LTP-like Plasticity of the Visual Evoked Potential

Robustness and Effects of Gender, Age, and Time-of-Day in a Large Sample of Healthy Volunteers

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Summary

This thesis was written by Ahsan Ali Rai under the supervision of Torbjørn Elvsåshagen and Torgeir Moberget, with the following title "LTP-like Plasticity of the Visual Evoked Potential: Robustness and Effects of Gender, Age, and Time-of-Day in a Large Sample of Healthy Volunteers." The data collected for this thesis is part of a larger study at the Norwegian Centre for Mental Disorders Research (NORMENT) group, of which the author is involved in. The majority of data was collected by Ahsan Rai and one other master's student. Alongside the guidance of the supervisors, development of the hypothesis, data refinement and analysis was performed independently by the author.

Background: Long-term potentiation (LTP) is the best characterized form of synaptic plasticity. Animal studies suggest that LTP is a central mechanism in learning, memory, and psychiatric illnesses. Despite these findings, there is a paucity of human LTP studies. The scarcity of human research is mainly due to a lack of methods for non-invasive measurements of LTP-like plasticity in the brain. However, plasticity of the visual evoked potential (VEP) has in recent years emerged as a promising non-invasive method for assessment of LTP-like plasticity in the human cortex. The method´s robustness and the effects of age, gender, and time-of-day on plasticity of the VEP remain to be clarified. **Objectives:** The present study had two main aims. First, we aimed to replicate VEP plasticity in the largest sample of healthy volunteers to date. Second, we aimed to assess the effects of age, gender, and time-of-day on plasticity of the VEP. **Method:** 119 healthy human control subjects between the ages of 18-65 were recruited to participate. VEP`s were elicited by checkerboard reversal stimulation before and after a modulation block of prolonged (10 mins) visual stimulation. Plasticity of the VEP was assessed by computing changes in C1, P1, N1 and P1-N1 peak-to peak amplitudes from pre- to post-modulation. **Results:** The modulation block induced significant changes in VEP amplitudes with the following *p*-values and effect sizes (Cohen`s *d*): C1 ($p < .001$, $d = .56$), P1 ($p < .001$, $d = .42$), N1 ($p = 0.002$, $d = .30$), and P1-N1 ($p < .001$, $d = .58$) peak-to-peak measures. There were significant effects of age, gender, and time-of-day on pre-modulation VEP amplitudes and latencies. There were no significant effects of age, gender, and time-of-day on plasticity of the VEP. **Conclusion:** We confirmed plasticity of the VEP in the largest sample of humans to date.

There were significant effects of age, gender, and time-of-day on pre-modulation VEP`s, but not on plasticity of the VEP. Together, these findings indicate that VEP plasticity is a robust and accessible method for non-invasive studies of LTP-like cortical synaptic processes in humans.

Acknowledgements

I would like to start by thanking my supervisors for providing the opportunity to be involved in the NORMENT group and contribute to the collection of clinical data in this new and promising field of research. Thank you to Torbjørn Elvsåshagen (Supervisor) for constantly taking the time out of your busy schedule to have engaging discussions while offering thought provoking insight into the current understandings of LTP with regards to the specifics of this experiment. I would like to thank Torgeir Moberget (Co-supervisor) for his instrumental role in data analysis as well as continual presence in the face of Matlab adversities. The practical and analytical skills I have acquired from being under the tutelage of you two will remain invaluable long after the completion of this research.

A special thanks to the participants for their involvement in the study, and to Anna Maria Matziorinis (Master's student) for assisting with data collection while providing feedback in continually improving upon the analysis of data. I would also like to thank all the researchers at NORMENT for facilitating a multidisciplinary and nurturing environment whilst always having a fresh pot of coffee ready for those sleep deprived mornings. Last but not least, I would like to thank my family and friends for making sure that I kept a balanced lifestyle between work and play, without whom this project may have been finished months in advance.

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1. Introduction

A widely-held theory in the field of neuroscience is that experiences lead to lasting changes in an individual's behaviour by modifying synapses. Working as ensembles in neural circuits, neurons can adjust the strength of their connections by synaptic plasticity processes. These mechanisms of synaptic modification were first postulated by Ramón y Cajal (Hebb, 1949) and later built upon by the works of Donald Hebb (Hebb, 1949). Hebbian theory postulated that the learning processes of the brain involve synaptic modifications: an enhancement in the strength of synapses take place when the pre- and post-synaptic activity co-occur (Hebb, 1949). In the present thesis, synaptic plasticity is defined as the capacity of synapses for functional and structural change. Synaptic plasticity is currently the leading candidate mechanism for learning and memory (Nicoll & Roche, 2013) and may play important roles in the etiologies and treatments of psychiatric disorders, including bipolar disorder, schizophrenia, major depressive disorder, and autism (Bourgeron, 2015; Duman, Aghajanian, Sanacora, & Krystal, 2016; Goto, Yang, & Otani, 2010; Harrison & Weinberger, 2005; Schloesser, Huang, Klein, & Manji, 2008).

1.1. Long-Term Potentiation

Empirical evidence supporting the theories of Cajal and Hebb (Hebb, 1949) was obtained through the discovery of long-term potentiation (LTP). As a result of the co-activation of neurons, Lømo first detected LTP in 1966 (Lømo, 2003). In a set of neurophysiological experiments exploring the role of the hippocampus in short term memory, presynaptic neurons of the performant pathway were stimulated and the responses of the postsynaptic cells in the dentate gyrus were recorded (Lømo, 2003). Lømo showed that high frequency electrical stimulation of the presynaptic neurons resulted in lasting increases - or long-term potentiation - of synaptic strength (Bliss & Lømo, 1973).

The discovery into mechanisms yielding LTP was succeeded by two important advancements. First, Schwartzkroin and Wester demonstrated that LTP could be induced in hippocampal slice preparations (Schwartzkroin and Wester, 1975). The fact that synaptic plasticity could be assessed *in vitro* substantially increased the accessibility of LTP studies. Second, Collingridge, Kehl, and McLennan (1983) showed that N-methyl-D-aspartate (NMDA)

receptors were critical for LTP induction in the hippocampus. Nicoll and Roche (2013) further built upon this by showing that LTP induction was blocked when NMDA receptors were deactivated.

There are many forms of synaptic plasticity and LTP is the best characterized type. Here, I briefly review the neural mechanisms underlying LTP. The synapse is the junction between neurons and allow for neural communication via chemical gradients. As such, it is the principal component involved in multiple functions from behaviour and cognition, to emotion, perception, learning and memory (Kandel, Schwartz, Jessell, Siegelbaum & Hudspeth, 2013). Glutamate is the main excitatory neurotransmitter of the nervous system. When sufficient presynaptic stimulation occurs, glutamate is released into the synaptic terminal and binds to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors on the postsynaptic membrane (Pankevich, Davis, & Altevogt, 2011) (Figure 1). Glutamate release leads to a depolarizing effect as a result of the sodium ions (Na^+) entering the postsynaptic membrane.

Provided that the electrical stimulus is strong enough or has the required frequency, AMPA receptors (AMPAR) will cause the postsynaptic membrane to depolarize. This allows NMDA receptors (NMDAR) to repel positively charged magnesium ions (Mg^{2+}) and leads to influx of calcium ions (Ca^{2+}) (Pankevich et al., 2011). Calcium ions are in turn vital secondary messenger involved in multiple postsynaptic intracellular signalling cascades. With calcium and glutamate being fundamental building blocks in the necessary steps required for synaptic transmission and thereby LTP, they are integral components of synaptic plasticity and learning (Wankerl, Weise, Gentner, Rumpf, & Classen, 2010).

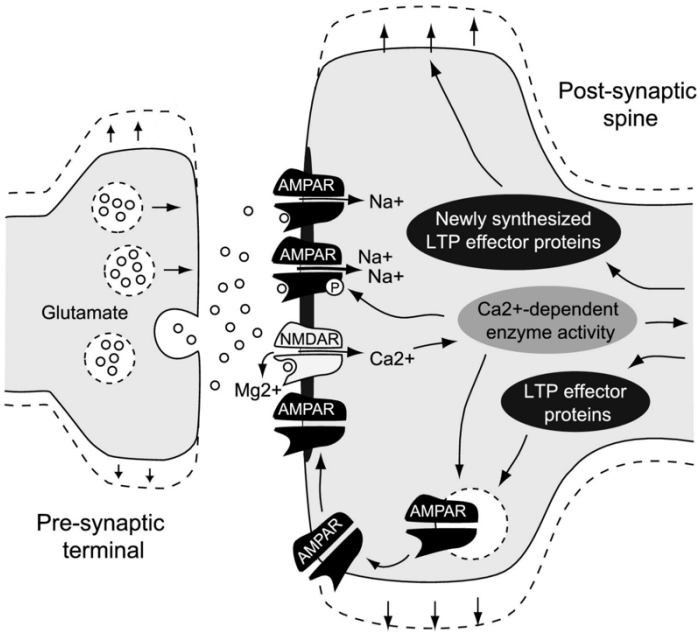


Figure 1. LTP mechanisms showing the pathway of sodium ions as well as glutamate binding to AMPAR and NMDAR leading to release of Mg^{2+} and influx in Ca^{2+} into the postsynaptic spine. Influx of calcium ions activates intracellular signaling cascades leading to integration of new AMPA receptors into the postsynaptic membrane. Adapted from "*Stimulus-selective response plasticity in the visual cortex: An assay for the assessment of pathophysiology and treatment of cognitive impairment associated with psychiatric disorders*." by Cooke, S.F. & Bear, M.F. (2012). *Biological Psychiatry, 71 (*6*),* 487-495.

A large number of molecules are responsible for the translation of intracellular signals and many of these have been shown to be integral in LTP (Malenka & Bear, 2004). In particular, calcium influx leads to phosphorylation of calmodulin dependent protein kinase II (CaMKII) (Barria, Muller, Derkach, Griffith & Sorderling, 1997). While the specific molecular components have yet to be determined in detail, the phosphorylated CaMKII facilitates increased AMPAR transport to the postsynaptic membrane (Citri & Malenka, 2008). Maintenance of LTP for hours is facilitated through the initial increase in AMPAR. The constant influx of Ca^{2+} leads to further activation of CaMKII related intracellular cascades (Citri & Malenka, 2008). This ensures a

lasting increase of AMPAR exocytosis and insertion into the postsynaptic membrane (Citri & Malenka, 2008).

1.1.1. Measuring LTP-like Plasticity

The CA1 region of the hippocampus, due to its involvement in the consolidation of long-term information, has been the focus of a large body of LTP research (Malenka & Nicoll, 1999). However, it is through acknowledgement of properties 'Hebbian' in nature and prototypic to information storage that LTP has become identifiable in animal studies on rodents (Malenka & Nicoll, 1999). These properties include *input specificity*, *associativity*, and *cooperativity* (Malenka & Nicoll, 1999).

Input specificity is the ability of individual synapses to be strengthened through repetitive stimulation while adjacent synapses are not affected. Associativity refers to 'strong' synapses being able to potentiate a 'weak' synapse input, given that excitation of the strong and weak synapses are in close temporal proximity. Cooperativity refers to the ability of a particular amount of depolarized synapses to be able to drive LTP if activated at the same time. Input specificity is inherently beneficial due to its ability to increase storage capabilities of the cell, whereas associativity and cooperativity are akin to the cellular mechanism involved in classical conditioning and therefore critical in Hebbian learning (Citri & Malenka, 2008).

Discovery of LTP and our ability to examine the molecular mechanisms responsible have been largely due to the excitation of neurons in excised cortical tissues, leading to succinct characterization (Clapp, Hamm, Kirk & Teyler, 2012). A key component in cognition and memory, previous LTP investigations have been limited due to a lack of ecological validity. Mainstream studies in the field have utilized methods from extracellular insertion of micro electrodes directly into the area of interest in lab animals (Bliss & Lømo, 1973), to exhibiting LTP effects on surgically resected brain tissue in the hippocampus (Beck, Goussakov, Lie, Helmstaedter & Elger, 2000) and temporal cortex (Chen et al., 1996) in humans. While the latter procedures confirms that LTP-like plasticity can be induced in brain tissues, it is imperative for future research to employ non-invasive methods. This would not only achieve high ecological validity and improved methods but further clarify the role of synaptic plasticity in human learning, memory, and psychiatric illnesses.

Results from animal studies using invasive procedures such as high frequency electrical stimulation have demonstrated LTP through direct measurements of synaptic strength (Cooke & Bear, 2013). However, to further advance our understanding of synaptic plasticity in human brain physiology and pathophysiology, techniques that enable non-invasive examinations of synaptic plasticity are required. To this end, two different methodological approaches have been recently employed: transcranial magnetic stimulation (TMS)-induced plasticity in the motor cortex (Esser et al., 2006) and modulation of auditory and visual evoked potentials (VEPs) by repetitive sensory stimulation (Kirk et al., 2010; Teyler et al., 2005). This thesis focuses on the latter methods.

In a sequence of experiments inducing LTP through visual (Teyler et al., 2005) as well as auditory stimulations (Clapp, Kirk, Hamm, Shepherd & Teyler, 2005), researchers demonstrated LTP-like effects within sensory cortices of healthy human subjects. These findings were further supported by studies by Cooke and Bear (2012) and strongly suggest that repetitive visual stimulation can induce synaptic plasticity in the visual cortices closely related to LTP (Clapp et al., 2012). Possessing similar characteristics as classical LTP, these non-invasive plasticity forms are considered to be 'LTP-*like*'.

Through the method of inducing LTP-like plasticity by repetitive visual stimulation, as is done in the present thesis, the non-surgical aspect of these techniques enable studies of synaptic plasticity in healthy humans and in individuals with psychiatric disorders. However, these techniques are new and whether and how factors such as gender, age, and the time-of-day affect LTP-like plasticity remain largely unknown.

1.2. Visual Evoked Potential (VEP) as a Non-Invasive Measure of LTP-like Plasticity

1.2.1. Visual Evoked Potential (VEP) and its Characteristics

In order to differentiate between spontaneous and induced EEG activity, ERP's (event related potentials) originally termed 'evoked potentials' (EPs) were labels referring to the induced potentials generated by external stimulation (Luck, 2005). The visual evoked potential (VEP) is the ERP evoked by visual stimulation (Luck, 2005). Measured by EEG techniques, the VEP mainly reflect postsynaptic potentials in the visual cortices (Luck, 2005).

When evoked by checkerboard reversal stimulation, the human VEP consists of positive and negative 'peaks' or 'components' termed C1, P1, and N1 (Figure 2). The numbers indicate

the position of the peak in the waveform while the letter preceding it indicates its polarity as a positive or negative deflection (Luck, 2005). According to Luck (2005), the C1 component is typically the first major peak observed with an onset time of 40 – 60 ms and a peak observed at 80 – 100 ms after presentation of stimulus. The C1 is not appropriately labelled with a 'P' or 'N', indicating that its polarity can vary. Among factors that affect the C1 polarity are the spatial location of the visual stimulus in the visual field and occipital cortex anatomy (Luck, 2005). Following the C1 component, the P1 (or P100) wave has an onset time of 60 – 90 ms, with a peak observed at 100 – 130 ms post stimulus (Luck, 2005). The final component of interest in this paper is the N1 (or N150) wave which consists of several subcomponents and peaks at 150 – 200 ms post-stimulus (Luck, 2005).

