Non-invasive brain stimulation of the primary visual cortex of healthy adults and individuals with amblyopia

by

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A thesis
presented to the University of Waterloo
in fulfillment of the
thesis requirement for the degree of
Doctor of Philosophy
in
Vision Science

Waterloo, Ontario, Canada, 2021

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AUTHOR'S DECLARATION

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

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Abstract

Introduction

Amblyopia is a neurological condition that affects the visual cortex and causes visual deficits, typically in one eye. Recovery from amblyopia requires significant visual cortex neuroplasticity to allow for changes in cortical processing. Non-invasive brain stimulation (NIBS) is a technique that can modulate cortical excitability and neurotransmitter concentration within superficial regions of the human brain, including the primary visual cortex. Recent studies have demonstrated that the application of NIBS to the visual cortex can promote neural plasticity and may enable recovery of vision. The overall goal of this thesis was to investigate whether repeated sessions of visual cortex NIBS could improve vision in adults with amblyopia. To achieve this goal, three specific objectives were pursued: (I) Identify the effective transcranial electrical stimulation (tES) protocol for modulating visual function in healthy adults, (II) determine whether primary visual cortex transcranial random noise stimulation (tRNS) alters cortical excitability in adults with normal vision as measured by VEPs, and (III) assess whether repeated primary visual cortex tRNS sessions could improve visual acuity and contrast sensitivity in adults with amblyopia.

Experiment – I

The purpose of this pilot study was to compare the effects of visual cortex anodal transcranial direct current stimulation (a-tDCS), tRNS and sham stimulation on contrast sensitivity in healthy adults. The objective was to identify the effective stimulation type for experiments II and III. The study also served as an initial pilot of a new NIBS system and contrast sensitivity measurement protocol. Within a small sample of healthy adults (n = 6 per

group), no differences were observed between stimulation conditions. tRNS was chosen for subsequent experiments based on trends in the data and the available literature.

Experiment – II

We tested the hypothesis that visual cortex tRNS would induce an acute increase in the amplitude of pattern-reversal VEPs in healthy adults (n = 10). The purpose of this experiment was to test for an electrophysiological effect of tRNS in healthy adults because experiment-I had not revealed a psychophysical effect. We found that for measurements made directly after active tRNS stimulation, significantly increased VEP amplitudes for parietal electrodes relative to sham treatment. Increased VEP amplitudes following tRNS suggest that tRNS acts to increase visual cortex excitability.

Experiment – III

We tested the hypothesis that five daily sessions of visual cortex transcranial random noise stimulation would improve contrast sensitivity, crowded and uncrowded visual acuity in adults with amblyopia. Nineteen adults with amblyopia were randomly allocated to active or sham tRNS of the visual cortex (active, n = 9; sham, n = 10). Sixteen participants completed the study (n = 8 per group). tRNS was delivered for 25 minutes across five consecutive days. Monocular contrast sensitivity, uncrowded and crowded visual acuity were measured before, during, 5 minutes and 30 minutes post stimulation on each day. Active tRNS significantly improved contrast sensitivity and uncrowded visual acuity for both amblyopic and fellow eyes whereas sham stimulation had no effect. An analysis of the day-by-day effects revealed large within session improvements on day 1 for the active group that waned across

subsequent days. No long-lasting (multi-day) improvements were observed for contrast sensitivity, however a long-lasting improvement in uncrowded visual acuity in amblyopic eyes was observed for the active group. No effect of tRNS was observed for crowded visual acuity in amblyopic eyes. In agreement with previous non-invasive brain stimulation studies using different techniques, tRNS induced short-term contrast sensitivity improvements in adult amblyopic eyes, however, repeated sessions of tRNS did not lead to enhanced or long-lasting effects for the majority of outcome measures.

Conclusion

Our findings buttress the existing reports of improved vision in amblyopia following NIBS of the visual cortex. In agreement with previous non-invasive brain stimulation studies, our results demonstrated considerable short-term plasticity within the visual cortex of human adults with amblyopia. Future research should integrate modification of stimulation parameters across repeated sessions aiming to increase the cumulative effects induced by NIBS.

Acknowledgements

First and foremost, I would like to thank my supervisor, Dr. Ben Thompson for his mentorship, guidance, encouragement throughout the course of my degree. I would like to thank my committee members Dr Daphne McCulloch and Dr. Michael Barnett-Cowan for their valuable comments and suggestions. Again, special thanks goes to Dr Daphne McCulloch for her constant guidance and always being available to help me. I would also like to thank members of Human Visual Neuroscience Lab. I would like to thank my good friend, Emmanuel Alabi Borquaye who has been with me throughout my ups and downs in my career in Canada. Finally, my beloved family members (wife, son and mom) for their love and support.

This work was supported by CIHR grant 390283, CFI grant 34095 and NSERC grants RPIN-05394 and RGPAS-477166 to BT.

Dedication

I would like to dedicate this thesis dissertation to my family members.

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Chapter 1: General introduction, aims and objectives

1.1 General Introduction

1.1.1 Non-invasive brain stimulation

Non-invasive brain stimulation (NIBS) techniques enable the modulation of neural activity by electrically stimulating targeted brain regions through the scalp. NIBS techniques can facilitate neuro-plastic changes and induce after-effects that last beyond the duration of stimulation [1,2]. NIBS techniques have been shown to modulate neural excitability in a wide variety of brain regions including the motor cortex [3–5], visual cortex [6–10] as well as oculomotor areas of the cerebellum [11]. The two main modalities of NIBS are transcranial magnetic stimulation (TMS) and transcranial electrical stimulation (tES). TMS can generate action potentials in stimulated neurons. For example, when TMS is applied to the motor cortex, it can generate peripheral muscle responses via de-polarization of motor neurons [12]. Similarly, stimulation of the visual cortex can induce the perception of phosphenes [10]. On the other hand, tES techniques involve the application of a weak electrical current to the brain through the skull, using two or more electrodes, and act to alter neuronal excitability. tES can be delivered as transcranial direct current stimulation (tDCS), alternating current stimulation (tACS) and random noise stimulation (tRNS). The mechanism underlying the effects of each tES technique is different [13] [See Chapter 2; and also see Paulus et al. 2011 and Antal et al. 2016 for a detailed description of the mechanisms of each tES technique]. In brief, tDCS alters cortical excitability through membrane polarization, whereby anodal-tDCS depolarizes whereas cathodal hyperpolarizes the resting membrane potential [7]. tRNS and tACS induce cortical excitability through interference

with ongoing brain oscillations [4,14]. Previous studies have reported that effects induced by tES techniques may be due to alterations in the homeostatic balance of neurotransmitters, strengthening of functional connectivity of local neuronal networks and promoting the expression of brain-derived neurotrophic factor (BDNF) [15–18]. For instance, tDCS was found to reduce concentration of the inhibitory neurotransmitter GABA [16] in human [19] and animal [16] studies. Previous studies have shown that repetitive transcranial magnetic (rTMS) and transcranial electrical (tES) visual cortex stimulation can transiently enhance contrast sensitivity and cortical activation in adults with amblyopia [10,20,21]. Recently, other studies have also demonstrated that non-invasive tES of the primary visual cortex modulates contrast sensitivity in healthy individuals and patients with amblyopia [21,22], perhaps by altering the concentration of GABA. These are important findings because previous studies in animal models indicated that GABA reduction promoted neural plasticity within the visual cortex and recovery of vision in animals with amblyopia [23].

Single and repeated sessions of NIBS have been demonstrated to modulate cognitive performance [24–26] and visual function [27–29] in healthy individuals. NIBS has also been proposed as a potential tool for the treatment of neurological conditions including stroke, depression and amblyopia [20,22,30–32]. NIBS techniques are shown to be safe and tolerable in humans [33–36].

1.1.2 Amblyopia

In a clinical context, amblyopia is defined as reduced visual acuity, despite a healthy eye and visual system and best optical correction[37,38] in one or both eyes. Amblyopia in one eye (unilateral amblyopia) is diagnosed when there is a 0.2 logMAR difference in visual acuity between the eyes measured using an optotype chart, without any pathological disease in the visual system. Conventionally, amblyopia can be defined as a neurologically based condition that affects visual and extrastriate cortices leading to visual functions deficits in one or both eyes[39,40]. Uniocular amblyopia occurs during childhood when both eyes see different images leading to abnormal development of the primary and extrastriate visual cortex. The cause of the abnormal visual cortex development is suppression of visual inputs from the amblyopic eye due to blurred image (anisometropia), visual blockade (deprivation) and/or misalignment (strabismus)[41,42]. Individuals with amblyopia suffer from a range of visual deficits including deficits of higher-order visual functions[43–47]. Studies assessing the social and psychological wellbeing of individuals with amblyopia have reported associated negative impacts of amblyopia on quality of life[48–51]. Amblyopia is the second leading causing of monocular visual impairment, following cataract, in adults, with a prevalence of 1-3%[52,53]. The underlying neural mechanism of amblyopia is attributed to an abnormal pattern of interocular suppression as well as neural function deficits in the visual cortex[39,40,54–59]. The dysfunction in the visual cortex is associated with an imbalance of neural excitation and inhibition[39,40,60]. In particular, neural inputs from the amblyopic eye elicit weaker neural responses within the visual cortex than inputs from the fellow eye. In addition, the strength of interocular suppression is positively associated with the severity of the amblyopic eye visual acuity loss[61]. The asymmetrically suppressed intercortical

neurons within the visual cortex hemispheres are associated with increased levels of GABAergic inhibition[23].

1.1.3 Treatment of Amblyopia

The efficacy of traditional amblyopia treatments that involve limiting the duration or quality of input from the fellow eye vary as a function of age, type of amblyopia and the extent of neural suppression. Recent animal models of amblyopia showed that visual cortex neuroplasticity still persisted within the adult brain leading to a discovery that a treatment regimen can be attempted in human adults with amblyopia[18,62–64]. The mammalian visual system has an inborn binocular circuit; hence, the bulk of the cortical neurons are binocular. Restoration of visual functions requires binocular stimulation of cortical neurons within the visual cortex.

The loss of visual functions following monocular deprivation is associated with decrease of visual input driven by imbalance between excitatory and inhibitory cortical neurons. Inhibition in the deprived eye dominates the drive of visual input whilst excitation from the non-deprived eye is increased. To promote neuroplasticity and restore visual function several methods have been attempted. For instance, the use of tDCS stimulation[65,66], pharmacological agents[67–71], environment enrichment[72–74] and visual perceptual learning[75–78] have been shown to reactivate ocular dominance plasticity, induce improvement in visual functions and induce morphological and neurochemical changes in animals[79–87].

In the past 2 decades, the application of non-invasive brain stimulation (NIBS) to a targeted area of the human cortex resulted in excitatory or inhibitory effects on binocular cortical neurons[7,88]. NIBS aims at modulating binocular cortical function in order to reduce interocular suppression. NIBS techniques facilitate neuro-plastic changes yielding enhanced neural processing in human visual[7,9,20,22,89–91] and motor cortices[13,88,92–97]. These effects last beyond the duration of stimulation. Although NIBS is a new field currently being explored extensively, it has gained much attention by demonstrating the possibility of brain plasticity in adulthood. Evidence indicates that NIBS enhances the reorganization of structural and functional abnormalities of the brain following neurological-based diseases such as depression[98–101], post-stoke aphasia[31,102,103] and traumatic brain injury[104–106]. Recent studies have also reported improvements in contrast sensitivity and visual acuity following application of human visual cortex NIBS in adults with amblyopia [20,22,107–109].

1.1.4 Mechanisms of Neuroplasticity

Neuroplasticity refers to structural and functional changes in the brain that occur in response to physiological changes, injuries, new environmental demands and sensory experiences[110,111]. Other sources simply define neuroplasticity as the ability of the central nervous system to perceive, respond and adapt to extrinsic or intrinsic stimuli[112,113]. The mechanism of neural plasticity can be explained at the cellular level of individual neurons and at the level of functional networks involving large groups of neurons. Cellular and molecular mechanisms that facilitate neuroplasticity include but are not limited to the

production of new neurons, growth of axons or dendrites, formation and reorganization of synapses and alterations in the speed of synaptic-transmission[112]. For instance, mitochondrial biogenesis (the growth and division of mitochondria) and varicosity within an individual cell regulate neuroplasticity[114,115]. Also, at the cellular level, neural plasticity is facilitated by alteration in the strength of synapses due to patterns of activity within that cell or from neighbouring cells connected to it. Activities such as the influx of calcium at the synapse regulate the alterations of synaptic strength. For instance, long-term potentiation (LTP), an increase in the strength of active synapses and long-term depression (LTD), a decrease in the strength of active synapses are activity dependant cellular processes that modulate the direction of neural plasticity[116]. Thus, promotion of neuroplasticity is dependent on efficiency of synaptic transmission. It has been reported that LTP and LTD are dependent on the expression of genes and the synthesis of proteins from a specific cells[117]. Among the genes, receptors for neurotrophic growth factors such as brain-derived neurotrophic factor (BDNF) regulate neurogenesis, the birth of new neuronal cells, leading to the formation of new local network connections. In particular, alterations of BDNF receptor gene expression contributes to the mechanisms that modulate synaptic configuration and function[112].

In human studies, short-term (~2 hours) monocular deprivation in healthy adults alters cortical responses showing that neural plasticity can be induced by temporarily altering afferent input to the visual cortex[111]. fMRI and TMS experiments have showed that monocular deprivation in healthy adults enhances the visual cortex response to inputs from

the recently deprived eye indicating the presence of short-term neuroplasticity[118–120]. For instance, Binda et al. used fMRI to demonstrate the neural correlation with BOLD signal levels in the visual cortex response to non-deprived and deprived eyes. BOLD responses to the high-spatial frequency stimulation of the deprived eye increased and showed the strongest cortical activation in V1, V2, V3 and V4. Also, during binocular rivalry performed outside the fMRI scanner, they showed that deprivation effects measured by BOLD responses correlated with perceptual effects, whereby mean rivalry phase durations were longer/shorter after deprivation for the deprived/non-deprived eye, respectively.

1.1.5 Promotion of Neuroplasticity using NIBS

Monocular deprivation is used to determine visual cortical plasticity. Monocular deprivation is created by occluding one eye for a few weeks after birth which causes structural and functional changes in the visual cortex. In animal models, monocular amblyopia can be induced by monocular deprivation within the critical period (a time of enhanced visual cortex neuroplasticity). Monocular deprivation leads to a shift in ocular dominance, structural changes (such as atrophy of the lateral geniculate nucleus (LGN) layers) and imbalance between inhibitory and excitatory neurotransmitters[121,122]. Earlier studies in amblyopic animals demonstrated that infusion of bicuculline or noradrenalin (GABA antagonists) significantly restored plasticity and improved visual responses from the deprived eye to the occipital cortex[123–125]. Interestingly, these studies showed that curtailing the effects of GABAergic inhibitory neurotransmitters restored neuroplasticity within the visual cortex.

Therefore, reducing the concentration of GABA within the visual cortex may play an essential role to neuroplasticity restoration in animal models[67,126].

Kasamatsu et al. demonstrated the use of electrical stimulation to restore neuroplasticity in monocularly deprived kittens with amblyopia[63]. They built on their previous model that electrical stimulation of Noradrenaline(NA)-containing cells in the locus coeruleus (LC) released endogenous NA which facilitated and restored neuroplasticity[123,127]. Mature animals were kept in the dark and briefly exposed to light with monocular vision (2 hours a day for 6 days) combined with 1.5mA LC stimulation. A restoration of visual neuroplasticity was observed that persisted for 3 weeks[63]. Their results paved the way for a new understanding of neurochemical level effects on neuroplasticity within visual cortex of amblyopic animals. Since then researchers have investigated multiple pharmacological agents (fluoxetine, acetylcholine, serotonin) to directly reduce intracortical inhibition and promote neuroplasticity in post-critical period animals with amblyopia following monocular deprivation [67,71,128–130]. However, translating the findings from animal studies of neuroplasticity-enhancing pharmacological agents to humans with amblyopia has many challenges including a large dosage parameter space and associated side effects[131].

Recently, researchers have revisited the use of electrical stimulation, as an alternative technique to pharmacological agents for regulating the balance between inhibitory and excitatory mechanisms to induce visual cortex neuroplasticity plasticity in animals with amblyopia. Castaño-Castaño et al. demonstrated that repeated sessions of visual cortex tDCS potentially reversed the effects of monocular deprivation on visual acuity and depth perception in Long-Evans rats[62,64,132]. The selected animal subjects in each study

experienced monocular deprivation for 48 days following monocular occlusion (eyelid sutured, and egg white was applied as tissue glue) on postnatal day 12. Prior to tDCS stimulation, animals were allowed to recover for 1 month after eyelid opening on day 60. They reported a decrease of visual acuity or depth perception in monocular and/or binocular viewing conditions following this monocular occlusion during the critical period. tDCS (current intensity: 2mA) was delivered for 20 mins per day for 8 consecutive days. The amblyopic animals that received the repeated sessions of anodal tDCS showed restored plasticity within the visual cortex relative to control animals. The common finding from Castaño-Castaño et al. is an acute improvement in visual function following repeated sessions of visual cortex tDCS treatment.

