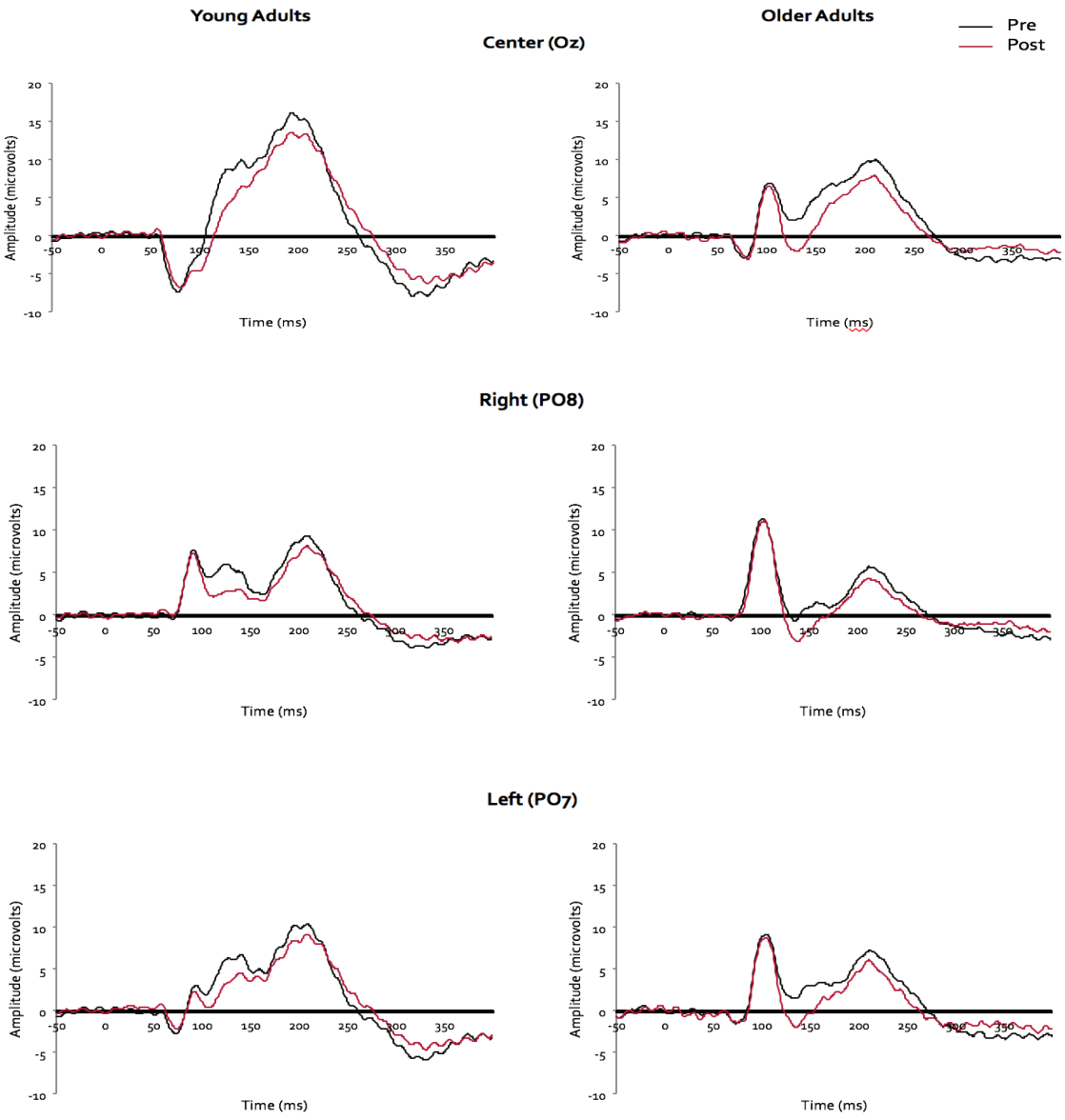

Figure 6.6: Average waveforms for the center, right and left channels across 29 young adults and 12 older adults.

6.3.1 VEP Latencies

The latencies of each measured peak were qualitatively similar (table 6.3). The standard deviations of each latency value are shown in table 6.4. Peak 3 exhibited a bimodal distribution (figure 6.7) due to the variation of waveforms as seen in figure 6.6.