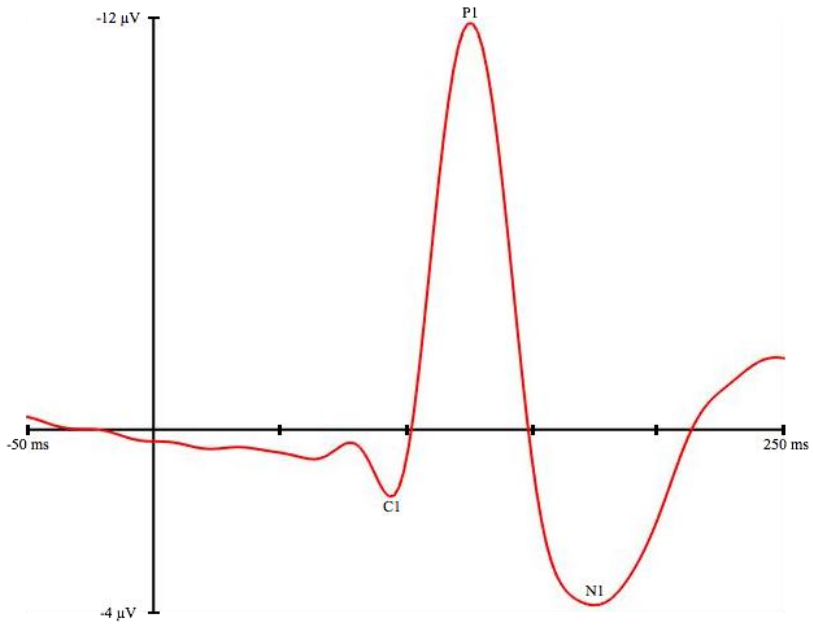


Figure 2. Expected visual evoked potential with C1, P1, and N1 components peaking at specific time windows in a healthy human of the present thesis study.

1.2.1.1. Animal Research

Heynen and Bear (2001) observed that *in vivo* theta-burst stimulation of the rat visual cortex induced NMDAR-dependent LTP and increased VEP amplitudes. To further explore the connection between VEP amplitude increases and LTP, Frenkel et al. (2006) designed a sophisticated experiment with repeated presentation of grated stimuli to awake mice. They

observed that repetitive visual stimulation potentiated the VEP and found that the increases could be best explained by LTP-like processes in the visual cortex. In particular, the VEP amplitude increases were blocked by delivery of NMDAR antagonists into the cortex, thus linking VEP plasticity to a core LTP characteristic (Frenkel et al., 2006).

Continuing the work of Frenkel et al. (2006), Cooke and Bear (2010) induced VEP plasticity by repetitive presentation of a sinusoidal grating to awake mice while measuring the VEP (Cooke & Bear, 2010). The observed VEP plasticity and LTP induced by electrical stimulation imitated and mutually occluded each other (Cooke & Bear, 2010). Furthermore, inhibition of a protein peptide, protein kinase M zeta, known to preserve NMDA receptor dependent LTP and memory in the rat hippocampus (Pastalkova et al., 2006), erased VEP plasticity (Cooke & Bear, 2010). Together, these recent findings in animals strongly suggest that VEP plasticity reflects synaptic processes closely related to LTP in the visual cortex.

1.2.1.2. Human Research

Teyler et al. (2005) were first to demonstrate LTP-like plasticity of the VEP after repetitive visual stimulation in humans. Electroencephalographic methods in the study employed a 128-channel electrode net on a group of 6 participants (Teyler et al., 2005). Following repetitive visual stimulation, the researchers observed no significant change in the P100, N1a, or P2 of the VEP while the N1b component of the VEP exhibited a significant increase (Teyler et al., 2005). Later VEP plasticity studies found a less negative N75 and a more positive P100 amplitude following repetitive visual stimulation (Elvsåshagen et al., 2012; Normann, Schmitz, Fürmier, Döing, & Bach, 2007)). Normann et al. demonstrated modulation of successive early components in the VEP of 74 healthy human controls: the P100 and N150 components of the VEP increased in amplitude and the effects lasted effect > 30 minutes (Normann et al., 2007). Results from human studies evoking VEP (Elvsåshagen et al., 2012; Normann et al., 2007; Teyler et al., 2005) show the ability to induce and measure modulations of the VEP noninvasively while observing properties parallel to that of LTP like plasticity and Hebbian learning.

Although, these initial human studies are promising, more research is needed to further characterize VEP plasticity in humans. For example, there are inconsistencies when it comes to which VEP amplitudes exhibit the most robust changes after repetitive visual stimulation

(Elvsåshagen et al., 2012; Teyler et al., 2005). Consequently, the present research is an extension of the paper by Elvsåshagen et al. (2012) which suggested that plasticity of the P1 and N1 amplitudes might be robust indices of LTP-like plasticity. In particular, the previous study (Elvsåshagen et al. 2012) was limited by a modest sample size and low number (15) of employed EEG electrodes.

1.3. Effects of Gender, Age and Time-of-Day on LTP

Proposed to form the principal neurobiological pathway for basic learning and memory (Nicoll & Roche, 2013), it is essential to have a brief review about gender, age and circadian variation effects on LTP.

Areas of the brain involved in the modulation of spatial learning and memory are theorized to employ LTP as the foremost cognitive mechanism (Bliss & Collingridge, 1993). Gender differences in humans have presented dissimilar performances on cognitive tasks. Sexual dimorphism research has previously found that men score higher on spatial ability tests, while women have scored higher on verbal ability tasks (Wegesin, 1998). Animal research has shown gender differences to exist within spatial learning as well: adult male rats and female rats were observed to use divergent learning tactics originating from different areas of the brain (Hawley, Grissom, Barratt, Conrad, & Dohanich, 2012). Male rats employed hippocampal-dependent strategies in comparison to female rats, who preferred a striatum-dependent learning strategy (Hawley et al., 2012). A look at the underlying mechanisms involved in visual and spatial learning may help to elucidate gender differences in LTP.

Ageing has been widely known to impair learning and memory. A molecular analysis of the effects of ageing indicates a shift from an NMDA-dependent LTP pathway to a voltage-operated calcium channels dependent mechanism (Ris & Godaux, 2007; Rosenweig & Barnes, 2003). Additionally, an increase in calcium loads within the synapse occurs from ageing effects on increasing calcium induced calcium release (Thibault, Gant, & Landfield, 2007). Taken together, ageing causes a shift to a voltage dependent calcium channel dependent LTP from a highly synapse specific NMDA-dependent LTP (Ris & Godaux, 2007). While the mechanism of LTP may function with ageing, one of the underlying criteria of LTP and Hebbian learning of input and synaptic *specificity* is compromised (Ris & Godaux, 2007). Animal research on rats has shown older rats to have impaired LTP initiation and maintenance with a complex

relationship governed regionally for different areas of the brain (Rex et al., 2005). An investigation exploring the effects of ageing on LTP would therefore require individual analyses of different brain regions.

Elucidating the time-of-day effect observed, researchers looked at circadian regulation of LTP in the hippocampus (Chaudhury, Wang, & Colwell, 2005). Chaudhury et al. (2005) were the first to provide evidence for changes in synaptic plasticity due to circadian oscillation. The change in strength of the post synaptic potential in the CA1 dendritic layer, as a result of high frequency stimulation, was much larger at night when compared to the same effect during the day (Chaudhury et al., 2005). In the hippocampus of nocturnal animals, LTP has been observed to change over the course of the day (Barnes, McNaughton, Goddard, Douglas, & Adamec, 1977; Chaudhury et al., 2005; Dana & Martinez, 1984; Nishikawa, Shibata, & Watanabe, 1995). This could indicate for synaptic efficiency to fluctuate based on the time of day at which a stimulus is presented. With animal research indicating for varying effects of gender, age and time-of-day on LTP, it would be interesting to study the impact such variables have on VEP plasticity.

1.3.1. Factors Affecting VEP

Previous studies examining VEP plasticity have shown modulation of the VEP via presentation of checkerboard reversals (Normann et al., 2007; Teyler et al., 2005). However, as a recent phenomenon observed non-invasively within humans, confounding factors leading to noteworthy changes in VEP plasticity between subjects have yet to be investigated in depth. By providing the P1-N1 peak-to-peak measure of the VEP as an effective quantification of LTP-like plasticity as shown through Elvsåshagen et al. (2012), variables leading to measurable alterations in the participants' respective plasticity have become a calculable task. Previous research as well as theoretical knowledge on what is expected to be observed has shown age, gender, and a time-of-day effect to convey a vital change in the results of such studies.

1.3.2. Gender and Age Effects on the VEP

Multiple studies have substantiated the use of the VEP in the analysis of decoding visual information (Allison, Wood, & Goff, 1983; Kubová et al., 2015; Porjesz, Begleiter, & Garozzo, 1980). While there exist variations in the observed VEP of individuals with respect to gender age (La Marche, Dobson, Cohn, & Dustman, 1986; Mitchell, Howe, & Spencer, 1987) most studies

have done so within the confines of small sample sizes and have not assessed VEP plasticity. La Marche et al. (1986) examined the effects of ageing and gender on pattern reversal VEPs. They found that older individuals had increased latencies across all VEP amplitudes while no significant latency effects of gender were observed (La Marche et al., 1986). However, females ranging from 55-70 years of age had a larger P100-N150 amplitude than men of the same age and younger females; this age effect on P100-N150 was not seen in males. Together, these findings indicate an age by gender interactions effect on VEP amplitudes.

In a manner of investigating the effects of similar paradigms but across the older population (40 – 80 years of age), Mitchell et al. (1987) looked at visual evoked potentials and the influence that age as well as gender may impart on the VEP. Comprised of 68 subject, 37 females, with a mean age of 61, latencies and amplitudes of the P100 and N150 components were measured while participants had their VEP elicited via use of a reverse checkerboard stimulus (Mitchell et al., 1987). Concurrent with the findings by La Marche et al. (1986), Mitchell et al. (1987) observed a significant increase in P100 latency with age effect, which the authors contributed towards an ageing in the neural pathways. However, unlike the significant change in amplitude detected in older females in the study by La Marche et al. (1987) this finding was not present in the study by Mitchell et al. (1987). Conversely Mitchell et al. (1987) with regards to gender differences observed a lower amplitude in males across all ages in comparison to females. A composition of the findings by Mitchell et al. (1987) argue for an age effect on the measurable latency of the P100 component in the occipital cortex for the VEP that is not effected by gender. However, the opposite holds true for amplitude, whereby, no observed effects of age on amplitude were detected, while gender differences presented significant variation.

Conflicting findings are observed between gender differences causing variations in the P100 amplitude of older women in the study by La Marche et al. (1986) in comparison to Mitchell et al. (1987). Research by Mitchell et al. (1987) could be improved upon to compare results across a younger age group while simultaneously recording for a longer period of time than what was previously capped at 15 minutes. Additionally, while the effects of the paradigm inciting VEP were measured and compared by gender and age after processing the test, it is noteworthy to include and compare the differences in resting state VEP prior to administering the test in both variables: age and gender.

Continuing along the work by La Marche et al. (1986), a paper written shortly after by Fein and Brown (1987), aimed to document differences in the P100 and N150 components of the VEP between genders. The researchers located a tendency in the P100 and N150 amplitudes of elderly females to be much larger, while latency to be smaller in comparison to elderly males, as analyzed through a peak-to-peak measurement (Fein & Brown, 1987). Latency effects observed in the N150 were significant even after the P100 latency effects were statistically removed. Furthermore, Fein and Brown (1987) observed gender variations in the P100 latency to subsist after age was statistically accounted for in the variation. This indicates distinguishable elements underlying differences in the VEP between genders. Replicating previous studies more recently, Sharma, Joshi, Singh, and Kumar (2015) focused particularly on the effects of gender and P100 amplitudes in 100 young adults from the ages of 17-20. Statistical significance between the two genders was observed with regards to latencies in the P100 and N150 peak as much higher in males, while P100 amplitudes as larger in females (Sharma et al., 2015). This agree with results presented in previous papers (Fein & Brown, 1987; La Marche et al., 1987).

An analysis of the methods in the previously mentioned papers, undertaken to explore variations that gender and age impart on the VEP, have limitations in their design. While there are generally consistent findings with regards to shorter latencies in the P100 and N150 VEP components as well as larger amplitudes in the older female group, subjects are split on either extreme of the age index as being very young (17-25) or very old (65+). This variability does not allow for a wholesome exploration of the effects of ageing upon components of the VEP, further effecting LTP-like plasticity. Additionally, a cross examination of age as a covariate of gender variations in VEP components has not been thoroughly scrutinized within all the previously mentioned papers in regards to this subject. As a result, a larger sample size of individuals with a diversification in the age range is necessary to probe for the precise effects that sex and age and any covariation the variables may convey on the P100 and N150 components of the VEP.

1.3.3. Time of Day Effect

It is commonly accepted that brain activity as monitored using EEG varies with the circadian rhythm. A paper by Cummings, Dane, Rhodes, Lynch and Hughes (2000) found the existence of time-of-day effects on healthy volunteers when collecting EEG data across a 24h period. Significant changes were recorded across the alpha, beta and theta wavebands denoting

discrepancy in the EEG data based on the time of day at which it was recorded (Cummings et al., 2000). Despite pre-existing knowledge on the time-of-day effects present in EEG and VEP components, very little research has been done with regards to the time-of-day at which electrical activity is evoked, more specifically synaptic plasticity, and the deviations that may arise because of it.

The synaptic homeostasis hypothesis was introduced by Tononi and Cirelli (2006) and claims that wake is associated with *net synaptic strengthening* in the brain. Tononi and Cirelli (2006) argue that wakefulness causes synaptic connections between neurons to strengthen (based on the concept of Hebbian learning) as a result of the exposure to the stimuli in an individuals' environment. The synaptic strengthening also results in less potential for change; thus, synaptic plasticity should be reduced after a day of wake, according to the hypothesis (Tononi & Cirelli, 2006). In addition, the hypothesis postulates that sleep is associated with *net synaptic weakening* in the brain and a normalization of synaptic plasticity (Tononi & Cirelli, 2006).

1.3.3.1. Time-of-Day Effects in Animal Studies:

Animals studies testing predictions from the synaptic homeostasis hypothesis have supported the theory. Maret, Faraguna, Nelson, Cirelli and Tononi (2011) observed that the number of cortical spines of adolescent mice increased after wakefulness and decreased after sleep. Gilestro, Tononi and Cirelli (2009) found strong evidence for net synaptic strengthening in the fly brain after 16 hours of wake and net synaptic weakening after sleep. Recently, de Vivo et al. (2017) measured synapses in the mouse motor and sensory cortices before and after sleep, showing a significant net down scaling of 18% in the axon-to-spine interface (an increase is correlated with synaptic potentiation). In the awake state, the mouse animal models presented a net increase in the axon-to-spine interface (de Vivo et al., 2017). Taken together, these results strongly support net synaptic strengthening during wake and synaptic renormalization after sleep.

1.3.3.2. Time-of-Day Effects on VEP in Human Studies:

In a study by Stolz, Aschoff, Born and Aschoff (1988) examining modulation in VEPs due to circadian variations over a 24h time period, participants had VEPs elicited while being presented with a checkerboard pattern reversal stimulus. Researchers saw significant effects from the data with increased latencies of the P1 (103ms) and N1 (138ms) wave between 2 - 5 am,

while shorter latencies were observed in the same wave at 5pm (P1 – 97ms / N1 – 130ms) (Stolz et al., 1988). Circadian variations leading to differences in the amplitude of the P1 and N1 peaks by Stolz et al. (1988) was observed to be consistent with the synaptic homeostasis hypothesis. Results demonstrated P1 and N1 amplitudes to increase from 6pm – 11pm while amplitudes were observed to have decreased during the night following a period of sleep (Stolz et al., 1988).

A separate study by Heninger, McDonald, Goff and Solberger (1969) analysing circadian effects conflicted with the results presented by Stolz et al. (1988). Heninger et al. (1969) observed increased latencies and decreased amplitudes in the VEP from measurements taken in the morning (9am) versus those taken in the evening (6pm). While presenting evidence for the theory of a time-of-day effect on the modulation of VEP and subsequently LTP-like plasticity, with a sample size of 6 individuals in the study by Heninger et al. (1969) and 8 male participants in the study by Stolz et al. (1988), there is much to be elaborated upon. Presumptive functions governing the time of day at which synaptic plasticity is evoked and its influence has remained largely unexplored through limitations in sample size.

Since the synaptic homeostasis hypothesis posits that wakefulness is associated with net synaptic strengthening and given that VEP amplitudes reflect strength of synapses in the visual cortex, wakefulness might lead to observable changes in the VEP amplitudes and latencies. For example, it can be hypothesized that individuals examined later in the day will have larger VEP amplitudes than participants examined earlier in the day. In addition, synaptic strengthening throughout the day may saturate synapses and thus make it more difficult to induce plasticity of the VEP later in the day.