Conclusions from Castaño-Castaño et al. postulated that amblyopia caused by monocular deprivation is associated with cortical disorganization that affects visual functioning. Their results support the evidence that functional, structural and neurochemical changes are dramatically affected in the amblyopic visual cortex following monocular deprivation. Finally, their data demonstrated that repeated sessions of tDCS treatment can potentially induce balance in ocular dominance and restore neuroplasticity within amblyopic visual cortex in animals.

Many studies have demonstrated the use of NIBS to promote neuroplasticity in the human brain using neurological based disorders as models[105,133,134]. TMS and tDCS are the most widely used NIBS techniques to enhance neuroplasticity in healthy and diseased adult brains through alteration of synaptic plasticity and restructuring of local neural

networks[133,135]. In healthy adults with amblyopia, the visual cortex was thought to lack adequate neuroplasticity[136] for treatment to be beneficial. Recently, reports of visual function improvement using NIBS have proven that neuroplasticity still exists in human adults with amblyopia. Thompson et al. demonstrated an enhanced contrast sensitivity in the amblyopic visual cortex following repetitive transcranial magnetic stimulation (rTMS)[10]. Again, NIBS, specifically anodal tDCS delivered to the primary visual cortex, induced balancing of cortical activation in response to inputs from the amblyopic and fellow eyes. In healthy adult subjects, anodal-tDCS increased BOLD signal levels in the primary motor cortex post stimulation[137], suggesting an increased cortical excitability, synaptic plasticity and reconfiguration of intrinsic brain activity networks.

1.2 Specific aims

1. Identify the most effective transcranial electrical stimulation (tES) protocol for modulating visual function in healthy adults.
2. Determine the effects of primary visual cortex transcranial random noise stimulation (tRNS) in adults using VEPs.
3. Assess the effects of repeated primary visual cortex tRNS sessions on contrast sensitivity and visual acuity in healthy adults with amblyopia.

1.3 Main objective

The overall objective of this research was to assess whether tES can be used as a treatment modality for amblyopia in adult humans. The first experiment involved piloting of tES stimulation protocols and to identify an effective transcranial electrical stimulation protocol for modulating contrast sensitivity in humans. We compared the aftereffects of visual cortex tDCS, tRNS and Sham stimulation on contrast sensitivity in healthy adults. This pilot study did not identify any differences between stimulation conditions. Therefore, tRNS was selected for subsequent experiments based on the existing literature. The second experiment sought to assess the aftereffects of tRNS using an objective (electrophysiological) method. We investigated whether visual cortex tRNS would induce excitability changes as evidenced by increased pattern reversal VEP amplitudes post-stimulation in healthy adults. Although a similar study had shown that anodal-tDCS transiently and significantly increased visual evoked potential (VEP) amplitudes and contrast sensitivity for amblyopic and control eyes [22], tRNS effects on VEP amplitudes had not yet been studied. The third experiment focused on investigating visual function changes before, during and after visual cortex tRNS in healthy adults with amblyopia. The aim was to investigate whether multi-section visual cortex tRNS would induce acute and long-lasting improvements in contrast sensitivity and visual acuity in adults with amblyopia.

1.4 Significance of the study

Amblyopia urgently requires new treatments for adult patients. Available treatments for amblyopia are focused on children and teenagers[138], but no treatments are widely available for adults. This is due to the (now disproven) idea that the adult brain lacks sufficient neural

plasticity to re-learn use of the amblyopic eye. This study expands the evidence base for the use of NIBS to treat amblyopia in adult patients.

Chapter 2: Non-invasive brain stimulation (NIBS) as a potential treatment for amblyopia: Literature review

2.1 Introduction

Amblyopia is a neurological condition that affects the visual cortex leading to visual function deficits in one or both eyes [39,40]. Amblyopia is caused by abnormal visual experience during childhood that is typically associated with visual deprivation (deprivation amblyopia), unequal refractive error between the two eyes (anisometropic amblyopia), ocular misalignment that disrupts binocular fusion (Strabismic amblyopia) or a combination of these factors[37,41,42]. The presence of one or more of these amblyogenic factors alters visual cortex development and, in the case of monocular amblyopia, leads to suppression of inputs from the amblyopic eye. Individuals with amblyopia experience a range of visual deficits including reduced contrast sensitivity, impaired high-contrast visual acuity, and an absence of stereoscopic depth perception [43–47]. In monocular amblyopia, vision deficits are also present in the non-amblyopic fellow eye [43]. Supra-threshold deficits such as reduced motion sensitivity [47,139], spatial distortions [140–142] and reduced fixational stability [143,144] also manifest in amblyopia (both eyes) to different degrees. . Amblyopia is the second leading cause of monocular visual impairment in adults with a prevalence of 1-3% [52,53]. Many studies that have assessed the social and psychological wellbeing of

individuals with amblyopia have observed a negative impact of amblyopia on quality of life [48–51].

The conventional treatment for amblyopia in children is occlusion therapy (patching, optical penalization and atropine penalization) [139,145] of the non-amblyopic eye combined with spectacle correction [146,147] and/or vision therapy [148] in both eyes. If over-administered, occlusion therapy may lead to the development of amblyopia in the previously non-affected eye [149]. In addition, occlusion therapy is often poorly tolerated by children, especially by children with poor visual acuity, increasing the rate of non-compliance and poor treatment effectiveness [150]. Regression of treatment effects is also common when occlusion therapy is terminated [149]. Other treatment approaches such as dichoptic training [151–157] and the use of pharmacological agents [158–161] have been reported to improve visual function in amblyopia. However, side effects with pharmacological agents as well as difficulties with adherence for dichoptic training represent a challenge to the effective treatment of amblyopia in children. Amblyopia treatments are available for children and teenagers, however, adults with amblyopia are typically left untreated due to the belief that the mature brain lacks the necessary plasticity to enable improvements in visual function [136].

Recovery from amblyopia requires the presence of neuroplasticity, a property of neural systems that decreases with age. The mature human visual cortex was previously assumed to lack neuroplasticity [136], however, recent reports have demonstrated that the mechanisms that modulate plasticity are retained throughout life [8,60,91,162]. This is supported by

studies in animals which demonstrated the presence and restoration of neuroplasticity in mature animals following monocular deprivation in infancy [163–165]. For instance, dark exposure for several days in mature animals induced significant visual cortex neuroplasticity as evidenced by an ocular dominance shift towards the previously deprived eye [163]. Additionally, it has been reported that the recovery of neuroplasticity in mature animals is associated with the balance of excitation and inhibition within visual cortex [166,167]. Van Versendaal et al.[167] showed that monocular deprivation caused an ocular dominance shift and a rapid loss of inhibitory synapses in pyramidal neurons within the primary visual cortex of kittens, thereby, shifting the bias of cortical responses to the nondeprived eye. Similarly, inhibitory synapse loss was associated with increased cortical responses to the deprived eye following binocular vision recovery and ocular dominance plasticity [167].

The evidence that neuroplasticity still exists in adulthood has opened up a new research area investigating different innovative treatment modalities for amblyopia. Once such modality is non-invasive brain stimulation. NIBS can be delivered to a targeted area of the human cortex and can modulate neural excitation and inhibition [7,88]. Particular types of NIBS alter the homeostatic balance of specific neurotransmitters such as Gamma aminobutyric acid (GABA) [168,169] and increase brain-derived neurotrophic factor (BDNF) secretion [18,170,171], enhancing neuroplasticity in the human adult brain. Therefore, NIBS may be clinically beneficial and is being studied as a potential treatment modality for cortical-based conditions such as chronic pain [172,173], tinnitus [97,174], stroke [31,175–180] and depression [32,100,101,181–186].

This review focusses on NIBS experimental studies in subjects with amblyopia. We discussed the significance of NIBS as a potential non-invasive treatment regimen aimed at improving primary visual functions and restoring neuroplasticity within the visual cortex of individuals with amblyopia. We also reviewed the effects of single and repeated sessions of visual cortex NIBS on visual cortex function and visual perception in amblyopia. Twelve studies published between 2008 and 2020 demonstrated that NIBS has the potential to be used as a new treatment regimen for adults with amblyopia **Table 1.**

Table 1:Summary of non-invasive brain stimulation studies in animal and human adults with amblyopia.

	Study	Method	No. of subjects	Age range/Mean	Etiology	NIBS Type/Session	Stimulation parameters	Main visual function outcome	Secondary test outcomes
	Animal								
1	Castaño-Castaño et al.,2017	Visual cortex anodal tDCS contralateral to the amblyopic eye in Long Evans Rats	32	60 days	monocular deprivation for 48 days	tDCS/repeated for 8 days	2mA;20 mins/day	-Decreased VA after MD -Increased monocular and binocular VA after tDCS	
2	Castaño-Castaño et al.,2019a	Visual cortex anodal tDCS contralateral to the amblyopic eye in Long Evans Rats	36	60 days	monocular deprivation for 48 days	tDCS/repeated for 8 days	2mA;20 mins/day	-Decreased VA after MD -Increased monocular VA after tDCS	-Decreased parvalbumin (PV)-positive cells after MD -PV positive cells increased after tDCS

3	Castaño-Castaño et al.,2019b	Visual cortex anodal tDCS contralateral to the amblyopic eye in Long Evans Rats	48	60 days	monocular deprivation for 48 days	tDCS/repeated for 8 days	2mA;20 mins/day	Increased stereoacuity	-Decreased Glucose (18F-FDG) uptake after MD -Glucose metabolism increased after tDCS
	Human								
4	Thompson et al.,2008	Visual cortex rTMS	9	20-45 yrs	3 strab and 6 mixed	rTMS/single	1 Hz (600 pulses, 10mins); 10 Hz (900 pulses) 100% phosphene threshold for 1Hz, 100% motor threshold for 10Hz	Increased high CS after 10 Hz rTMS in amblyopic eye	
5	Spiegel et al.,2013	Tetris dichoptic training combined with visual cortex anodal tDCS	16	18-31/22.07 yrs	3 strab, 9 aniso and 1 mixed. 5 subjects had previous patching therapy. 11 subjects had no previous patching therapy	-tDCS/single -Dichoptic training (5 sessions active and 5 sessions sham)	2mA, 15mins	-Greater stereoacuity increase with combined tDCS and dichoptic training No effect of tDCS on visual acuity improvements	
6	Clavagnier, 2013	Visual cortex cTBS	5	18-60 yrs	2 strab; 1 aniso; 2 mixed. 3 subjects had previous patching.	cTBS/repeated for 5 days	Three bursts of 50 Hz in 200ms (600 pulses; 40s) Theta burst stimulation was	Long-lasting and sustained high CS improvement after cTBS.	

					2 subjects had no previous patching		delivered at 41% MSO intensity.		
7	Spiegel et al.,2013	Visual cortex anodal or cathodal tDCS	13	19-69 yrs	10 strab and 3 aniso	tDCS/single	2mA, 15mins	-Increased high CS after anodal-tDCS in amblyopic eye. - Decreased high CS in fellow eye after cathodal-tDCS	BOLD level signal showed balancing of the cortical response to the amblyopic and non-amblyopic eyes.
8	Campana 2014 et al.,2014 (Pilot study)	Short perceptual training combined with hf-tRNS	7	26-52/39.20 yrs	7 aniso	100-640Hz tRNS/single. -Perceptual training (8 sessions)	1.5mA; 25mins	Increased VA and CS following perceptual training and hf-tRNS in trained and untrained eyes.	
9	Moret et al.,2018	Short perceptual training combined with high frequency-tRNS	20	27-58/44 yrs	Not specified	100-640Hz tRNS/single. -Perceptual training (8 sessions)	1.5mA; 25mins	-Increased in low and high CS in trained and untrained eyes for both hf-tRNS groups. -Increased VA in hf-tRNS group in trained and untrained eyes.	
10	Bocci et al.,2018	Visual cortex cathodal tDCS contralateral to the amblyopic eye	24	24-44/26.1 yrs	12 amblyopia (2 patching;7 aniso; 4 strab; 1 mixed). 12 healthy subjects	tDCS/single	1.5mA; 20mins	VA improved following cathodal-tDCS over occipital hemisphere contralateral to the amblyopic eye	-VEP amplitudes reduced in contralateral hemisphere.

11	Tuna et al.,2020 (Pilot study)	Visual cortex cTBS	13	19-24 yrs	6 strab,5 aniso, 2 mixed and	cTBS/single	Three bursts of 50 Hz (600 pulses; 40s)	Increased VA, stereoacuity and reduced suppressive imbalance after cTBS treatment.	
12.	Donkor et al., 2021	Visual cortex tRNS	16	19-59/44.2 yrs	2 strab, 10 aniso and 4 mixed	0.1-640Hz tRNS/repeated sessions for 5 days	2mA; 25mins	-Acute improvement in CS and uncrowded VA in amblyopic and fellow eye after active tRNS group. -Crowded VA improved in only fellow but not amblyopic eye after active tRNS -Successive and cumulative tRNS effects were not found after repeated tRNS sessions.	

MD = monocular deprivation; VA = visual acuity; CS = contrast sensitivity; strab = strabismus; aniso = anisometropia; MSO = maximum stimulator output.

2.2 Types of NIBS used in amblyopia studies

2.2.1 Transcranial magnetic stimulation (TMS)

TMS (**Figure 1**) uses an insulated coil of wire placed over the scalp to produce a transitory magnetic field which induces an electric current within the underlying cortex. TMS devices include a stimulating coil, a capacitor, a resistor and a thyristor switch. These components form a RIC (resistor, inductor and capacitor) circuit capable of producing an oscillatory sinusoidal (biphasic) current pulse. The stimulating coil also contains a diode which reduces coil heating and power utilization. TMS requires a high-voltage (400V– 3kV) and high current (4kA-20kA) pulse generator to induce a brief, strong magnetic field perpendicular to the stimulating coil [187].

When the TMS coil is positioned at a targeted area on the scalp, the induced magnetic field is attenuated by the external tissues of the head before reaching the neuronal cells. The diminished magnetic field is capable of inducing an electric current that is sufficient to depolarize the superficial axons and stimulate neuronal systems within the cortex [188].

When applied to the primary motor cortex, single pulses of TMS can cause muscle twitches, recorded as motor evoked potentials, that are a result of neural depolarization. The mechanisms underlying the effects of TMS on the motor cortex have been explored in detail [189,190]. TMS effects are most pronounced for neurons with axons that are parallel to the magnetic field. Non-parallel axons are less likely to be depolarized by TMS pulses. Thus, TMS favourably stimulates cortical neurons that are positioned horizontal to the brain surface, thereby, when TMS is applied to the primary motor cortex, pyramidal cells and axons that connect to the corticospinal tract are most likely to depolarize [12]. For a homogeneous system, the direction of induced electrical current and the plane of the TMS coil are parallel, and the distribution of current is easy to predict. However, the human brain is heterogeneous, and therefore, there is a distortion of the distribution of induced electrical current within the intracranial tissue. This means that the prediction of the distribution of the electrical current requires extensive modelling with internal and external factors considered. For example, Roth et al.[191] used numerical computation to show that the magnitude of the electric field generated by TMS is a function of the position, shape and orientation of the

magnetic coil. Barker et al.[3] recognized that the depth of cortical neurons or peripheral nerve cells also determines the probability that the cells will be depolarized by TMS.

Figure 1: Transcranial magnetic stimulation is being demonstrated by researchers from the University of Waterloo.

Transcranial magnetic stimulation can be applied to both the central nervous system and peripheral nerves. Baker et al.[3] were the first to introduce the application of painless non-invasive TMS to the human motor cortex, and showed that a single TMS pulse induced movement in muscles that received impulses from the corticomotor neurons under the site of

stimulation. They then suggested that TMS function and efficacy should be tested in neurological conditions. Since then the use of TMS has evolved to include the evaluation of inhibitory or excitatory neuronal populations [190,192], the study of neuroplasticity mechanisms [190,193,194] and the exploration of treatments options for a wide range of neurological conditions [30,179,195–197]. TMS is also used to study the relationship between specific brain areas, behavioral performance and perception [11,24,198].

