Modulation of visual evoked potentials as a measure of LTP-like synaptic plasticity

Relationship to stress, cortisol and physical activity

Christopher Laugsand-de Lange &
Daniel Løke

Hovedoppgave

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Abstract

Authors: Christopher Laugsand-de Lange & Daniel Løke

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Supervisor: Professor Stein Andersson at the Psychological Institute, UiO.

This study is part of an ongoing research project in collaboration between the Psychological Institue, UiO and Rikshospitalet, OUS. The authors collected all the data used in this paper themselves.

Long-term potentiation (LTP) is a model that explains the neural basis for Hebbian learning and synaptic plasticity. Measuring LTP has traditionally demanded invasive techniques, and has therefore, until recently, almost exclusively been studied in animals. The use of high frequency sensory stimulation could open for the possibility to induce and observe LTP-like plasticity non-invasively.

Hypotheses: This study is focused on three hypotheses: 1. Replicating previous research showing that high-frequency visual stimulation will yield LTP-like plasticity. 2. There is a positive correlation between level of cortisol and LTP-like plasticity in healthy participants. 3. There is a positive correlation between level of physical activity and LTP-like plasticity.

Design: This study uses a vertical sine wave grating stimulus paradigm to measure visual evoked potentials (VEP) in 38 healthy adults, using electroencephalogram to measure cortical electrical activity. Testing involved VEP registration for 48 minutes, during which time the participants observed two baseline blocks at 2 and 8 minutes into the paradigm, one modulation block at 10 minutes into the session, and six post-modulation blocks 2, 8, 12, 18, 22, and 28 minutes subsequent to the modulation block of the sine wave grating. In addition, participants responded to questionnaires regarding mood, level of stress and physical activity. Participants also delivered saliva-samples to measure level of cortisol.

Results: This study replicates earlier findings confirming modulation of visual evoked potentials as a valid method for studying LTP-like synaptic plasticity. The results show significant increases of the P1 and P1-N1 peak-to-peak amplitudes of the VEP, signifying underlying LTP-like plasticity, thus supporting hypothesis 1. This study demonstrates a positive correlation between level of cortisol and LTP-like plasticity, thus hypothesis 2 is

supported. Level of physical activity and LTP-like plasticity did not demonstrate a positive correlation, thus hypothesis 3 is not supported.

Conclusions: We conclude that VEP registration of high frequency visual stimulation can be a valid method for inducing and observing LTP-like plasticity in vivo in humans, thus replicating earlier studies. In addition the positive correlations found between level of cortisol and LTP-like plasticity indicate an underlying inverted U-relationship between these variables. The null-finding of physical activity related to LTP-like plasticity indicates that there either is no correlation between these variables, or that our method for measuring physical activity lacked criterion validity.

Preface

This study is part of an ongoing research project in collaboration between the Psychological Institute, UiO and Rikshospitalet, OUS. Our contributions began in May 2013, when we conducted preliminary pilot testing of two potential visual paradigms for use in the main study. Due to technical difficulties with the EEG laboratory, the main testing for the project had to be postponed until the summer of 2014. In addition to the main study, the authors of this paper also performed additional retests of fourteen subjects from the main study, using an alternate visual paradigm used in previous studies on the subject matter. The data from these retests were not used in this study, but may contribute to a better understanding of the differences between the visual paradigm used in our study and the one primarily used in earlier studies of the subject matter.

We would like to thank Torbjørn Elvåshagen, postdoctoral fellow at Rikshospitalet, and our supervisor, professor Stein Andersson at the Institute of Psychology, UiO, for giving us the opportunity to participate in this exciting and promising new field of research, and not least for providing us with much needed guidance and motivational support. We would also like to thank Torgeir Moberget, PhD candidate at UiO, for his indispensible guidance in the use of Matlab and EEGlab for data filtration and analysis. We would also like to thank Markus Handal Sneve, postdoctoral fellow at UiO, for creating the visual paradigms used in this project. Finally, we would like to thank the Psychological Institute, UiO for awarding us with a much needed summer scholarship for work on this study in the summer of 2014.

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1 Introduction

It is a fascinating hallmark of human nature that we are able to change our behaviour. We can alter the way we think through learning, experience and conscious choice. William James is one of many to show interest in our ability to change, learn and develop. According to William James, this behavioural plasticity, like all behaviour and thoughts, had to have its basis in the brain, meaning that the brain has to somehow be "plastic" (Cotman & Berchtold, 2002). Cajal speculated, in the beginning of the 1900s, that the basis for learning lay in increases in synaptic strength (Nicoll & Roche, 2013). However, there was no concrete model for how this could occur until Donald Hebb proposed his model of "Hebbian learning" in 1949 (Nicoll & Roche, 2013), which basically states that "neurons that wire together fire together". In other words, if you do an activity a lot, the neurons associated with that activity will become more efficient at communicating – you get better at the activity in question. Neurons that are stimulated a lot together will become more effective. Conversely, if you don't use it, you lose it - if you stop doing an activity, the neurons associated with that activity will gradually become less efficient at communicating. Although this idea makes intuitive sense as a theoretical explanation for why "practice makes perfect," the actual neural basis was not demonstrated until Bliss and Lømo induced long-term potentiation (LTP) in the dentate-area of a rabbit-hippocampus in 1973 (Bliss & Lømo, 1973). Bliss and Lømo demonstrated that electrical stimulation of neuron A with a tetanus shock activated neuron B. Repeated stimulation led to neuron B becoming more effective; more excitable. This is what one calls "potentiation"; that the synaptic activity becomes more potent, or powerful. "Long-term" potentiation means that this potency is long lasting – in Bliss and Lømo's case they found that neurons remained potentiated for 30 minutes to 10 hours after the initial electric stimulation. Long-term potentiation can therefore be defined as *"an enduring, activity-dependant increase in synaptic efficacy that is the principal candidate synaptic mechanism underlying learning and memory formation"* (Clapp, Eckert, Kirk, Teyler & Abraham, 2006). LTP is a precise neural mechanism that demonstrates the ideas of Donald Hebb very accurately and is believed to reflect the principal neurobiological mechanism of learning. In this paper we aim to induce and measure LTP-like plasticity in a population of healthy, adult participants. We also aim to explore possible effects of stress and level of physical activity on LTP-like plasticity.

1.1 Long-term potentiation

Bliss and Lømo's discovery of LTP led to extensive research into the underlying mechanisms and physiology of potentiation and neural plasticity. The neurotransmitter directly associated with LTP is glutamate. Two major discoveries in early LTP-research were that LTP could be induced in a hippocampal slice, and that the N-methyl D-aspartat (NMDA) subtype of glutamate receptor was necessary and adequate for hippocampal LTP to occur. This means that glutamate is the neurotransmitter that starts the process, and that LTP does not occur if the NMDA-receptors are deactivated in some way (Nicoll & Roche, 2013).

LTP occurs when neuron A is stimulated to fire multiple action potentials, thus releasing a shower of glutamate molecules into the synaptic cleft between neuron A and neuron B (figure 1). Two of the post-synaptic receptors are the α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor and the NMDA receptor. When the postsynaptic AMPA receptor is bombarded with glutamate-molecules, the AMPA receptors allow a stream of positively charged natrium (Na+) ions to flow into the postsynaptic neuron B. This creates a local depolarizing effect. Glutamate also binds to the NMDA-receptors, but these are blocked by a positively charged magnesium ion Mg2+. If a sufficient amount of Na+ ions enter neuron B through the AMPA-receptors, causing a sufficient depolarization, the Mg2+ blocking the NMDA-receptors leaves, and ceases blocking the receptor. This opens the pathway for positively charged calcium ions (Ca2+) to flow into neuron B, causing even more depolarisation. The NMDA receptors therefore only open to allow Ca2+ into the cell when the cell has already been depolarized to -35mV and glutamate has bound itself to the NMDA-receptors. The downstream target for Ca2+ is CaMKII-protein, which leads to new AMPA-receptors being transported into the post-synaptic membrane through exocytosis. The mechanisms behind these new AMPA-receptors finding their way to the dendritic membrane are not fully understood, and go beyond the scope of this paper. The new post-synaptic AMPA-receptors potentiate the excitability of neuron B – it becomes more effective. More AMPA-receptors mean that depolarisation can occur more rapidly, thus leading to the NMDA-receptors being opened more easily, which again leads to even more AMPA-receptors being installed in the post-synaptic membrane. It's called "long-term potentiation" because the new AMPA-receptors remain in the dendritic membrane for an extended length of time (Gazzaniga, Ivry & Mangun, 2009).

Lømo (2012) claims that LTP is absolutely necessary for our ability to learn and remember. It is therefore safe to say that LTP is an important part of what makes us who we are and has a functional role in our everyday lives.

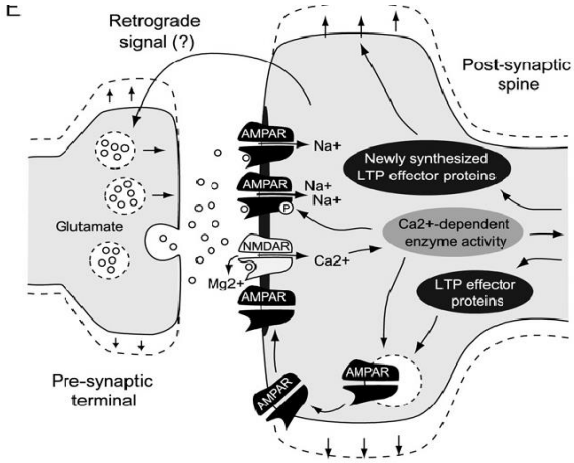

Figure 1. Shows glutamate binding with the AMPA and NMDA recepors, the magnesium ion that initially blocks the NMDA receptors, until depolarization reaches -35mV, thus allowing Ca2+ ions to enter the cell. This starts a series of events, the end result of which is new AMPA-receptors being installed in the membrane, creating a more efficient post-synapse (Cook & Bear, 2012)

1.2 Modulation of visually evoked potentials - non-invasive measure of LTP-like plasticity

Until recent years, measuring neuroplasticity demanded an invasive process of directly stimulating parts of the brain with tetanus shocks (Ehlers, 2012). LTP in animals has been studied and understood for many years, and there is a lot of literature supporting this model of neural learning (Ehlers, 2012.) The observed LTP in animal models is indeed "Hebbian", in that it can be long lasting (up to several months in the right conditions), it is input-specific and it is cooperative and associative – meaning that "weak" synapses can undergo potentiation, making them powerful enough to drive action potentials (Cooke & Bear, 2012). Typical methods have been studying LTP with micro-electrodes directly inserted into the brains of lab-animals, or by electrically stimulating brain samples, e.g. slices of hippocampi (Clapp et al. 2006). It is reasonable that most studies have been conducted in the hippocampal regions, given this area's role in memory formation and learning. A fair criticism against the invasive methods is that this sort of stimulation has limited ecological validity – rats in nature don't have electrodes in their heads creating tetanus-shocks, thus inducing LTP (Clapp et al. 2006).