1.4. Aims and Hypotheses

Use of the checkerboard reversals paradigm may provide for a non-invasive index to measure LTP like plasticity in humans. However, with very few studies evaluating the paradigm alongside the modest sample sizes employed (Elvsåshagen et al., 2012; Normann et al., 2007) it is important to examine the results within a larger group of participants. Simultaneously scrutinizing for effects that factors such as the gender, age, or the time-of-day at which participant data was collected may have.

Luck (2005) specifies a variation in polarity of the C1 component between individuals based on the exact location from which the scalp electrode detects the evoked potential. Current

research suggests that the P1 and N1 peaks to display the largest reliable modulation of the VEP from presentation of visual stimuli (Elvsåshagen et al., 2012; Normann et al., 2007). As a result, this study will focus on a comprehensive analysis of the P1 and N1 components.

The two focal aims of the present study with relevant hypotheses are as follows:

Aim 1: To replicate VEP plasticity and assess its robustness in the largest sample of healthy humans to date.

Aim 2: To investigate the effects of gender, age and time-of-day on pre-modulation VEP and VEP plasticity.

Hypotheses:

1. Based on previous literature (Elvsåshagen et al., 2012; Normann et al., 2007) it is hypothesized that 10 minutes of continuous visual stimulation will increase the P1, N1 and P1-N1 peak-to-peak amplitudes.
2. Consistent with previous literature, we expect to observe significant effects of gender on pre-modulation VEP amplitudes and latencies, including higher P1 amplitudes in females.
3. It is hypothesized that VEP plasticity does not vary by gender.
4. An increase in pre-modulation P1 latency with increasing age is expected.
5. Relative changes in VEP plasticity from pre- to post-modulation due to presentation of visual stimuli is not expected to be influenced by age
6. Pre-modulation VEP amplitudes and latencies are larger in individuals with increasing wakefulness
7. Individuals examined later in the day will have less VEP plasticity than individuals examined earlier in the day.

2. Methods and Materials

2.1. Participants

119 healthy control subjects (mean [SD] age, 36.58 [9.40]*;* 58 women) were recruited from ongoing recruitment of new participants in the larger Norwegian Centre for Mental Disorders Research (NORMENT) group pool of which the author is involved in. Written informed consent was obtained prior to each participants' involvement in the experiment. All work related to the project by NORMENT was approved by the Regional Committee for Medical Health and Research Ethics (REK Sør-Øst: 2014/664) and all Biobanks were approved. Subjects from the healthy control pool received 500 NOK as compensation for their participation throughout the entire NORMENT project.

The exclusion criteria for subjects included an intelligence quotient score of less than 70; record of severe brain or skull injury; parents, offspring or siblings with schizophrenia; pregnancy; disorders to the nervous system or other conditions relating to severe somatic symptoms; disorders related to mood; existing or prior use of mind-altering drugs; and recent abuse of alcohol, medication or any other drugs. Subjects between the ages of 18 and 65 with no existing or former diagnosis of a mental disorder were included in the sample. All subjects had normal or corrected-to-normal visual acuity.

A visual assessment of the data collected and the subjects' respective ability to complete the task as per instructed was performed. Based on an epoch rejection threshold > 25%, 6 subjects were rejected because of incomplete or noisy data due to an inaccurate assessment of the paradigm and an unbalanced weighting of stimuli that would subsequently occur in data analysis. The remaining sample included data from 113 subjects, 57 of whom were men (mean [SD] age, 35.67 [9.45]) and 56 women (mean [SD] age, 36.39 [9.90]).

2.2. Experimental Paradigm

The experimental paradigm employed was a modified version of the one used by Elvsåshagen et al. (2012). Due to the projects' larger involvement in the NORMENT group, additional mismatch negativity (MMN) and pre-pulse inhibition (PPI) paradigms for separate studies were implemented within the timeline of the data recording (Figure 3). MMN and PPI information will not be assessed further as it is outside the scope of this paper.

The paradigm consisted of checkerboard reversal stimulation (check size =.5°) with interstimulus interval varying randomly between 500 and 1500 ms. The visual stimuli were presented binocularly in the two blocks (pre-modulation blocks) prior to and eight blocks (post-modulation blocks) following the plasticity invoking block (modulation block) as shown in Figure 3. Forty checkerboard reversals were presented in the pre- and post-modulation blocks within a timespan of approximately 40sec. Continuous checkerboard reversal stimulation was employed in the modulation block with two reversals per second and a total of 1200 reversals for 10min, presented binocularly.

To allow for greater subject attention to the paradigm and to maintain focus on the paradigm, participants were monitored and instructed to fixate on to the 0.1° filled red circle located in the center of the screen. They were to press a button (green triangle) on a PlayStation 3 console handheld controller each time the circle changed colour to green. The pre-modulation phase consisting of baseline blocks 1 and 2 were presented at 1 and 2 min respectively following the initiation of the experiment while in the post modulation phase, post-modulation blocks 1-8 were performed following the 10 min plasticity inducing block at 2min / 3min 40 sec / 6min 20sec / 8min / 25min 50 sec / 27min 30 sec / 48min 50 sec / 50min 30sec, respectively. The screen displayed a neutral grey colour prior to and after checkerboard reversals.

Visual stimulation for evoking VEPs was done through presentation of a standard checkerboard reversal paradigm while participants were seated 60cm from a AOC G2460PQU 24” LCD monitor with a horizontal screen size of 53.3cm, a vertical screen size of 30.4cm, a screen resolution of 1920 x 1080 and a refresh rate of 144 Hz. Matlab R2015a (TheMathWorks Inc., 2015) alongside Psychtoolbox-3 (Wolf, 2013) was used to present visual stimuli while JoytoKey version 5.8.2 (JTKSOFT, 2015) and DS3 Tool version 0.6.0.3 (MotionInJoy, 2010) were used to register and record button pressing for the Playstation 3 console handheld controller.

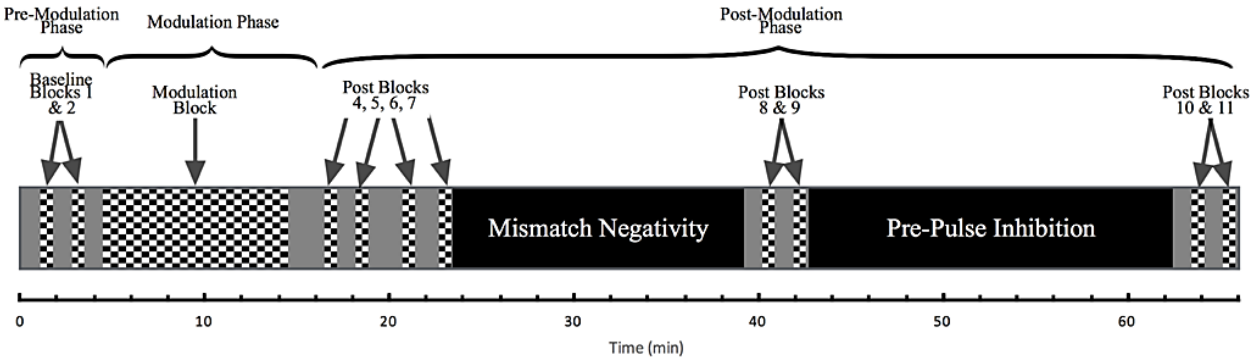


Figure 3. Experimental Procedure: Experimental paradigm adapted from Elvsåshagen et al. (2012) with additional data collection for a separate study using the mismatch negativity and pre-pulse inhibition paradigm. Visual evoked potentials were induced through checkerboard reversals in two baseline blocks (pre-modulation phase) followed by a plasticity inducing block of 10 minutes (modulation phase) and 8 post modulation blocks (post-modulation phase). Pre modulation (baseline) blocks 1 and 2 were 40 seconds in length and initiated at 1min and 2min 40sec respectively. Post modulation blocks 1 through 8 were initiated, at 2min / 3min 40 sec / 6min 20sec / 8min / 25min 50 sec / 27min 30 sec / 48min 50 sec / 50min 30sec, respectively following post-modulation block with a grey screen displayed between modulation intervals.

2.3. Recording and Analysis of the VEP

Scalp EEG was continuously recorded at a sampling rate of 2048 Hz, positioned according to the international 10/20 layout using a 64-channel-system Active electrode setup from BIOSEMI with 64 electrodes possessing a sintered silver-silver chloride electrode tip. Common Mode Sense (CMS) active electrode and Driven Right Leg (DRL) passive electrode are replacements in the BioSemi setup to the typical "ground" electrode. Performing a constant feedback loop, these electrodes are responsible for decreasing the average potential in the participant to zero (BioSemi, 2013). Therefore, eliminating the need for a ground or reference electrode. Actiview version 6.05 (BioSemi, 2013) software acquisition program was employed to continuously record EEG scalp data while no online filtering occurred.

EEG analysis of data after collection occurred offline using Matlab R2014a (TheMathWorks Inc., 2014) alongside EEGLAB 13.3.2b (Delorme & Makeig, 2004). Continuous EEG data from pre-modulation, modulation, and post-modulation phases was

merged and downsampled from 2048Hz to 512Hz followed by a re-reference to the average of all 64 cap electrodes. Identification of bad channels and the calculation of a robust average reference was administered to the data using PREP pipeline (Bigdely-Shamlo, Mullen, Kothe, Su, & Robbins, 2015), an EEG processing pipeline that relies on Matlab and EEGLAB. Data was subsequently high-pass filtered at 0.1 Hz and then low-pass filtered at 40 Hz followed by segmentation into epochs starting at 200 msec before to 500 msec after the commencement of checkerboard reversal patterns. An independent component analysis was applied across all 64 electrodes while, in an effort to produce manageable outputs, 35 principal components explaining for the largest subset of the data were specified. Subjects' independent component analysis was manually inspected with any components representing eye blinks discarded from the data. Following eye-blink components rejection, epochs were further reduced in time (-50msec to 350msec) and those containing amplitudes larger than 100V or smaller than -100V across the 64 electrodes were rejected. Subjects who had larger that 25% of their total epochs (1600) rejected were removed from the dataset (6 participants) as well. Data was re-referenced to scalp channel 37 (AFz) and baseline correction was applied from 50msec to 0msec prior to onset of checkerboard reversals.

Block specific averages were successively composed of the remaining data to generate VEP's that subsequently underwent extraction of amplitude and latency measures of the C1, P1, and N1 peaks from the Oz electrode. A Matlab script was used to extract information regarding peak amplitudes and their corresponding latencies of the components of interests. It specified time windows after onset of checkerboard reversals based on where one would expect to observe the components as per previous research (C1: Most negative value between 60-115 msec, P1: Most positive value between 90-150 msec, N1: First most negative peak following P1 between 130-200 msec). Following extraction, values were manually assessed and visually inspected for consistency with block specific VEPs. Any erroneous peaks recorded within the time window were corrected with the precise peaks of interest.

2.4. Statistical Analyses

Matlab R2015a (TheMathWorks Inc., 2015) alongside EEGLAB 13.3.2b (Delorme & Makeig, 2004) were used for generation of evoked potentials while SPSS statistical analysis software (Version 24.0 for Macintosh; SPSS, Chicago, Illinois) was the statistical program used

for analyses of data. Two-tailed t-tests were implemented with a *p* value less than or equal to .05 indicating significance in the tests. Post hoc statistical power analysis were conducted using GPower (Faul, Erdfelder, Lang, & Buchner, 2007), a software program. The measure of effect used was Cohen's *d* while effect sizes were computed by calculating the standardized mean difference between the two groups.

To examine the effects of the modulation phase on the post modulation blocks, the VEP amplitudes of the first two baseline blocks were combined as a collective baseline VEP. All VEPs from the blocks in the post-modulation phase (1-8) were averaged for a standardized approach to compare the baseline VEP against. C1, P1, N1 and P1-N1 peak-to-peak amplitudes from averaged post-modulation blocks were compared against the associated VEP components in the averaged pre-modulation blocks in a paired sample *t*-test to yield the effect of the modulation block on VEP in the entire group of subjects.

To assess for inherent differences present in the VEP because of gender, age, and the time-of-day at which participants were tested, averaged VEPs were extracted from the modulation phase. These VEPs contained the amplitude and latencies of the C1, P1, N1, and P1-N1 components. The modulation phase was selected for an indication of overall data as it contains the largest number of checkerboard reversals (1200) and therefore presents the most reliable estimate of the VEP, whereas the pre-modulation and post-modulation blocks individually contain 35 checkerboard reversals. Statistical analysis looking at inherent differences present in VEP amplitudes and latencies of the modulation block was done via Student *t* tests between male and female subjects while a Spearman's correlation was computed for the effects of age, gender by age, and the time of day at which participants were tested.

To measure the effect of the modulation phase on VEP, relative changes in VEP component amplitudes and latencies from the pre-modulation to the post-modulation phase were computed by subtracting the averaged C1, P1, N1, and P1-N1 peak-to-peak amplitudes and latencies in the post-modulation blocks from the related amplitudes and latencies in the pre-modulation blocks. The effect of gender on modulation of VEP component amplitudes and latencies was done by computing Student *t* tests. The effects of age and time-of day on relative changes in VEP component amplitudes and latencies was done by subjecting the data to a Spearman's correlation. Effects of age and time of data collection on relative changes in VEP as a result of the modulation looked at all subjects.

A non-parametric locally weighted polynomial regression (loess) model was employed to test for non-linearity present in the datasets and a tricube weight function with a span window accounting for 75% of neighbouring values was used to plot line fits in the scatterplots.

3. Results

3.1. Aim 1: Pre-modulation and Post-Modulation Phase VEPs

Checkerboard reversal stimulation produced the expected VEP amplitudes in the pre-and post-modulation blocks: a negative C1-peak at 94.19 ± .99 msec pre- and 93.65 ± .83 msec post-modulation, a positive P1 peak at 125.84 ± .54 msec pre- and 127.28 ± .50 msec post-modulation, and a negative N1 peak at 172.44 ± 1.44 msec pre- and 175.89 ± 1.15 msec post-modulation (Figure 4).

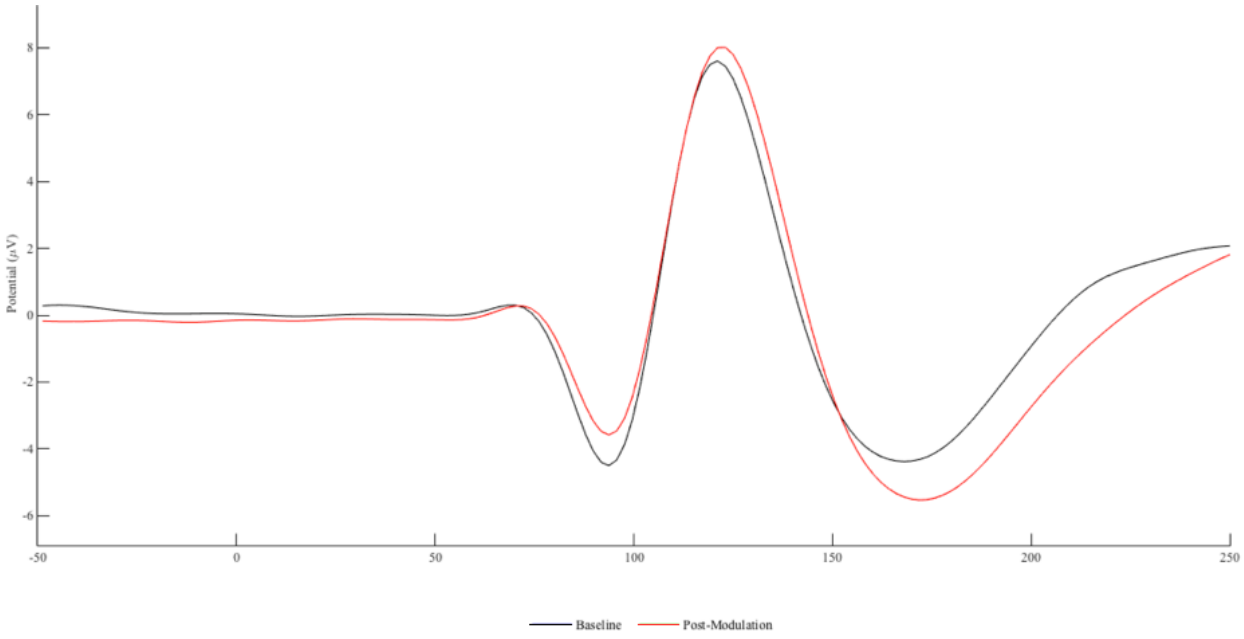


Figure 4. Grand averaged visual evoked potential in healthy subjects (n = 113) at the pre-(black) and post-modulation blocks (red).