2.2.1.1 Repetitive Transcranial magnetic stimulation (rTMS)

rTMS refers to the application of recurring TMS pulses to a targeted brain area. Rapidly repeated TMS pulses induce changes in cortical excitability, possibly by changing the strength of synaptic connections in a manner similar to long-term depression (LTD) or long-term potentiation (LTP) [96,192]. The direction of the change in cortical excitability induced by rTMS depends on the frequency, intensity of stimulation and coil orientation. Rossi et al.[199] and Machii et al.[36] reported stimulation frequency ranges that are safe for the human brain, and further established that low frequencies (below 1-5 Hz) induced inhibitory (LTD) whereas higher frequencies (5 Hz and higher) induced excitatory (LTP) effects when applied to the motor cortex [199]. In addition to the motor cortex, the efficacy of rTMS has been extensively studied in a number of human brain areas especially in the parietal [200–202], visual [10,203,204] and extrastriate [205–207] cortices. There is initial evidence suggesting that rTMS can be used as a potential treatment option for neurological conditions (stroke-rehabilitation, migraine, Alzheimer's disease, multiple sclerosis, traumatic brain injury and schizophrenia) [32,96,105,106,179–181,187,208,209] and psychiatric disorders

(obsessive-compulsive disorder, panic disorder and post-traumatic stress disorder) [188] [see Lefaucheur 2014 for a detailed review].

rTMS applied over primary visual cortex of adults with amblyopia has been shown to induce improvements in visual function, providing evidence for a restoration of neuroplasticity. Thompson et al.[10] were the first to investigate the effects of single sessions of visual cortex rTMS in adult humans with amblyopia. Low-frequency 1 Hz stimulation was performed on 9 subjects with amblyopia and 6 out of the 9 subjects with amblyopia were also tested with higher-frequency 10 Hz stimulation. Contrast sensitivity for low-spatial-frequency and high-spatial-frequency gratings was measured directly before, directly after, and 30 min after occipital rTMS. The effects of 10 Hz visual cortex rTMS on contrast sensitivity was also tested for a control group of 5 subjects with normal vision. For high and low spatial frequency viewing conditions, the study demonstrated that 1 Hz visual-cortex stimulation had no effect on contrast sensitivity for the amblyopic grouped data. However, considering only responders to rTMS treatment (7 out of the 9 subjects), there were significant effects of rTMS at post 30mins for high spatial frequency contrast sensitivity. All amblyopic subjects who received 10 Hz stimulation showed a significant improvement in high spatial frequency contrast sensitivity of the dominant eye immediately and 30mins after stimulation. Additionally, control data showed that 1Hz motor cortex rTMS had no reliable effects on contrast sensitivity for the subjects with amblyopia. In subjects with normal vision, 10 Hz rTMS over the visual cortex induced a significant improvement in contrast sensitivity immediately after stimulation. Comparing the relative effects induced by 1 Hz and 10 Hz

visual cortex rTMS in adults with amblyopia, 10 Hz showed enhanced contrast detection for high spatial frequencies for the group as a whole whereas 1 Hz showed improved contrast detection only for a subset of participants. This suggested that 10Hz may be effective stimulation protocol. In agreement with motor cortex rTMS studies [32,199], visual cortex rTMS has the possibility of inducing neuroplasticity. There are a few limitations that make it difficult to extrapolate the results from this study: first, the sample size was small which may have impeded reliable results from the group data due to insufficient statistical power; and second, the sample size was too small to assess whether the rTMS effect varied across amblyopia subtypes. Other limitations that the authors recognized were that other types of visual functions as well as multiple sessions of rTMS were not assessed.

2.2.1.2 Theta burst stimulation (TBS)

More recently, another rTMS technique called theta burst stimulation (TBS), has been introduced [210,211]. TBS uses a lower stimulation intensity and a shorter duration of stimulation which have be shown to produce robust and long-lasting effects on cortical excitability. However, the main advantage of TBS is the speed of application, whereby TBS takes a maximum of 2-3 mins while 1 Hz rTMS takes 20-30mins. Aftereffects of both standard rTMS and TBS on cortical neurons have been demonstrated using electrophysiology [212–214] and functional magnetic resonance imaging (fMRI) studies [215–218]. TBS stimulation protocols are based on in vitro studies of hippocampus cells indicating that bursts of rapid electrical stimulation repeated at 5-10 Hz can induce long-term potentiation (LTP) and promote neuroplasticity [219]. TBS be can be delivered as continuous (cTBS) or intermittent (iTBS) pulse sequences. cTBS and iTBS have opposite effects on cortical

excitability when applied to the motor cortex. cTBS induces cortical inhibition whereas iTBS induces excitation. Specifically, in the first demonstration of cTBS, Huang et al.[210] reported decreased MEP amplitude (inhibition) after motor cortex cTBS and increased MEP amplitude (facilitation) after motor cortex iTBS. The effects lasted for more than 20 mins. They also reported that the duration of the stimulation aftereffects was affected by the number of TMS pulses applied. Their study showed that 40 sec of cTBS with a total of 600 TMS pulses (i.e., 40 s cTBS) decreased MEPs for 60 min, whereas 300 TMS pulses decreased MEPs for only 20 min. Subsequent studies have reported enhanced motor learning in healthy subjects following motor cortex TBS [211,220,221].

With regard to amblyopia, Clavagnier et al.[20] applied five repeated daily sessions of cTBS over the primary visual cortex, and assessed both acute and long-lasting effects on contrast sensitivity in adults with amblyopia. Subjects viewed high contrast stimuli with the non-amblyopic eye while visual cortex cTBS was delivered. The rationale for this was that neurons responding preferentially to the fellow eye would be active during stimulation and therefore be more affected by the inhibitory effects of cTBS. Preferential inhibition of neurons responding to the fellow eye would, in turn, reduce suppression of the neurons driven by the amblyopic eye. Contrast sensitivity for a range of spatial frequencies (0.5, 1, 2, 5 and 10 cpd) was measured before, directly after, and 30 min after cTBS. The cTBS protocol consisted of 600 pulses per session which were delivered as bursts of three 50 Hz pulses for 200 ms over a 40 s stimulation duration. Both single and repeated sessions of cTBS were delivered to 5 and 4 subjects respectively, whereas 3 out of the 5 subjects

participated in follow-up visits after the last stimulation session. Significant improvements from baseline in amblyopic eye contrast sensitivity were observed for high spatial frequencies on days 1-3 and there was a cumulative improvement in baseline contrast sensitivity from one day to the next. The cTBS-induced improvements on contrast sensitivity were sustained 8, 19 and 78 days after the final stimulation session in three participants who were available for follow-up testing.

The major limitation of this study is the small sample size and lack of assessment of cTBS effects on different visual functions, however, the data demonstrate that the use of cTBS as a treatment regimen in adults with amblyopia is potentially promising. In agreement with previous studies [10], rTMS and cTBS enhanced high spatial frequency contrast sensitivity in the amblyopic eye of adults with amblyopia. Thus, cTBS results replicated the single session 1 Hz rTMS results from Thompson et al.[10].

Recently, Tuna et at.[222] explored the effects of cTBS on visual acuity, suppressive imbalance (related to interocular suppression), and stereoacuity in adults with amblyopia. Their cTBS protocol involved 600 pulses continuously delivered in bursts of 3 pulses at 50 Hz and repeated at 5-Hz for 40 sec. A single session of cTBS was applied at a location on the occipital cortex where phosphenes were induced in 13 subjects with amblyopia (8 subjects received active cTBS treatment whilst 5 received sham cTBS treatment). The results showed reliable improvements in visual acuity, stereoacuity and suppressive imbalance in the active cTBS treatment group relative to the sham group although there was considerable inter-

individual variability. This study provides further evidence that neuroplasticity can be restored in the visual cortex of adults with amblyopia following application of cTBS.

Possible mechanisms for improved vision in amblyopia following visual cortex rTMS or cTBS include a balancing of the cortical response to input from each eye. Under normal circumstances, the visual cortex response to stimulation of the fellow eye is much larger than the response to stimulation of the amblyopic eye [21,223–227]. Because the effects of rTMS and cTBS are state- dependent [228,229], they may act to preferentially increase excitability of the less active amblyopic eye dedicated cells or reduce excitability of the fellow eye dedicated cells therefore allowing a more balanced cortical responses to input from each eye. In addition, rTMS and cTBS may alter cortical inhibition which is key factor in the regulation of visual cortex neuroplasticity [23,230,231].

2.2.2 Transcranial electrical stimulation (TES)

Brain alterations in adult humans induced by small polarizing currents were described in 1964 by Lippold and Redfearn [232,233]. Subsequent studies showed long-lasting therapeutic effects of brain polarization in neurological and psychiatric disorders [233] such as depression [234,235]. For example, Costain et al.[236] reported that brain stimulation using a weak direct current (100-250μA) improved the symptoms of depression. In earlier animal work, Bindman et al.[237] reported excitatory after-effects of surface-positive cortical polarization in rats. Later, the effects of electrical stimulation applied to the locus coeruleus (LC) were assessed within the parietal cortex *in vitro* by Semenyutin et al.[238]. The results

showed that the stimulation caused neural hyperpolarization and an inhibition of background activity.

Following this earlier work, the application of tES to the human brain was revisited by Nitsche and Paulus (2000) who applied a weak electrical current to the scalp through a pair of surface electrodes (**Figure 2)**. The electric current generated an electric field that caused modification of membrane polarization resulting in cerebral excitation or inhibition depending on the current polarity (anodal vs. cathodal stimulation). They showed that the aftereffects of tES lasted longer than the stimulation duration by varying the current intensity and duration [88]. The changes in cortical excitability were explained in the context of LTP and LTD-like mechanisms that altered synaptic strength [239]. Further investigations have reported other underlying mechanisms of tES effects such as alterations in the concentration of the inhibitory neurotransmitter GABA [15,168,169], BDNF secretion, Tropomyosin receptor kinase B (TrkB) activation within the cortex [18,170] and changes in regional neuronal activity [240,241].

tES can be delivered as transcranial direct current stimulation (tDCS), transcranial random noise stimulation (tRNS) or transcranial alternating current stimulation (tACS) to the human cortex.

Figure 2: tES apparatus. Two surface electrodes (red: active electrode; blue: reference electrode) placed on the scalp with the reference electrode at CZ and the active electrode at either midline (left panel) or lateral occipital cortex (right panel).

2.2.2.1 Transcranial direct current stimulation (tDCS)

tDCS is a non-invasive brain stimulation technique that applies a mild (1-2 mA) [7,88] direct electrical current through the scalp to modulate neuronal excitability. Antal et al., 2001;2004 demonstrated anodal (a-tDCS) and cathodal (c-tDCS) direct stimulation over the motor cortex enhanced or reduced cerebral excitability in healthy humans [7,9]. The effects of tDCS are potentially promising for neuroplasticity. tDCS studies have reported improvements in patients suffering from neurological-based disorders including stroke [176–

179], chronic pain [172] and tinnitus [174], and tDCS can be considered as a tool for activating the processes of cortical reorganization and functional neurorehabilitation [60,185,208].

Long-lasting effects of tDCS can be achieved when tDCS is combined with peripheral stimulation methods. Hesse, 2007 and Nair, 2011 showed that the combination of motor cortex a-tDCS and peripheral nerve stimulation increased synaptic plasticity and potentiated the relearning of motor skills [242,243]. a-tDCS has also been combined with perceptual learning and virtual reality therapy [244] to assess whether the stimulation can augment other treatment approaches. The mechanism of enhanced synaptic plasticity following augmented treatments may be due to the fact that a-tDCS effects enhance brain excitability whereas peripheral therapy alters LTP/LTD trans-synaptic strength [245].

There is a growing body of evidence indicating that visual cortex neuroplasticity can be restored by reducing cortical inhibition [10,23,61,125,151]. Spiegel et al.[21] investigated whether a single session of visual cortex a-tDCS (2mA; 15minutes) would reduce inhibition, facilitate cortical excitability and enhance contrast sensitivity in adult patients with amblyopia. To determine the effects of a-tDCS on cortical responses to visual inputs from amblyopic and fellow eyes, fMRI measures of the BOLD signal level were compared between a-tDCS and s-tDCS for each visual area (V1, V2, V3, V3a, and V4). Contrast sensitivity was assessed before, during, immediately after and 30 mins after a-tDCS or c-tDCS in 13 adult subjects with amblyopia, and 5 out of the 13 subjects who responded to a-

tDCS treatment participated in the fMRI investigation. Considering the whole data set, the study reported no reliable improvement in contrast sensitivity in the amblyopic eye following either a-tDCS or c-tDCS. However, c-tDCS significantly improved contrast sensitivity in the fellow eye immediately after stimulation. A cluster of "responders" (N = 8) showed a significant improvement in amblyopic eye contrast sensitivity immediately after and 30 minutes post a-tDCS. The fact that the effects of visual cortex tDCS on visual function were not observed in all subjects is in agreement with other studies that reported inter-individual variability of tDCS effects in the human brain [246–248]. A larger sample size and clustering of subjects into amblyopia subtypes may have enabled the observation of greater tDCS effects.

fMRI measures were obtained from a subset of responders to assess whether tDCS induced any measurable cortical changes that could explain the contrast sensitivity improvements. When sham (placebo) tDCS was applied to the visual cortex, fMRI measurements showed asymmetrical activation within visual cortex areas whereby significantly higher activation was observed for visual stimulation of the fellow eye. This bias in visual cortex activity towards the fellow eye was significantly reduced by visual cortex a-tDCS. Balancing of the cortical response to each eye, possibly due to reduced cortical inhibition, may underlie the amblyopic eye vision improvements that can be induced by a-tDCS.

Building on the study conducted by Spiegel et al.[21], Ding et al.[22] explored the effect of visual cortex a-tDCS in older teens and adults with amblyopia using monocular pattern

reversal visual evoked potentials (VEPs) and psychophysical measures of contrast sensitivity. An amblyopia group (n = 21) and a control group (n = 27) completed six study sessions, three involving VEP measures and three involving psychophysical measures. For each measure, participants completed one real a-tDCS session (2mA; 20minutes), one cathodal tDCS condition and one sham tDCS condition. VEP recordings and contrast sensitivity measurements were made for amblyopic and fellow eyes, but only one randomly selected eye for the control subjects. They reported an increased VEP amplitude for amblyopic, non-amblyopic and control eyes directly after and 30 minutes post a-tDCS. As expected, c-tDCS reduced the VEP amplitudes and sham stimulation had no effect. Anodal and cathodal tDCS effects did not affect peak latencies. For contrast sensitivity, there was a reliable and transient contrast sensitivity improvement following a-tDCS whereas c-tDCS had the opposite effect. Further VEP baseline measurements were carried to investigate long-lasting a-tDCS effects, and the results showed a reliable increase in VEP amplitudes 48 hours after the last a-tDCS session. Sham stimulation had no effect for any condition.

Together, the results from Spiegel et al. [21] and Ding et al.[22] demonstrate that a single session of a-tDCS can transiently enhance contrast sensitivity in the amblyopic eyes of adult patients. The effects of a-tDCS on cortical activation also suggest that that the underlying mechanism of a-tDCS effects involves a reduction of cortical inhibition within the visual cortex. The cumulative effects of tDCS over several days, if any, should further be investigated using multiple sessions of visual cortex tDCS.

Recent reports on amblyopia treatment have suggested that enhancement of visual functions in adults with amblyopia can be achieved by reducing suppression of visual inputs from the amblyopic eye using dichoptic video games [151,154,157,249,250]. Spiegel et al.[89] investigated whether visual cortex a-tDCS (2mA; 15minutes) could augment the effects dichoptic video play game on stereopsis and amblyopic eye visual acuity in adults with amblyopia. Within the study, sixteen adults with amblyopia (5 out of 16 subjects had previous patching treatment) participated in a crossover-design intervention. The participants were split into two equal groups. A-tDCS of the visual cortex was delivered to group 1 during the first 5 dichoptic training sessions whereas s-tDCS of the motor cortex was given to the same group during the second 5 dichoptic training sessions. The order of tDCS conditions was reversed for group 2. The study reported greater and long-lasting improvements in stereopsis in both groups when a-tDCS was combined with dichoptic treatment relative to dichoptic treatment administered alone. However, although long-lasting improvements in amblyopic eye visual acuity were observed in both groups, there was no additional effect of a-tDCS. This suggests that the augmenting effect of a-tDCS on dichoptic treatment may be specific for binocular visual functions. The dichoptic treatment was designed to reduce suppression of the amblyopic eye by exposing the visual system to stimuli that could be binocularly combined [151,155], whereas a-tDCS was hypothesized to reduce GABA mediated inhibition, thereby reducing suppression of the amblyopic eye [15,21,22,168]. The study results suggested that these two approaches are additive, thereby demonstrating a promising, preliminary treatment regime for adults with amblyopia.