Table 6.3: Average latency of measured peaks for each channel

		Center (Oz)		Right (PO8)		Left (PO7)	
		Pre	Post	Pre	Post	Pre	Post
Young	Peak 1	82	83	84	85	81	83
	Peak 2	134	134	116	117	121	122
	Peak 3	174	174	145	148	160	161
Older	Peak 1	84	85	83	88	87	90
	Peak 2	117	120	116	116	124	124
	Peak 3	156	154	156	157	157	161

Table 6.4: Standard deviations of latencies of measured peaks for each channel

		Center (Oz)		Right (PO8)		Left (PO7)	
		Pre	Post	Pre	Post	Pre	Post
Young	Peak 1	13	13	18	17	13	13
	Peak 2	42	42	27	27	37	37
	Peak 3	82	83	39	41	70	71
Older	Peak 1	11	9	16	14	11	11
	Peak 2	23	26	22	23	27	27
	Peak 3	39	39	29	30	37	42

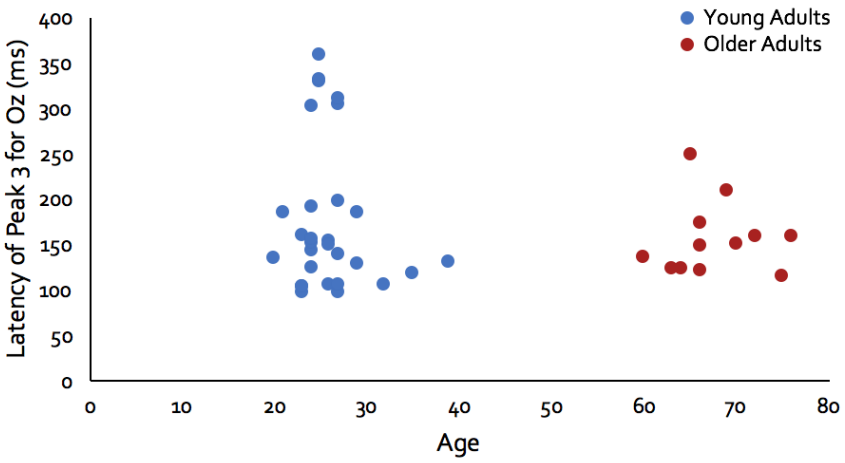

Figure 6.7: Peak 3 latencies of the pre Oz waveform plotted against age.

A repeated measures ANOVA (2 x group, 2 x time, 3 x electrode) on the pre and post latencies was performed separately for each peak. The results show a significant main effect of time for Peak 1 only ($F_{1,1}$=13.438, p=0.001). Post measure latencies were slightly higher than the pre measures. However the averages show that the peak measurements for at approximately the same latency. No significant main effects of electrode or group were found and no significant interactions between time, electrode and group was found. For both Peak 2 and Peak 3, main effects and interactions were not significant.

6.3.2 VEP Amplitudes

Amplitude 1 and 2 values varied considerably between participants, as expected, depending on the signal-to-noise ratio as well as the maximum potentials of each individual participant (table 6.5). The statistical method of choice was a repeated measures ANOVA, performed on the pre and post absolute values of the differences between each peak.

Table 6.5: Mean absolute values of the peak to peak amplitudes and the percent of positive polarity for amplitudes 1 and 2

		Center (Oz)			Right (PO8)			Left (PO7)		
		Polarity	Pre	Post	Polarity	Pre	Post	Polarity	Pre	Post
Young	Amp 1	13.8	24.28 ±15.64	20.09 ±13.97	44.8	12.80 ±9.89	11.96 ±9.45	24.1	14.58 ±8.49	12.95 ±8.01
	Amp 2	86.2	13.09 ±13.11	11.01 ±11.08	58.6	10.79 ±8.11	10.27 ±8.46	75.9	9.38 ±5.97	8.36 ±6.10
Older	Amp 1	16.7	16.34 ±11.02	15.53 ±11.78	33.3	15.59 ±11.80	15.60 ±12.70	33.3	15.55 ±9.80	15.86 ±11.29
	Amp 2	83.3	10.67 ±8.36	13.14 ±11.34	66.7	11.34 ±10.29	13.04 ±13.70	66.7	10.06 ±6.83	12.32 ±9.83