3.1.1. Hypothesis 1: VEP Modulation

Figure 4 indicates averaged baseline blocks 1 and 2 VEPs graphed against post-modulation blocks 4-11 VEPs, of all subjects showing negative C1, N1 components and a positive P1 component. A significant effect was observed with a more positive C1 (t (112) = -5.988, p < .001) and P1 (t (112) = -4.425; p < .001) amplitude, and more negative N1 (t (112) = 3.3243; p =.002) amplitude between baseline and post-modulation blocks (Figure 5). A significant effect was observed within the differences of the P1-N1 peak-to peak amplitudes (t

(112) = -6.149*; p* < .001) from the pre-modulation to the post-modulation phase (Figure 5). Changes in amplitude relative to baseline in the 4 components measured (C1, P1, N1, P1-N1 peak-to-peak) survived Bonferroni correction for four tests (α /4 = .0125, with α = .05) with the largest measurable effect of the modulation block present in the P1-N1 peak-to-peak amplitude.

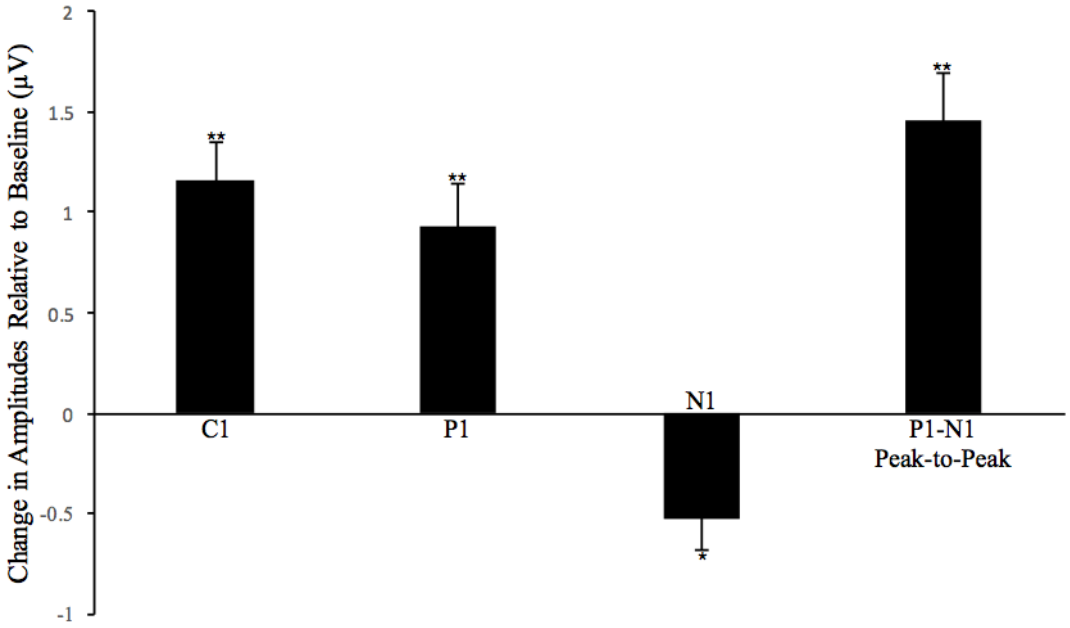


Figure 5. Modulation block of the experiment resulted in significant increases of the C1, P1, N1, and P1-N1 peak-to-peak amplitudes. Changes in the amplitudes of all components from the pre-modulation to post-modulation phase subsisted through Bonferroni correction (α /4 = .0125, with α = .05) for the four components tested with the largest measurable effect of the modulation block in the P1-N1 peak-to-peak amplitude. *p = .002, ** = p < .001. Error bars represent the SEM.

Figure 6 shows the change in P1-N1 peak-to-peak amplitudes as a result of the modulation block (i.e., relative to the mean of the pre-modulation blocks). With the exception of post-modulation blocks 5 and 7, the effect measured as a change in the relative component amplitudes, was observed as significant in each of the blocks, manifesting itself until the very last post-modulation block.

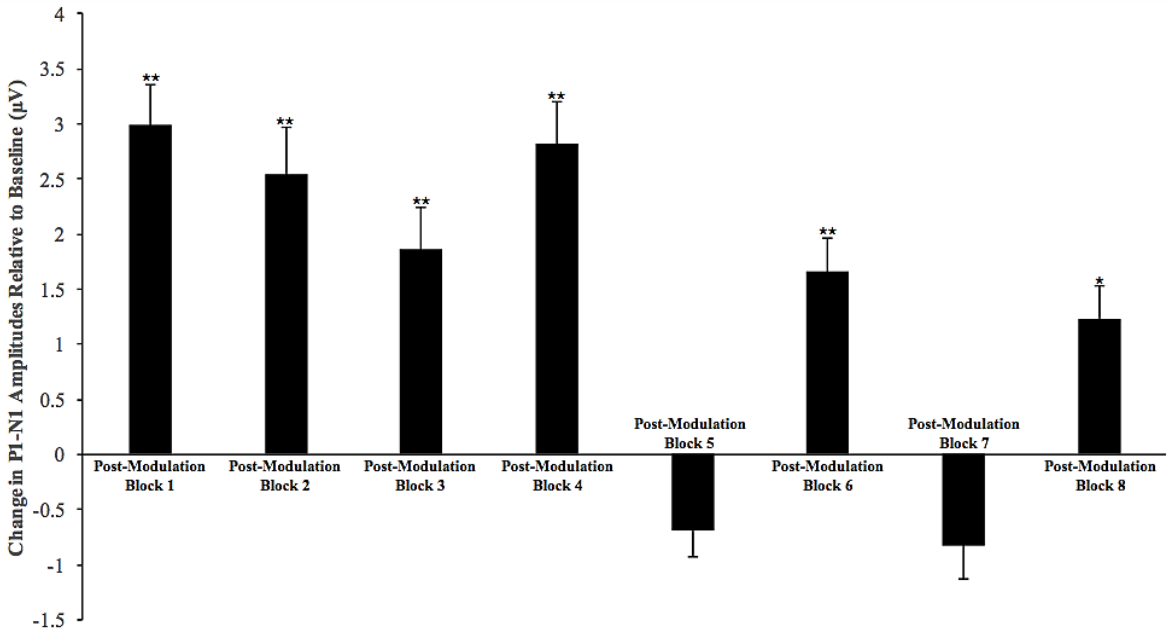


Figure 6. Relative change in P1-N1 peak-to-peak amplitudes in each post-modulation block from averaged baseline blocks. Statistical significance was observed from modulation of the change in all post modulation block (except for 5 and 7) until the final block. *p = .002, ** = p < .001. Error bars represent the SEM.

A detailed examination of the specific component changes leading to the observed effects in Figure 6, identifies individual changes observed in the post-modulation phase of the P1 and N1 amplitudes relative to the pre-modulation blocks (1 and 2) (Figure 7). P1 component amplitude increases in post-modulations blocks 1-4 as well as N1 amplitude increase in post-modulation block 6 were statistically significant from the averaged baseline blocks' P1 and N1 amplitudes. Thus, P1 and N1 appear to react differently to the modulation block.

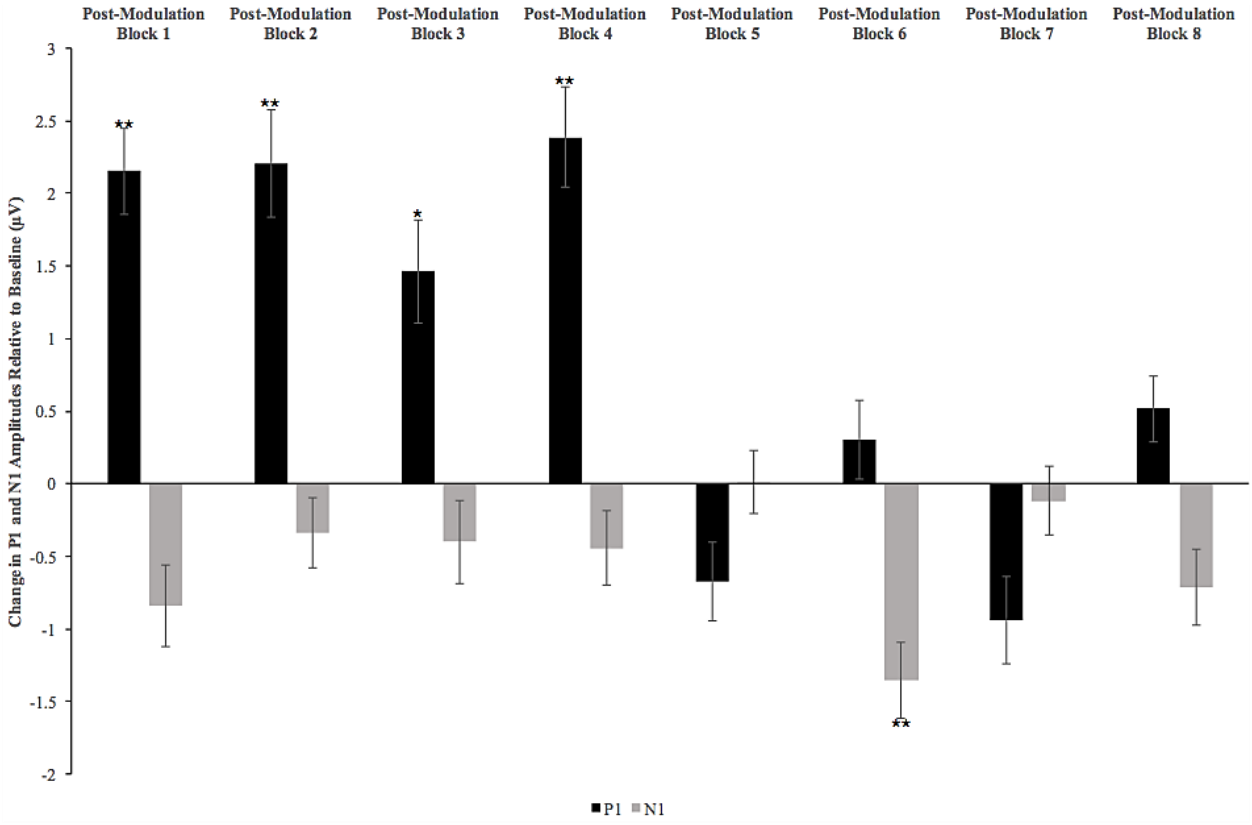


Figure 7. Relative change in P1 and N1 amplitudes of each post-modulation block from averaged baseline blocks. P1 amplitude change in post-modulation blocks 1-4 as well as N1 amplitude increase in post-modulation block 6 were statistically significant. *p = .003, ** = p < .001. Error bars represent the SEM.

3.1.1.1. Post Hoc Statistical Power Analyses of Modulation in VEP

C1 Amplitude Modulation: A post hoc power analysis using GPower (Faul et al., 2007) with α = .05, two-tailed, revealed a statistical power of 1.00 based on the effect size observed (d =0.56) from the means and standard deviation (M = -5.64, SD = 4.45) of the pre-modulation phase C1 amplitudes to its respective post-modulation phase values (M = -4.49, SD =3.28). For future studies, a minimum sample size of 27 would be necessary to achieve 80% power to detect a C1-modualtion effect of this magnitude.

P1 Amplitude Modulation: Post hoc power analyses using GPower (Faul et al., 2007) with α = .05, two-tailed, revealed a statistical power of 0.99 based on the effect size observed (d =0.42) from the means and standard deviation (M = 8.37, SD = 5.13) of the pre-modulation

phase P1 amplitudes to its respective post-modulation phase values (M = 9.30, SD =5.05). In order to achieve an identical effect size at a high statistical power advocated as .80 (Cohen, 1988), a sample size of 48 would be necessary to observe modulations in P1 peak amplitudes as a result of the modulation block.

N1 Amplitude Modulation: Post hoc power analyses using GPower (Faul et al., 2007) with $\alpha = .05$, two-tailed, revealed a statistical power of 0.89 based on the effect size observed (d =0.30) from the means and standard deviation (M = -6.79, SD = 3.55) of the pre-modulation phase N1 amplitudes to its respective post-modulation phase values (M = -7.31, SD = 3.55). In order to achieve an identical effect size at a high statistical power advocated as .80 (Cohen, 1988), a sample size of 87 would be necessary to observe modulations in P1 peak amplitudes as a result of the modulation block.

P1-N1 Peak-to-Peak Amplitude Modulation: Post hoc power analyses using GPower (Faul et al., 2007) with $\alpha = .05$, two-tailed, revealed a statistical power of 1.00 based on the effect size observed (d =0.58) from the means and standard deviation (M = 15.16, SD = 6.22) of the pre-modulation phase P1-N1 peak-to-peak amplitude measurements to its respective post-modulation phase values (M = 16.13, SD =6.49). In order to achieve an identical effect size at a high statistical power advocated as .80 (Cohen, 1988), a sample size of 26 would be necessary to observe modulations in P1-N1 peak-to-peak amplitudes as a result of the modulation block.

3.2. Aim 2 – Gender, Age, Time-of-Day

3.2.1. Effects of Gender on VEP

3.2.1.1. Hypothesis 2: Inherent Differences Between Genders in VEP Amplitude and Latency

A significant difference was observed in the P1 peak amplitude and latency scores for males (n = 57) (P1 amplitude: M = 8.835, SD = 4.379 / P1 latency: M = 129.627, SD = 6.667) and females (n = 56) (P1 amplitude: M = 10.782, SD = 5.017 / P1 latency: M = 126.447, SD = 6.931); (P1 amplitude: $t(111) = -2.199$, p = .030 / P1 latency: $t(111) = .738$, p = .014).

Post hoc power analysis using GPower (Faul et al., 2007) with $\alpha = .05$, two-tailed, revealed a statistical power of 0.59 based on the effect size observed (d = 0.413) from the means of the between groups comparison in P1 amplitudes, while a statistical power of 0.99 was observed based on the effect size (d = 1.37) from the means of the between groups comparison in

P1 latencies (i.e. a medium effect, according to Cohen, 1988). To achieve an identical effect size at a high statistical power, advocated as .80 (Cohen, 1988) with an allocation ratio of 0.98 females to males, a sample size of 186 would be necessary for P1 peak amplitudes while a sample size of 20 would be required for P1 peak latencies.

3.2.1.2. Hypothesis 3: Effects of Gender on VEP Modulation

Figure 8 shows averaged VEPs of females and males at the pre-modulation phase (pre-modulation blocks 1 and 2) and then again of the post-modulation phase (post-modulation blocks 1-8). As is expected, the VEP of both genders is characteristic of an averaged VEP response elicited from the presentation of reverse checkerboard visual stimuli showing negative C1 and N1 components as well as a positive P1 component.