Neural interactions within visuo-cortical areas in the two hemispheres are facilitated by fibers that pass through the corpus callosum [251,252]. In animal studies, it has been reported that callosal connections are responsible for suppression of the amblyopic eye following early monocular deprivation [253,254]. Therefore, dampening the effects of callosal interhemispheric inhibition may decrease the level of interocular suppression and potentially enhance cortical responses and improve visual function in amblyopia. In agreement with this hypothesis, there is initial evidence that the corpus callosum may modulate both inhibition and excitation of the contralateral hemisphere [251,255,256]. Bocci et al.[90] investigated the effects of visual cortex c-tDCS (1.5mA; 20mins) on interhemispheric cortical responses and visual function changes in adults with amblyopia. Cathodal tDCS was applied over the occipital cortex contralateral to the amblyopic eye, and visual acuity and VEP amplitudes (induced by 90% and 20% contrast grating stimuli) were measured. The results showed a reliable improvement in amblyopic eye visual acuity following c-tDCS, and the aftereffects lasted for about 1 hour. Sham stimulation had no effect. Therefore, the results indeed demonstrated that the cortical inhibitory effects induced by c-TDCS have the potential to be used as a treatment for amblyopia in human adults.

Regarding the VEP results reported by Bocci et al., at both low and high-contrasts, VEP amplitudes increased post c-tDCS for the occipital hemisphere ipsilateral to the amblyopic eye relative to control subjects with normal vision. The effects lasted for up to 1 hour. In contrast, a reduction of VEP amplitude was observed immediately after c-tDCS, for high and low-contrasts, at the occipital hemisphere contralateral to the amblyopic eye (the hemisphere

that received c-tDCS). The authors propose that this pattern of VEP amplitude change indicates a reduction of interhemispheric inhibitory effects within the visual cortex.

In agreement with studies in humans, recent animal studies [62,64,132] showed that tDCS has the potential to enhance visual functions in adult amblyopia. Castaño-Castaño et al.[62,64,132] demonstrated that repeated sessions of visual cortex a-tDCS reversed the effects of early monocular deprivation on visual acuity and depth perception in Long-Evans rats. Across three separate studies, the animals experienced monocular deprivation for 48 days following monocular occlusion (eyelid suture, and egg white was applied as tissue glue) on postnatal day 12. Prior to tDCS stimulation, animals were allowed to recover for 1 month after eyelid opening on day 60. The monocular deprivation induced dense monocular deprivation amblyopia associated with impaired monocular and binocular visual acuity along with absent stereoscopic depth perception. tDCS (2mA) was delivered to the visual cortex contralateral to the amblyopic eye for 20 mins per day for 8 consecutive days. Amblyopic animals that received repeated sessions of anodal tDCS experienced restoration of plasticity within the visual cortex relative to control animals.

Prior to a-tDCS, two of the studies reported decreased parvalbumin (PV)-positive cells and Glucose (18F-FDG) uptake within the visual cortex following monocular deprivation respectively [64,132]. Parvalbumin cells are calcium-binding proteins present in some GABAergic interneurons in the cortex. Functionally, parvalbumin is involved in a variety of physiological processes including cell-cycle regulation, second messenger production,

muscle contraction, microtubule organization and vision [257]. Therefore, an increase in PV-positive cells may contribute to functional organization and restoration of neuroplasticity within the visual cortex. Indeed, the effects of repeated sessions of tDCS resulted in an increased and balanced PV-positive cell level within the visual cortex areas. Similarly, following repeated a-tDCS there was balanced cortical activation within the visual cortex areas. Previous studies have also used 18F-FDG to evaluate cortical activations in the brain especially in neurological diseases [258,259] including amblyopia [223,224,226].

Overall, the results of the rat studies conducted by Castaño-Castaño et al. indicate that repeated sessions of daily tDCS over the primary visual cortex induce a period of neuroplasticity that enables a restoration of ocular dominance balance in amblyopic animals. However, the authors observed the following study limitations: (i) visual function progression following each day's tDCS treatment was not tested, and (ii) sustained long-term tDCS aftereffects were not assessed.

2.2.2.2 Transcranial random noise stimulation (tRNS)

tRNS modulates cortical excitability by interfering with the continuing neural oscillations in the brain through the delivery of an electrical current with a randomly oscillating frequency and amplitude [4,260]. tRNS induced greater and reliable effects when compared with tDCS and tACS [13,97,261] [See Paulus, 2011; for detail description of each stimulation method]. For instance, in healthy individuals, Inukai et al.[261] showed that motor cortex tRNS induced the largest significant increase in MEPs relative to sham stimulation of the various stimulation types tested. Similarly, Vanneste et al.[97] reported a larger transient suppressive

effect on the tinnitus loudness and tinnitus related distress when tRNS was applied over the auditory cortex of tinnitus patients compared to other stimulation protocols.

Within tRNS protocols, the spectrum of different of frequencies can either cause a reduction or enhancement of motor cortex excitability [4,262]. Thus, effect of low- frequency tRNS (lf-tRNS; 0.1–100 Hz) induced no or reduced cortical excitability whilst high-frequency tRNS (hf-tRNS; 100–640 Hz) induced an opposite effect [4,108,263]. However, Fertonani et al.[91] reported that the effects of visual cortex tRNS on visual perceptual learning enhancement showed no statistically significant difference between the effect of high frequency (101-640 Hz) and low frequency (0.1-100 Hz) tRNS.

Following the previous work of Spiegel et al.[89] showing that combined perceptual learning and a-tDCS treatment enhanced visual function in adults with amblyopia, Moret et al.[108] adopted the same principle and combined high-frequency transcranial random noise stimulation (hf-tRNS) with a short perceptual training, and reported an improvement in visual function in adults with amblyopia. They administered visual cortex hf-tRNS (1.5mA; 25mins) during 8 perceptual training sessions in 10 adult subjects with amblyopia, and compared the results to a sham stimulation group. Both groups received 8 sessions of monocular perceptual training using the amblyopic eye during which subjects performed a contrast detection task by detecting the presence of a central Gabor patch flanked by two high-contrast collinear Gabor patches. Hf-tRNS was delivered within the first 5 blocks of each training session. Contrast sensitivity (measured with different spatial frequencies; 0.8,

2.9, 5.8, 9.7 and 14.5 cpd) and visual acuity were assessed before and after monocular perceptual training with or without visual cortex hf-tRNS. The study reported, for all the spatial frequencies tested, that perceptual training alone enhanced contrast sensitivity in the amblyopic/trained eye and non-amblyopic/untrained eye for both the active and sham tRNS groups. The effect of perceptual learning on contrast sensitivity was not reliably improved by hf-tRNS, however, the magnitude of improvement in both the trained and untrained eyes was larger in the hf-tRNS group. Similarly, for both trained and untrained eyes, there was a significant enhancement of visual acuity in the hf-tRNS group only. The results of this study are consistent with the authors' previous pilot study [264] in which visual acuity and contrast sensitivity improved following visual cortex hf-tRNS (1.5mA, 25mins) combined with short perceptual training in adults with amblyopia (N = 7). It remains to be seen whether the effects of combined perceptual learning and NIBS are long lasting in adults with amblyopia and whether the effects can be replicated in larger samples within randomized clinical trial designs.

To investigate acute and long-lasting effects of tRNS, we [see experiment 3] tested whether five daily sessions of visual cortex tRNS (2mA; 25 minutes) would improve contrast sensitivity, crowded and uncrowded visual acuity in adults with amblyopia. the effects of active or sham tRNS of the visual cortex was assessed in 16 adults with amblyopia (active, n = 8; sham, n = 8). Visual function outcomes were measured before, during, 5 minutes and 30 minutes post stimulation on each day, and baselines were again measured on day 28 after the last day of stimulation. The results showed that repeated sessions of visual cortex tRNS

induced an acute improvement of visual functions in adult patients with amblyopia. Specifically, tRNS improved contrast sensitivity and uncrowded visual acuity relative to the sham group for both amblyopic and fellow eyes. tRNS improved crowded visual acuity in the fellow but not the amblyopic eyes. However, we did not find successive and cumulative tRNS effects on all visual function outcomes. Therefore, repeated sessions of tRNS using the same stimulation parameters for each day, may not induce long-lasting tRNS effects on visual function in amblyopia. It is possible that increasing the sample size and changing stimulation parameters for each day may elicit greater reliable long-lasting tRNS effects [See Chapter five for more details about experimental procedure].

2.3 General summary

All the recent animal studies discussed in this review investigated the effects of repeated sessions of visual cortex tDCS with the aim of restoring neuroplasticity in monocularly deprived amblyopic rats. Using the same stimulation protocol, visual cortex tDCS significantly improved visual acuity and depth perception. Similarly, tDCS application boosted physiological processes and enhanced symmetric cortical responses between occipital hemispheres within the visual cortex of the animals. In human studies, there was a reliable improvement in visual functions in adults with amblyopia following single or repeated sessions of visual cortex TMS. In particular, the effects of cTBS reliably improved visual acuity, suppressive imbalance, and stereoacuity. With regards to visual cortex tES, a-tDCS and/or c-tDCS induced enhancement of contrast sensitivity and visual acuity. Direct neurophysiological and electrophysiological measurements (fMRI and VEP) demonstrated that tDCS enhanced cortical responses to inputs from the amblyopic eye, and balanced

cortical activation in response to inputs from the amblyopic and fellow eyes. Lastly, both tDCS and tRNS have been found to enhance the effects of perceptual learning on visual function in adults with amblyopia. Whether repeated sessions of NIBS alone (i.e., in the absence of perceptual learning) can lead to cumulative effects remains uncertain. Initial evidence for cumulative effects of visual cortex cTBS were reported by Clavagnier et al.[20].

We observed some study limitations with the papers reviewed. Most studies had a small sample size, which may have reduced statistical power and masked any subtle effects of NIBS. Almost all studies did not perform a cluster analysis for subtypes of amblyopia among the selected cohort. Whether the effects of NIBS vary depending on amblyopia subtype is an important clinical question related to the potential use of NIBS for amblyopia treatment. Also, the effects of TMS and tES were mostly tested on one visual function for the majority of studies. Each visual function is processed differently within the visual cortex and, therefore, testing other functions may have revealed greater or more reliable effects. Lack of repeated sessions and assessment of long-lasting effects of NIBS were observed in most of the human studies. Lastly, none of the studies were conducted using randomized control trial (RCT) methodology to reduce various forms of bias, and to provide a rigorous measure of the effectiveness of NIBS for the treatment of amblyopia in human adults**.**

2.4 Conclusion and outstanding research questions

Transcranial magnetic and electrical stimulation studies have demonstrated remarkable improvements in visual functions and restored neuroplasticity in adult humans and animals with amblyopia. Both single and repeated sessions of TMS or tES can be used to induce

neuroplastic effects within the visual cortex of amblyopic adults. Finally, tES augmentation with perceptual learning can be a potential treatment option for adults with amblyopia. Many areas recognized as weaknesses in previous studies need to be explored regarding the effects of visual cortex TMS and tES in healthy adults with or without amblyopia. The most effective form of tES for inducing visual cortex neuroplasticity requires further investigation. In addition, direct objective assessment of the effects of visual cortex tES in humans needs further investigation. This is because the majority of studies focussing on tES mechanisms focus exclusively on the motor cortex and it is unknown whether the results of these studies also apply to the visual cortex. A deeper understanding of tES mechanisms will enable the development of more effective tES protocols for amblyopia treatment. Additionally, cumulative and long-lasting effects of visual cortex tES in humans should be investigated. This issue is critical for the clinical use of tES for amblyopia treatment. The experiments reported in this thesis were designed to address these questions. Specifically, a pilot study was conducted to investigate the application of visual cortex tRNS in healthy adults and assess its effects on contrast sensitivity as compared with a-tDCS and sham stimulation. A larger experiment was then conducted to objectively assess the effect of tRNS on visual cortex excitability using EEG in participants with normal vision. Finally, an experiment was conducted to investigate the effects of repeated visual cortex tRNS, over several days, on contrast sensitivity and visual acuity in adults with amblyopia.

Chapter 3: Identification of the effective transcranial electrical stimulation technique for modulating contrast sensitivity in humans: a pilot study

3.1 Introduction

The three tES methods (tDCS, tRNS and tACS) have the capability to induce cortical excitability and enhance neuroplasticity in the human brain [4,13,208]. tDCS induces cortical excitability through membrane polarization, whereby anodal-tDCS depolarizes whereas cathodal-tDCS hyperpolarizes the resting membrane potential. tRNS and tACS induce cortical excitability through interference with ongoing brain oscillations. The after-effects induced by each tES method vary and maybe dependent on factors such as the stimulation parameters used and inter and intra-individual variability [88,247,248]. It is important to find the most reliable tES technique for inducing cortical plasticity before conducting treatment studies for individuals with neurological disorders. Currently, only a few studies have compared the efficacy of tES methods using the same stimulation parameters in the same cohort of subjects[97,261,262]. All the three studies reported that tRNS was the most effective tES method for modulating cortical function. For instance, Vanneste et al.[97] and Moliadze et al.[262] applied 1 mA of a-tDCS, full spectrum tRNS and tACS over primary motor cortex for 10 minutes. MEP amplitudes increased post stimulation showing an increase in cortical excitability. Among the stimulation methods, tRNS induced the greatest effect on MEPs, however, Moliadze et al. reported that a-tDCS reliably produced the longest MEP increase compared to sham stimulation. Different studies have applied different tES methods (non-comparative studies) over the primary visual cortex to modulate phosphene threshold and visual function in normally sighted individuals and to improve vision in brain based

vision disorders such as amblyopia[9,21,22,90,109,265–267]. However, at present, there are no studies comparing tES methods when applied over the visual cortex using the same stimulation parameters and visual function outcome measures.

The major aim of this pilot study was to identify the effective tES protocol (tDCS or tRNS) for modulating visual function (contrast sensitivity) in healthy adults while applying 2mA current to the visual cortex for 15 mins. We hypothesized that a single session of visual cortex tRNS would induce a larger improvement in contrast sensitivity that a-tDCS or sham. The secondary purpose of this pilot study was to assess the feasibility of the use of our stimulation apparatus on subjects, specifically, to test the tolerability of different tES techniques using tES apparatus that had recently been acquired by the laboratory. Lastly, this study aimed to assess the feasibility of measuring tES effects using contrast sensitivity for Landolt-C optotypes (a clinically-relevant outcome measure) as previous studies used Gabor patches that lack clinical acceptability [29,89,107,108]. We did not test tACS because the available stimulator did not have this functionality.

3.2 Materials and methods

3.2.1 Subjects

We used convenience sampling to recruit 18 healthy adults (26.7 ± 5.2yrs, 10 female) from graduate students in the School of Optometry and Vision Science, University of Waterloo. Each participant was randomized into one of three groups and underwent single session of visual cortex tES (anodal-tDCS, n = 6; tRNS, n = 6 and sham, n = 6). Participants had best corrected visual acuity of 6/7.5 or better in each eye and had no contraindication to tES

[33,261]. The purpose of the study and tES protocol were explained to all participants prior to administering treatment. Our tES protocol and procedures conformed to safety guidelines for tES application in humans [34]. Written informed consent was obtained from all participants.

3.2.2 Instrument and stimuli

The pilot study employed a Landolt-C contrast sensitivity test (LCST) presented within a set of computer-based, automated vision tests (AVT) developed by the Operational Health and Performance Research Division, Aeromedical Research Department, U.S. Air Force School of Aerospace Medicine and Wright Patterson Air Force Base, Dayton Ohio[268,269]. The configuration and calibration of the NEC MultiSync P232W monitor used to display the stimuli (21.3 inches, 1920x1080 resolution, non-stereo display) was conducted using a X-Rite i1 Display Pro colorimeter. The contrast of the Landolt-C (three stimuli: 166.67, 83.33, 12.25 and 6.25 Arc Mins) (**Figure 3**) was varied (40 trials each) according to an adaptive procedure[270].

3.2.3 Experimental Design

Participants sat in dark room at a 4-meter viewing distance from the monitor (**Figure 3**). Participants were instructed to fixate at the center of the screen when performing the task. The dominant eye was tested with the non-dominant eye occluded. The Landolt-C (gap in C oriented at four possible positions: left, right, top, or bottom) appeared on white/gray at the center of the screen during the contrast test and the participant's task was to identify the gap location using the keyboard arrows to respond following a four alternative forced choice procedure. Correct and wrong responses were followed by distinct sounds, and every 10^{th} trial was 50% bigger than previous trial in order to maintain attention. LCST tests were

administered before tES (tRNS, a-tDCS or sham), during tES, 5 min and 30 min after stimulation. Each LCST session took 7-9 mins to complete. All participants performed practice blocks to familiarize them with the stimuli and task. Each participant spent between 60-80 mins to complete each experiment including practice blocks.