It has therefore been important to find non-invasive methods for inducing and observing LTP, both to lend ecological validity to the invasive methods, and to study this phenomenon in humans. Because of the invasiveness of LTP-research, traditional studies on human brains are rare. According to Teyler, Hamm, Clapp, Johnson, Corballis & Kirk (2005) the only studies on the human brain were, until recently, samples taken from brain-surgery patients. These studies revealed exactly the same properties as the hippocampal studies on animal brains, implying that the mechanisms underlying LTP are the same in humans and animals, and that LTP is not limited to the hippocampal regions (Teyler et al. 2005). However, in recent years evidence has accumulated for the possibility of non-invasive research of LTP-like plasticity. One such method is the use of high-frequency visual stimulation.

1.2.1 Visual evoked potentials (VEP)

A visually evoked potential (VEP) is an event-related potential induced by some form of high-frequency visual stimuli (Luck, 2005). Cortical neuroelectric activity is recorded by electroencephalography (EEG) using scalp electrodes. High-frequency visual stimulation alters the neuroelectric activity, especially over occipital regions. This altered activity can be measured and manipulated. The rationale is that repeated sensory stimulation causes multiple excitatory potentials in specific brain regions, with similar properties to the potentials that are created with micro electrodes inserted in the brain (Teyler et al. 2005). This altered neuroelectric activity is revealed by significant increases in the amplitudes of the measured VEP-components and mainly reflects post-synaptic activity. A VEP gives a typical waveform (see figure 2) that represents the averaged neuroelectric activity induced by the repeated visual stimulation. The earliest peak is designated “C1”, a negative amplitude occurring approximately 70-90 milliseconds (ms) after stimulus onset. The next peak is positive, designated P1, usually about 90-120 ms after stimulus onset, and is followed by a negative amplitude designated N1, occurring about 130 – 170 ms post stimulus onset (Luck, 2005).

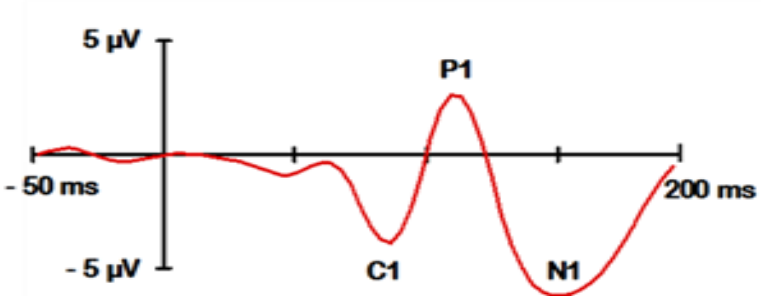

Figure 2. A VEP pattern produced by checkerboard reversals showing typical VEP waveform with C1, P1 and N1 components (Elvsåshagen et al., 2012).

1.2.2 Modulation of visual evoked potentials

Teyler et al. (2005) was, to the authors' knowledge, the first study demonstrating that a VEP-paradigm can be used to induce and observe LTP-like plasticity in human subjects. In this study 6 adult participants were shown a reversing black and white checkerboard image on a computer screen – a photic tetanus with periods of a grey screen between each set of stimuli.

In the post stimulation VEPs there was a clear enhancement of an early component of the VEP. According to the authors, this effect is best explained with the mechanisms of LTP (Teyler et al. 2005). More recent evidence that LTP can be induced through sensory stimuli was provided by Clapp, Hamm, Kirk & Teyler (2012). They also used a reversing checkerboard as a visual stimuli, presented to either the left or right visual hemisphere. Using EEG they measured changes in VEP amplitudes, showing significant potentiation of especially the N1 component compared to baseline measures. Clapp et al. (2012) compared the EEG-findings to functional magnetic resonance imaging (fMRI), using the same checkerboard paradigm, to show that increased BOLD signal was indeed concentrated to occipital areas, associated with visual processing. This indicates that visual stimulation can be used to induce and record LTP (Clapp et al. 2012). Using auditory stimulation, Clapp et al. (2012) were able to demonstrate that LTP-like plasticity can also be induced non-invasively in the auditory system. The Auditory Evoked Potential (AEP) showed a significant increase in N1-amplitude. Using fMRI this effect was localized to the auditory cortex (Clapp et al. 2012). Cooke and Bear (2012) use the term "stimulus-specific response potential" (SRP) to describe the observed plasticity from sensory stimulation. There are several indications to suggest that SRP represents an LTP-like process. Both SRP and LTP are prevented with the application of the NMDAR antagonist 3-(2-Carboxypiperan-4-yl) propyl-1-phosphonic acid (CPP) (Cooke,

2010). VEP measured with occipital scalp electrodes appears to record plasticity effects exclusively in V1 suggesting stimulus-specificity (Cooke & Bear, 2012). Normann, Schmitz, Fürmaier, Döing & Bach (2007) used modulation of VEPs, to study LTP-like plasticity in individuals with major depression compared to healthy control subjects. They used checkerboard reversals as stimulation and showed that the stimulation produced clear modulation of the measured amplitudes in the VEPs in healthy subjects. This means that the measured amplitudes of the VEPs were significantly stronger when subjects were presented with the same stimuli over and over again. These findings were replicated in a study by Elvsåshagen et al. (2012) using the same VEP paradigm as in the Normann et al. (2007) study.

Little research has been focused on the cognitive and functional correlates of VEP-modulation. One study by Waage (2012), measured VEP-modulation with the use of a checkerboard paradigm in both a bipolar II disorder-group and a control group, and also tested participants with an expanded version of Brief Visuospatial Memory Test – Revised (BVMT-R). There were significant associations in the patient group between modulation effects in P1-N1 peak-to-peak amplitude and visual learning, and between N1-modulation and delayed recall of visual information. In the control group, there was a significant association between P1-modulation and delayed visual recognition. Although research into this matter is sparse, these findings indicate a possible association between LTP-like plasticity in VEP paradigms and functional measures.

Inducing and observing LTP-like synaptic plasticity through high-frequency visual stimulation is the main focus of this study, thus replicating earlier research by, amongst others, Elvsåshagen et al. (2012).

1.3 Influences on LTP

The basic mechanisms of LTP, as described in the previous paragraphs, are fairly well understood. LTP is ubiquitous throughout the brain (Normann, 2006) and this plasticity plays a crucial role in how we function in our lives (Lømo, 2012). It is safe to say then, that a reduction in the brain's plasticity, or ability to exhibit plasticity, will be accompanied with challenges of some sort. Indeed, there is evidence that LTP is not a constant, like for example our ability to hear or see. Several factors have been identified that alter and influence both

LTP and LTD (Long-term Depression; the opposite of LTP; beyond the scope of this paper.) These factors broadly fit under the term metaplasticity. This term was first coined by Abraham and Bear in 1996 (Schmidt, Abraham, Maroun, Stork & Richter-Levin, 2013), and can be understood as "the plasticity of plasticity." Müller-Dahlhaus & Ziemann (2014) describe it as the ability of neurons to exhibit plasticity. In other words, many factors can influence neuroplasticity. Research on metaplasticity focuses on neuronal or behavioural stimulation before the induction of LTP or LTD, and what the effects are on the neural ability to exhibit plasticity. Enriched environments, or stressful events, have been shown to have powerful effects on synaptic plasticity in animal research (Abraham, 2008). Metaplasticity is critical for human cortical physiological function, and aberrant metaplasticity can therefore lead to a variety of neurological and psychiatric disorders (Abraham, 2008).

1.3.1 LTP and cortisol

Level of cortisol is one factor known to influence plasticity. Cortisol is the main hormone associated with elevated levels of stress. Periods of elevated stress, for example examination periods, are known to coincide with severely elevated levels of cortisol (Singh, Goyal, Tiwari, Ghildiyal, Nattu & Das, 2012). Elevated levels of cortisol over time can disrupt several functions, including plasticity, which can also disturb cognitive functions. Yuen et al. (2012) showed that chronic stress leads to prolonged periods of elevated corticosteroids, which inhibits glutamatergic synaptic transmission in the prefrontal cortex of rats. Exposure to stress is an important risk factor for the development of a broad array of psychopathology in vulnerable individuals (Timmermanns, 2013). However, mild stress can have several beneficial effects on cognitive function and performance (Singh et al. 2012).

Stress, measured as level of cortisol, appears to have potentially positive and negative effects on the way humans function. Initial responses to stress can be highly adaptive. For example, the activation of the autonomic nervous system, with subsequent release of noradrenaline, can make us more awake and alert in a challenging situation (Timmermanns, 2013). However, extreme, acute stress can lead to PTSD (Timmermanns, 2013). Stress is one of the most important factors predicting depression (Holderbach et al. 2007). Prolonged elevated levels of stresshormones have also been shown to have a detrimental effect on hippocampal function (Ivy et al., 2010 in Timmermanns, 2013). Timmermanns' review can be understood in light of a Yerkes-Dodson curve, where the effects of stress depend on many factors, such as level of

stress, whether it is chronic or not, and on individual susceptibility. Diamond, Bennet, Fleshner & Rose (1992) demonstrated an inverted-U relationship between levels of corticosterone (a steroid released during stress) in rats and hippocampal primed burst potentiation (PB; a lasting increase in the amplitude of the CA1 population spike). Small increases in corticosterone gave a moderate positive correlation with enhanced PB, intermediate levels of corticosterone gave the strongest, positive correlation with PB, whereas high levels of corticosterone correlated negatively with PB, indicating an inverted U-relationship, adding support to Yerkes-Dodson's law, first put forward in 1908 (Diamond, et al. 1992).The Yerkes-Dodson law (figure 3) can be characterized as an inverted U-shaped relationship where low dose stimulates, but high dose inhibits function (Calabrese, 2008). Diamond, Campbell, Park, Halonen & Zoladz (2007) discuss many studies on the relationships between LTP and stress in a comprehensive review. It seems that stress and stress hormones have varying effects on brain function, depending on location in the brain, and intensity of the stress-inducing experience. Cortisol can block LTP in the prefrontal cortex, whereas it can both enhance and impair LTP in the amygdala and hippocampus, depending on cortisol levels (Diamond et al. 2007). It seems that elevated levels of stress can enhance LTP in the amygdala, but when level of stress is too great it can inhibit LTP. A stressful event can be virtually impossible to forget, as in PTSD, but at the same time elevated levels of cortisol over time is known to disrupt learning and memory (Diamond et al. 2007).