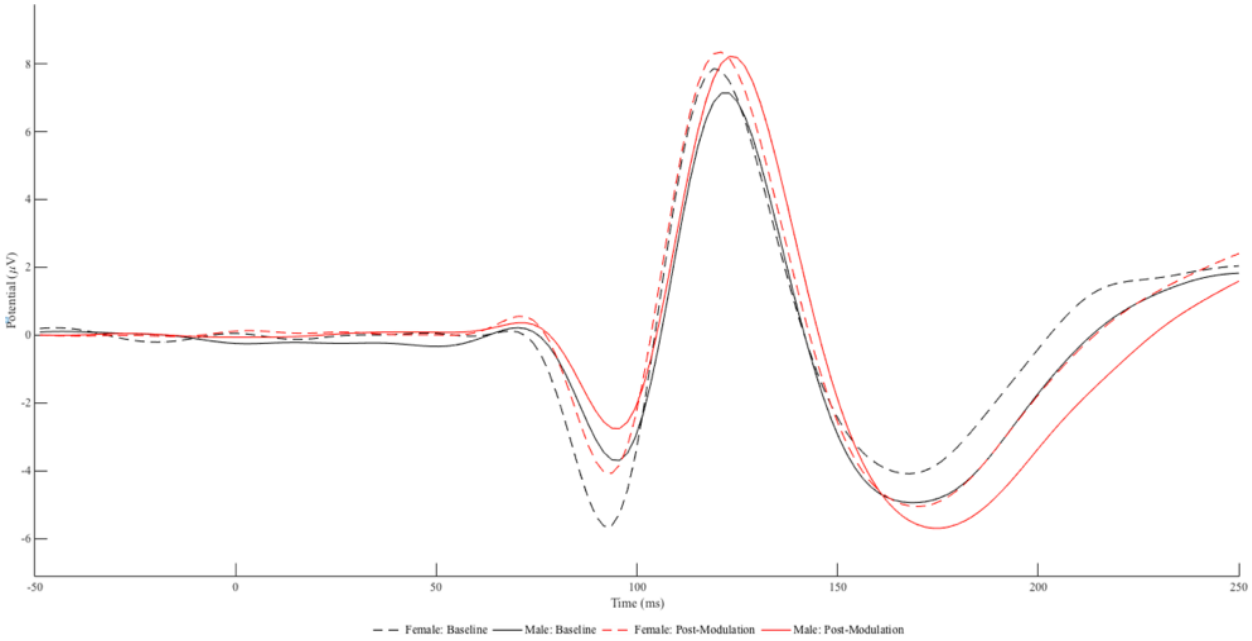


Figure 8. Grand averaged VEP of visual evoked potential in males (solid line, n = 57) and females (hashed line, n=56) at pre-modulation phase (black) and after post-modulation phase (red).

An independent-samples t-test was conducted to compare relative changes from pre- to post-modulation in C1, P1, N1 and P1-N1 peak-to-peak amplitudes and latencies between male and female subjects. This analysis failed to reveal a significant difference between the two

groups on relative changes in C1 ($t(111) = -1.138$, p = .257), P1 $t(111) = 1.209$, p = .229), N1 ($t(111) = -.042$, p = .966), P1-N1 peak-to-peak ($t(111) = .198$, p = .273) amplitudes or P1 ($t(111) = 1.402$, p = .164), and N1 ($t(111) = .678$, p = .499) latencies.

There was a significant difference in the scores for relative changes in C1 latency ($t(111) = 2.064$, p = .041), between males (M = .9467, SD = 8.522) and females (M = -2.0579, SD =6.8481). The sample means are displayed, in Figure 9a and 9b, for relative changes in amplitude and latencies respectively from pre- to post-modulation. Except for C1 latencies, Figure 9a and 9b show relative changes in amplitude and latency from pre- to post-modulation, between males and females in C1, P1, N1, and P1-N1 peak-to-peak measures, to be similar to each other.

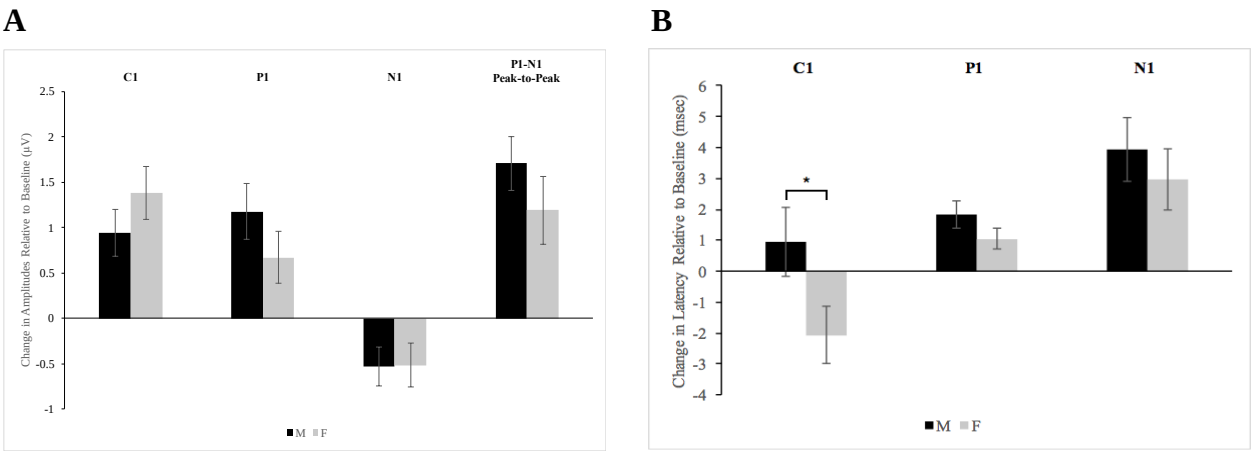


Figure 9. Mean levels of relative change in VEP from pre- to post modulation in male (n = 57) and female (n = 56) subjects. (*A*) C1, P1, N1, and P1-N1 peak-to-peak amplitude change from pre-to post-modulation. (*B*) Relative change in C1, P1, and N1 peak latency from pre- to post-modulation. Error bars represent the SEM.

3.2.2. Effects of Age on VEP

3.2.2.1. Hypothesis 4: Inherent Differences in VEP Amplitude and Latency Due to Ageing

A scatterplot summarizes the results of C1 (Figure 10a), P1 (Figure 10b), N1 (Figure 10c), and P1-N1 peak-to-peak (Figure 10d), amplitudes at the modulation block. Results of C1 (Figure 11a), P1 (Figure 11b), and N1 (Figure 11c) peak latencies at the modulation block are

summarized in Figure 11. Visual inspection of the data presented no apparent trend with age. As a result, a non-parametric locally weighted polynomial regression (loess) model was employed to look for non-linearity present in the datasets. The tricube weight function with a span window accounting for 75% of neighbouring values was used.

Spearman's correlation was computed for an examination of the effects of age on inherent differences in the VEP components of interests and their respective amplitudes and latencies in the modulation block. A negative correlation was observed in the latency of the N1 peak with increasing age (r_s = -.230, p = .014), while the data presented no strong correlates with age for other VEP component (C1, P1, N1, P1-N1 peak-to-peak) amplitude or latency.

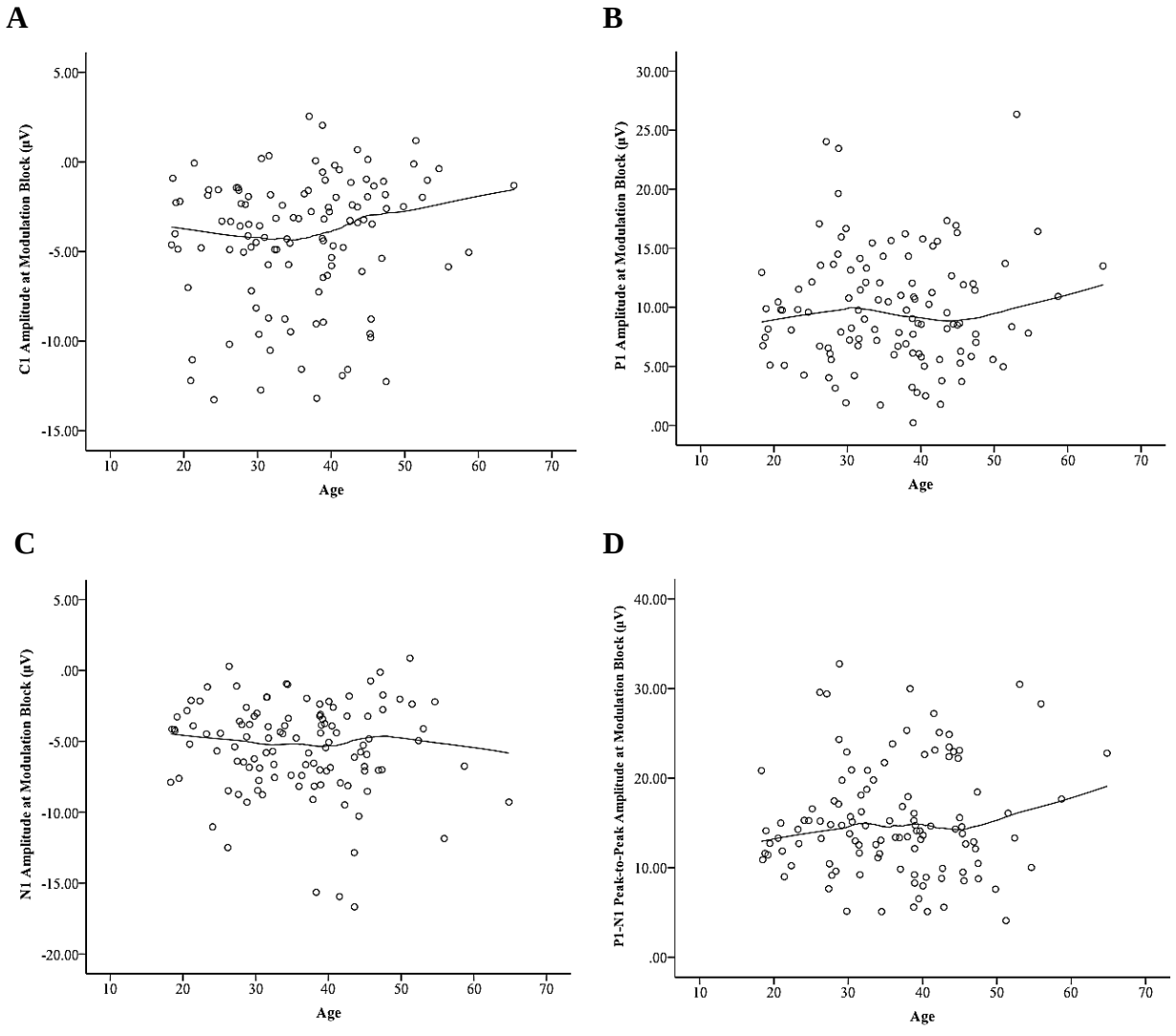


Figure 10. Component amplitudes of the VEP with age at the modulation phase (n = 113). (*A*) C1 peak amplitudes. (*B*) P1 peak amplitude. (*C*) N1 peak amplitude. (*D*) P1-N1 peak-to-peak amplitude. The line of best fit demonstrates a non-parametric LOESS regression model with a tricube weight function and a span window accounting for 75% of neighbouring values.

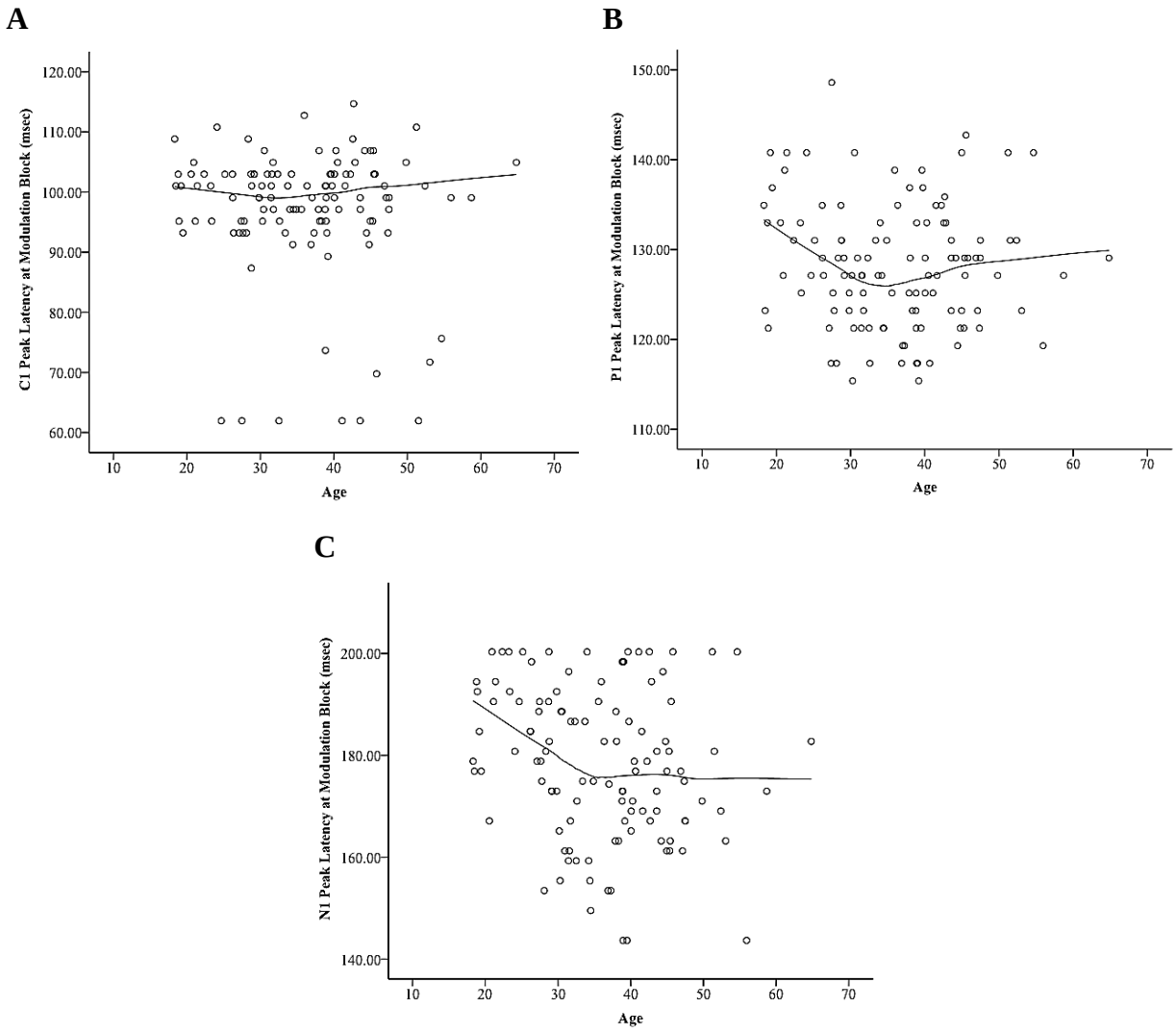


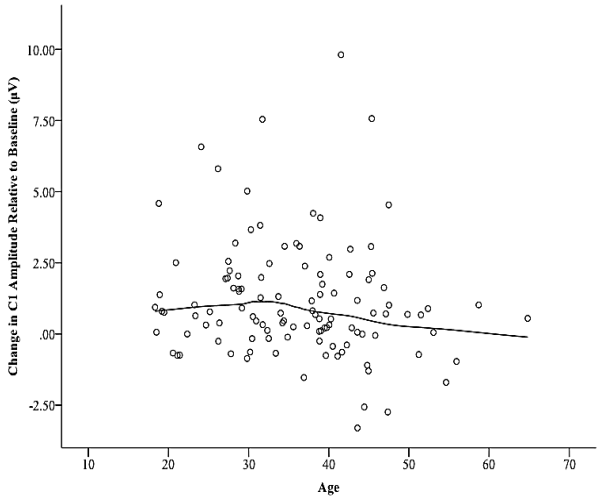
Figure 11. Component latencies of the VEP with age at the modulation phase (n = 113). (*A*) C1 peak latency. (*B*) P1 peak latency. (*C*) N1 peak latency. The line of best fit demonstrates a non-parametric LOESS regression model with a tricube weight function and a span window accounting for 75% of neighbouring values.

3.2.2.2. Hypothesis 5: Effects of Ageing on VEP Modulation

A scatterplot summarizes the results of relative changes in C1 (Figure 12a), P1 (Figure 12b), N1 (Figure 12c), and P1-N1 peak-to-peak (Figure 12d), amplitudes from pre-modulation to post-modulation. Results of relative changes in C1 (Figure 13a), P1 (Figure 13b), and N1 (Figure 13c) peak latencies from the pre- to post-modulation block are summarized in Figure 13. Visual inspection of the data presents no apparent trend with age. To look for non-linearity present in the datasets, a non-parametric locally weighted polynomial regression (loess) model was employed. The tricube weight function with a span window accounting for 75% of neighbouring values was used.