6.3.3 VEP Amplitude 1

A repeated measures ANOVA (2 x time, 3 x electrode, 2 x group) was chosen for analysis of amplitude 1. The three-way interaction between time, electrodes and group was non-significant ($F_{1,2}$=1.579, p=0.213). However, significant two-way interactions were observed between time and group ($F_{1,1}$=5.596, p=0.023) (figure 6.8) as well as time and electrode ($F_{1,2}$=5.292, p=0.007) (figure 6.9). The interaction between electrode and group was not significant ($F_{1,1.748}$=3.269, p=0.051). Between participant analysis showed no significant main effect of time ($F_{1,1}$=3.811, p=0.058) or age group ($F_{1,1}$=0.065, p=0.800). However, a significant main effect of electrode was found ($F_{1,1}$=3.811, p=0.044). Post-hoc analyses of the significant time x group interaction showed no significant main effects between groups for neither the pre ($F_{1,39}$=0.201, p=0.657) nor the post measures ($F_{1,39}$=0.045, p=0.834). Post-hoc analysis with a Bonferroni correction of the significant time x electrode interaction showed the amplitude of the center channel for the pre waveform is significantly different from the right (p=0.004) and the left (p=0.019) channels. A post-hoc t-test between pre and post measures for each channel showed significance only in the center channel (t_{40}=5.089, p<0.001). See Appendix D for plots of non-significant interactions.

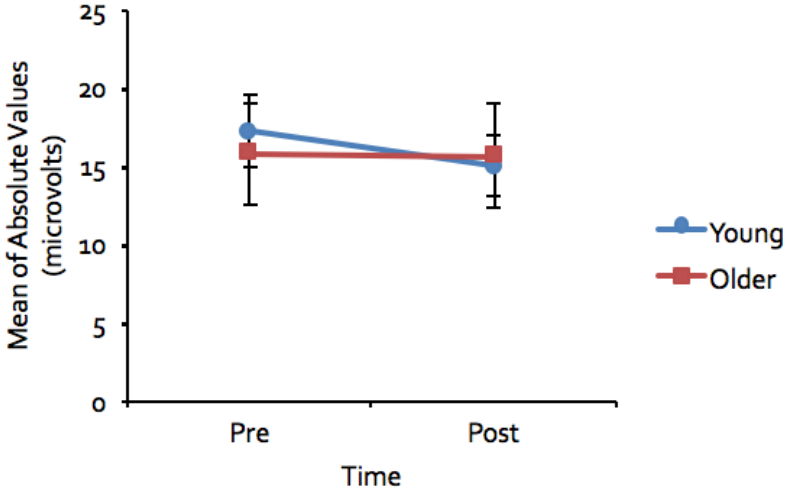

Figure 6.8: The interaction between amplitude 1 pre and post values and age was significant. Main effects were not significant. Error bars = SEM.

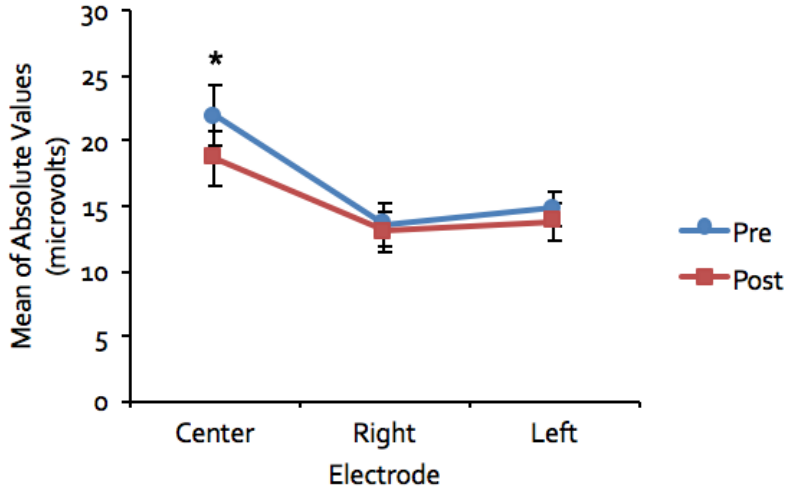

Figure 6.9: The interaction between pre/post amplitudes and channels was significant. Main effects were not significant. Error bars = SEM.

6.3.4 VEP Amplitude 2

A repeated measures ANOVA (2 x time, 3 x electrode, 2 x group) was chosen for analysis for amplitude 2. As with amplitude 1, the three-way interaction between time, electrodes and group was non-significant for amplitude 2 ($F_{1,2}$ =1.706, p=0.188). However the interaction between time and group was significant ($F_{1,1}$=10.847, p=0.002) (figure 6.10). The young group exhibited depotentiation after tetanization whereas the older group exhibited potentiation (figure 6.6). No significant interaction was found between electrode and group ($F_{1,1.998}$=0.235, p=0.791) and time and electrode ($F_{1,2}$=0.366, p=0.695). No significant main effect of time ($F_{1,1}$=0.944, p=0.337), electrode ($F_{1,1.998}$=0.671, p=0.514) or age group was found ($F_{1,1}$=0.138, p=0.712). Post-hoc analyses of the significant time x group interaction showed no significant main effects between groups for neither the pre ($F_{1,39}$=0.028, p=0.868) nor the post measures ($F_{1,39}$=1.126, p=0.295). See Appendix D for plots of non-significant interactions.