3.2.4 Transcranial Random Noise Stimulation

Single session anodal-tDCS (2.0 mA, current density: 0.10 mA/cm^2) or tRNS (2.0 mA, current density: 0.10 mA/cm^2, frequency range 0.1-640 Hz) or placebo (sham) stimulation of the primary visual cortex for 15mins was delivered using a DC-STIMULATOR PLUS (Eldith, NeuroConn GmbH, Germany) through a pair of saline-soaked surface sponge electrodes (5 cm x 5 cm, 25 cm^2)[261]. For tDCS, the current was linearly ramped up over 30sec to 2 mA, maintained at 2 mA for 15 minutes, and then linearly ramped down to 0mA over 30sec[33]. For tRNS, the AC current was initially ramped up to a maximum of 2mA over 30 sec and ramped down to 0mA over 30 sec at the end of the stimulation session. During sham stimulation, the 30 sec ramp-up was immediately followed by the ramp-down out. For each stimulation protocol, the reference electrode (cathodal electrode for tDCS) was placed at Cz while the active electrode (anodal electrode for tDCS) was placed at Oz (~10% above the inion) (**Figure 3**).

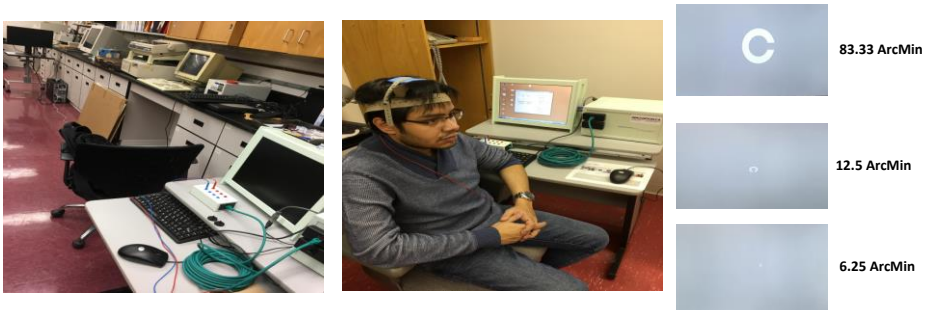


Figure 3:Set up of experiment showing distance of monitor and sitting chair for participants (left image). Monitor tES stimulation using DC-Stimulator-MC (middle image), and the 3 Landolt-C optotypes displayed on NEC MultiSync P232W monitor (right image).

3.2.5 Data analysis

Statistical analyses were performed using SPSS 19 (IBM Corp., Armonk, NY). Contrast sensitivity was recorded in log units. A mixed-effects analysis of variance (ANOVA) with a within-subjects factor of Time (pre, during, post 5 and post 30), between subject factor of Stimulation Type (a-tDCS, tRNS and Sham) and within subject factor of Stimulus Size (83.3 ArcMin, 12.5 ArcMin and 6.25 ArcMin) was conducted for the contrast sensitivity data. Pairwise comparisons were conducted to explore significant interaction effects. A significance threshold of $p < 0.05$ was adopted for all analyses.

3.3 Results

The mean age distribution for each group was 27.6 ± 4.11yrs with 4 females for anodal-tDCS, n = 6; 25.47 ± 4.10yrs with 3 females for tRNS, n = 6; and 26.26 ± 4.85yrs with 3 females for sham, n = 6. The total mean distributions of LCST (Log Threshold) for 83.3 ArcMin, 12.5 ArcMin and 6.25 ArcMin target sizes recorded 5 minutes before and 30 minutes after stimulation were -1.7399 ± -0.7082 and -1.7178 ± -0.6987, -1.0113 ± -0.6634 and -1.0848 ± -0.7175 and -0.5080 ± -0.4523 respectively. A repeated measures ANOVA conducted on the log contrast sensitivity thresholds showed no statistically significant interaction between Time (pre, during, post 5 and post 30), Stimulation Type (a-tDCS, tRNS and Sham) and Stimulus Size (83.3 ArcMin, 12.5 ArcMin and 6.25 ArcMin) (**Figure 4 & 5**).

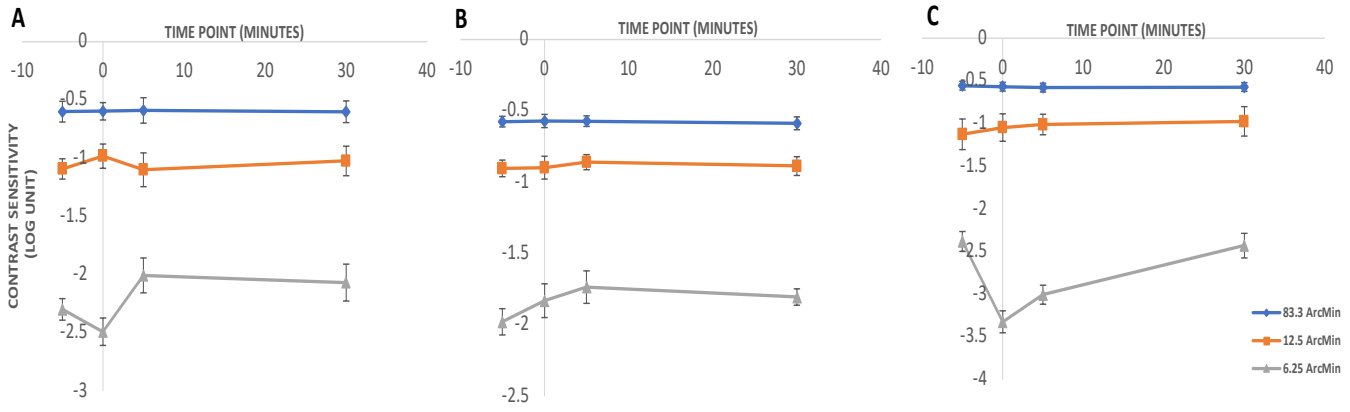


Figure 4: Contrast sensitivity (Log threshold) measured at baseline (-5 mins), during (0 min), immediately after (5mins) and 30mins after stimulation. Small and medium stimuli showed an increase in contrast sensitivity 5mins and 30mins respectively after tDCS (A), tRNS (B) and Sham (C) stimulation but this effect was not statistically significant. Larger (less negative) y-axis values indicate improvement in contrast sensitivity. Error bars shows standard error of the mean.

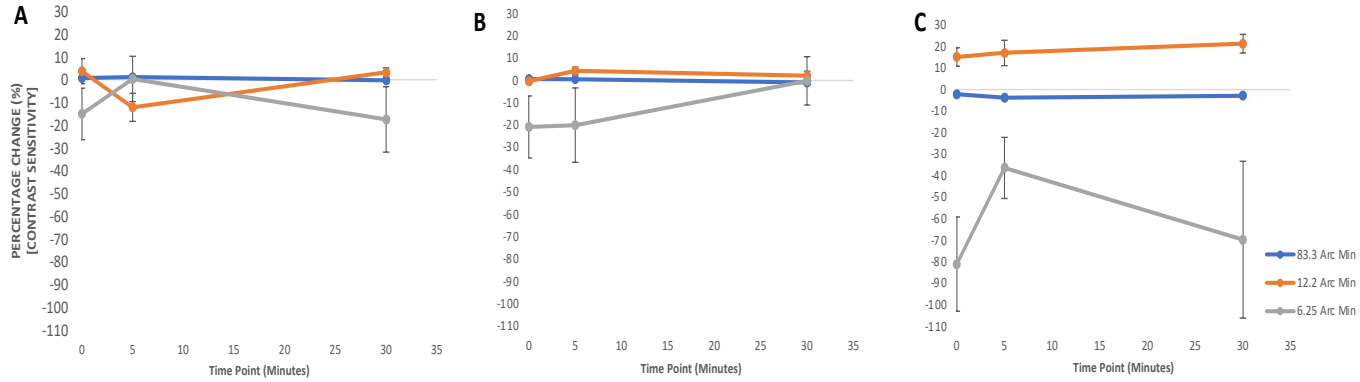


Figure 5: Percentage change of contrast sensitivity (Log threshold) from baseline at 3 different time points. Small and medium stimuli showed an increase in contrast sensitivity 5mins and 30mins respectively after tDCS (A), tRNS (B) and Sham (C)

stimulation but this effect was not statistically significant. Larger y-axis indicates improvement in contrast sensitivity. Error bars shows standard error of the mean.

3.4 Summary and Conclusion

We tested the hypothesis that visual cortex tRNS would induce the largest improvement in contrast sensitivity compared to visual cortex tDCS and sham in healthy adults. However, this pilot study did not find any reliable difference between tES protocols for effects on contrast sensitivity for healthy adults. Our results showed small improvements for all tES methods for small and medium size stimuli immediately and 30 mins after stimulation, but these effects were not reliably different from baseline measurements. This may be due to the small sample size and lack of a within-subjects test of stimulation types employed in this pilot study. Alternatively, healthy participants may already have effective contrast sensitivity for the stimuli we used. Future experiments will assess whether measurable effects occur in patients with amblyopia who have contrast sensitivity deficits. This study was the first to test effects of tES methods on contrast sensitivity using Landolt-C outcome measure. The use of Landolt-C plays a significant role in clinical assessment of primary visual function. Previous studies have used gabor patch as contrast detection assessment of the effects of tES methods [29,89,107,108], however, the Gabor patch is not generally used clinically for assessing visual function of patients. Therefore, we demonstrated that the Landolt-C potentially can be used as an outcome measure in determining the effects of tES methods in healthy adults, and possibly in patients with amblyopia in our subsequent experiment [see chapter 5].

Finally, our stimulation protocol conformed to the safety guidelines, and subjects tolerated the stimulation well without reporting adverse effects. The majority of the subjects spontaneously reported mild tingling sensations under the electrodes.

Chapter 4: Effects of transcranial random noise stimulation on Primary visual cortex in adults using EEG

4.1 Introduction

Transcranial random noise stimulation (tRNS) is a non-invasive brain stimulation technique that involves the delivery of a randomly oscillating alternating current through two head mounted electrodes [4,13]. tRNS has attracted interest in the fields of neurostimulation and neurorehabilitation because it appears to induce larger increases in neuroplasticity within stimulated neural systems than other transcranial electrical stimulation techniques such as transcranial direct current stimulation (tDCS) [97,261,271,272]. In addition, recent tRNS studies in tinnitus[97], visual perceptual learning [91,273] and myopia [109] have reported tRNS-induced enhancements in sensory function.

When applied to the motor cortex, tRNS induces a lasting increase in cortical excitability measured as an increase in the amplitude of motor evoked potentials induced by motor cortex transcranial magnetic stimulation [262,274]. A similar effect has been observed when tRNS is applied to the visual cortex. Herpich et al.[275] observed a prolonged reduction in the intensity of visual cortex TMS required to induce the perception of a phosphene (the phosphene threshold) following 20 min of visual cortex tRNS. Reduced phosphene thresholds represent increased visual cortex excitability.

The majority of visual cortex tRNS studies have employed high frequency tRNS (hf-tRNS; alternation frequency between 101 and 640 Hz) [4,92,275–277]. However, previous studies have found no statistically significant difference between the effects of low frequency (0.1 – 100 Hz) and high frequency tRNS applied to the visual cortex on visual perceptual learning [271]. Therefore, in this study, with visually normal controls, we investigated the effect of full spectrum tRNS on visual cortex excitability using visually evoked potentials (VEPs). We chose to use VEPs as our outcome measure because many individuals do not experience TMS-induced phosphenes [275,278] and visual electrophysiological measures are sensitive to the effects of other types of transcranial electric stimulation such as tDCS and transcranial alternate current stimulation (tACS) when applied to visual cortex [9,22,279–282].

VEPs are visually evoked electrophysiological signals obtained from electroencephalographic activity within the visual cortex [283,284]. VEPs are used clinically as a diagnostic tool for diseases that affect the retina [285,286], optic nerve [287,288], optic

radiations [289–291] and occipital cortex [292–294]. In human research, VEPs are often used to study the functional integrity of the visual system. Many studies have investigated the cortical sources that generate the major components of pattern-reversal VEPs [295–297]. A number of these studies have used magnetoencephalography (MEG) [298,299] and/or functional magnetic resonance imaging (fMRI) [300] to identify the cortical areas that generate pattern-reversal VEPs. There is a general consensus that the N75 component of the pattern reversal VEP waveform is generated in V1[298,299,301,302]. However, V1, V2-V3 and V3-V4 have all been associated with both the P100 and N135 [302,303].

Recently, VEPs have emerged as an investigative tool to explore, electrophysiologically, the effects of occipital cortex TMS [212–214], tDCS [22] and tACS [304] in humans [212] and animals [305]. For instance, Antal et. al. (2004) applied 10-15mins tDCS over the occipital cortex and recorded VEPs in response to sinusoidal luminance gratings presented in an on/off mode in healthy human adults. They reported a decrease in the amplitude of the N70 component with cathodal stimulation whereas anodal stimulation increased the amplitude of the N70 component. Contrarily, the P100 waveform component increased in amplitude with cathodal stimulation whilst anodal stimulation had no effect [9].

The efficacy of tRNS in modulating cortical activity has been demonstrated, by implication, from perceptual and behavioral effects [109,275,276,306–309]. For instance, Herpich et al.[275] showed increased visual cortex excitability after high frequency tRNS. There is no reported objective electrophysiological evidence of the effects of tRNS in healthy human

occipital cortex using VEPs. Therefore, we tested the hypothesis that active tRNS would induce an acute increase in visual cortex excitability as evidenced by increased VEP amplitude.

To provide a within-session control, we applied tRNS to only one cerebral hemisphere and presented hemifield pattern reversal stimuli to both the stimulated and unstimulated hemisphere. We anticipated tRNS effects to be apparent only when VEP stimuli were presented to the stimulated hemisphere. Although it is generally expected that hemi-field stimulation would induce a bigger scalp response from the contralateral hemisphere, a phenomenon referred to as paradoxical localization often occurs whereby the largest VEP responses are recorded from the hemisphere ipsilateral to the hemifield VEP stimulus. This effect was first identified by Halliday and Michael [310] who demonstrated that the scalp responses to hemi-field pattern stimulation were distributed asymmetrically. Thereafter, Barrett et al.[311] and Towle et al.[312] reported reliably greater amplitude of evoked potentials over the ipsilateral occipital cortex in response to hemi-field pattern stimulation. This paradoxical laterization was due to medial orientation of cortical generators within the visual cortex but diagonally positioned relative to the scalp surface. We anticipated that paradoxical localization would be apparent in our VEP datasets.

Variations in peak latencies of cortical responses to pattern stimulation are dependent on pathological (migraine [313], optic neuritis [314], retinal disease [285,286], multiple sclerosis [315]) and non-pathological (age and sex [316], visual acuity [317,318], contrast

and luminance [317], check and field size [319–321]) conditions. Previous studies have reported the effects of non-invasive brain stimulation on amplitudes [9,22,212,213,280,322–324] and peak latencies [9,281] of evoked potentials. Despite the changes of amplitudes induced following NIBS, Antal et al. (2004) and Accornero et al. (2007) showed that peak latency was statistically unchanged or not affected by NIBS, specifically tDCS. Adding to the body of evidence, we tested our third hypothesis that visual cortex tRNS will not affect peak latencies.

In this study, to assess the acute effects of visual cortex tRNS, we investigated pattern-reversal VEP waveforms (in particular the N75, P100 and N135 waveform components) in response to hemifield stimulation with high-contrast, black and white checkerboard reversal stimuli before and after active tRNS or sham treatment delivered to the left or right occipital cortex. We selected the pattern reversal stimulus because the waveform and timing are less variable than for other stimuli; hemifield stimulation allowed selective testing of the stimulated and non-stimulated occipital cortices.

4.2 Materials and methods

4.2.1 Subjects

Ten healthy subjects were recruited from the University of Waterloo campus (6 females; mean age = 27.10, range 20-37 years). All subjects had no history of neurological disorders and no contra-indications to non-invasive brain stimulation. All subjects had normal or corrected-to-normal visual acuity (VA $\leq$ 0.1 logMAR in each eye). This study was approved by the University of Waterloo Office of Research Ethics, and was carried out in agreement

with the principles of the Declaration of Helsinki. Written informed consent was obtained from all subjects.

4.2.2 Experimental Design

All subjects attended two sessions to receive both active tRNS and sham treatment on different days, however, each treatment was randomized for each participant. There was a one week interval between active and sham data collections to eliminate any stimulation after-effects [35,325]. Subjects were naïve to both active and sham tRNS procedures. Each session included baseline (pre) and post electroencephalographic (EEG) recordings. Pre-post measurements were made for right and left hemi-field checkerboard stimuli as well as for right and left eyes (**Figure 6**). The right eye (left eye occluded) viewed the right hemi-field checkerboard stimulus which was always presented first followed by the left hemi-field checkerboard stimulus. The same procedure was repeated for left eye (right eye occluded).