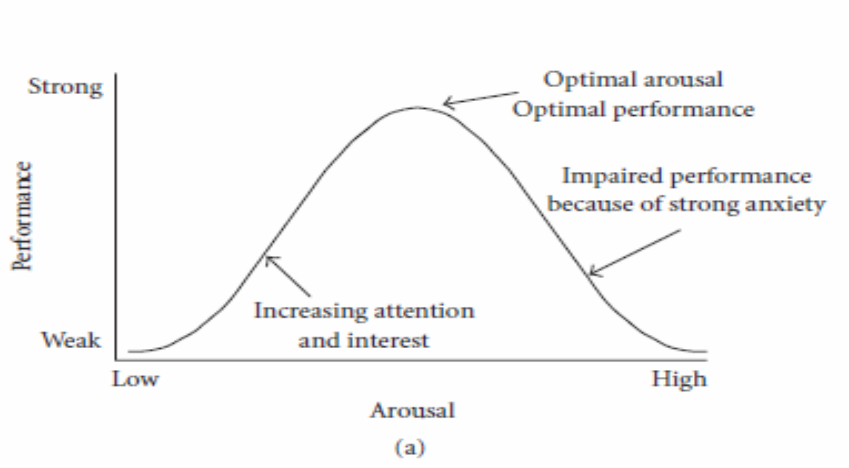

Figure 3. Illustrates how stress/arousal can enhance or inhibit performance, depending on the level of stress (Diamond et al. 2007).

Animal studies

It isn't easy to induce extreme levels of cortisol in humans to study the effects on the brain, for obvious ethical reasons. However, several animal studies have studied the effect of stress on cognition and plasticity. Sousa et al. (2014) used the maternal separation paradigm (MS) on post natal rats to study how extreme stress early in life can affect late-life cognitive function by measuring hippocampal LTP. They found that cognitive decline of memory in older age and decline of LTP was significantly greater in the MS-rats than in the control rats. They measured LTP using hippocampal samples from the rats, and found significantly lower LTP in the hippocampi of aged MS-rats.

Using a model of animal depression, Holderbach et al. (2007) studied the effects of chronic mild stress (CMS) on synaptic plasticity in adult rats. After three weeks of CMS, plasticity was definitely disturbed. Rats that had been in the CMS condition showed high rates of LTD. Although there was no significant reduction in LTP, the results still show that chronic stress has significant effects on plasticity (Holderbach et al. 2007).

It appears then that marked increases in level of cortisol has a disruptive effect on plasticity and cognitive function, whereas little is known concerning the possibility that low or moderate elevated levels of cortisol might have beneficial effects.

Human studies

Human studies tend to give similar results as the animal studies, although the level of experimental control is altered in human research. According to Schmidt, Abraham, Maroun, Stork & Righter-Levin (2013) high levels of stress has the metaplastic effect of reducing LTP, and enhancing LTD, irrespective of whether the stress is chemically induced through the administration of stress hormones, or environmentally induced. Normann et al. (2007) used modulation of VEP to study LTP-like plasticity in individuals with major depression. They found significant differences in the plasticity of VEP amplitudes of healthy control subjects compared to subjects with major depression. The depressed subjects showed significantly less plasticity-effects in the VEPs. According to Normann et al. (2007) depression might therefore be a basic disorder of brain plasticity. These results imply that down-regulated synaptic transmission and plasticity are basic pathophysiological properties of depression (Normann et al. 2007). The idea that depression is the result of a dysfunction of neural plasticity was tested

by Nissen et al. (2010). They used various learning conditions as a model for synaptic plasticity, hypothesizing, amongst other things, that patients with major depression disorder would show reduced declarative memory, indicating reduced hippocampal plasticity. Their results indicated a clear reduction of learning and long term plasticity in humans with major depressive disorder in several memory and learning conditions (Nissen et al. 2010).

Elvsåshagen et al. (2012) used a VEP paradigm similar to Normann et al. (2007) to show that neocortical synaptic plasticity was reduced in patients with bipolar type-II disorder, a mood disorder characterized by recurring depressive episodes and hypomania. They also mention a general lack of human evidence for reduced plasticity being a basis of mood disorders – most studies are conducted on animal models. Elvsåshagen et al. (2012) found systematic plasticity effects in healthy controls, but these effects were significantly lower in subjects with bipolar II disorder. Elvsåshagen et al. (2012) and others, have tended to find the strongest, most robust plasticity effects reflected in altered amplitudes of the P1, N1 and peak-to-peak P1-N1 components of the VEP (see figure 2). In a follow-up study using partially the same study population, Elvåshagen et al. replicated earlier findings, but additionally also measured cortisol in saliva. The results published as a conference abstract (Elvåshagen et al., 2013) showed that level of cortisol measured in the saliva of healthy controls correlated positively with VEP-plasticity.

Human studies confirm that cortisol has an effect on a broad array of brain functions. However, human studies on cortisol tend to be focused on clinical populations. An exception is the study by Singh et al. (2012) who examined the effect of stress, measured with cortisol in saliva, in medicine students during examination. These were clinically healthy subjects. While cortisol did have an effect on mood, it did not have a significant effect on performance.

It seems safe to say then, that the relationship between level of cortisol and various cognitive functions is complex. It makes intuitive sense to claim that stress is disruptive to many functions. However, it also makes intuitive sense to say that a moderately heightened level of stress during demanding cognitive tasks can enhance performance, hence the Yerkes-Dodson curve.

The relationship between level of cortisol and LTP-like plasticity is the second focus of this study.

1.3.2 Physical activity and LTP

Another factor that appears to influence plasticity and learning is physical activity. In the course of the last century the many beneficial effects of physical exercise became more and more recognized (Praag, 2009). Exercise is not only good for our somatic well-being, it is also increasingly evident that exercise has beneficial effects for our mental health, with benefits to cognition and memory, and it is even used in the treatment of anxiety and depression disorders (Nadel, Huang, Xia, Burlin, Zametkin & Smith, 2013.) Brain-derived neurotrophic factor (BDNF) is associated with neural plasticity, protein synthesis and synaptic strengthening, and the link between exercise and increases in BDNF is well established Nadel, et al. (2013). There is substantial evidence indicating that physical activity has a significant beneficial effect on cognition and age-related memory decline in older adults, including individuals who have started showing early signs of Alzheimer's disease (Hillman, Erickson & Kramer, 2008). There has been more research into the effects of exercise for older adults than young people, but according to Hillman, Erickson & Kramer (2008) there is evidence to suggest that exercise is beneficial to brain health in a life-span perspective, not just in old age. Pinpointing direction of causality in the relationship between physical activity and enhanced cognitive abilities can be difficult. The beneficial effects of exercise may be due to an overall healthy lifestyle in already cognitively well-functioning individuals. However, longitudinal studies have shown that subjects improve their performance on cognitive tests after some months of physical activity, indicating causality (Colcombe & Kramer, 2003). Several studies have shown that physical activity has a positive effect on brain activity and learning. In a meta-analysis where 42 studies were analyzed, Shoshanka, Hindin & Zelinski (2012) looked at the effects of cognitive practice and aerobic exercise on cognitive functioning on untrained cognitive outcomes in humans. They found that both extended cognitive practice and aerobic exercise produced statistically significant improvements on cognitive tasks (Hindin & Zelinski, 2012).

Animal studies

Running has been identified as an enhancer of neurogenesis, learning and LTP in mice (Praag, Christie, Sejnowski & Gage, 1999). Mice who were assigned to the "runner condition" showed significantly greater LTP in the dentate gyrus compared to their sedentary peers. Nichol, Deeny, Seif, Camaclang & Cotman (2009) studied the effects of exercise on

hippocampal plasticity in apolipoprotein ε4 (Apo-E 4) mice. Humans with the Apo-E 4 allele have a significantly higher risk of developing Alzheimer's disease, although it is important to note that not everybody with this allele develop Alzheimer's (Nichol et al. 2009). Exercise has been shown to be a protective factor against the development of Alzheimer's disease and dementia in humans (Nichol et al. 2009). Nichol et al. (2009) compared Apo-E 4 and Apo-E 3 mice. Mice with the Apo-E 3 allele are at a low risk of developing Alzheimer's type dementia. They found that exercise "significantly increased accuracy on the radial-arm water maze task. They also reported dramatic changes in plasticity in the hippocampus due to physical exercise, bringing the Apo-E 4 mice up to the same levels of learning as the Apo-E 3 mice.

Fares et al. (2013) designed a special environmental-enrichment cage for rats called the Marlau cage. The rats in the Marlau cage had unstressful social interactions, various mazes, running wheels for voluntary exercise and various forms of cognitive stimulation, in contrast to environmentally impoverished rats. Rats in the Marlau cage showed, amongst other things; increased cortical thickness and hippocampal neurogenesis. Fares et al. (2013) found that environmental enrichment has a positive influence on neurogenesis, plasticity and protects against brain insult. In fact, LTP was increased in rats in the Marlau cage after as little as one week. Voluntary physical activity on a running wheel was an important factor in the Marlau cage.

Human studies

The broad effect of exercise on human learning and cognitive abilities has been extensively researched. Physical exercise can actually enhance mental health (Gomez-Pinilla & Hillman, 2013). Exercise influences synaptic function (Gomez-Pinilla &Hillman, 2013), which one could extrapolate to imply that exercise can enhance neuroplasticity in humans. The hippocampus, one of the main regions associated with cognitive decline in ageing, is also one of the regions most positively influenced by exercise (Gomez-Pinilla & Hillman, 2013).

In a comprehensive meta-analysis comprising 59 studies from 1947 to 2009, Fedewa & Ahn (2011) concluded that physical activity has a significant positive effect on children's cognition and academic achievements. In fact, children with cognitive or physical disabilities showed greater benefits from physical activity than their peers. Their findings were also

robust; physical activity had a positive effect on cognitive and academic outcomes regardless of who was directing the physical activity intervention (Fedewa & Ahn, 2011).

Cross-sectional studies have shown that individuals who exercise regularly report higher levels of mental health and well-being (Salmon, 2001). It would appear that intense exercise can have positive effects in the present as well, not just in the future. Winter et al. (2007) found that highly intense physical exercise had immediate benefits on learning and cognition. Subjects in an intense sprint condition showed higher levels of catecholamines in the blood immediately after running, and their vocabulary learning was 20% faster than both their sedimentary peers, and a "moderate-group" who had participated in low-intensity running. The results from Winter et al. (2007) suggest that elevated levels of catelochalmines and BDNF explain the immediate benefits to learning.

Physical exercise has special benefits on the cognitive functions of older adults (Hillman, Belopolsky, Snook, Kramer & McAuley, 2004). They measured event related potentials (ERPs) with scalp electrodes to study reaction times and P3 amplitude and latencies in three groups of older adults (high, low and moderately physically active), plus a group of young adults as control subjects. The physically fit older adults showed significantly shorter reaction times than their less fit peers. They also found that the amplitude of the P3-component of the ERPs was significantly increased in the physically fit older adults, although the amplitude was strongest for the young control subjects. It seems that the physically fit older adults showed greater benefits from physical fitness on the more demanding tasks.

The relationship between level of physical activity and LTP-like plasticity is the third focus of this study.