Spearman's correlation was computed to assess for the relationship between age and its relative effect on peak amplitudes as a result of the modulation block. Although there was a tendency towards a negative effect on all relative changes in amplitude and latency with increasing age except for in N1 latency, there was no significant correlation observed in any of the relative changes in amplitude or latency from pre- to post-modulation with age.

A



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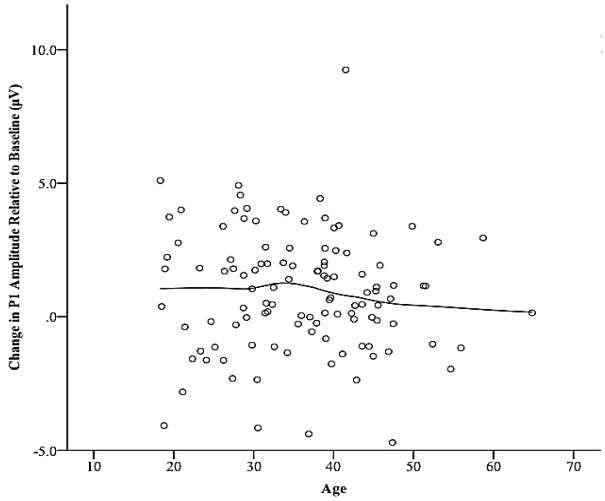


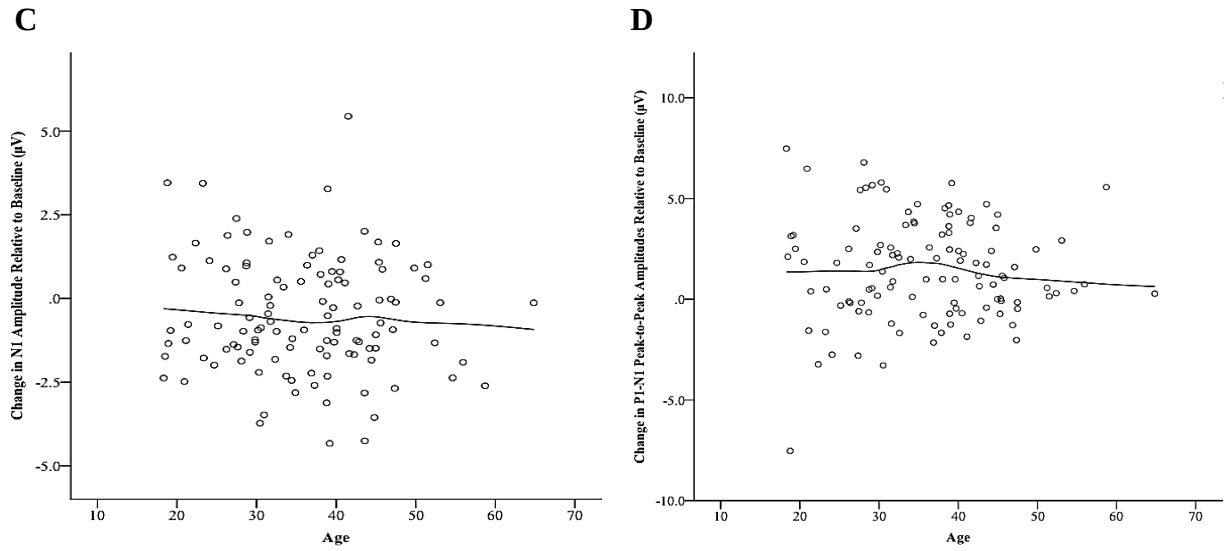
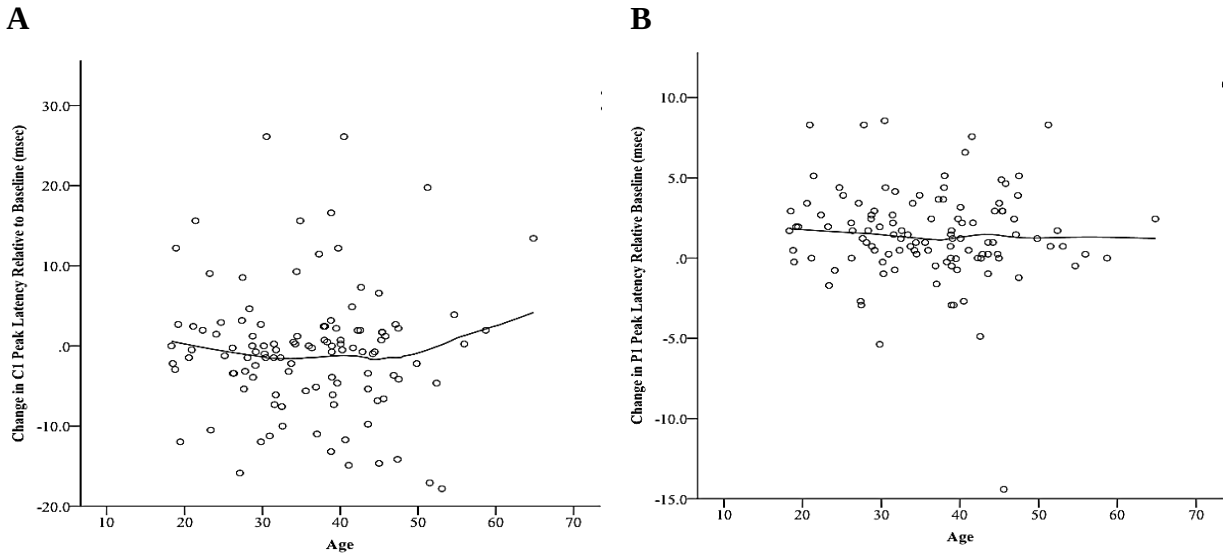


Figure 12. Relative changes in VEP component amplitudes of the VEP with age, from pre-modulation phase to post-modulation phase in all subjects (n = 113). (*A*) Relative change in C1 peak amplitudes. (*B*) Relative change in P1 peak amplitude. (*C*) Relative change in N1 peak amplitude. (*D*) Relative change in P1-N1 peak-to-peak amplitude. The line of best fit demonstrates a non-parametric LOESS regression model with a tricube weight function and a span window accounting for 75% of neighbouring values.



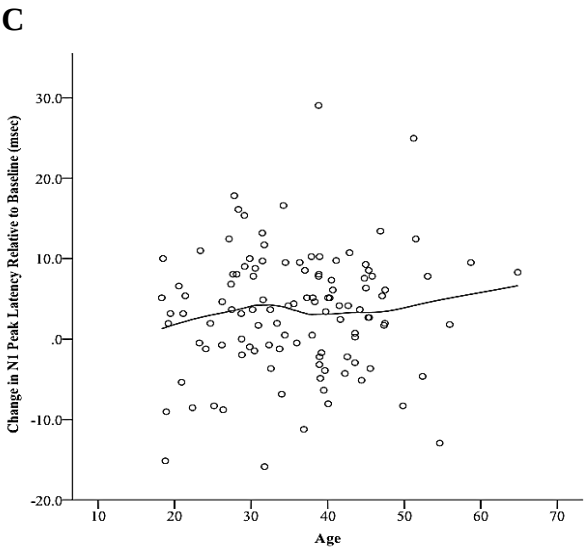


Figure 13. Relative changes in VEP component latencies (msec) of the VEP with age, from pre-modulation phase to post-modulation phase in all subjects (n = 113). (*A*) Relative change in C1 peak latencies. (*B*) Relative change in P1 peak latencies. (*C*) Relative change in N1 peak latencies. The line of best fit demonstrates a non-parametric LOESS regression model with a tricube weight function and a span window accounting for 75% of neighbouring values.

3.2.3. Gender by Age Interaction and VEP

Within the modulation block, a negative correlation with statistical significance was observed in the latency of the N1 peak with increasing age for females (r_s = -.388, p = .003). Evaluating for inherent differences in the amplitude and latency measures for the VEP components of the modulation block, the data presented no other statistically significant correlates of an age by gender effect in C1, P1, N1, P1-N1 peak-to-peak amplitude or C1, P1 peak latency measures.

Spearman's correlation was computed for an examination of an age by gender effect on modulation of the VEP from the pre- to post-modulation phase. Tests revealed statistically insignificant findings for all relative changes in latency and amplitude of C1, P1, N1 and P1-N1 peak-to-peak measures.

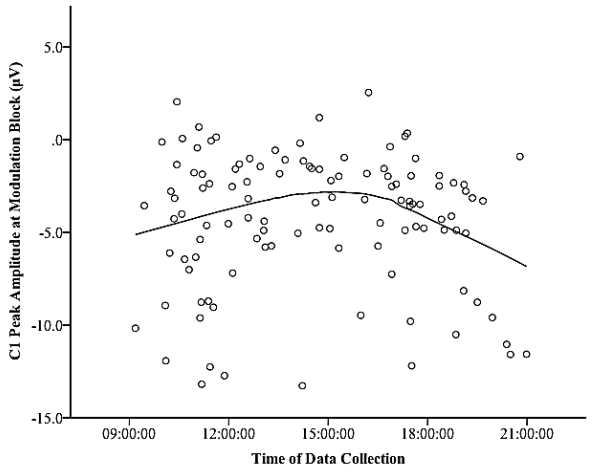
3.2.4. Effects of the Time-of-Day on VEP

3.2.4.1. Hypothesis 6: Time-of-Day Effect on Inherent Differences in the Amplitude and Latency of the VEP Peaks

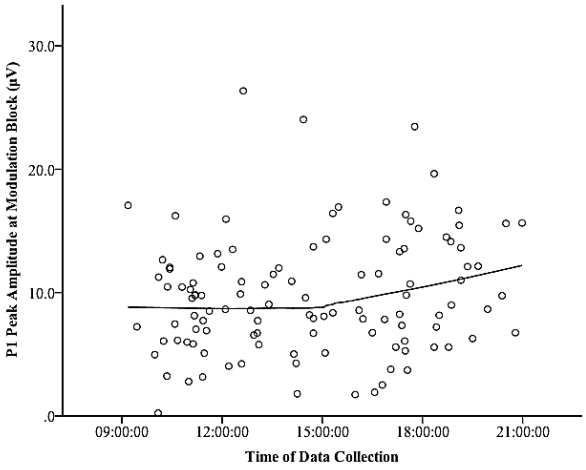
A scatterplot summarizes the results of C1 (Figure 14a), P1 (Figure 14b), N1 (Figure 14c), and P1-N1 peak-to-peak (Figure 14d), amplitudes at the modulation block plotted against the time of data collection. Results of C1 (Figure 15a), P1 (Figure 15b), and N1 (Figure 15c) peak latencies at the modulation block and time of data collection are summarized in Figure 15. Visual inspection of the data presents no apparent trend with the time-of-day at which data was collected.

Spearman's correlation was computed for an examination of the effects of the time-of-day at which participants were tested on inherent differences in the VEP components of interests (C1, P1, N1, P1-N1 peak-to-peak amplitudes and latencies) in the modulation block. While the fitted curves might suggest an association between amplitudes or latencies and the time of data collection, none of these, except for P1 peak amplitude, was significant. A positive correlation with statistical significance was observed in the amplitude of the P1 peak as time of data collection progressed later in the day ($r_s = .187$, $p = .047$).

A



B



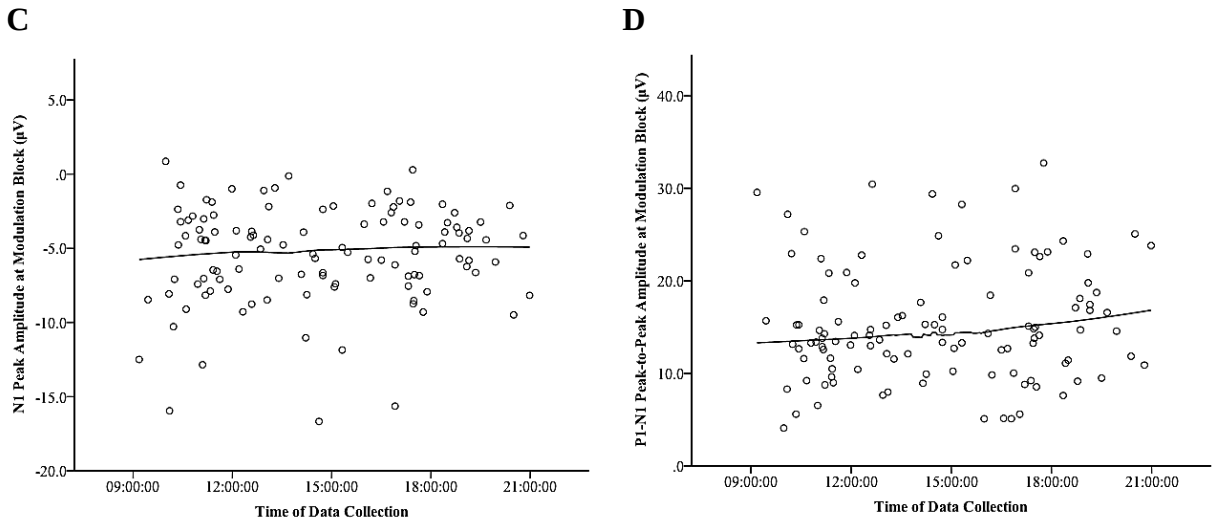
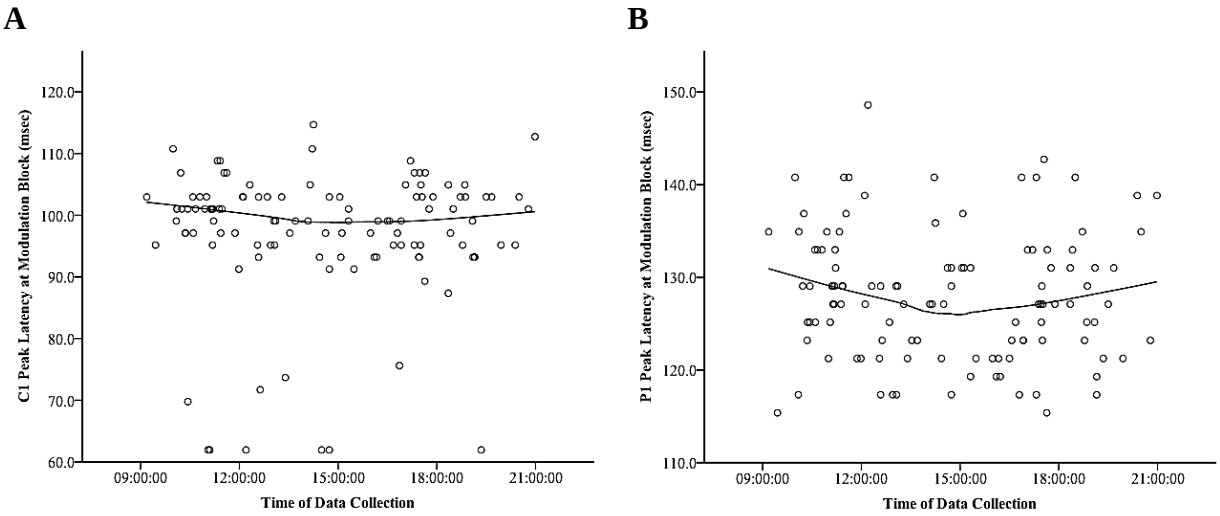


Figure 14. VEP component amplitudes of the VEP at the modulation block, with time-of day at which participants were tested (n = 113). (*A*) Averaged C1 peak amplitudes at modulation phase. (*B*) Averaged P1 peak amplitudes at modulation phase. (*C*) Averaged N1 peak amplitudes at modulation phase. (*D*) Averaged P1-N1 peak-to-peak amplitudes at modulation phase. The line of best fit demonstrates a non-parametric LOESS regression model with a tricube weight function and a span window accounting for 75% of neighbouring values.