4.2.3 Experimental paradigm

Four (4) EEG recordings (right eye – right hemi-field stimulus; right eye – left hemi-field stimulus; left eye – right hemi-field stimulus and left eye – left hemi-field stimulus) were collected during the pre-session and another 4 EEG recordings were collected during the post-session. In all sessions, the subject was seated comfortably at a desk while fixating a red fixation cross in the center of a computer screen, at 1-meter distance, in front of them in a dark room. After the baseline EEG recordings, each subject then received either active or sham tRNS over randomized right or left occipital hemisphere. This was followed by post EEG recordings. Each hemi-field checkerboard stimulus was presented for 3 mins, totaling 12 mins of recordings for both eyes. Five minutes were used to remove the EEG cap and set

up the tRNS system followed by 20 mins of tRNS. Another 5 minutes was used to remove tRNS system and set up the EEG cap followed by 12 mins of post EEG recordings (60 minutes in total for each subject per day session). The checkerboard stimulus was a black-and-white checkerboard with individual check widths of 1°. The contrast between black and white squares was set to 100% with mean photopic luminance of 50 cd/m2. The pattern reversal rate was 2 reversals per second and the diameter of the square hemi-field was 15°. Our recording parameters generally conformed to ISCEV standards for clinical VEP recording [283].

4.2.4 EEG parameters

We followed the international 10–20 system for electrode placement to measure the EEG activity from the surface of the scalp. Recording references were linked mastoids. A 32-channel EEG cap (Quick-Cap, Neuroscan, Compumedics, NC USA) was used to record EEG data. Continuous EEG data were gathered, filtered with a band pass of (0.2–1000 Hz), digitized at 1000 Hz (Neuroscan 4.5, SynAmps2, Compumedics, NC USA) and stored on a personal computer for offline analysis. The continuous EEG data from Neuroscan were imported and analyzed in EEGLAB (EEGLAB v2019.4.31). Channel labels were automatically allocated. In order to eliminate unnecessary noise in the data, we re-referenced all channels to the vertex electrode (Cz). To further reduce high-frequency noise, the data were filtered with a bandpass filter with a range of 1Hz and 50Hz and a sample rate of 512Hz. Next, the pre-processed data underwent linear decomposition using independent components analysis (ICA). ICA decomposes the data collected at single scalp channels to a

spatially transformed "virtual channel" basis. After ICA, the data were epoched and the processed data were exported for data analysis.

4.2.5 Transcranial Random Noise Stimulation

Subjects either received active tRNS (2.0 mA, current density: 0.10 mA/cm, frequency range 0.1-640 Hz) or placebo (sham) stimulation of the primary visual cortex for 20 mins. Stimulation was delivered using a DC-stimulation MC device (Eldith, NeuroConn GmbH, Germany). The stimulation was delivered via a pair of saline-soaked surface sponge electrodes (5 cm x 5 cm, 25 cm^2) placed at Cz (blue) and either at O1 or O2 (red) (**Figure 6**). The AC current was initially ramped up to a maximum of 2mA over 30 sec and ramped down to 0mA over 30 sec at the end of the stimulation session. During sham stimulation, the 30 sec ramp-up was immediately followed by the ramp-down out[33,308]. All subjects participated in both active and sham tRNS experimental sessions with a minimum of one-week interval. Subjects were entirely naïve to non-invasive brain stimulation, hence, ensuring that subjects remained masked to their treatment allocation. Our application of tRNS conformed to tDCS safety guidelines [35,325,326].

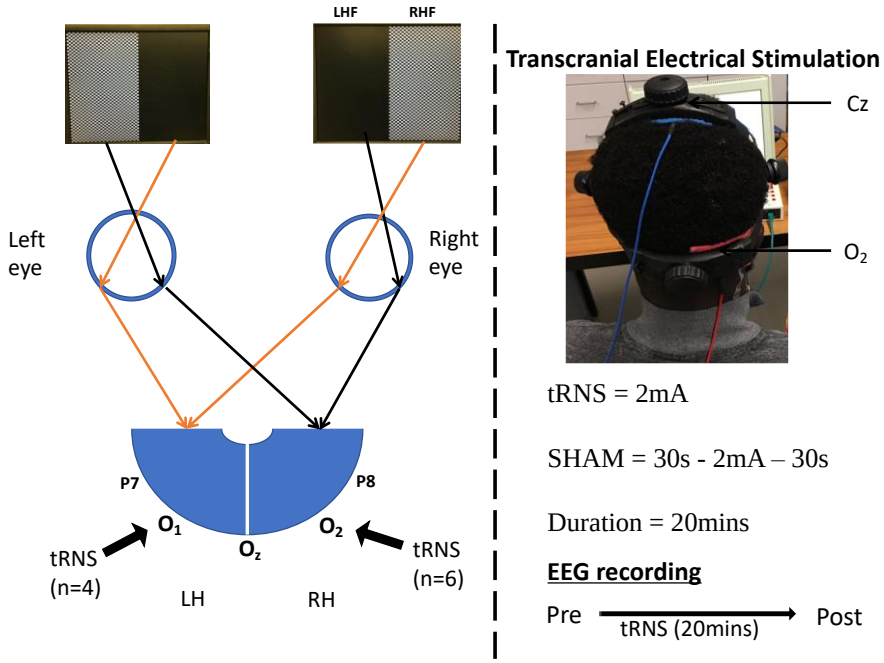


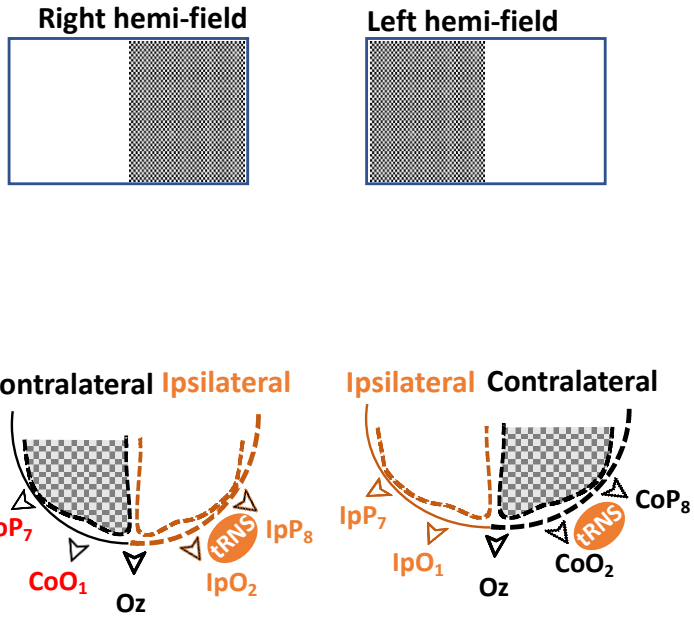
Figure 6: Schematic presentation of the EEG recording and tRNS protocol. The left panel shows the right hemi-field (RHF) visual stimuli processed in the left cortical hemisphere (orange arrows) and left hemi-field (LHF) visual stimuli processed in the right cortical hemisphere (black arrows). Six subjects received tRNS at O2 (right occipital lobe) and 4 subjects received tRNS at O1 (left occipital lobe). The right eye always viewed the RHF stimulus first (left eye occluded) for 3mins, followed by the LHF stimulus for 3mins. The same procedure was repeated for the left eye (right eye occluded). The right panel depicts the tRNS electrode montage.

4.2.6 Data analysis

The peak-to-peak amplitudes of the VEPs were measured within the first 400ms after pattern reversal. The VEP amplitudes were calculated by (i) subtracting the first negative deflection (N75) from the first positive deflection (P100), and (ii) subtracting the second negative deflection (N135) from first positive deflection (P100). We also defined each peak latency

(N75, P100 and N135) as the time from stimulus onset to the point of maximum positive or negative amplitude within the latency window (0-400ms).

We performed a repeated measures analysis of variance (ANOVA) on the VEP amplitude and latency data for each electrode separately using SPSS (IBM, Version 25). The ANOVA model had within-subject factors of CONDITON (Active vs Sham) and TIME (Pre vs Post). We also compared mean amplitudes for EYE (Right Eye vs Left Eye) and ELECTRODE ((CoP7/8, CoO1/2, Oz, IpO1/2 and IpP7/8) - **Figure 7**)). Statistical significance was set at p = 0.05.



	Visual stimulus	No visual stimulus
tRNS	CoP8, CoO2	IpO2, IpP8
No tRNS	CoP7, CoO1	IpO1, IpP7

Figure 7: Schematic of the relationship between tRNS location, cerebral hemisphere and EEG electrode position for tRNS of the right hemisphere. Electrode notations were reversed for subjects who received tRNS over the left hemisphere. Each occipital hemisphere is described as either ipsilateral (orange) or contralateral (black) to the hemi-field checkerboard stimulus. We analyzed 5 electrodes placed over the occipital cortex (CoO1/2 – Contralateral O1 or O2; Oz; IpO1/2 – Ipsilateral O2 or O1; CoP7/8 – Contralateral P7 or P8; and IpP7/8 – Ipsilateral P7 or P8). The 2x2 table shows the relationship between electrodes, tRNS and visual stimulation.

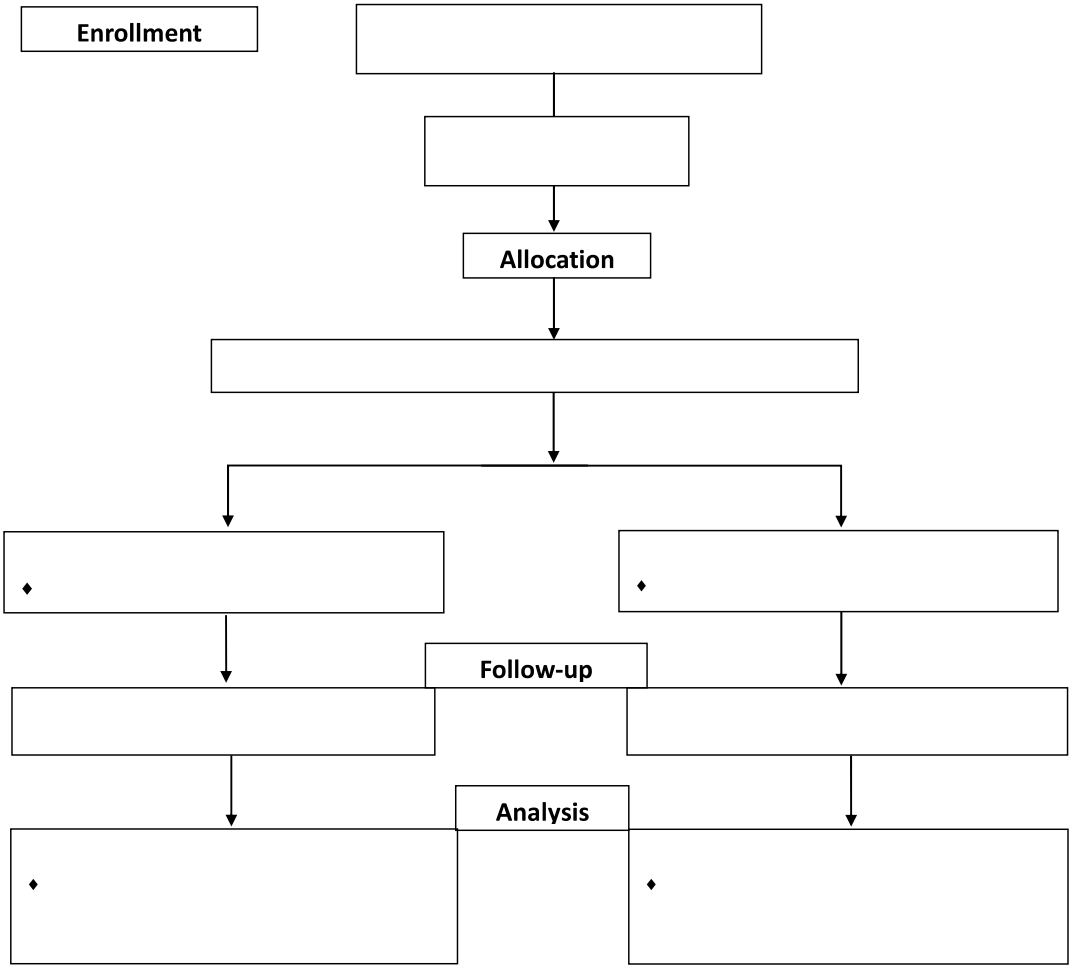


Figure 8: Flowchart detailing the enrolment protocol of study subjects.

4.3 Results

There were no adverse effects of tRNS, however, a majority of the subjects reported mild tingling sensations under the electrodes during both active and sham sessions. The VEP amplitudes for right and left half-field pattern reversals were recorded in ten subjects, however, one subject was excluded from the data analysis due to the presence of high-frequency noise in the EEG data (**Figure 8**).

Example individual (blue) and grand averaged (black) waveforms at the midline (Oz) from all 9 subjects are presented in **Figure 9**. Each individual waveform represents a standard pattern reversal VEP recorded after at Oz after active tRNS for the right eye viewing condition.

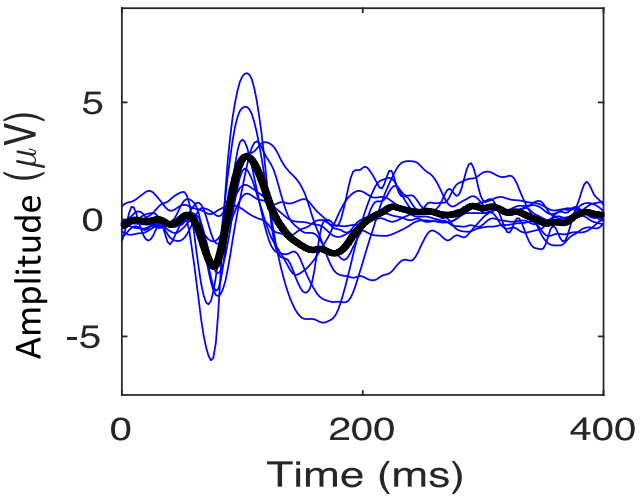


Figure 9: Individual (blue waveform) and averaged (black waveform) VEP recorded by the Oz (midline) electrode for all 9 subjects after active tRNS.

4.3.1 Paradoxical localization of hemi-filed pattern-reversal VEPs

For both active and sham conditions and for both eyes pre and post tRNS, we observed paradoxical localization of the hemi-field VEPs. Specifically, there was an asymmetric distribution of N75-P100 and -N135 amplitudes across the 5 electrodes. The largest amplitudes were at Oz with a sharp decline for the electrodes contralateral to the hemi-field pattern-reversal visual stimulus. A similar pattern was evident for the latency data, whereby longer latencies were observed ipsilateral to the hemi-field VEP stimulus (**Figures 10 to 14)**.

4.4 VEP checkerboards presented to the occipital hemisphere that received tRNS

Significant effects of tRNS were observed for data from the lateral scalp electrodes ipsilateral to the VEP hemifield stimulus for the right eye viewing condition only. For the N75-P100 amplitude data, a significant interaction between Condition and Time ($F_{1,8}$ =18.451, p = 0.003) was observed (**Figure 10A & B**). The same interaction was also observed for the P100-N135 amplitude data ($F_{1,8}$ =10.195, p = 0.013). A significant main effect of Condition ($F_{1,8}$ = 9.708, P = 0.014) was also present whereby amplitudes were larger for the active tRNS session at both timepoints. No other main effects or interactions reached significance. **Figure 12** illustrates group averaged VEP waveforms for the lateral electrodes placed ipsilateral to the hemi-field stimulus for the right eye viewing condition. There were no interactions or main effects for the VEP latency data (all p > 0.05; **Figures 11**).