1.4 Aims and hypotheses

We aim to replicate earlier studies indicating that high-frequency visual stimulation is a valid method for inducing and observing LTP-like plasticity. This is the main aim and focus of this study. Based on earlier findings of Elvsåshagen et al. (2012), we expect to find the most robust results indicating LTP-like plasticity in the P1 and P1-N1 peak-to-peak components of the VEP.

Hypothesis 1: We hypothesize that the visual stimulation in our paradigm will induce observable, significant LTP-like plasticity in our participants on a group level.

Furthermore we aim to explore the relationship between stress and LTP-like plasticity. Level of stress is gauged by measuring level of cortisol in 5 saliva samples over a 24 hour period from each participant. In addition each participant will report perceived stress on questionnaire and a visual analog scale (VAS). We expect our population to have a relatively homogenous distribution of level of cortisol, well within what is considered normal, or healthy.

Hypothesis 2: Based on preliminary findings reported by Elvsåshagen et al. (2013) we expect that level of stress in our participants will correlate positively with plasticity effects. Participants with higher levels of cortisol will show greater LTP-like plasticity. Conversely, participants with the lowest levels of cortisol will show less powerful LTP-like plasticity. This is based on our understanding of the Yerkes-Dodson law, as discussed in Diamond et al. (2007).

Lastly, we explore the relationship between LTP-like plasticity and physical activity. Previous research cited in this paper has shown numerous positive effects due to physical activity, including positive effects on learning and neural plasticity as well as general mental and physical health.

Hypothesis 3: We expect to find a positive correlation between level of physical activity and LTP-like plasticity. We expect that the participants who are more physically active will show stronger LTP-like plasticity. Conversely, participants who report the low levels of physical activity will show less powerful LTP-like plasticity.

2 Methods

2.1 Participants

The participants in the following study were recruited through advertisement/flyers and personal contacts. The criteria for inclusion were: 1) between 18 and 50 years of age; 2) no known neurological or psychiatric condition; 3) no current use of psychopharmaceuticals; 4) no current drug dependence; 5) normal or corrected-to-normal vision.

A total of forty subjects were tested. Two of these were subsequently rejected due to failure to meet inclusion criteria. The final sample consisted of 38 participants, whereof 23 females (60.5%) and 15 males (39.5%). The mean age of the sample was 26.58 (SD = 5.32).

All participants received a gift card (500 NOK) as compensation for their participation. The study was approved by the Regional Ethics Committee (REK, approval no. 2009/2297).

2.2 Visual evoked potential paradigm

The paradigm used to evoke visual evoked potentials (VEPs), consisted of two inverted images of vertical oriented black and white sine wave gratings, each with a spatial frequency of 0.8 cycles per degree. Stimuli subtended 52 degrees of visual angle and were presented on a 24" LCD computer screen with a resolution of 1376 x 768 pixels using E-Prime 2.0. The images were inverted two times per second, and contained a centered grey fixation point (0.5 cm in diameter), which the subjects were instructed to fixate on throughout the experiment.

The entire paradigm lasted for 48 minutes, and consisted of two 20 seconds baseline recordings (40 stimulus reversals) separated by one modulation phase lasting ten minutes (1200 reversals), and 6 post-modulation recordings (equal to baseline recordings) at 2, 8, 12, 18, 22, and 28 minutes subsequent to the end of the modulation block. See figure 4 for a graphic depiction of the paradigm. The subjects were given an auditory signal ten seconds prior to each recording block.

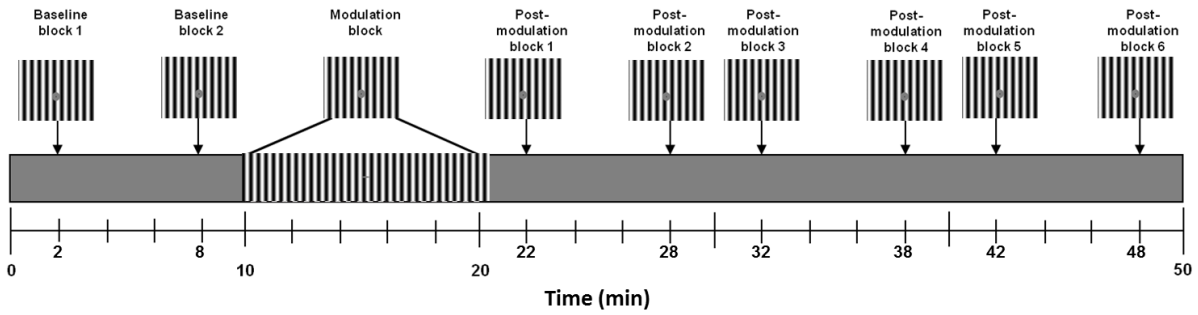

Figure 4. An illustration of the VEP paradigm used in this experiment.

2.3 EEG recordings

The EEG recordings were conducted using a 64 channel NeuroScan Synamp 2. Twenty-six scalp Ag/AgCl electrodes fixed on an EasyCap net (FP1, FPz, FP2, F7, F3, Fz, F4, F8, C3, Cz, C4, T3, T4, T5, T6, M1, M2, P3, Pz, P4, PO7, POz, PO8, O1, Oz & O2), positioned according to the 10-20 system, and referenced to the AFz electrode, were used for recording. Four additional Ag/AgCl electrodes were used for the bipolar HEOG and VEOG channels for detecting ocular activity, in addition to the ground electrode placed on the forehead. The EEG was recorded at 1000 Hz, with no online filtration. The impedance of all electrodes was maintained below 10 KΩ, and below 5 KΩ for the occipital electrodes. Curry Scan 7 Neuroimaging Suite was used as the data acquisition software.

Each subject placed their head on a chin rest throughout the experiment, with a fixed distance to the screen of 57 cm. The lighting in the test room was kept constant throughout the entire subject pool.

2.4 ERP analysis

The ERP analysis and identification of peak amplitude values for each epoch were conducted using Matlab R2013B (The MathWorks Inc., 2013) and the open source software package EEG lab Version 13.3.2B (Delorme & Makeig, 2004). The continuous EEG was filtered offline with a High Pass filter at 1 Hz, and then analyzed with an Independent Component Analysis. Using the generated component array, components deemed to be artifactual of either ocular or muscular origin were rejected and removed from the data. The criteria used for identifying artifactual components were provided in an instructional guide on the website of Swartz Center for Computational Neuroscience (2013).

The resulting data was then run through a Low Pass filter at 30 Hz. The continuous EEG was epoched into segments of -50 to 350 ms in relation to stimulus onset. Bad segments were subsequently rejected, and the remaining segments from the nine epochs were averaged and segmented into separate ERPs. For our purpose only the Oz electrode is used in further analyses. A Matlab script was used to extract the peak amplitude and peak latency values from the ERP data files. For the C1 component, the script extracted the lowest value between 65 and 110 milliseconds following stimulus presentation. For the P1 component, the script extracted the highest value between 90 and 150 milliseconds following stimulus presentation. For the N1 component, the script extracted the lowest value between 130 and 190 milliseconds following stimulus presentation. All the extracted values were then manually checked for consistency with the ERPs, and corrected if erroneous.

Previous research indicates that the P1 and N1 VEP amplitudes and the P1 to N1 peak-to-peak value in particular tend to show modulation effects, and these will therefore be the main focus of our study.

2.5 Questionnaires

The participants responded to several questionnaires and visual analog scales (VAS). For our purpose the International Physical Activity Questionnaire Short Form (IPAQ-SF), the Perceived Stress Scale (PSS), and VAS measures pertaining to subjective experience of stress, anxiety and depression will be the used for further analyses.

Physical activity: Levels of physical activity was measured using the International Physical Activity Questionnaire Short Form (IPAQ-SF), a self-report measure of physical activity within the preceding week. IPAQ has been shown to have an acceptable level of test-retest reliability in a study conducted throughout several countries (Craig et. al, 2003). It has been proposed as the most viable questionnaire for measuring levels of physical activity in a review of the literature regarding 85 different physical activity questionnaires (Poppel, Chinapaw, Mokkink, Mechelen & Terwee, 2010).

We chose to score the results from IPAQ according to the official IPAQ scoring protocol (The IPAQ group, 2005), calculating continuous Mean Exercise Time (MET)-minutes scores in the three categories “walking”, “moderate-intensity activity” and “vigorous-intensity activity”, as well as a weighed composite score for total physical activity.

Visual Analogue Scales: For simple measures of subjectively perceived stress, anxiety, and depression, participants responded to 10 centimeter visual analogue scales. Within each variable of interest, the participants were asked to rate themselves "today", "this week", "this month" and "this year".

2.6 Cortisol measurement

Saliva cortisol levels were measured using Salivette cotton swabs. Saliva specimens provide a reliable and non-invasive method of measuring cortisol levels (Kirschbaum & Hellhammer, 1989). The saliva specimens were collected immediately before (T1) and after (T2) the EEG registration session, and the subjects were instructed to collect three samples the following day. These three samples were to be collected immediately upon waking (T3), half an hour after waking (T4), and lastly at noon/12 pm (T5). All subjects were instructed on the use of the cotton swabs. Four participants did not return their saliva specimens despite reminders, and three participants had too little saliva in their specimens for analysis, leaving 31 participants with complete cortisol analyses. For further analyses, mean cortisol value from immediately before and after EEG recording, mean cortisol values from the next day samples, mean cortisol values of all five samples, and the individual cortisol values are used.

The saliva specimens were analyzed at The Hormone Laboratory, Oslo University Hospital - Aker Sykehus.

2.7 Statistical methods

The statistical analyses were performed using IBM SPSS Statistics version 22 (IBM Corp., 2013). Results with a two-tailed p value of < 0.05 were considered significant. The use of the more stringent Bonferroni adjustsments to control for multiple tests was considered. However, as is pointed out by Perneger (1998), while the Bonferroni adjustment for multiple tests decreases the likelihood of a Type I-error, it greatly increases the likelihood of a Type II-error. Considering the limited sample of our study, and the fact that this area of research is still quite new, we decided to minimize the chance of rejecting the hypotheses erroneously, and therefore not use the Bonferroni adjustments in this study.

As there was no statistically significant difference between baseline 1 and baseline 2 on the amplitudes of interest (P1 and N1), these VEPs were averaged to a single baseline measure

for further analyses. In addition to the VEPs from the six different post modulation blocks, average amplitudes across the six post modulation blocks were calculated for the P1, N1, and P1-N1 peak-to-peak values to be used in further analysis.

Repeated measures one way analyses of variance (ANOVA) were performed on the component peak amplitude variables between the baseline and post-modulation blocks to measure plasticity effects. The Greenhouse-Geisser correction was used for these analyses. Paired samples t-tests were used for post hoc analyses between baseline and the separate post-modulation blocks.

All variables were tested for normality using the Shapiro-Wilk test. These tests showed that cortisol T5 (cortisol measurement at noon), all VAS ratings, and the MET scores from IPAQ all differed significantly from a normal distribution, and as such, all analyses pertaining to associations between modulation effect and cortisol T5, VAS ratings and MET scores were performed using Spearman's rank-order correlation test. All other analyses of relationships between modulation effects and cortisol were performed using Pearson's correlation test.