C

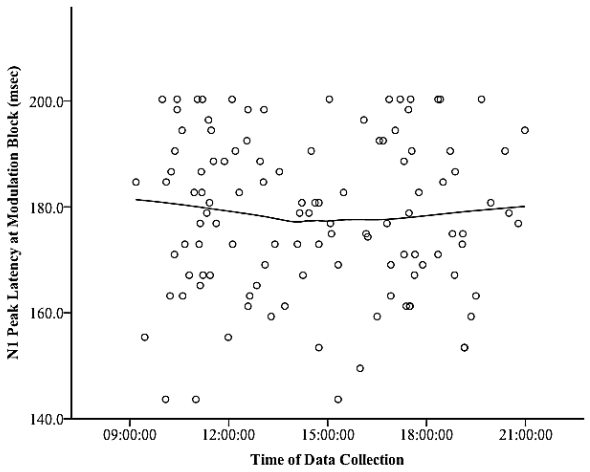


Figure 15. VEP component latencies (msec) of the VEP at the modulation block, with time-of day at which participants were tested (n = 113). (*A*) Averaged C1 peak latencies at modulation phase. (*B*) Averaged P1 peak latencies at modulation phase. (*C*) Averaged N1 peak latencies at modulation phase. The line of best fit demonstrates a non-parametric LOESS regression model with a tricube weight function and a span window accounting for 75% of neighbouring values.

3.2.4.2. Hypothesis 7: Time-of-Day Effects on VEP Modulation

Figure 16 summarizes the results of relative changes in C1 (Figure 16a), P1 (Figure 16b), N1 (Figure 16c), and P1-N1 peak-to-peak (Figure 16d) amplitudes from an average of the pre-modulation to the post-modulation blocks plotted against the time at which VEP recording started in the experiment. Figure 17 summarizes the results of relative change in C1 (Figure 17a), P1 (Figure17b), and N1 (Figure 17c) peak latencies from the pre- to post-modulation phase.

Visual inspection of the data in Figures 16 and 17 suggest an association between amplitude, latencies, and time of data collection for the VEP component of interests. Spearman's correlation was calculated individually for relative change in averaged peak amplitude and latency from pre-modulation to post-modulation on C1, P1, N1 and P1-N1 peak-to-peak measures. There was no significant correlation between the time of day and relative changes in C1, P1, N1, P1-N1 peak-to-peak amplitudes and latencies from pre- to post-modulation.

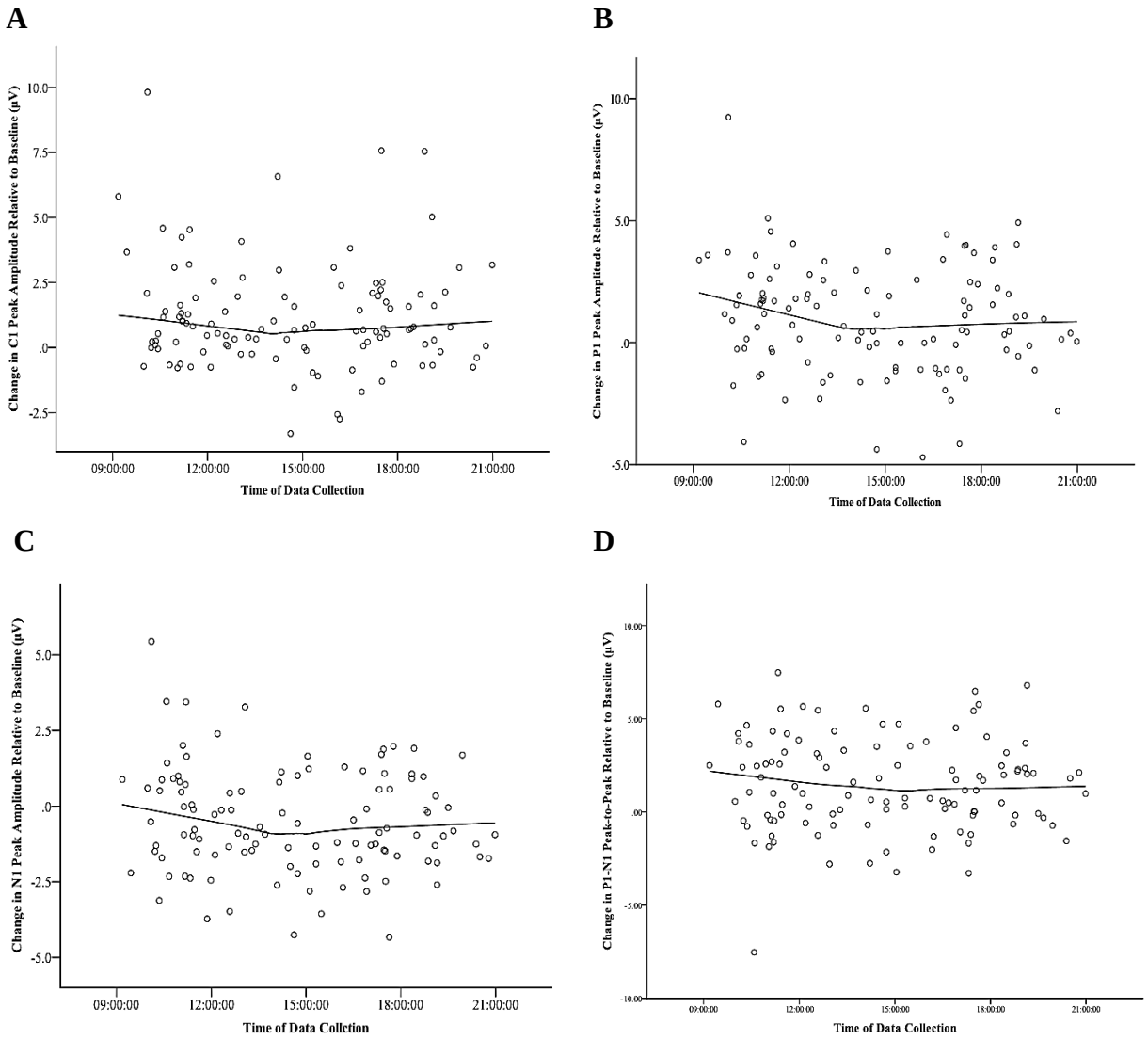


Figure 16. Relative changes in VEP component amplitudes of the VEP, from pre-modulation phase to post-modulation phase with time-of day at which participants were tested (n = 113). (*A*) Relative change in C1 peak amplitudes. (*B*) Relative change in P1 peak amplitude. (*C*) Relative change in N1 peak amplitude. (*D*) Relative change in P1-N1 peak-to-peak amplitude. To test for non-linearity in the dataset, the line of best fit demonstrates a non-parametric LOESS regression model with a tricube weight function and a span window accounting for 75% of neighbouring values.

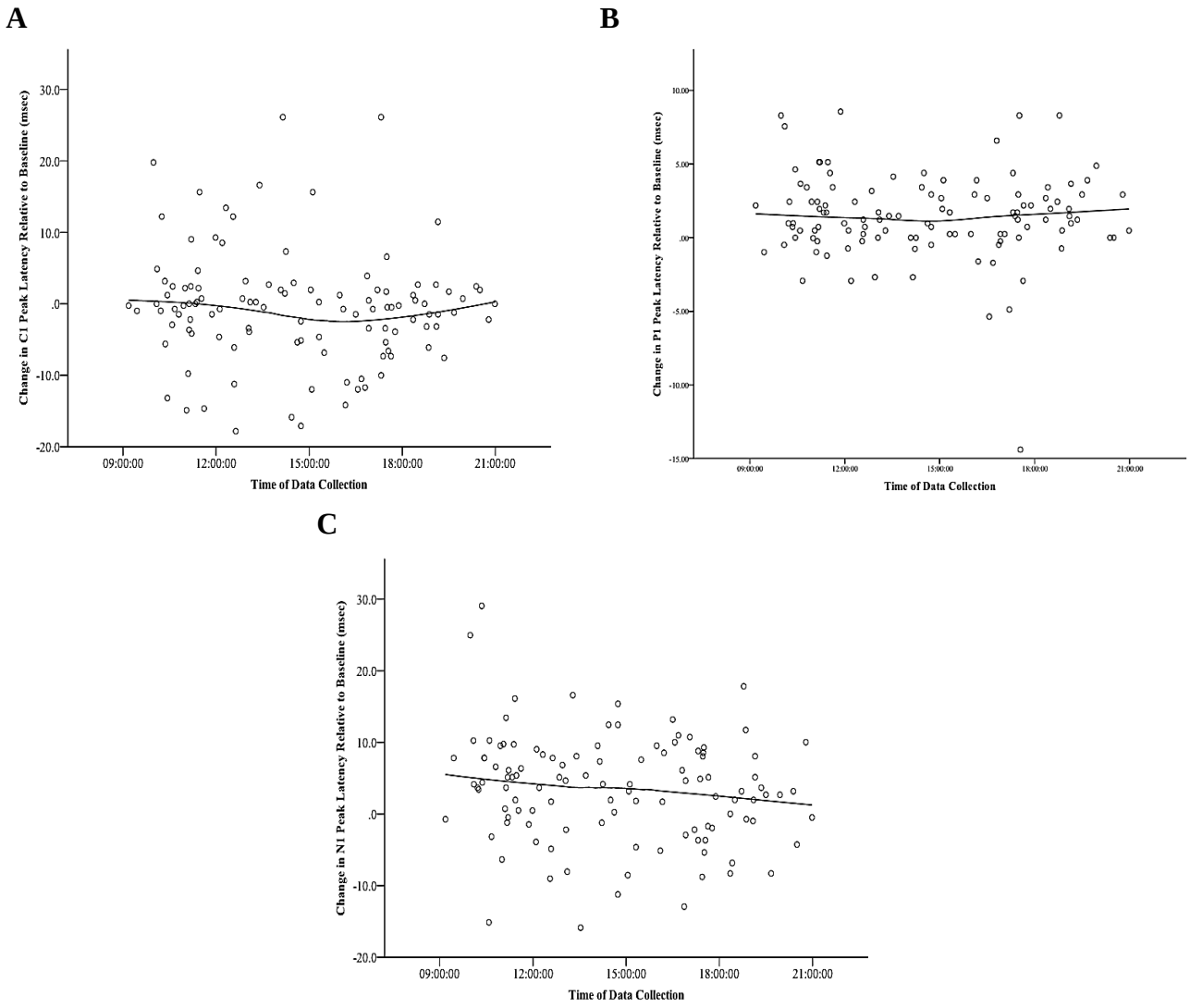


Figure 17. Relative changes in VEP component latencies (msec) of the VEP, from pre-modulation phase to post-modulation phase with time-of day at which participants were tested (n = 113). (*A*) Relative change in C1 peak latencies. (*B*) Relative change in P1 peak latencies. (*C*) Relative change in N1 peak latencies. To test for non-linearity in the dataset, the line of best fit demonstrates a non-parametric LOESS regression model with a tricube weight function and a span window accounting for 75% of neighbouring values.

4. Discussion

The aim of the current study was twofold. First, we aimed to reproduce VEP plasticity in the largest sample of healthy volunteers to date. The second aim was to examine the robustness of the paradigm by assessing the effects of gender, age and time-of-day on plasticity of the VEP.

First, we found significant plasticity of the VEP after repetitive checkerboard reversal stimulation, consistent with previous studies (Elvsåshagen et al., 2012; Normann et al., 2007). We also found significant effects of gender, age and time-of-day on VEP amplitudes. However, there were no significant effects of gender, age, or time-of-day on VEP plasticity.

4.1. VEP as a Measure of LTP-like Plasticity

Previous studies induced LTP-like plasticity by high-frequent and invasive electrical stimulation of thalamocortical visual pathways of adult rats (Heynen & Bear, 2001). Teyler et al. (2005) were first to report non-invasive LTP like VEP-plasticity in humans after repetitive visual stimulation. Subsequent reports on the non-invasive manner of studying VEP as a measure of LTP-like plasticity came from Normann et al. (2007) and their observation on being able to induce plastic changes in neural responses of healthy subjects: modulation of the VEP resulted in significant increase in P1 and N1 amplitudes with effects lasting over 20 minutes. This discovery was followed closely by Elvsåshagen et al. (2012): results confirmed reports of significant amplitude changes in P1 and N1 components as well as overall peak-to-peak P1-N1 amplitude measures.

Previous studies suggested that plasticity of the P1, N1, and P1-N1 of VEP is a robust phenomenon (Elvsåshagen et al., 2012; Normann et al. 2007). As a result, this study examined amplitude plasticity of the P1, N1, P1-N1 peak-to-peak. We confirmed significant increases in the P1, N1, and P1-N1 peak-to-peak amplitudes following the modulation phase. Modulation of the P1-N1 peak-to-peak amplitude from the pre- to post-modulation phase was more robust than P1 or N1 plasticity. Furthermore, modulation of the P1-N1 peak-to-peak measure was observed to be significant in the final post-modulation block, approximately 49 minutes following visual stimulus presentation in the modulation block. The “long-termness” of LTP is commonly considered to mean >30 minutes (Abraham, 2003), thus it is encouraging to see that VEP plasticity lasted at least 49 minutes.

To remain consistent with previous literature (Elvsåhagen et al., 2012; Normann et al., 2007) changes in the P1-N1 peak-to-peak amplitude from pre- to post modulation blocks were used as a measure of plasticity. Results from the current study presented modulation in the P1-N1 peak-to peak amplitudes as the most robust change in data with the largest effect size (d =0.58), in comparison to P1 (d =0.42) or N1(d =0.30) amplitude modulations and effect sizes, individually. An indication of long term synaptic potentiation as a result of this paradigm can be hypothesized from the effect remaining significant and observable until post-modulation block 8 of the study. This data suggests repeated visual stimulation over an extended period of time to non-invasively modulate VEP plasticity in healthy human subjects. Consequently, robustness of the P1-N1 peak-to-peak amplitude in VEP plasticity from this study, may indicate for its consideration in future studies exploring plasticity of the VEP.

A decrease in the amplitude measurements presented at post modulation blocks 5 and 7 followed by a reversal in the opposite direction for the proceeding blocks (6 and 8) was observed. This indicates for a return to baseline in synaptic potentiation after presentation of checkerboard reversals. Given the setup of the experiment, with an approximate 20-minute gap preceding post-modulation block 5 and 7, to accommodate for the testing of other paradigms (MMN and PPI, for the larger study in which the author is involved), it is possible that the P1-N1 peak-to-peak modulation change in post-modulation blocks 5 and 7 are the result of external visual stimulation. This may have subsequently influenced the potentiated VEP of the subject. Future studies could assess whether skipping the PPI and MMN paradigms would lead to more lasting amplitude increases. However, studies on VEP plasticity in animals have shown the VEP response to be extremely stimulus specific after training to a specific criterion: presentation of a differently oriented sinusoidal grating did not lead to further long-term potentiation (Cooke & Bear, 2010). A reappearance of the familiarly oriented stimulus demonstrated a return of the visually potentiated response (Cooke & Bear, 2010). This is in agreement with the current study and the re-emergence of a significantly potentiated P1-N1 peak-to-peak amplitude following initial presentation of the familiar stimuli in post-modulation blocks 6 and 8.

A closer inspection of directional changes in P1 and N1 amplitudes individually across the post-modulation block indicate for a general decrease in the relative change of P1 and N1 amplitudes from the pre-modulation phase. A trend of the amplitudes returning to baseline levels is observed. However, an interesting effect of a significant increase in N1 amplitudes was

observed at post-modulation block 6. Research looking at factors influencing early components of the VEP have discovered the length of time between the presentation of matching stimuli to affect the respective VEP components' properties (Steiner, Barry, & Gonsalvez, 2014). Increasing temporal length between stimulus-to-matching-stimulus presentation led to an increase in the amplitudes of the N1 in the VEP extracted (Steiner et al., 2014). While it has been shown previously that a systematic increase in the target-to-target stimulus (exposure to familiar stimuli over increasing intervals of time) have caused an augmentation of the P300 amplitude (Croft, Gonsalvez, Gabriel, & Barry, 2003), Steiner et al. (2014) provide evidence towards an increase in intervals between familiar stimulus presentation subsequently predicting a larger N1 amplitude. This is in line with the current study as we observe an increase in N1 amplitudes following an approximate 20-minute stimulus-to-matching-stimulus interval preceding post-modulation block 5 and 6. This observation is defined once more in post-modulation block 8 with an increase in N1 amplitude following presentation of the familiar reverse checkerboard stimulus after a stimulus-to-stimulus interval gap. Given the increase in N1 amplitudes to a familiar stimulus alongside P1-N1 component modulations in the final block of the paradigm, changes in the VEP from the pre-modulation to post-modulation phase indicate for LTP-like plasticity of the VEP.