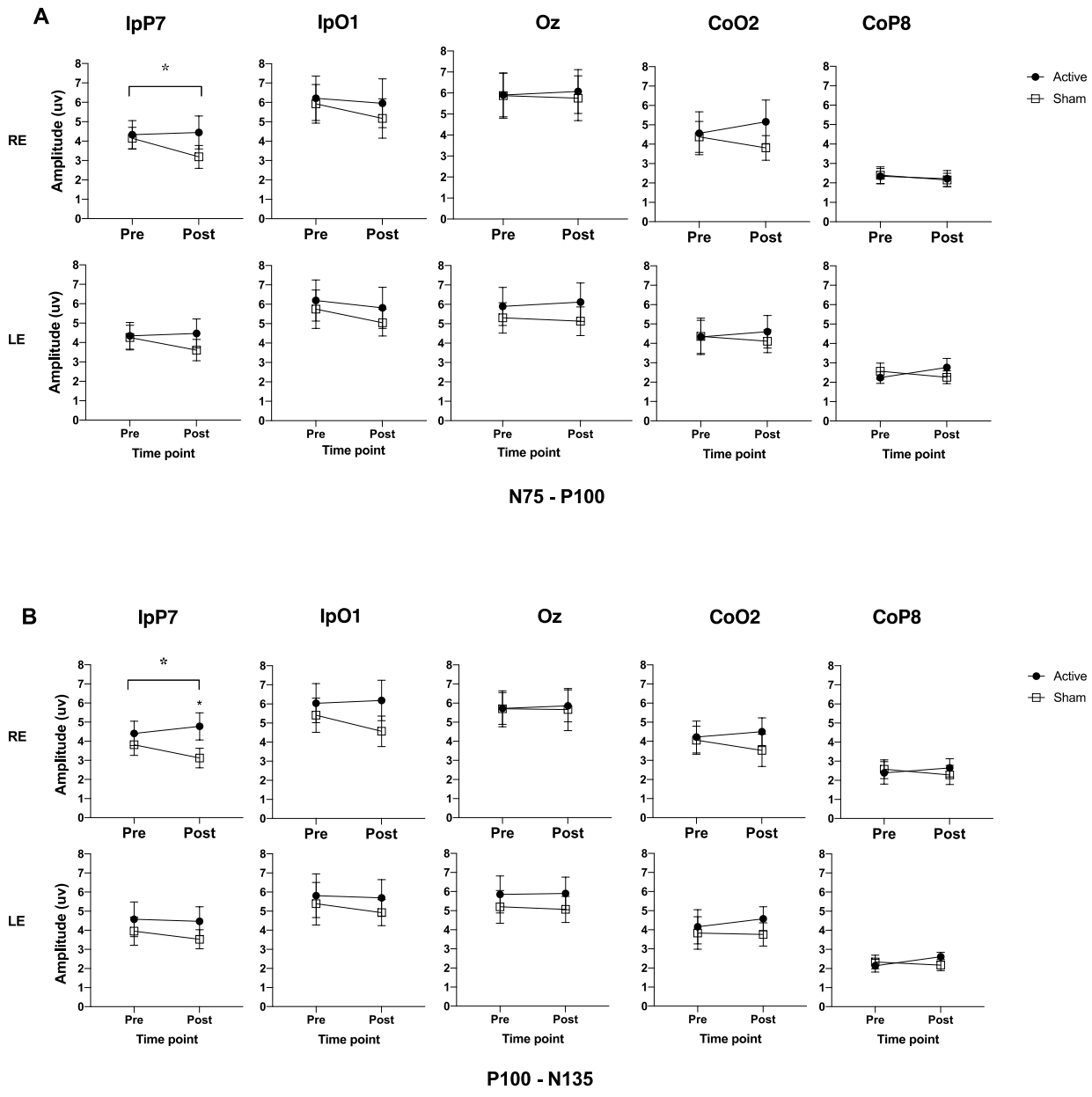


Figure 10: Amplitudes of VEPs to the right eyes (RE) and left eyes (LE) when VEP checkerboard stimuli were presented to the occipital hemisphere that received active / sham tRNS. A (upper panel):VEP amplitudes for N75-P100 (mean ± within subject standard error of the mean (SEM)). B (lower panel): VEP amplitudes for P100-N135). Asterix (*) indicate significant interactions ($p<0.05$) between Condition and Time.

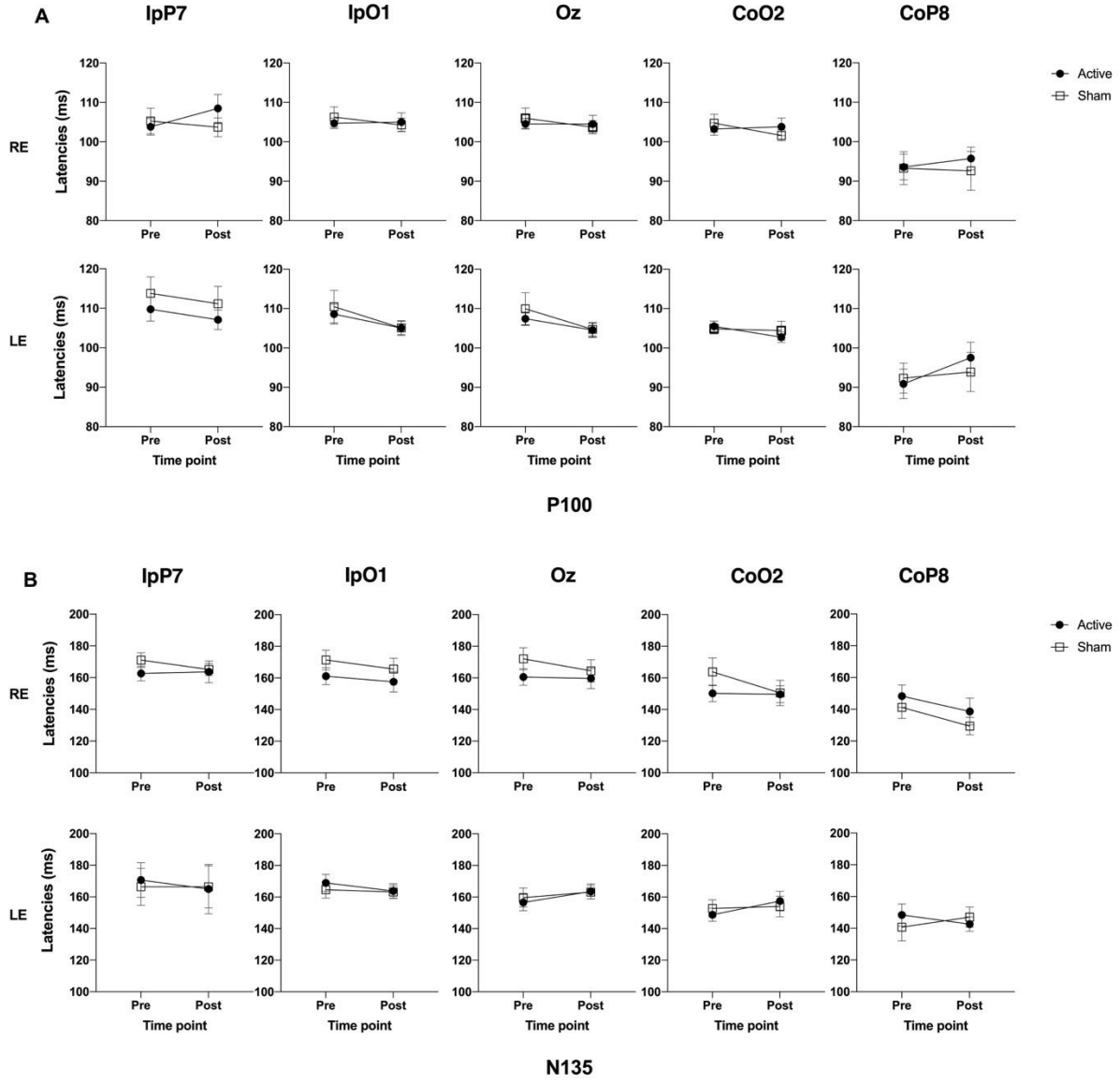


Figure 11: Peak latencies of VEPs when VEP checkerboard stimuli were presented to the occipital hemisphere that received active / sham tRNS. Panel A shows P100 peak latencies and panel B shows N135 peak latencies (mean ±SEM). *Statistically significant difference from baseline ($p < 0.05$). Larger y-axis values indicate delayed timing.

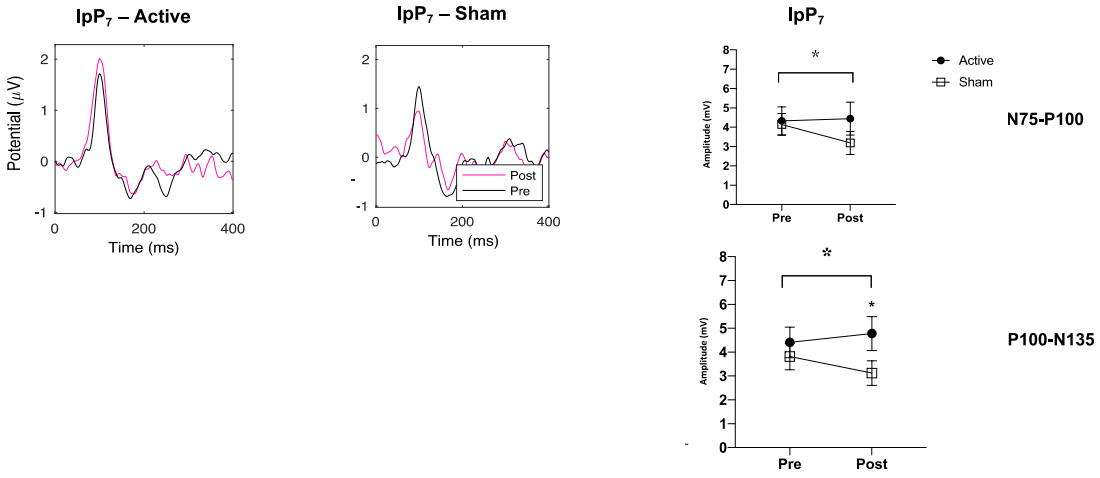


Figure 12: Group averaged VEP waveforms illustrating the Condition x Time interaction effects for electrode IpP7. Averaged VEP waveforms for other electrode sites can be found in supplementary figures: Appendix 1)

4.5 VEP checkerboards presented to the occipital hemisphere that did not receive tRNS

No significant interactions or main effects were observed for the VEP amplitude or latency datasets when the VEP checkerboard stimulus was presented to the occipital hemisphere that did not receive active / sham tRNS (all $p > 0.05$; **Figures 13 & 14**).

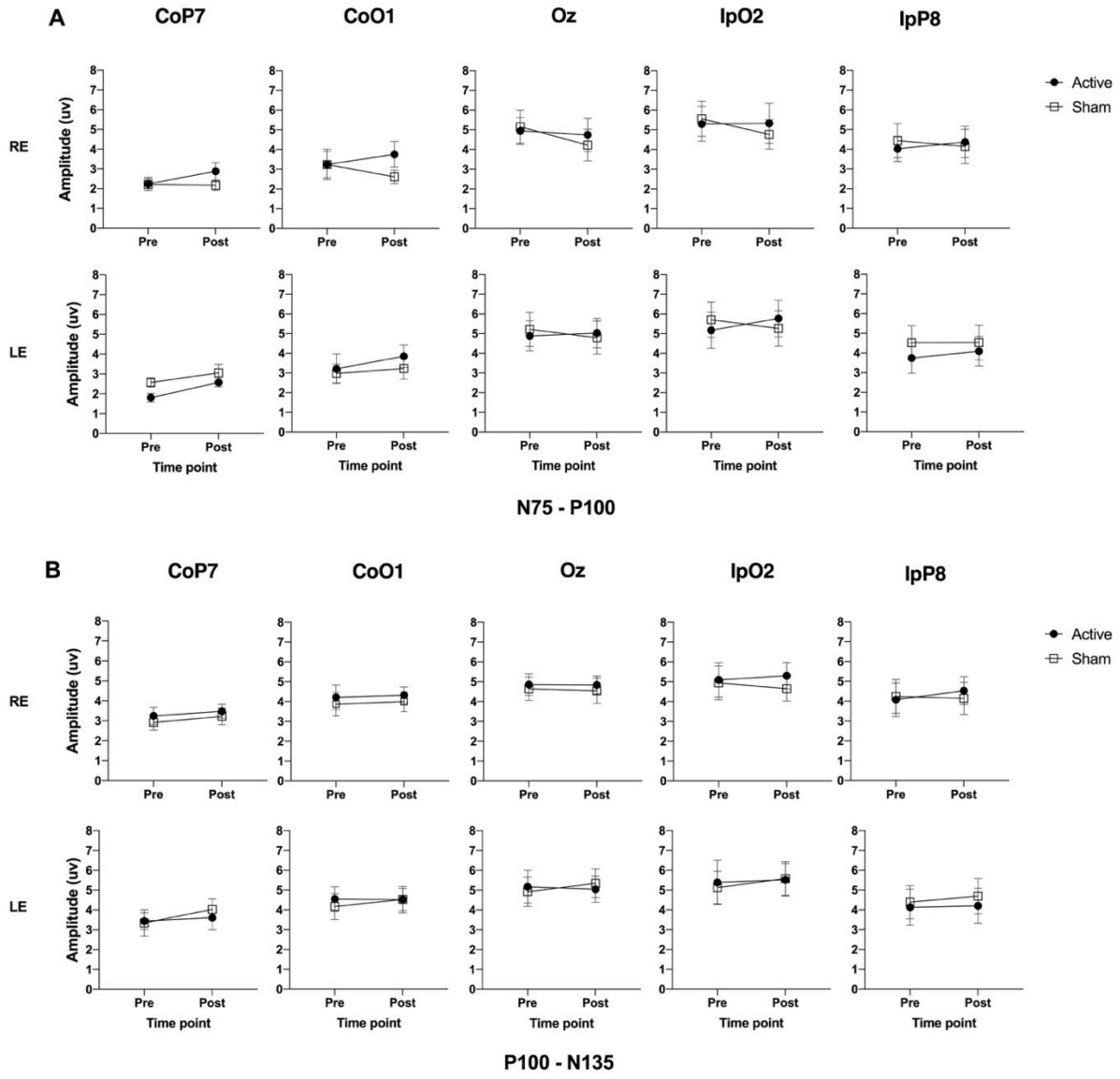


Figure 13: Amplitudes of VEPs when VEP checkerboard stimuli were presented to the occipital hemisphere that did not receive active / sham tRNS. A (upper panel) shows VEP amplitudes for N75-P100. B (lower panel) is VEP amplitudes for P100-N135 (mean ±SEM).

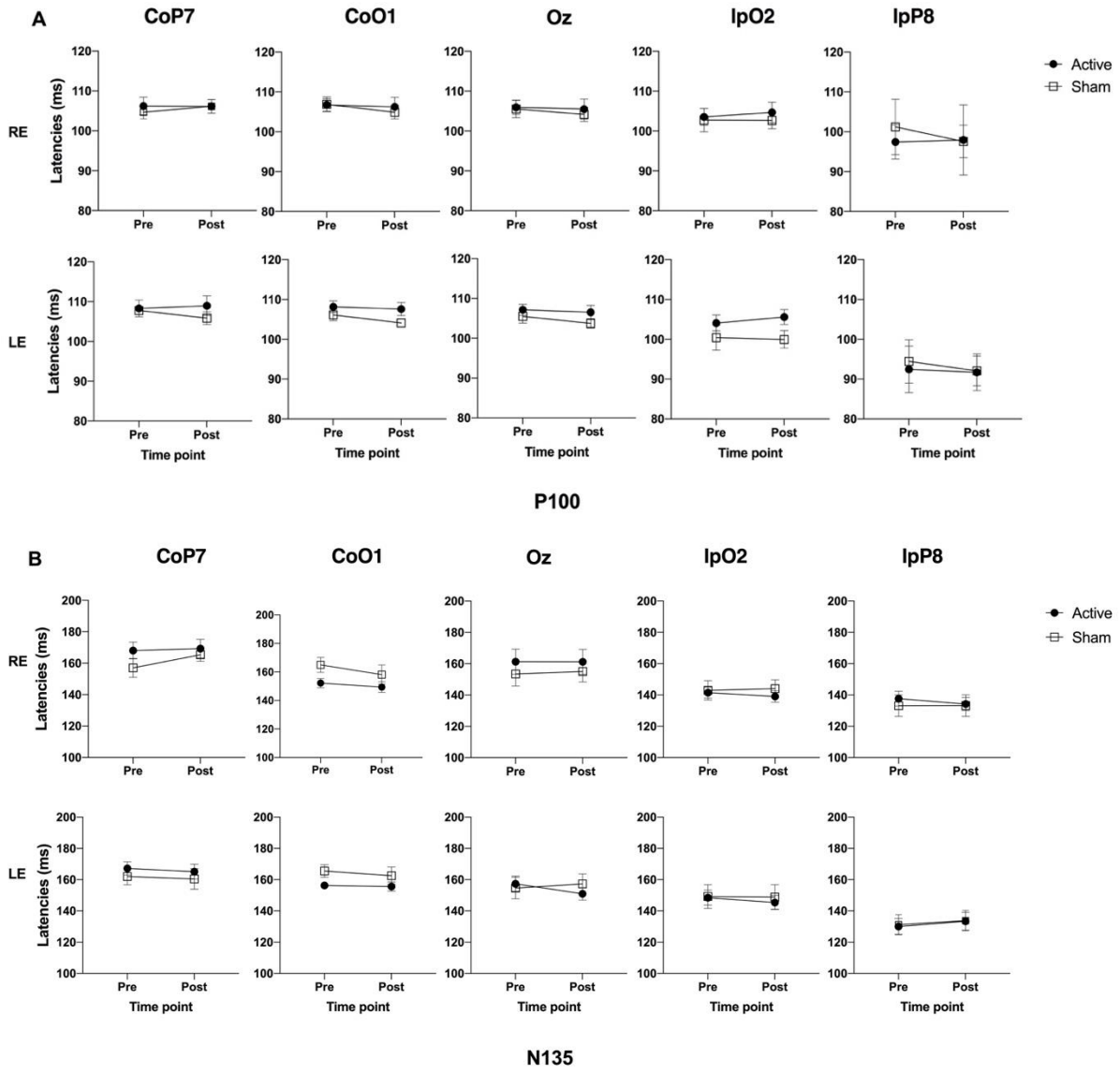


Figure 14: Peak latencies of VEPs when VEP checkerboard stimuli were presented to the occipital hemisphere that did not receive active / sham tRNS. Panel A shows P100 peak latencies and panel B shows N135 peak latencies (mean ±SEM).