3 Results

3.1 VEP amplitude modulation

The various components will be presented in the order they appear in the VEP. Figure 5 shows the grand average ERPs of each separate measurement block.

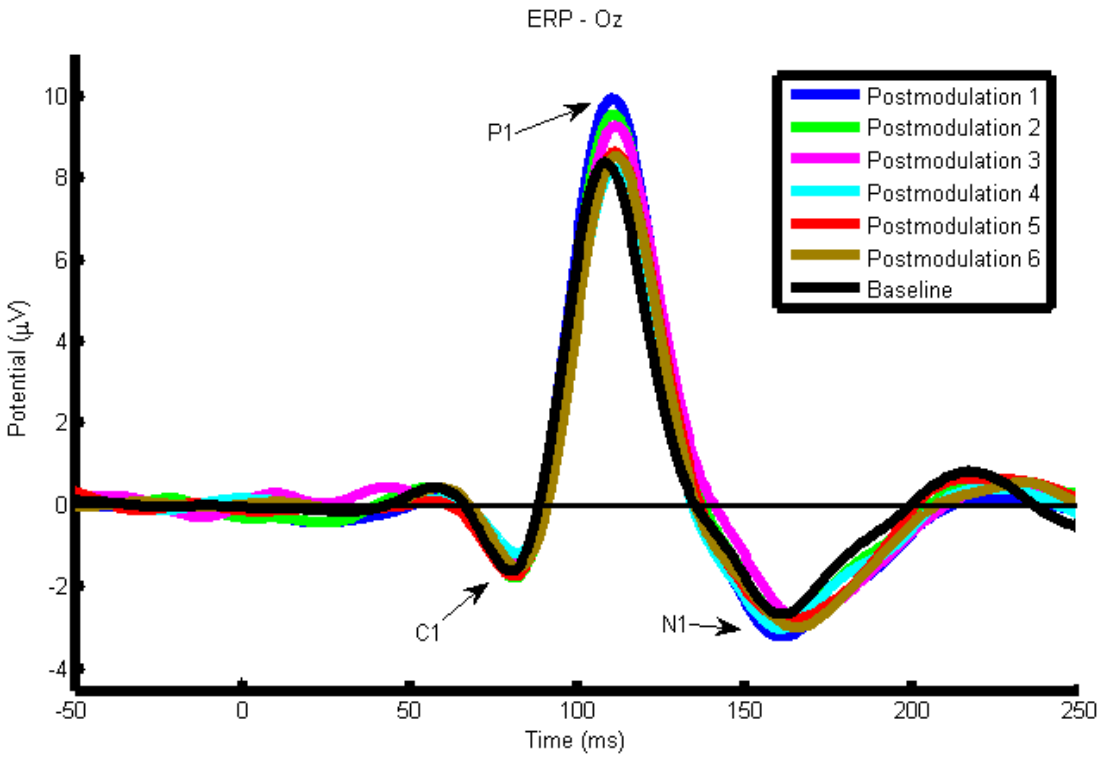

Figure 5. The grand averages ERPs of the all separate measurement blocks.

The C1 component. Repeated-measures ANOVA with the mean baseline and the six post-modulation amplitudes shows no statistically significant change across baseline and post-modulation blocks, F (6, 37) = 0.684, p = 0.624. There is a decrease in amplitude averaged across all post-modulation blocks, and the mean decrease is 0.14 µV (SD = 1.25). Post hoc paired sample t-tests reveal no statistically significant changes in amplitude from baseline to separate post-modulation blocks.

The P1 component. Repeated-measures ANOVA with the mean baseline and the six post-modulation amplitudes shows a statistically significant difference across baseline and post-modulation blocks, F (6, 37) = 9.42, p = 0.000. The mean increase in amplitude between baseline and mean post-modulation blocks is 0.89 µV (SD = 1.93). Post hoc paired sample t-

tests reveal statistically significant increase in amplitude from baseline to the first post-modulation block ($t(37) = 5.305$, $p \leq 0.000$), the second post-modulation block ($t(37) = 3.144$, $p = 0.003$) and the third post-modulation block ($t(37) = 3.038$, $p = 0.004$), but no significant increases in the subsequent blocks.

The N1 component. Repeated-measures ANOVA shows no statistically significant difference across baseline and post-modulation blocks, $F(6, 37) = 0.727$, $p = 0.583$. The mean increase in amplitude from baseline averaged across all post-modulation blocks is 0.39 μV (SD = 1.88). Post hoc paired sample t-tests reveal no statistically significant differences in amplitude between baseline and the separate post-modulation blocks.

The P1-N1 peak-to-peak amplitude. Repeated-measures ANOVA shows a statistically significant difference across baseline and post-modulation blocks, $F(6, 37) = 8.57$, $p = 0.000$. The mean increase from baseline averaged across all post-modulation blocks is 1.27 μV (SD = 2.45). Post hoc paired sample t-tests reveal statistically significant increases in P1-N1 peak-to-peak value from baseline to the first post-modulation block ($t(37) = 5.096$, $p \leq 0.000$), the second post-modulation block ($t(37) = 2.667$, $p = 0.011$) and the third post-modulation block ($t(37) = 3.035$, $p = 0.004$), but no significant changes in the subsequent blocks.

In summary, figure 6 shows the modulation effects between baseline and mean post-modulation blocks, as well as the first post-modulation block, for the various VEP components.

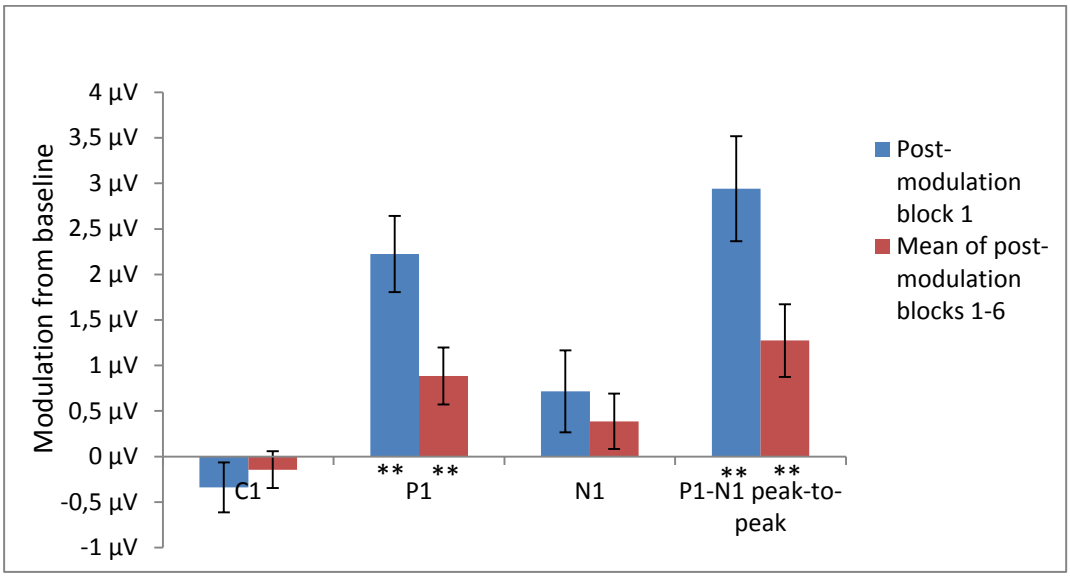

Figure 6. The mean modulation effects from baseline to the first post-modulation block and mean of post-modulation blocks 1-6 for each component, including the standard error range of the mean. ** = $p \leq 0.005$.

3.2 Modulation, stress and cortisol

There were no significant correlations between VEP amplitude modulation measures and individual cortisol samples. There were, however, statistically significant correlations between modulation of the N1 component, P1-N1 peak-to-peak amplitude, and the mean cortisol value of the three morning specimens and the mean of all five cortisol specimens, for modulation values between baseline and first post-modulation block, and between baseline and all post-modulation blocks. Table 1 shows the significant findings. There were no significant correlations between P1 modulation effects and cortisol values.

Correlational analyses between baseline amplitudes and cortisol levels were performed to investigate a possible confounder in the observed correlations between cortisol and modulation effects. No significant correlations could be detected.

Scatterplots of the significant relationships can be seen in figure 7. Considering that all the values were within the normal range of morning salivary cortisol levels, which is between 3.5 and 27 nmol/l (Norsk Elektronisk Legehåndbok, 2013), no subjects were excluded from this analysis.

Table 1. Correlations between salivary cortisol and modulation effects from baseline in N1 component and P1-N1 peak-to-peak values. The two cortisol values displayed are T345, which is the mean of the two morning and the noon sample, and All Mean, the mean of all five cortisol values.

		Cortisol T345 Mean (N=31)	Cortisol All Mean (N=31)
N1-Postmodulation block 1	r	.421	.343
	p	.018	.059
N1-Mean of postmodulation blocks 1-6	r	.446	.292
	p	.012	.111
P1N1-Postmodulation block 1	r	.405	.399
	p	.024	.026
P1N1-Mean of postmodulation blocks 1-6	r	.252	.194
	p	.172	.295

There were no statistically significant correlations between the self-reported VAS stress values and modulation of any of the VEP components. There were, however, statistically significant negative correlations between VAS stress ratings in the preceding month and the average of the two morning cortisol levels ($r_s(29) = -0.458$, $p = 0.010$) as well as the average of morning and noon cortisol levels ($r_s(29) = -0.455$, $p = 0.010$).

Besides the results directly associated with the main hypotheses, a statistically significant negative correlation could be observed between P1 modulation from baseline to the first post-modulation block and VAS depression ratings within the preceding month ($r_s(36) = -0.366$, $p = 0.024$). There were no significant correlations between N1 or P1-N1 modulation and VAS depression ratings, and no significant associations were found between VEP modulation and VAS measures for anxiety.