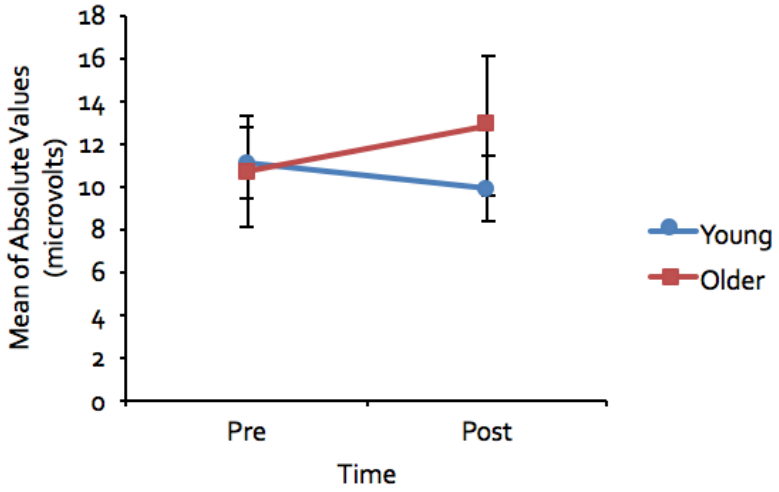

Figure 6.10: The interaction between amplitude 2 pre and post values and age was significant. Error bars = SEM.

6.4 Alternation Rates, VEP Amplitudes and the Effect of Age

A linear regression analysis was chosen to investigate whether alternation rates could predict the change in VEP amplitudes for each age group. The difference between pre and post was quantified as percent change for both amplitude 1 and amplitude 2. No significant association was found between alternation rates and either amplitude when controlling for both electrode (right and left electrode only) and group (table 6.6). The percent change in amplitude 1 is equal to 16.435 - 44.194(AR) + 1.166(Electrode) +4.516(Group), where electrode is coded as 1=right and 2=left and group is coded as 0=young adults and 1=older adults. The percent change for amplitude 2 is equal to -3.031 +3.360(AR) -

0.921(Electrode) + 19.417(Group) where the variables are defined as in amplitude 1. A Pearson correlation between alternation rates and amplitude 1 or 2 was not significant (amplitude 1: R^2=0.081, p=0.071; amplitude 2: R^2=0.048, p=0.160).

Table 6.6: Univariate regressions between alternation rate, electrode and age (dependent variables) for amplitude 1 (independent variable)

Factor	B [95% CI]	R^2	p
AR	-44.184 [-97.449, 9.081]	0.061	0.103
Electrode	1.166 [-14.632, 16.963]	0.061	0.884
Group	4.516 [-16.234, 25.265]	0.064	0.666

Table 6.7: Univariate regressions between alternation rate, electrode and age (dependent variables) for amplitude 2 (independent variable)

Factor	B [95% CI]	R^2	p
AR	3.360 [-59.202, 65.921]	0.101	0.915
Electrode	-0.921 [-19.476, 17.633]	0.010	0.922
Group	19.417 [-4.954, 43.789]	0.041	0.117

6.5 Summary

Alternation rates differed between the groups as young adults had faster alternation rates than did older adults. The VEP waveforms for both age groups appeared to be qualitatively different; however no main effect of group was found. Alternation rates were not significant predictors of either amplitude 1 or amplitude 2. Pre and post amplitude

measures were significantly different in the young age group but the difference indicated depotentation. This difference was not statistically significant in the older age group.

Chapter 7

Discussion and Conclusions

7.1 Objective 1

The first objective of this study was to identify the best method of LTP induction measured by visually-evoked potentials (VEPs) in humans. The original pattern onset method[16] and pattern reversal method[38] have been modified slightly throughout the years[15,51,58], however a reliable protocol for practical and clinical use in the future has yet to be established. Clinical systems typically record up to 4 channels while studies in the literature use a 64 or 128 electrode net, which requires expensive equipment and is not practical for clinical use[16,51,55,58]. The few studies which used only one channel, namely Oz, had short pre and post measures that lasted only 20 seconds[15,38]. With the large variability in VEPs between individuals, we found that 20 seconds did not provide a strong enough average for all participants, particularly for those with a low signal-to-noise ratio