While confirming results from previous studies of a potentiated P1 and N1 as well as P1-N1 peak to peak amplitude, significant potentiation was found in the C1 peak amplitude previously not confirmed in similar studies. Based on the moderate effect size (d =0.56) present in this study, a post hoc statistical power analysis revealed a sample size of 27 as necessary to achieve 80% power in detecting modulations of the C1 peak amplitude as a result of the modulation block. While both studies (Elvsåshagen et al., 2012; Normann et al., 2007) possessed 40 and 74 healthy controls respectively, studies looking at potential generators of early components in the VEP have identified C1 activity to heavily rely on the retinotopic organization of the striate cortex. In a study by Clark, Fan and Hillyard (1995), varying stimulus position led to a consistent change in the polarity and topography of the C1 based on the retinotopic mapping of the visual stimuli on the striate cortex. Although the neural sources of the pattern-reversal VEP remain to be fully clarified, previous studies suggest that the C1 mainly reflects postsynaptic activity in V1, whereas P100 and N145 are likely generated in both striate and extrastriatal cortex (Di Russo et al., 2005; Fuglø, Pedersen, Rostrup, Hansen, & Larsson, 2012;

Novitskiy et al., 2011; Tobimatsu and Celesia, 2006; Whittingstall, Stroink, & Schmidt, 2007). Taken together, these studies reveal multiple neural generators from different areas in the brain, contributing to the potentiation of the VEP.

Following previous reports, Martinez et al. (1999) investigated neural pathways and anatomical generators of visual spatial attention. Using fMRI retinotopic mapping, Martinez et al. (1999) observed a lack of modulation in very early VEP components of the striate cortex (50-55 msec) succeeding sensory input due to attention. However, attention was measured to be modulating VEP components starting from 70 milliseconds following stimulus presentation in the extrastriatal visual areas (Martinez et al., 1999). These findings regarding the cortical sources of the C1 component in the striate cortex alongside the lack in modulation in early components of VEP from attentional changes, indicate for plasticity of the VEP to involve extrastriatal areas. With multiple neural generators influencing potentiation of the VEP, the C1 component measures may not pose to be a primarily ideal indicator of learning through visual stimulation.

4.2. Gender, Age and Age-Dependent Gender Effects on VEP

An independent-samples t-test was conducted on data in the modulation block to examine for inherent differences present in the VEP components of interest (C1, P1, N1, P1-N1 peak-to-peak) amplitudes and latencies between male and female subjects. The present study confirms previous reports of a larger P100 amplitude and shorter latency in females in comparison to males (Fein & Brown, 1987; La Marche et al., 1987; Mitchell et al., 1987; Sharma et al., 2015).

Spearman's correlation was computed for an examination of the effects of age on inherent differences in the VEP components of interests and their respective amplitudes and latencies. Inherent differences present in the VEP of individuals due to age, as evaluated through the modulation block revealed a statically significant modulation in the N1 peak latency. However, further analysis into ageing-dependent gender effects presented a significant effect of ageing on the latency of the N1 component solely within female subjects. This confirms previous literature observations of a gender specific effect on N1 peak latency (Fein & Brown, 1987). However, while Fein and Brown (1987) observed a significantly shorter latency of the N1 component in older female subjects in comparison to males, the present study having conducted a non-parametric regression analysis across the continuous variable of age detected the measurable effect across all ages.

Having confirmed inherent differences in VEP present between individuals as a result of age and gender, an exploratory analysis of the data in this paper pursued examination of the effect of such variables on the potentiation of VEP and LTP-like plasticity.

Student *t* tests and non-parametric regression analyses on gender and age variables respectively, found no significant effect from the variability in age or gender of subjects on the relative changes in C1, P1, N1, or P1-N1 peak-to-peak amplitude or latency measures from pre-modulation to post-modulation blocks. Previously, studies comparing the effects of gender or age on VEP (La Marche et al., 1986; Sharma et al., 2015) did not employ a modulation paradigm. Due to a lack in studies utilizing a modulation paradigm in the investigation of the effects of gender and age on VEP plasticity, one cannot positively confirm the effects a variable like gender or age may impart on differences between individuals resulting in varying potentiation of the VEP.

In the present study, relative changes from the pre-modulation to the post-modulation phase in the components of interest were extracted for each subject, followed by a comparison across groups. Having accounted for the pre-existing variances present as a result of age and gender, the effect these variables have on potentiation of the VEP can be measured via relative changes in C1, P1, N1, P1-N1 peak-to-peak amplitudes and latencies leading to a more accurate inspection of the effects of age and sex on VEP plasticity. While the data did not present any significant differences in the effect of age or gender on relative changes in the VEP of subjects, it can be assumed that potentiation of the VEP through the presentation of checkerboard reversals remain consistent across all healthy subjects.

4.3. Time-of-Day Effects on VEP

The synaptic homeostasis hypothesis claims that wake is associated with net synaptic strengthening (Tononi & Cirelli, 2006). Thus, we hypothesized the VEP amplitudes – reflecting synaptic strength in the visual cortices – would be larger in individuals that were examined later in the day when compared to amplitudes of participants examined earlier in the day. To test for this hypothesis, a non-parametric regression analysis was used to explore changes in amplitudes and latencies of the C1, P1, N1, and P1-N1 peak-to peak measures over the course of the day within the modulation block. Averaged P1 amplitude measures from the modulation block were extracted as this encapsulates 1200 epochs and therefore contains the largest sampling of VEP in

a specific block; compared to 35 epochs within any given block of the pre- or post-modulation phase. Significant time-of-day effects were subsequently observed in the modulation block as positively correlated with the time of day at which participants were tested and P1 amplitudes.

Human studies exploring time-of-day effects on the VEP have observed an increase in the latency of the P1 and N1 wave in groups tested at 5pm to those tested at 2am - 5am (Stolz et al., 1988). Furthermore, the results from the current study are in direct conflict with a previous study (Heninger et al., 1969), whereby decreased VEP amplitudes and increased latencies were observed for individuals tested at 6pm in comparison to those at 9am. While the two studies previously mentioned, were within-subject designs, they contained no larger than 8 individuals as the entire sample size. With a particularly small amount of literature available regarding the effect of the time at which a participant is measured and its corresponding influence on VEP, much is left to be elaborated upon.

The second postulate concerning time-of-day effects on VEP was an analysis of a variation in the time of day at which the participant was measured and its effect on plasticity of the VEP. Results focused on the relative changes observed from the pre-modulation to post-modulation phase through assessment via non-parametric regression of C1, P1, N1 and P1-N1 peak-to-peak amplitudes as well as their corresponding latencies against subjects' corresponding VEP recording time. No significant relationship was observed with regards to the time-of-day at which participants were tested leading to a difference in modulating the components of interest. This indicates for a lack of relationship between VEP potentiation change as a result of time-of-day variation.

There are two fundamental concerns with regards to observations made on the effect of the time-of-day on VEP plasticity. Firstly, the modulation block contains the largest dataset of VEP measures and therefore provides validity with regards to a reliable source on which to investigate the time-of day effects on the VEP. However, the repetitive visual stimuli presented within the modulation block lead to a net increase in P1 amplitudes, as observed in the post-modulation phase. Due to very nature of the modulation block in leading to P1 amplitude increases, for a thorough exploration of inherent differences present within the VEP of individuals throughout the day, the results dictate for a larger dataset containing baseline VEP activity. Secondly, while the synaptic homeostasis hypothesis postulates a net synaptic strengthening during wakefulness, we do not know if that is the case in the visual cortex. Animal

studies (Cirelli & Tononi, 2011; Gilestro et al., 2009; de Vivo et al., 2017; Maret et al., 2011) have used homogenates of the entire cortex or other areas than the visual cortex. As no previous studies have looked at the time-of-day effect on the visual cortex, the fact that very few significant changes are observed is not a strong argument against the synaptic homeostasis hypothesis.

4.4. Limitations to the Present Study

Foremost limitations of the present study stem from the timeline parameters provided towards measurement of the VEP and the resulting extrapolated data. With the primary goal of potentiating VEP through presentation of checkerboard reversals, the setup of the experiment does not allow for a superlative exploratory analysis of the secondary and tertiary hypothesis looking at age, gender as well as time-of-day effects.

It has been observed in literature that a deterioration of VEP components, specifically P1 amplitudes and latency occur with increasing drowsiness (Skuse & Burke, 1992) which may negatively impact accurate assessment of the VEP in the current study. However, alertness was maintained during recording of the paradigm through the engaging activity of pressing a specified button each time the dot, in the center of the screen, changed colour. Additionally, by varying the recording of data, from participants, throughout the day, effects of circadian rhythms and drowsiness experienced as a confounding variable were minimized. Provided for the fact that a robust measure of LTP-like plasticity was observed from modulation in VEP components regardless of the maintaining a consistent time quantification, further validity in data could be achieved through stricter controls on such variables.

Given for the largest sample size in a study looking at VEP plasticity through presentation of repetitive visual stimuli, methodological limitations with regards to the age range do not permit for a greater analysis of the variables' effects on the present data. With a mean age of 36.03 and a standard deviation of 9.64 years, the group is not distributed in a manner to investigate polarized effects of age on VEP from either extremes of the populations' age. To contest against such limitations, previous studies (La Marche et al., 1986; Mitchell et al., 1987; Sharma et al., 2015) have isolated variations in VEP modulation as a result of age through the comparison of young adults (18-25) against much older individuals (65+). Furthermore, while having a relatively consistent gender ratio of 57 males to 56 females in the present dataset,

gender effects due to age have been largely observed in subjects above the age of 60 (La Marche et al., 1986, Mitchell et al., 1987).

With regards to modulations observed in VEP as a result of the time-of day at which participants had undergone the experiment, a time range of 12 hours severely restricts the ability to entirely detect within and between subject variations. To correct for this specific methodological constraint, future studies are urged to split participants along a 24h cycle so to reduce between-subject effects, as was done by Cummings et al., 2000. While within-subject variables could be reduced by having the same subject tested for in each time block.

Previous studies have shown numerous other factors such as physical fitness levels (Smallwood et al., 2015), cortisol levels (Ponomareva, Fokin, Pavlova, Androsova, & Selezneva, 1998), alcohol doses (Colrain et al., 1993), nicotine (Beer, Vartak, Greenlee, 2013) and caffeine intake (Landolt et al., 2004) to modulate VEP components and LTP-like plasticity, which in turn may have led to varying results between participants in the current study. Although subjects were instructed to restrain from drinking beverages containing caffeine 3 hours prior to the start of data collection, there were no directions provided to control for the other variables listed above. As the participation from subjects was entirely voluntary, with a lack of strict control over the specific variables previously observed in changing VEP data leading to dissimilarity between subjects, even after providing for directions, mandatory adherence to the protocol cannot be ensured and therefore may have influenced the results observed.

4.5. Strengths and Future Directions:

Having considered sources of weakness within the design of the current study, it is practical to acknowledge strengths as well as prospective directions to further expand upon. A closer inspection of the strengths in the study presents the principal asset from the design to be the largest sample size on which modulation of VEP amplitudes has been explored to date. With an approximately equal group split by gender, careful attention was paid to the EEG and VEP data analyses. Use of the PREP pipeline toolbox as a standardized and sophisticated pre-processing measure in the early stages of the analysis led to an increase in the signal-to-noise ratio. This also identified channels producing noise and completed a robust referencing to exclude the amount of influence they had over the data. In addition, by completing an independent component analysis while manually going through each participants' recording to

reject for eye blink components, greater quality of data was achieved through the steps taken. Face and construct validity of the paradigm in modulating VEP remain after rigorous data cleaning and analysis, indicating for the test and data analysis steps taken as suitable in measuring of the paradigm.

Concurrent with previous studies, inherent differences in the VEP because of gender, age, and time of day at which participants are tested, are present within the paradigm. While studies have explored the effect of these variables, they have not been looked at within a modulation paradigm; investigating how these variables might influence VEP plasticity. For plasticity of the VEP to be a robust paradigm, one would have to know how influential these factors are, and in which direction they may potentially effect this measure. In the current study, plasticity of the VEP via replication of previous results in expected P1, N1 and P1-N1 amplitudes as well as a lack of modulation in the VEP from age, gender or the time-of-day at which participants were tested indicates for a robust and effective measure regardless of these variables.

Given the robust observations made regarding potentiation of the VEP through repetitive visual stimulations while replicating previous studies, an evaluation of the variables confounding VEP modulation, e.g. age, gender and time-of-day effects, requires caution. The age range of the subjects in the present study does not sufficiently allow for an overarching hypothesis across all individuals and therefore lacks population validity. To explore time-of-day effects, future methods should encapsulate time windows larger than 12 hours as well consider re-testing the same subject at multiple time blocks to effectively probe for variation in the VEP. Literature about LTP-like plasticity as evaluated through VEP modulations has isolated numerous other variables, as previously mentioned, influencing specific components within the VEP, thereby taking away from the construct validity of the initial test. For the purpose of evaluating VEP modulation, it is essential in future studies to standardize a method in which as many of these variables can be regulated and accounted for, such that the results produced can be generalized to a larger population.

As examined previously, inherent differences are present within the specific VEP components based on gender, age and time-of-day variation, however, the extent to which they further modulate potentiation of the VEP was shown to be non-significant. These results remain inconclusive due to the small amount of sampling in VEP data prior to the modulation block and dictate for a larger sampling of the visual evoked potential prior to presentation of the visual

stimuli. To avoid inducing some sort of modulation within the pre-modulation block and to establish a baseline measure, future studies could use a different stimulus, as plasticity of the VEP has been shown to be stimulus specific (Cooke & Bear, 2010).

Furthermore, focus on the neural sources leading to potentiation of the VEP and its respective components via neurobiological and imaging approaches is required to enhance understanding on the topic. The observed VEP waveform consists of underlying components that are summed together in what is detected as a reflection of overall voltage activity measured on the scalp at a specific time point. The averaged amplitude and respective latency properties of the underlying components form the observed VEP (Luck, 2005). Change in the observed VEP waveform is the result of potentiation within specific underlying components leading to an overall change in the amplitude and latency of the modulated VEP (Luck, 2005). Although one could hypothesize that the underlying VEP components giving rise to a change in latency shifts of the observed VEP are different from those that give rise to an increase or decrease in the amplitude, according to Occam's razor, the simpler explanation would be that the same underlying VEP component being potentiated and leading to a rise in the amplitude of the observed VEP is also the same one that is occurring later in time and therefore modulating the components of the observed VEP shift in amplitude and latency. While research has focused on evaluating modulation in amplitude of the observed VEP waveform components as a measure of plasticity, if the previous case holds true, changes in the latency of peaks of the observed VEP could be established as a measure of LTP-like plasticity given that the underlying neural source influencing the change in VEP within the modulation block is the same.

5. Conclusion

The present thesis replicates significant plasticity of the VEP in the largest study of healthy humans to date. While the study detected significant effects of gender, age, and time-of-day on pre-modulation amplitudes, there were no significant effects of these variables on VEP plasticity. The latter finding supports the notion that VEP plasticity is a robust phenomenon in healthy humans.

Applications of the VEP extend into areas of clinical and diagnostic functions. For example, integrity of the optic nerve and visual pathways can be assessed by VEP examinations (Odom et al., 2010) and previous studies observed impaired VEP plasticity in schizophrenia, bipolar disorder and major depressive disorder (Elvsåshagen et al., 2012; Normann et al., 2007). In conclusion, the present thesis and previous research together suggest that VEP plasticity is a robust paradigm for non-invasive studies of LTP-like plasticity in humans and may help elucidate the pathophysiology of psychiatric disorders.

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