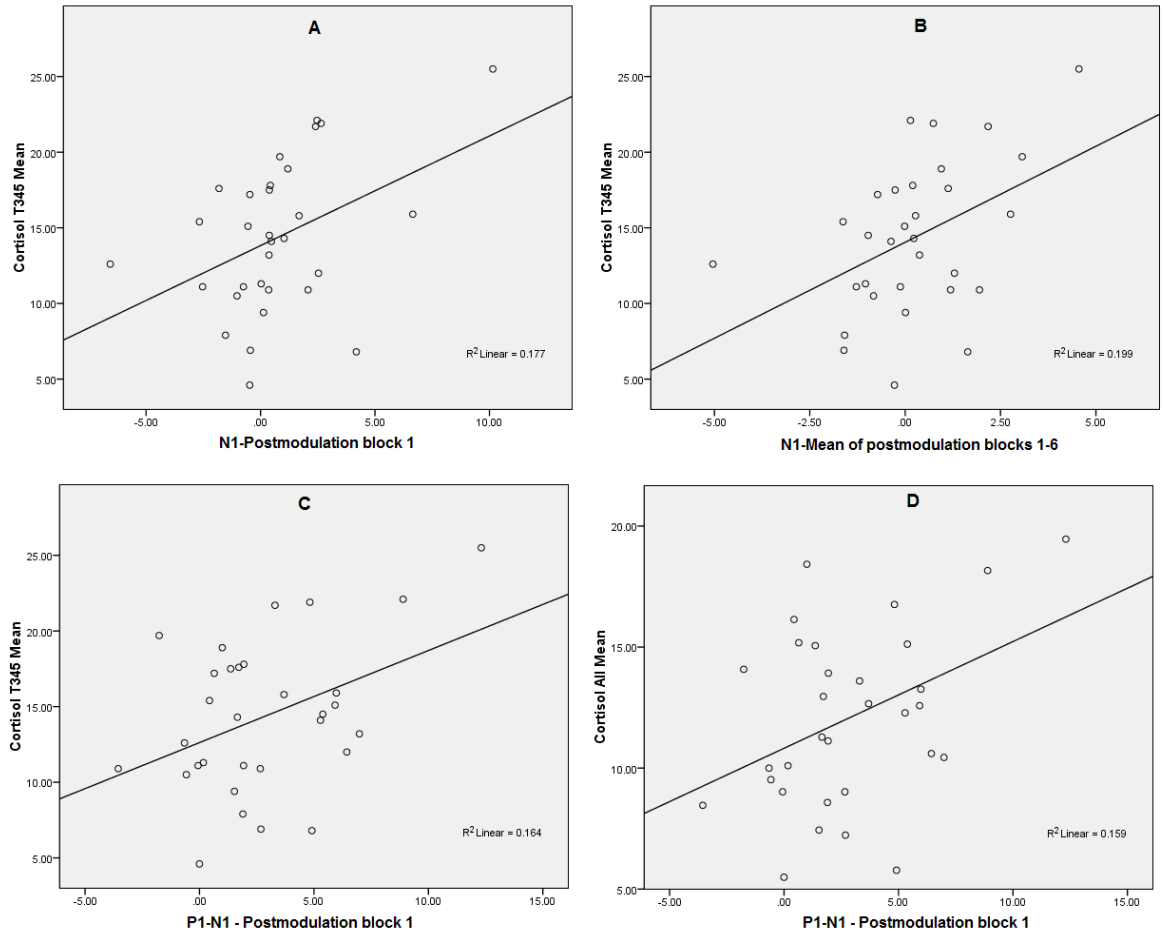

Figure 7. Scatterplots showing the significant relationships between plasticity in N1 and P1-N1 peak-to-peak values, and cortisol values. Scatterplot A shows the relationship between the mean cortisol value of the two morning and the noon samples, and N1 modulation from baseline to postmodulation block 1. Scatterplot B shows shows the relationship between the mean cortisol value of the two morning and the noon samples, and the N1 modulation from baseline to the mean postmodulation blocks 1-6. Scatterplot C shows the relationship between the mean cortisol value of the two morning and the noon samples, and the P1-N1 peak-to-peak modulation from baseline to postmodulation block 1. Scatterplot D shows the relationship between the mean cortisol value of all five samples, and P1-N1 peak-to-peak modulation from baseline to postmodulation block 1.

Gender specific effects appear to occur in the correlations between cortisol levels and modulation effects. By splitting the data according to gender, the previously discussed correlations disappear when only considering females. When only considering the males, however, the effects are augmented, and new ones appear. See figure 8 for a scatterplot between the average of the two morning and the noon cortisol levels, and the modulation effect of N1 between baseline and all post-modulation blocks. Table 2 contains the significant correlations between cortisol levels and modulation effects for males.

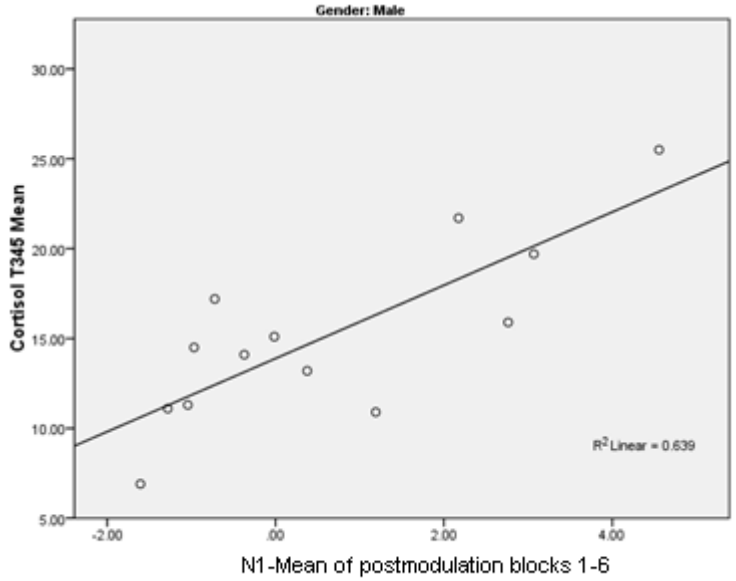

Figure 8. A male-specific scatterplot showing the distribution of mean cortisol value of morning and noon samples and modulation in N1 component between baseline and all post-modulation blocks, as well as the derived regression line.

There were observed differences in average salivary cortisol levels for the two genders. Although not statistically significant differences in independent samples t-tests, there are trends towards lower cortisol levels for females than males. On the mean value of the morning and noon samples (T345), males have an average salivary cortisol value of 15.16 nmol/l (SD=4.98), whereas women have an average value of 13.73 nmol/l (SD=5.03).

3.3 Modulation and physical activity

There were no statistically significant correlations between P1, N1 or P1-N1 modulation effects and measures of physical activity, neither for the MET-minutes scores from IPAQ, nor the self-reported VAS physical activity ratings.

Table 2. Male-specific effects between N1- and P1-N1 modulation effects from baseline and cortisol. The cortisol values used are T3, which is cortisol level immediately upon waking, T34, which is the average of the two morning samples, and T345, which is the mean of the two morning and the noon sample, as well as a mean value of all cortisol samples.

Gender: Male		Cortisol T3 (N=11)	Cortisol T34 Mean (N=13)	Cortisol T345 Mean (N=13)	Cortisol All Mean (N=13)
N1-Postmodulation block 1	r	.711	.439	.688	.669
	p	.014	.133	.009	.012
N1-Mean of postmodulation blocks 1-6	r	.704	.712	.799	.609
	p	.016	.006	.001	.027
P1N1-Postmodulation block 1	r	.401	.152	.454	.573
	p	.222	.620	.119	.041
P1N1-Mean of postmodulation blocks 1-6	r	.379	.139	.461	.443
	p	.251	.652	.113	.130

4 Discussion

4.1 Hypothesis 1: LTP-like plasticity

The main focus of this study was to replicate earlier research results, indicating LTP-like plasticity represented in the potentiation of VEP components, as demonstrated in healthy control subjects in Elvsåshagen et al. (2012). Based on earlier research, our first hypothesis is that we expect to find significant modulation effects, indicating LTP-like plasticity in terms of increased amplitudes specifically of the P1 and P1-N1 components of the VEP, as it is these components that have typically shown the most robust effects. Our results show a clear, significant potentiation of the P1 and P1-N1 peak-to-peak amplitudes of the VEP, but no significant potentiation of the N1 component. This indicates that the visual stimulation paradigm has induced modulation of the response potentials. These data alone are sufficient to support and confirm our first hypothesis. Significant plasticity-effects in the P1 and P1-N1 peak-to-peak components are as predicted.

Typically, research into LTP has demanded invasive methods, using direct, stimulation of various brain regions in lab animals. The advantage of these traditional methods is that one can induce and observe plasticity directly in a brain sample. One can observe the cascade of reactions following tetanus shock stimulation, as described in this paper. The disadvantage is that these methods are unsuitable for human research. Our visual paradigm is based on previous research by Normann et al. (2007) and Elvsåshagen et al. (2012), although Teyler et al. (2005) were, to the authors' knowledge, the first to use a VEP-paradigm to study plasticity. The advantages and disadvantages of using EEG-based methods, e.g. a VEP-paradigm, are the exact opposite of traditional LTP-research methods; namely that a VEP-paradigm is non-invasive, and thus suitable for human research, but one doesn't have the possibility of actually observing the mechanisms of LTP. One must infer what the underlying mechanisms are, based on what is known about neurophysiology, parallel animal studies etc. The question is whether the potentiation found in research based on a VEP-paradigm truly represents underlying LTP-like plasticity?

According to Teyler et al. (2005) LTP is the best and most parsimonious explanation for the observed enhancement of stimulus-specific responses. In lab-animals the effects of visual stimulation have been reversed by the administration of protein-kinase M inhibitors (Lømo,

2012), a protein necessary for LTP, which clearly shows that certain of the same mechanisms are responsible for the observed effects. On the one hand, further research is needed before one can claim beyond reasonable doubt that the potentiation of the VEP-components represents LTP-like plasticity. On the other hand, LTP-like plasticity appears to best explain the results we have observed. Since it is ethically impossible, with today's technology, to observe the mechanisms of LTP directly in the living human brain, we think it is safe to conclude, if tentatively, that the modulations found in this VEP-paradigm do indeed constitute LTP, or at least LTP-like plasticity. Furthermore, clinical studies, using a VEP-paradigm, have already started giving promising results, adding knowledge to the possible neural bases of psychiatric disorders like schizophrenia and major depression Normann, et al. (2007). This research could in future lead to advances in medical and therapeutic treatment of many disorders. Our results, therefore, confirm the first hypothesis of this study, and thus add validity to the model that repeated visual stimulation constitutes a valid method for inducing and observing LTP-like plasticity, non-invasively in humans.

4.1.1 Replication of Elvsåshagen et al. (2012)

There are certain aspects to discuss regarding our results compared to the results in Elvsåshagen et al. (2012). While Elvsåshagen et al. (2012) used a reversing checkerboard image, with two reversals per second; we used vertically oriented black and white sine wave gratings, also with two reversals per second. Results in a small pilot study that we conducted prior to starting this study, indicated a stronger immediate plasticity effect using the vertical black and white sine wave grating. It was thought that both visual stimulations would influence area V1 of the visual cortex, and we decided to use the vertical sine wave gratings to induce LTP-like cortical plasticity.