and therefore may not be reliable for clinical use. The chosen protocol for the main study was an attempt to adapt published research protocols to a clinical setting[16,38]. VEP pilot data waveforms showed unexpected variability and did not qualitatively look as published in previous literature[16,38]. A large negative peak at approximately 175ms after the stimulus onset was found to potentiate significantly in the past[16] however our waveforms did not consistently have a prominent negative peak for the same results to be found. The prolonged onset stimulus parameters (on for 490ms and off for 510ms) were thought to eliminate any possible masking of the negative peak by the larger positive peak preceding it and was therefore chosen as the main protocol. The main study results show considerable variability between participants within each age group along with differences between the younger and older adult groups. Contradictory to previous results, amplitudes 1 and 2 did not change significantly for the young population[16,38]. The older population pre and post amplitudes of amplitude 2, however, showed a mean potentiation although non-significant. Original papers establishing the protocol reported results with as little as 6 subjects. This study had a sample size of 30 young and 14 older participants, providing further insight into the application of LTP on the general population. The timeframe of this study prevented a more detailed exploration of optimal LTP induction and measurement paradigms. Therefore, further investigation of stimulus parameters, protocol timing and the practical use of this measure of visual cortex plasticity across all age groups is necessary for optimization.

7.2 Objective 2

The second objective of this study was to investigate whether or not a relationship exists between GABA levels–assessed indirectly using binocular rivalry alternation rates–and the extent of LTP induction in the human visual cortex. Previous studies have shown that potentiation is found in the second negative component of the waveform[16,38]. Additionally, results from similar VEP protocols demonstrate that potentiation occurs laterally 10-15% away from Oz[16]. Most studies have used dipole measures that select optimal signal location from one participant to the next[92]. However this is not possible with a clinical system. Our results show that alternation rates cannot significantly predict the potentiation of either amplitude. Although both VEP and AR measures show differences across both age groups, the two were not correlated. Based on previous literature, we hypothesized that slower alternation rates will be correlated with higher GABA levels[18]. This implies that the older age group–who had slower alternation rates than the young population–have higher GABA levels. This contradicts other literature which hypothesizes decreased GABA levels as a cause of the cognitive decline that occurs with age[65]. Both binocular rivalry and LTP were indirect measures of plasticity in the adult brain. Perhaps a stronger statistical power would reveal significant relationships between the two measures. Although differences in alternation rates and changes in VEPs exist between both age groups, the two measures were not significantly correlated.

7.3 Objective 3

The final objective of this study was to investigate the effect that age may have on alternation rates and LTP. Older adults had significantly slower alternation rates than the younger population. Research on GABA levels and bistable perception such as binocular rivalry demonstrated a correlation between the two[18]. Higher GABA levels resulted in slower alternation rates[18]. We therefore used binocular rivalry as a behavioural measure and inferred that our older adults population have higher GABA levels than do the younger adults. While an increase in GABA levels is necessary for the opening of the critical periods and further increases contribute to their closure, studies have shown that a decrease in GABA with age may play a role in cognitive decline[93–95]. Based on GABA levels being inversely correlated with alternation rates, the results demonstrate that the older adults should have higher GABA levels. Although consistent with van Loon and colleagues' correlation of GABA and bistable perception[18], this outcome was unexpected given contradictory evidence for a general reduction of GABA in older age[75,93–95]. The correlation found may not be a result of higher GABA levels, but rather an imbalance of excitatory and inhibitory interactions within the brain. Previous studies have hypothesized that binocular rivalry may be a result of excitation and inhibition within the primary visual cortex[18,96]. Changes to the amount of excitatory and inhibitory activity may have an effect on cognitive function and conscious awareness. One study in cats showed that a decrease of GABAergic neurons by about half occurs with age whereas no change was found in the number of excitatory neurons within the visual cortex[65]. Slower alternation rates in older adults may be one aspect of the cognitive decline that occurs with age, not due to lower

GABA levels but rather the disproportion of inhibition and excitation. It is still unclear, however, whether these age-related changes contribute to changes in cognition, plasticity or perception.

An interesting result of the study was the difference in waveforms between the two age groups. Waveform patterns for both amplitude 1 and 2 were qualitatively different between the young and older populations. Changes in latencies with age have been reported in the past with pattern reversal stimuli and may be attributed in part to a random loss of neurons within the visual pathway[97,98]. As pattern onset waveforms are more variable, there are no reported changes between young and older adults. The young age group showed a qualitative overall depotentiation of both amplitudes while the older population showed no significance for either. Nonetheless, a potentiation was is qualitatively visible in the second amplitude for most participants in the older age group and an increase in statistical power may reveal significance.