In addition to the actual visual stimulation being different in the two studies, there are subtle differences between our results and the results in Elvsåshagen et al. (2012), although both studies show the same, basic effects. Our results show a clear increase in the P1 and P1-N1 peak-to-peak amplitudes of post-modulation blocks 1, 2 and 3. The strongest potentiation was in post-modulation block 1, with reducing effects in post-modulation blocks 2 and 3. In the remaining post-modulation blocks, the potentiation effect was no longer statistically significant. Elvsåshagen et al. (2012) found statistically significant plasticity effects on P1, N1 and P1-N1 peak-to-peak amplitude, although the effects on the N1 component did not

survive the conservative Bonferroni-correction used. In addition, the effects in Elvsåshagen et al. (2012) lasted longer, and the P1-N1 peak-to-peak amplitude showed significant plasticity effects in all the post-modulation blocks except for the fourth, although they also report that potentiation showed a reducing trend in the latter post-modulations. This indicates that the LTP-like plasticity in our results was not as long lasting as in the results from Elvsåshagen et al. (2012). This could mean that our paradigm yielded a narrower cortical stimulation, leading to a shorter, faster LTP-like plasticity in a smaller cortical region. A checkerboard includes both vertical and horizontal stimulation, which should theoretically stimulate a broader set of neurons. This is not possible to prove on the basis of our data, and remains open to interpretation and further research.

Furthermore we found the most statistically robust potentiation in the P1 component, although the mean increase was greater in the P1-N1 peak-to-peak amplitude, while Elvsåshagen et al. (2012) found the most robust potentiation in the P1-N1 peak-to-peak component. Elvåshagen et al. (2012) also found significant modulation effects in the N1 component, although weaker than the effects seen on P1 and P1-N1, which we failed to replicate. It is not easy to pinpoint the reasons for these differences. Again, it is possible that the checkerboard visual stimulation induces broader stimulation, with broader neural activity, which could also theoretically explain the longer lasting plasticity effects, and more robust modulation of P1-N1 peak-to-peak. Given the possibility that our simpler visual paradigm induces a narrower stimulation of area V1, this could explain both the stronger, more robust changes found in the P1-component, and the quicker reduction in plasticity effects across the post-modulation phases. In our opinion, the fact that we implemented a different visual stimulation, yet still found statistically significant, robust plasticity effects adds validity to paradigm of non-invasively induced LTP-like plasticity.

Participants were asked to self-report experienced depressed mood over the last month. Based on clinical research one would expect to find tendencies towards associations between depressed mood and LTP-like plasticity in a healthy population. Indeed, there was a significant negative association between subjectively experienced depression and LTP-like plasticity in P1. This indicates that depressive mood in our clinically healthy sample has the same tendency as seen in previous research on clinical populations. Elvsåshagen et al. (2012) did not find a significant association between depression scores and plasticity effects in the healthy control sample. However, they did report a significant negative correlation between

depression scores and P1-N1 plasticity in the clinical sample, if two outliers in the sample were removed. Our results strengthen the idea that disrupted plasticity could be part of the underlying neural correlates of depressive mood, even in a clinically healthy sample. To the authors' knowledge, this is the first time the relationship between depressed mood and LTP-like plasticity has been demonstrated in a clinically healthy sample.

4.1.2 Methodological considerations

Certain methodological issues should be mentioned. Our goal was that EEG-testing should be conducted at roughly the same time of day for each participant, and participants would be well rested and alert. However, in practice, we found that this level of experimental control was not realistically attainable. Some participants were tested early in the day, while others were tested after a full day of work. Although we did not measure alertness in this study, variation in alertness might impact the results. This could, theoretically, have a confounding effect. Although there could be individual differences in alertness, it is uncertain whether this plays a significant role in LTP-like plasticity in the visual cortex. Regarding testing each participant at the same time of day, this could possibly increase validity and quality of data. However, even testing each participant at exactly the same time would not take individual differences in circadian rhythms into account, giving variations in individual alertness. The fact that we found robust plasticity-like effects indicates that variation in time of testing and individual alertness has had little effect on our study, although stricter experimental control may yield more powerful effects.

Another possible weakness is that we lacked control over whether our participants were actually looking at the computer screen as instructed, or not. Elvsåshagen et al. (2012) were able to observe each participant on a small monitor screen. This let them observe whether participants looked away from the screen during test-duration. However, each participant sat with his/her chin resting on a chinrest 57cm from the computer screen and received instructions to keep staring at the fixation point on the screen. This reduces the likelihood of participants looking away during any of the stimulation blocks.

In summary, our results replicate the findings from Elvsåshagen et al. (2012) in a clinically healthy sample, and support the first hypothesis.

4.2 Hypothesis 2: LTP-like plasticity, stress and cortisol

4.2.1 LTP-like plasticity and cortisol

Based on research literature on the association between cortisol levels, stress and plasticity, our second hypothesis was that we would find significant positive correlations between LTP-like plasticity, and salivary cortisol levels. As our sample consisted of healthy individuals, all with cortisol levels within the referential normal range, these correlations were expected to be positive, showing increased VEP-modulation with increased levels of cortisol. This was theorized based on the assumption that our sample would primarily represent the first half of the Yerkes-Dodson law of arousal. One would not expect to find as strong effects of cortisol in a clinically healthy sample, as reported in clinically focused research. There is a paucity of research on salivary cortisol and LTP in clinically healthy populations. Animal and humans studies tend to focus on unhealthy levels of cortisol, demonstrating disruptive effects on various brain functions, including LTP, indicating that populations with extremely high levels of cortisol represent the latter half of a Yerkes-Dodson curve. However, as mentioned previously in this paper, the relationship between cortisol and brain function is complex. Diamond et al. (2007) mention how cortisol can enhance or inhibit LTP in humans, depending on factors such as cortisol level, duration of elevated levels and individual susceptibility.

In this study, there were significant positive correlations between LTP-like plasticity effects of the N1amplitude, P1-N1 peak-to-peak amplitude and salivary cortisol levels, thus supporting hypothesis 2. There are, however, reasons to be cautious in interpreting these findings.

As mentioned in the previous section, there were no statistically significant modulation effects seen on the N1-component. There is, on average, an increase in amplitude, but not strong or consistent enough to reach statistical significance. While we failed to demonstrate significant modulation effects in the N1 component, it has to be considered that baseline amplitudes of the N1 component did not correlate with cortisol values. This might, in turn, indicate that the correlations between the statistically non-significant modulation effects in the N1 component and cortisol values are not merely due to variation in baseline amplitudes. As

such, there is reason to not negate these findings despite the apparent lack of statistically significant modulation effects in the N1 component.

As mentioned, there is very little existing research on the effects of level of cortisol on VEP-modulation in healthy populations. Elvsåshagen et al. (2013) found an association between cortisol levels and VEP-modulation in a healthy control sample. Besides this study, there are several studies showing varying results of cortisol on learning and memory. A meta-analysis of the research into the effect of cortisol administration on memory in humans (Het, Ramlow & Wolf, 2005) found varying effects of cortisol depending on when cortisol was administered in the learning and memory consolidation process, and the time of testing in the day. Although there did seem to be mostly detrimental effects on memory retrieval by administration of cortisol either before learning or before retrieval, time of testing did seem to impact the effects of cortisol to some degree. Testing in the afternoon tended to show beneficial effects of cortisol administration before learning, while testing in the morning tended towards detrimental effects of cortisol administration before learning. Het, Ramlow & Wolf also remark that few studies investigate dose-dependent effects of cortisol on learning in humans, and as such, not much can be said with certainty regarding the possibility of a Yerkes-Dodson inverted U-curve between level of cortisol and learning in humans.

Our results indicate enhanced modulation of certain VEP-components in subjects with slightly elevated levels of cortisol. It is possible that our sample of participants represents a statistical floor-effect, with relatively few participants showing cortisol-levels in the upper region of the normal range, which is where one would expect the main correlational relationship to be found. This is consistent with our hypothesis, based on earlier research and the Yerkes-Dodson law. More research is needed, both into the underlying processes of the modulation effects of the individual VEP-components, and the effects of cortisol in clinically healthy populations.

4.2.2 Gender specific effects

The results indicate that there is a distinction between females and males in the relationship between VEP-modulation and salivary cortisol levels. While there was no semblance of such an association for females, there was a strong association for males, suggesting that the male subjects may provide much of the strength in the correlations for the entire sample. Thus, the

male participants clearly illustrate what might appear to be the first half of the Yerkes-Dodson effect underlying hypothesis 2 (See figure 8).

The menstrual cycle is known to affect salivary cortisol levels in women. In a review by Kudielka, Hellhammer and Wüst (2009), results from several studies show that salivary cortisol levels in females in the luteal (premenstrual) phase of the cycle are comparable to those of males, whereas the cortisol levels are significantly lower for women in the follicular (postmenstrual) phase or for women using oral contraceptives. Considering the slightly lower salivary cortisol values of females in this study, this might indicate that some of the women were in the follicular phase of the menstruation cycle, or were using oral contraceptives.

Kudielka, Hellhammer and Wüst (2009) also emphasize that a vast number of studies show that males tend to show immediate increases in salivary cortisol levels in response to induced stress in laboratory settings, whereas women tend not to show this effect. Gender differences in autonomic stress regulation may therefore provide yet another confounder in our analyses of the relationship between cortisol and VEP-modulation.

Finally, the number of male participants in our study is sparse, and despite significant, strong correlation coefficients between cortisol and modulation effects for males, these results should be considered with the restraints of generalizability in mind.

4.2.3 LTP-like plasticity and subjective stress

No significant relationship could be found between VEP-modulation and subjective experience of stress. We did, however, find a significant negative correlation between cortisol levels and VAS stress ratings within the last month, which may provide insight into the lack of direct associations between VEP-modulation and subjective experience of stress.

All subjects in this study had cortisol levels within the normal range. Operating within the theoretical assumption that our sample represented the first half of the Yerkes Dodson curve regarding level of stress or activation, higher cortisol levels might indicate performance-beneficial levels of arousal. This might in turn mean that higher cortisol within the normal range might be conducive to better every day functioning, and thereby less subjectively experienced stress. Little research has been performed on the relationship between cortisol

levels and perceived stress in healthy samples, and as such, this finding remains open to interpretation.

4.2.4 Methodological considerations

Several factors have been shown to affect salivary cortisol levels in healthy samples. Hansen, Garde & Persson (2008) provide a checklist for research conducted using salivary cortisol samples, based on the existing studies of possible methodological caveats. Between-subjects time variation of cortisol samples is perhaps the most concerning of these which might be relevant to our study, and was also discussed in a review by Kudielka, Hellhammer & Wüst (2009). Subjects in our study were instructed to collect one sample immediately upon waking, another thirty minutes later, and one at noon. Although all subjects were instructed to specify the time for each sample taken, few subjects did, and it is likely there was some variation in administration time for the morning samples. One solution to this problem might have been to specify the time for the first two samples to be taken, although this solution would ignore the problem of individual variation in circadian rhythms, which may also affect cortisol levels.

Physical exercise has also been shown to have an immediate effect on salivary cortisol levels, as well the use of alcohol or nicotine and possibly caffeine consumption. (Hansen, Garde & Persson, 2008). Food consumption and tooth brushing may also cause gum bleeding, which may in turn contaminate the saliva specimens with blood. All subjects were instructed to avoid food, drinks, nicotine and brushing their teeth within half an hour of saliva testing, as well as to avoid alcohol for 24 hours, although there were no instructions regarding physical exercise. As with all voluntary research without a strict experimental control of variables, uncertainties regarding adherence to instructions cannot be determined, and may well be a factor in the results relating to cortisol measurement from this study.

Adhering to the checklist provided by Hansen, Garde & Persson (2008), this study does appear to have several strengths in regards to proper saliva collection procedures. All cortisol samples were collected at the same time of year, using the same collection procedure, stored appropriately, and analyzed at the same laboratory, and contaminated specimens were excluded from analyses, which should eliminate some of the possibility of confounder bias from the cortisol data.

In summary, the relationship between cortisol and various brain functions is complex. Despite some possible methodological confounders, results from this study indicate an association between LTP-like plasticity and salivary cortisol levels.

4.3 Hypothesis 3: Physical activity and LTP-like plasticity

The third and final hypothesis is that participants who report higher levels of physical activity will also show stronger plasticity effects. Research on animals and humans, cited in this paper, highlights a broad array of positive effects that exercise leads to. On a neurophysiological level, physical exercise increases levels of BDNF, with many positive effects, including enhanced neuroplasticity, synaptic strengthening and neurogenesis (Nadel et al. 2013). Thus, level of physical activity would be expected to correlate positively with LTP-like plasticity. Indeed, animal studies have demonstrated enhanced LTP in mice after just one week of exercise and environmental enrichment (Fares et al. 2013). The hippocampal region of the brain appears to be particularly positively influenced by physical exercise. Our results, however, show no statistically significant effect of exercise on LTP-like plasticity. Participants who reported higher levels of physical activity did not demonstrate more powerful amplitudes in the VEP-data. Hypothesis 3 is therefore not supported by our data. There are many possible explanations for why our results did not demonstrate the expected correlations. Firstly, it could be that synaptic LTP is not affected by level of physical activity in humans. Given our null-findings, this could be a valid explanation. To the authors' knowledge, there is little research into the effects of physical activity on LTP, as measured with a VEP-paradigme.

4.3.1 Methodological considerations

One possible reason why our study failed to support hypothesis 3 could be that our study was not designed to measure the effects that exercise might, or might not have on LTP-like plasticity, per se. The main objective of this study was to replicate earlier findings demonstrating and strengthening the validity of using a VEP-paradigm for studying LTP-like plasticity in humans. The exercise variable was included as an exploratory hypothesis, aimed at registering self-reported level of physical activity to see if this was positively correlated with LTP-like plasticity in a clinically healthy sample of participants. This means that there

was a low degree of experimental control related to each participant's true level of physical activity. Although each participant filled out the IPAQ-SF questionnaire, which is largely considered to be the best self-report questionnaire for measuring level of physical activity (Poppel, Chinapaw, Mokkink, Mechelen & Terwee, 2010**),** there was no control over whether participants over or under-reported their level of physical activity. A higher degree of control, as described in studies focused directly on physical exercise, might have given different results.

Also, defining what should constitute "physical activity" isn't necessarily straight forward. Most of the research related to physical activity cited in this paper includes a high degree of experimental control over type and intensity of physical activity. Several animal and human studies mentioned in this paper include strict experimental manipulation of physical activity, e.g. with one group of mice allocated to a "running" condition, with vigorous running every day, while a control group is "sedentary." Human research cited in this paper, for example Winter et al. (2007), allocated participants into inactive, moderately active, and highly active groups. Focused studies into level of physical activity, with adequate levels of experimental control, tend to show positive effects from exercise in multiple areas, including effects on plasticity and learning. Again, our design does not include that level of control, even though the IPAQ-SF includes questions aimed at these areas. Studies of physical exercise and its effect on plasticity and other brain functions have tended to focus on aerobic exercise. Our study has not controlled what type of exercise participants partake in. Again, this leaves room for subjectivity in self-report. We have not defined whether running to the bus, or Yoga-class or taking an evening walk qualify as physical activity. Also, the IPAQ-questionnaire gives little opportunity to check and control objective exercise intensity. Again, this is left to the subjective experience of each participant.

Another factor to consider is the participants in our study. We included only healthy adults between the ages of 18 and 50. There is evidence to suggest stronger effects of exercise in older individuals. The total scores on the IPAQ-SF questionnaire indicate a certain degree of variation in reported amount of physical activity, but the lack of experimental control of what each participant actually defines as exercise, means that the variation in reported amount of physical activity could still hide a potential floor or ceiling effect.

Studies evaluating the IPAQ-questionnaire's criterion validity using concurrent activity monitors or fitness measurements have shown varying results. A review of twenty-three

studies researching the criterion validity of the questionnaire (Lee, Macfarlane, Lam & Stewart, 2011) found that the results were generally below the acceptable range, with a great deal of variability between studies. Correlations between IPAQ-SF and objective measures were acceptable only for the subscales walking and vigorous activities, although results proved more valid in studies using narrower, categorical definitions than the ones proposed in the official scoring protocol. One study conducted with doubly labeled water as a measure of concurrent validity (Ishikawa-Takata, et al., 2008), found that the IPAQ-SF proves useful to discriminate low from high levels of physical activity, although the differentiation of low and moderate levels was observed to be poor. This measurement problem has been observed through several studies, as discussed by Lee, Macfarlane, Lam & Stewart (2011).

Taking into consideration these methodological issues with the use of the IPAQ, the lack of significant effects of physical activity in our study may, in part, be explained by the lackluster criterion validity of the questionnaire used.

In summary, our data does not support the third hypothesis. However, one must also consider the possibility that level of physical activity in fact does not have an effect on LTP-like plasticity as measured by modulation of VEP amplitudes in a healthy population.

4.4 Strengths and limitations

The results of this study have to be considered in line with the strengths and limitations of our design and methods. The limited size of the subject sample proved sufficient to demonstrate plasticity effects, but suggests caution in generalizing findings from this study to a broader population. Several participants also failed to return both salivary cortisol samples and the physical activity questionnaire, constraining the statistical power of the study. In addition, there are indications in the literature that variation in time of testing in the day might impact results with regards to especially level of cortisol. This suggests that future research could benefit from standardizing when testing is performed.

As discussed, our study also demonstrated considerable gender differences in the association between salivary cortisol and plasticity effects. This may, in part, be due to the influence of the menstrual cycle on cortisol levels in women, and gender differences in autonomic stress regulation. In our study, female participants were asked to state the start date for their previous menstruation, but no questions were asked as to the use of oral contraceptives, thus

we could not control for this factor. Future research should possibly either focus mainly on males, or use sound methods for statistically controlling for menstrual cycle fluctuations in level of cortisol. Future research could also benefit from experimentally manipulating levels of cortisol prior to the EEG measurement, thus eliminating several confounders which might have influenced cortisol measurement in this study, and possibly generating greater variation in cortisol levels.

The criterion validity of the physical activity questionnaire used has been discussed, and provides reason to be cautious in rejecting the third hypothesis. It is possible that a greater degree of experimental control over level of physical activity would have contributed to finding the expected effects. It is also possible that a study including a focused manipulation of physical activity would also find the expected effects, especially when bearing in mind the positive effects of exercise that have been found in clinical populations.

Having considered some possible limitations, the strengths of this study also deserve consideration. Pilot testing was performed subsequent to the initiation of the main study, which resulted in the choice of the vertical sine wave grating paradigm for use in our study based on the preliminary findings. The quality of the EEG data for all participants was good, and no subjects had to be excluded due to poor data quality. Both data collection and data analyses were performed by the authors, and all EEG and ERP data was manually inspected to ensure no errors had occurred in the collection or analysis process. In addition, ocular and muscular artifacts were removed using the time-consuming and sophisticated filtration method of Independent Component Analysis, to ensure ideal data quality for the study.

5 Conclusion

In conclusion, this study adds validity to the still rather novel method of inducing and measuring LTP-like plasticity, non-invasively, in humans. Research cited in this paper indicates that these plasticity-effects constitute Hebbian-type LTP. This study also delivers new insight regarding the role of cortisol in relation to LTP-like plasticity in healthy control subjects, indicating a possible Yerkes-Dodson relationship. As mentioned, this study did not find any significant relationship between physical activity, as measured in this study, and LTP-like plasticity. This can either mean that physical activity doesn't have an effect on LTP-like plasticity, as measured with VEP-modulation, or that the method we used to measure level of physical activity has weak validity. As mentioned, this study has certain strengths and weaknesses. However, the results of the testing and analyses indicate a reliable and valid experimental design, with adequate control during testing and rigorous statistical analyses.

There are many ways in which this area of research is proving useful. Studies have demonstrated basic differences between healthy controls and psychiatric patients with various diagnoses in relation to neuroplasticity. It seems then, that reduced or impaired neuroplasticity might be part of the neural basis for many clinical conditions, as mentioned in Elvsåshagen et al. (2012). The functional implications of this are still speculative, and more research is demanded before definite conclusions can be reached. However, Waage (2012) reported a relationship between LTP and visual memory, indicating that LTP might have measurable, functional implications. Increased knowledge of metaplasticity, and how plasticity can be enhanced in clinical populations, might play an increasingly important role in our future understanding of various clinical conditions. In any case, greater insight into the neural basis for various clinical conditions, such as bipolar disorders, depression, and schizophrenia can lead to more targeted medications and treatment programs. Longitudinal studies have demonstrated that exercise has beneficial effects for depressed and anxious individuals (Salmon, 2001). Although our study failed to find a relationship between physical activity and plasticity, this path of research can, with increased experimental control, shed light on why exercise shows beneficial effects in the treatment of depression.

The association found in our results between cortisol and plasticity-effects confirms the complicated relationship between cortisol and various brain functions. In clinical populations, where cortisol levels are typically above what is considered normal, one has found reduced

LTP-like plasticity. This demonstrates the Yerkes-Dodson understanding of the effects of cortisol. The effects of cortisol depend on many factors. Our study sheds light on this relationship. This study is, to the authors' knowledge, the first to study the relationship between cortisol and LTP-like plasticity in a clinically healthy population, indicating that slightly elevated cortisol enhances LTP-like plasticity.

Further research is needed to fully understand the functional mechanisms of LTP and the implications this might have. LTP is considered to be the principal neural mechanism underlying learning and memory consolidation. Therefore further research could shed light on the relationship between LTP and cognitive functions, especially learning and memory in healthy individuals. Further studies on the modulation of sensory evoked potentials as a method of studying LTP-like plasticity should focus on refining the paradigm with regard to experimental factors such as stimulus property and length of modulation phase in order to optimize the plasticity effects. Further research, using clinically healthy samples, is also needed to draw parallels to clinical populations. As is the case in all cognitive neuroscience; one must understand the normal function of a given mechanism before one can understand impairments in the mechanism. This study constitutes a small contribution in that direction.

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