VEPs have been shown to vary slightly by gender. Females typically have shorter latencies and larger average VEPs than do males[98]. Gender differences may also be attributed to anatomical differences, skull thickness, possible differences in visual information processing as well as hormonal levels–although the later is speculative[39,98]. We did not collect data relating to the menstrual cycle for our younger female participants and therefore we do not currently have data to test this hypothesis. It is also possible that the group difference in qualitative VEPs be due to lower GABA levels in the older group. Lower inhibition in the visual cortex may be causing an increase in VEP potentials as compared to more inhibition in the young adults. It is still unclear however what the reason may be and whether GABA levels significantly influence VEPs.

7.4 Strengths and Limitations

Although the VEP protocol design was limited by technical restrictions of the available clinic system, it revealed intriguing differences in waveforms that occur with age and demonstrated the need for a reliable, clinical protocol. One limitation of the study was the smaller population of older adults recruited for the study. As some correlations were approaching significance, a larger study may reveal more significant changes between channels and pre and post measures as a result of improved statistical power. Additionally, attentional resources in older adults have been found to be weaker than younger adults. The slower alternation rates may not be solely a result of changes in GABA levels, rather to the amount of attention given to the ambiguous stimulus during passive viewing[83]. While this phenomenon has been studied, further investigation is needed to understand the mechanisms of attention and the differences that occur with age. Future studies might focus on the optimization of a clinical protocol that is fast, reliable and practical for patient studies. Additionally, further investigation of the variations in waveforms as well as the differences in potentiation between different VEP components and age groups is needed to understand the mechanisms that contribute to the changes in plasticity.

7.5 Conclusion

Recent studies have shown that the adult brain is in fact capable of forming and strengthening new connections, albeit at a slower rate than in children[1]. These findings have paved the way for new research to understand the mechanisms that underlie

adult cortical plasticity, and how these mechanisms can be controlled and manipulated to increase plasticity following the critical period. With our current understanding of age-related GABA, it is unclear whether binocular rivalry can in fact be used as an indirect measure of GABA. Further, we cannot conclude that binocular rivalry is correlated to the proportional increase in plasticity. Additional investigation is required to understand the differences in binocular rivalry, changes in plasticity as well as VEP waveforms that occur with age. Further research on the underlying mechanisms that drive these changes will provide an understanding of the neurochemicals necessary for neuroplasticity and the external influences that can modulate them. The changes that occur in the brain with age have a large impact on cognitive abilities as well as perception and can alter many aspects of daily life. The results may therefore have implications in the treatment and neurorehabilitation of developmental disorders as well as damage to the brain that can occur with age[9–12].

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APPENDICES

Appendix A

Pilot Data Significance Values

Test Statistics[a]

	sf1 - sf0.5	sf1.5 - sf0.5	sd2 - sf0.5	sf1.5 - sf1	sd2 - sf1	sd2 - sf1.5
Z	-1.811[b]	-3.119[b]	-4.429[b]	-1.962[b]	-3.008[b]	-2.786[b]
Asymp. Sig. (2-tailed)	.070	.002	.000	.050	.003	.005

a. Wilcoxon Signed Ranks Test

b. Based on negative ranks.

Figure A.1: The significance values of the non-parametric Wilcoxon tests between alternation rate and spatial frequency.

Appendix B

Exclusion Criteria Questionnaire

Questionnaire:

- Date of birth? _____
- Do you wear glasses/contact lenses? Yes/No
- Do you have good vision in each eye with/without the glasses? Yes/No
- Do you happen to know your prescription for each eye? Yes/No _____
- Do you have any eye problems? Yes/No
  - ☐ Glaucoma
  - ☐ Macular degeneration
  - ☐ Cataracts
  - ☐ Diabetic retinopathy
  - ☐ Stroke
  - ☐ Diabetes
- Medications for depression/anxiety? Yes/No

Appendix C

Consent Form

The following form was provided for each participant prior to beginning the study.

UNIVERSITY OF WATERLOO

INFORMATION CONSENT LETTER

Mechanisms of Brain Plasticity

Faculty Supervisors
Ben Thompson, PhD, University of Waterloo, Department of Optometry and Vision Science
519-888-4567 Ext. 39398
Daphne McCulloch, PhD, OD, University of Waterloo, Department of Optometry and Vision Science
519-888-4567 Ext. 37940

Student Investigators
Dania Abuleil, MSc Candidate, University of Waterloo, Department of Optometry

INTRODUCTION
You are being invited to take part in a research study for Dania Abuleil's master's thesis. Before agreeing to participate in this study, it is important that you read the study procedures. The following information describes the purpose, procedures, benefits, discomforts, risks, and precautions associated with this study. It also describes your right to refuse to participate or withdraw from the study at any time. In order to decide whether you wish to participate in this research study, you should be aware of its risks and benefits to be able to make an informed decision. This is known as the informed consent process. Please ask the study staff to explain any words that you do not understand before signing this consent form. Make sure all your questions have been answered to your satisfaction before signing this form.

PURPOSE
The purpose of this work is to further understand the mechanisms underlying plasticity (ability to change) in the adult brain. Such understanding has potential future advances in our knowledge of how and why it becomes increasingly more difficult to restructure the connections of our brain as one ages.

PROCEDURES
If you agree to participate, all assessments will be performed in one session in the Optometry Building OR the Applied Health Science (AHS) Building at the University of Waterloo. Four clinical screening procedures will be performed in order to determine eligibility for participation in the study. The screening procedures are as follows:

1. You will be asked to indicate the direction of motion (left or right) of the majority of moving dots presented on a 3D screen. The task will become difficult as increasingly more dots will move randomly rather than coherently throughout the tests. The task will take up to ten minutes.

2. You will be asked to indicate the direction of the letter "C" using a keypad at a distance of 3m from the screen with each eye individually, as well as with both eyes.
3. This task involved wearing 3D glasses. A book will be held up in front of you and you will be asked to indicate which image is coming out at you, or 3D, for 10 different levels of difficulty.
4. A basic clinical procedure, known as a cover test will be performed. You will be asked to focus at a distant object while one eye will be covered then uncovered. This will be repeated while focusing at a near object.

Following the screening, you will take part in two tasks for this study.
For the first task, you will be asked to wear 3D glasses. A red/green stimulus will be presented on a 3D monitor in front of you. You will be asked to indicate which colour you are seeing at a given time using keys on a keyboard. There will be 10 trials, each trial 60 seconds long. You may take breaks between each trial.

The second task involves a method of recording brain waves called electroencephalography – commercially produced equipment - that will be performed in a specially equipped room in the School of Optometry and Vision Science clinic or the EEG room in AHS. Electrodes will be placed on your head; 3 at the back of your head, 1 just above your forehead, and one on your right earlobe. A swab will be used to first clean the area where the electrode will be placed using NuPrep Skin Prep Gel. Ten20 Conductive paste will be used to hold the electrode in place. * Electrodes are washed and sanitized using alcohol-based wipes and left to dry between uses. You will be asked to focus on the cross presented on the screen in front of you while a checkerboard stimulus flashes on and off. This task will last for approximately 45 minutes.
* Note: If you have any allergies or sensitivities, please advise the researchers. You may want to consider not taking part in this study

Time Commitment:
The session will last up to 1.5 hours. You can request to stop for a rest at any time.

INCLUSION AND EXCLUSION CRITERIA
To participate in this study, you must be at least 18 years old and less than 80 years old with normal vision with or without glasses/contact lenses and with good health. You must also have the ability to acknowledge instructions and provide informed consent. Adults with cognitive impairments will be excluded from the study. We will also be excluding anyone currently taking medications such as antidepressants, anti-anxiety or antipsychotics as these medications alter the levels of a specific chemical within the brain (GABA) vital to the results of the study. Additionally, those with lazy eye (amblyopia) or those prone to seizures will also be excluded as they may have difficulty completing the assigned tasks.

There are no known or anticipated risks associated with this study. Participants will feel skin being gently rubbed during electrode placement however this procedure is pain-free.

BENEFITS
By participating in this study, you will benefit by furthering your knowledge of experimental procedures commonly used in neuroscience and vision science research. Your help will contribute to our knowledge about how and why children have a much greater capacity for their brain to change, learn and grow than do adults.

CONFIDENTIALITY AND SECURITY OF DATA
All information you provide is considered completely confidential; indeed, your name will not be included or in any other way associated, with the data collected in the study. Furthermore, you will not be identified individually in any way in any written reports of this research. Paper records of data collected during this study will be retained for a minimum of 7 years in a locked filing cabinet in OPT 220, to which only researchers associated with this study have access. Electronic data will be kept for a minimum of 7 years on a secure computer in OPT 220. The collected data will be coded with participant numbers (not names). All identifying information will be removed from the records prior to storage.

PARTICIPATION
Participation in this study is entirely voluntary and you may refuse to participate or you may withdraw at any time without loss of benefits that you are otherwise entitled.

ETHICS CLEARANCE
As with all University of Waterloo projects involving human participants, this project was reviewed by, and received ethics clearance through a University of Waterloo Research Ethics Committee. Should you have any comments or concerns resulting from your participation in this study, please contact the Chief Ethics Officer, Office of Research Ethics, at 1-519-888-4567 ext.36005 or ore-ceo@uwaterloo.ca.

QUESTIONS
Any questions with regard to this research should be directed to Dr. Ben Thompson, 519-888-4567 Ext. 39398

UNIVERSITY OF WATERLOO

B. Thompson, D. McCulloch
Student Researcher: D. Abuleil

CONSENT FORM

Mechanisms of Brain Plasticity

I, _____

have been informed and I am aware of the aim of this study, and have read the information consent letter and the clinical information form. I acknowledge that I am under no obligation to take part and may withdraw from the study at any time.

I acknowledge that I am free to ask questions and to withdraw from this study at any time. I also acknowledge that if I feel uncomfortable, I may ask the researcher to stop it immediately.

____ I agree to take part in the study. I will receive a copy of the information consent letter and signed consent form.

In no way does signing this consent form waive your legal rights, nor does it relieve the investigators or involved institution from their legal and professional responsibilities.

PARTICIPANT

NAME _____ SIGNATURE _____ Date _____

INDIVIDUAL OBTAINING CONSENT

NAME _____ SIGNATURE _____ Date _____

Would you like to receive a summary of the results for the experiment performed? Yes No

If yes: Email: _____ (provide email address)

OR Mail: _____ (provide mailing address)

Appendix D

Non-Significant Interactions

D.1 Amplitude 1

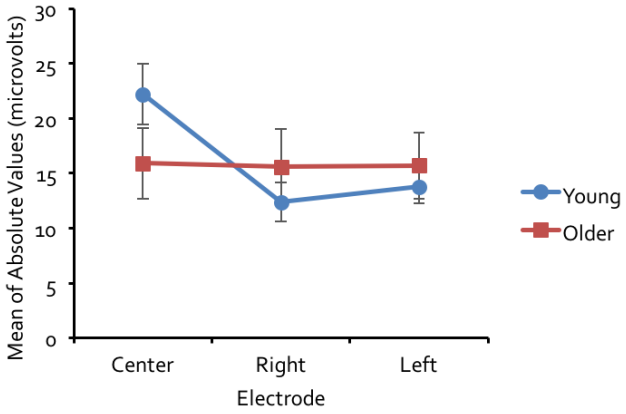

Figure D.1: The interaction between electrode group was not significant for amplitude 1. Error bars = SEM.

D.2 Amplitude 2

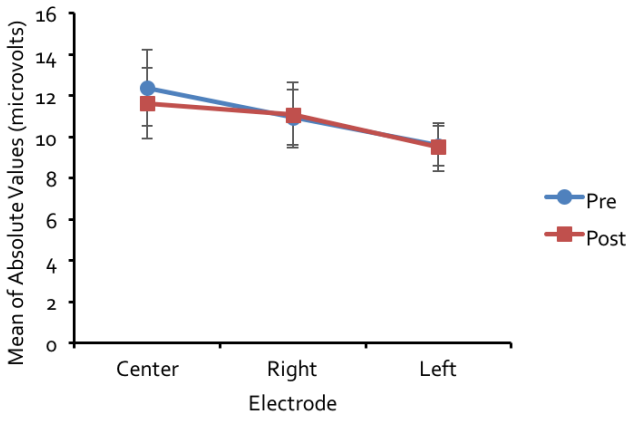

Figure D.2: The interaction between pre and post values and electrode was not significant for amplitude 2. Error bars = SEM.

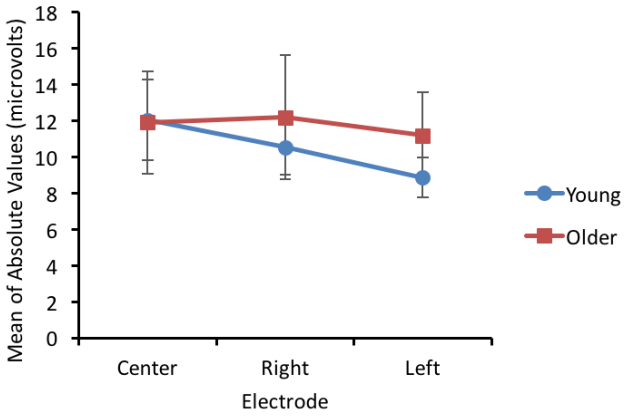

Figure D.3: The interaction between electrode and age group was not significant for amplitude 2. Error bars = SEM.