4.6 Discussion

The aim of this study was to investigate the effects of tRNS on visual cortex excitability using pattern reversal hemifield VEPs. Active tRNS induced increases in VEP amplitude

relative to sham tRNS for both N75 to P100 and P100 to N135 amplitude components when visual stimuli were presented to the hemisphere that received tRNS. Our results supported our hypothesis that visual cortex tRNS would enhance VEP amplitudes in healthy adults. Our observation that visual cortex tRNS increased the amplitudes of VEPs is consistent with other studies reporting enhanced amplitudes of motor evoked potentials (MEPs) following motor cortex tRNS [4]. Increased MEP and VEP amplitudes are consistent with increased excitability within the cortical neurons.

When the VEP right eye checkerboard stimulus was presented to the occipital hemisphere that received active / sham tRNS, ipsilateral occipital electrodes exhibited significant tRNS induced changes in VEP amplitude that were driven by a small increases post-stimulation in the active condition and a larger drop for the sham condition. The relative decrease in VEP amplitude post tRNS for the sham condition maybe attributed to adaptation and/or fatigue effects [181,327]. Several studies have reported VEP habituation in control subjects as a result of repeated checkerboard visual stimulation [313,328–330]. For instance, Áfra et al.[313] observed a progressive decrease in VEP amplitudes in control subjects following 15 min of repeated pattern reversal stimulation. Interestingly, normal VEP habituation is not present in patients with migraine [331,332] or chronic pain [313,330]. In this study, we observed habituation of the VEP response in the sham tRNS condition and habituation probably caused an underestimation of the VEP increase induced by active tRNS.

The VEP results were similar for the right and left eye viewing conditions, however, there were no significant tRNS induced changes in VEP amplitudes for any electrode recordings for the left eye viewing condition. VEPs during the left eye viewing condition were recorded 12 mins after the right eye viewing condition (right eye viewing condition's recordings lasted for 12 mins) or 17 mins after occipital tRNS (5 mins was used to switch from removing tRNS stimulation apparatus/setup to EEG recording setup). We therefore postulate that the acute effects of tRNS were evident for the right eye viewing condition but were attenuated for the left eye viewing condition.

Recently, a number of publications have reported that visual cortex tRNS improves contrast detection [37,264,333], visual acuity [107,109], and perceptual learning [91,109,276]. The potential mechanism of tRNS aftereffects within the cortical neurons is unclear. tRNS aftereffects may be explained by repeated opening and closing of sodium channels leading to membrane depolarization and an increase in cortical excitability that outlasts the stimulation itself [4]. Chaieb et. al., 2015, demonstrated that tRNS aftereffects are independent of NMDA-receptors, but dependent on modulation of gated sodium-ion channels. This implies that tRNS aftereffects may not rely on LTP-like mechanisms but rather on mechanisms related to sodium channel function.

Earlier studies reported cortical sources that generate major components of pattern reversal VEPs (29–31). The N75 component of pattern reversal VEPs is generated in V1 (32,33,35,36) whilst P100 and N135 are generated in V1 to V4 (6,37). Our observation that

active tRNS increased the amplitude of both the N75 to P100 and P100 to N135 VEP components suggests that tRNS influenced excitability in neurons located in V1 to V4. Our results are similar to studies that used VEPs to investigate the aftereffects of tDCS within the occipital cortex. Antal et. al., 2004 reported anodal and cathodal visual cortex tDCS increased the amplitude of the N70 and P100 components respectively [9]. Similarly, Ding et. al., 2016 showed that the aftereffects of visual cortex anodal tDCS increased VEP amplitudes for amblyopic, fellow and control eyes whilst cathodal tDCS decreased these amplitudes [22].

We observed paradoxical localization of the hemi-field VEPs, specifically an asymmetric distribution of N75-P100-N135 amplitudes across the occipital electrodes with largest amplitudes at the midline electrode (Oz) and with a sharp decline on the same side as the processing hemisphere (contralateral to the hemi-field pattern-reversal) and a more gradual decline opposite to the hemisphere processing the pattern-reversal. The data presented here are consisted with those of Barret et al.[311] who reported that higher amplitude visually evoked responses occurred at the occipital electrodes placed over the hemisphere ipsilateral to the pattern reversal stimulus. Paradoxical laterization is explained by the locations of cortical generator regions which are mainly positioned on the medial and posteromedial surface of the occipital cortex. The occipital electrodes placed over the ipsilateral hemisphere record optimal evoked responses from these cortical generators, however, cortical electrodes placed over the contralateral hemisphere are almost vertical to the axis of the cortical generator region, hence recording minimal evoked responses [311,312,334].

We also investigated the aftereffects of tRNS on peak latencies, and we observed that visual cortex tRNS did not induce statistically significant early or prolong peak latencies for either P100 or for N135 VEP components. N75 was either absent (not below baseline) or very low amplitude, rendering the peak latency unreliable and, therefore, N75 data were not analyzed. We do not have available evidence that tRNS aftereffects shift the timing of peaking latencies. VEP peak latencies are delayed by obstructions in the visual pathway[288,289,292,335] and by retinal diseases[285,286]. In this study, timing of the peak latencies may not have been affected by tRNS effects because we recruited healthy subjects. Another possible explanation is that tRNS aftereffects are not known to change the speed of action potential conduction. However, tRNS aftereffects directly affect the membrane potential of the neuronal cells leading to increase firing rates and subsequently resulting in an increase in excitability within the neural circuits[4].

4.7 Study limitations

There are two major limitations in this study that could be addressed in future research. First, there was non-randomization of the testing order of the eyes when viewing the hemi-field checkerboard stimuli. Our left eye data did not show significant differences in VEP amplitudes. If the effects of tRNS are brief, then a stronger effect for the eye tested first is would account for the effect in the right eyes only as these were recorded first for both pre and post treatment. Second, the small sample size in this study may have not provided sufficient statistical power to observe reliable effects of tRNS at all the scalp electrodes placed ipsilateral to the checkerboard stimulus for both right and left eye viewing conditions.

Finally, because this was an exploratory study with a large number of variables, the statistical results reported were not corrected for multiple comparisons and therefore there is the risk of type 1 error. Based on the current results, future studies can identify a more targeted set of variables and outcome measures to avoid a large number of statistical comparisons.

4.8 Conclusions

Visual cortex tRNS induced excitability changes in healthy human adults. Our results are consistent with previous studies that used VEPs to investigate the effects of transcranial direct current stimulation. This study supports the evidence that tRNS can induce neuroplastic changes within the occipital cortex of healthy adults.

Chapter 5: Repetitive visual cortex transcranial random noise stimulation improves visual function in adults with amblyopia

This chapter has been accepted for publication as:

Donkor R, Silva AE, Teske C, Wallis-Duffy M, Johnson A, Thompson B. Repetitive visual cortex transcranial random noise stimulation in adults with amblyopia. *Scientific Reports* volume 11, Article number: 3029 (2021).

Author contribution statement

Conceptualization RD and BT; experimental design RD, BT, CT, MWD; data collection RD, CT; data analysis RD, AS, AJ, BT; data interpretation RD, AS, AJ, BT; manuscript writing RD, BT, revision and review of manuscript all authors. Richard Donkor total contribution: 70%. Link<< https://www.nature.com/articles/s41598-020-80843-8>>

5.1 Introduction

Amblyopia is a developmental disorder of the visual cortex, with a prevalence of approximately 1-5% [52,53,336]. Amblyopia causes a wide range of vision deficits [41,47] including a monocular loss of high-contrast visual acuity [42,45] that is particularly pronounced for crowded optotypes [337,338], reduced contrast sensitivity in the affected eye [42,339–341], and impaired or absent stereopsis [342,343]. Amblyopia is also associated with chronic suppression of the affected eye [39,56] that may play a key role in the etiology of the disorder [59].

Amblyopia involves abnormal processing within the primary and extrastriate visual cortex [344] and therefore recovery from amblyopia requires a change in cortical function. Current amblyopia treatments achieve this by directly manipulating visual input to the brain. For

example, the most common amblyopia treatment involves the provision of a clear retinal image in the amblyopic eye using refractive correction followed by occlusion of the non-amblyopic eye. This treatment improves amblyopic eye visual acuity, but has drawbacks in terms of compliance [150] and reduced efficacy with increasing age [345].

Transcranial electrical stimulation (tES) refers to a suite of non-invasive neuro-modulation techniques including transcranial direct current stimulation (tDCS), transcranial alternating current stimulation (tACS) and transcranial random noise stimulation (tRNS) that may enhance plasticity in targeted regions of the human brain [4,13,261], including the visual cortex [89,108,109]. Currently, tES methods are being investigated as a potential neurorehabilitation tool for disorders including stroke [175,177–179], chronic pain [172,173] and tinnitus [97,174] and there is growing interest in the use of tES and transorbital stimulation to treat disorders of vision (see [208,209,346,347] for recent reviews). Following early work that reported improved contrast sensitivity in adults with amblyopia after visual cortex transcranial magnetic stimulation [10,20], a number of studies have investigated the application of anodal tDCS to amblyopia. A single session of anodal tDCS improves amblyopic eye contrast sensitivity [21,22], increases visually evoked potential (VEP) amplitudes induced by stimuli presented to the amblyopic eye [22], and balances the response to inputs from each eye within visual cortex [21]. Furthermore, anodal tDCS enhances the effect of perceptual learning (PL) in adults with amblyopia [89] and recent studies have revealed that visual acuity, detection thresholds, and stereopsis improve in mature amblyopic rats following anodal tDCS [62,64,132]. One potential mechanism for

anodal tDCS effects in adults with amblyopia is a reduction in GABA-mediated inhibition [168] within the visual cortex. GABA has been associated with interocular suppression in strabismic cats [57] and may act as a “break” on visual cortex plasticity [60].

A recently developed tES technique, tRNS, involves an alternating current that randomly changes in frequency and amplitude [260]. tRNS may have larger effects on cortical activity than other tES protocols. For example, tRNS induced significantly greater improvements in tinnitus symptoms [97] and larger increases in motor evoked potential amplitude (MEP) [261] compared to either tDCS or tACS. Furthermore, visual cortex tRNS enhanced visual perceptual learning in adults with normal vision to a greater extent than anodal tDCS [91,272] and the combination of tRNS and perceptual learning enhanced the transfer of learning to non-trained visual tasks in adults with amblyopia [24]. tRNS has also been reported to enhance visual perceptual learning in adults with cortical blindness [272]. Potential mechanisms for these effects include an acute enhancement of the signal-to-noise ratio within the visual cortex due to stochastic resonance [28,308,348] and longer-lasting alterations in neural membrane function due to repetitive opening and closing of sodium channels [14].

Within this single-blind, between subjects, randomized, sham-controlled study, we tested the hypothesis that five daily sessions of visual cortex tRNS alone would lead to improved amblyopic eye contrast sensitivity, crowded and uncrowded amblyopic eye visual acuity in adult patients.

5.2 Methods and materials

5.2.1 Participants

Amblyopia was defined as reduced best corrected visual acuity (> +0.3 logMAR) in one eye in the absence of ocular pathology and at least a 0.2 logMAR acuity difference between the eyes. Anisometropia was defined as a difference in spherical equivalent between the two eyes of ≥ 0.50 Diopters (D), or a difference of astigmatism in any meridian ≥ 1.50 D [155]. Initial visual acuity was measured using an M&S logMAR chart. Inclusion criteria were: (i) presence of strabismic and/or anisometropic amblyopia; (ii) 0.0 logMAR visual acuity or better in the fellow eye (FE). Exclusion criteria [34,325,326,349] were: (i) presence of a scalp skin condition that contraindicated tRNS; (ii) history of neurological or psychiatric disorders, such as seizures; (iii) current medication for the treatment of neurological or psychiatric disorders; (iv) a history of brain injury; (v) implanted medical devices. Potential participants were contacted following a search of the clinic's patient database. Interested participants completed telephone screening to determine eligibility. The experimental procedures were approved by the Ethics Review Board of the University of Waterloo, Canada and were consistent with the declaration of Helsinki. Written informed consent was obtained from all participants. All participants were remunerated for their time. Two subjects in the sham group were excluded due to loss of follow up after first day (**Figure 15**).

Table 2: Participant details. M = male, F = female, patching = previous history of occlusion therapy, mixed = mixed amblyopia, aniso = anisometropic amblyopia, strab = strabismic amblyopia, stereoacuity is presented in seconds of arc, AME = amblyopic eye, FFE = fellow fixating eye, add = near power addition.

ID	Age/ sex	Patching	Type of amblyopia	Stereoacuity	Visual acuity (logMAR)		Current refraction		
					AME	FFE	AME	FFE	ADD
AG1	27/ M	Yes	Mixed	<800	+1.40	0.00	+2.00 -0.50 x 155	+0.50 DS	
AG2	20/ M	Yes	Aniso	400	+0.50	0.00	+1.00 -1.50 x 175	Plano	
AG3	37/ M	Yes	Aniso	<800	+0.30	0.00	-1.00 -1.00 x 100	-0.25 -2.50 x 090	
AG4	45/F	Yes	Aniso	200	+0.30	0.00	+2.75 -0.75 x 080	+2.00 -0.25 x 159	+1.50
AG5	59/ M	No	Aniso	<800	+0.70	0.00	+8.25 DS	+8.25 -1.25 x 105	+2.50
AG6	41/F	Yes	Mixed	<800	+0.50	0.00	+2.00 DS	+0.25 -3.75 x 179	+0.75
AG7	46/ M	No	Aniso	<800	+0.40	0.00	+0.75 DS	+4.25 -0.50 x 165	
AG8	52/F	No	Aniso	400	+0.70	0.00	+0.50 -0.50 x 175	+2.00 -0.50 x 075	+2.25
SG1	51/F	Yes	Mixed	<800	+0.30	0.00	+3.25 -0.50 x 165	Plano	
SG2	58/ M	Yes	Aniso	400	+0.30	0.00	+4.00 -0.25 x 180	+1.50 -0.50 x 180	+2.25
SG3	52/F	No	Aniso	200	+0.40	0.00	-2.50 -0.50 x 155	-1.50 DS	+2.75
SG4	19/ M	No	Aniso	<800	+0.30	0.00	+2.25 DS	+0.75 DS	
SG5	55/ M	No	Aniso	<800	+0.30	0.00	+1.75 -0.75 x 085	+1.75 -0.75 x 090	+2.00
SG6	49/ M	No	Strab	100	+0.30	0.00	+1.75 DS	+1.50 DS	
SG7	24/ M	No	Mixed	<800	+0.30	0.00	+8.25 -2.00 x 160	+6.50 -1.25 x 0.40	

SG8	58/F	No	Strab	<800	+0.50	0.00	+4.50 -0.25 x 176	+3.50 -0.50 x 165	+2.75

5.2.2 Procedure

A single-blind, sham controlled, between-subjects design was adopted. Randomization followed allocation concealment procedures and was conducted by an experimenter who was not involved in data collection or eligibility assessment using a random number generator. Randomization occurred after participants had met the eligibility criteria and completed study enrolment. Participants completed 5 consecutive daily stimulation sessions and a follow-up session 28 days after the final stimulation session. Outcome measures were completed by the participants using automated computer programs with no input from the experimenter. This procedure was designed to mitigate against experimenter bias.

5.2.3 Transcranial Random Noise Stimulation

Subjects either received tRNS (2.0 mA, current density: 0.08 mA/cm, frequency range 0.1-640 Hz) or placebo (sham) stimulation of the primary visual cortex for 25 min over 5 consecutive days. Stimulation was delivered using a DC-stimulation MC device (Eldith, NeuroConn GmbH, Germany). We chose a relatively long and high intensity stimulation protocol because we planned to measure 6 outcomes (contrast sensitivity, crowded and uncrowded visual acuity for each eye) and therefore attempted to maximize the duration of the stimulation aftereffects [88,237]. There is no statistically significant difference between the effect of high frequency (101-640 Hz) and low frequency (0.1-100 Hz) visual cortex tRNS on visual perceptual learning enhancement [91]. Therefore, we chose to deliver the full

frequency range. The stimulation was delivered via a pair of saline-soaked surface sponge electrodes (5 cm x 5 cm, 25 cm^2) placed at Cz and Oz [7], as determined by the international 10/20 electroencephalogram system. The AC current was initially ramped up to a maximum of 2mA over 30 s and ramped down to 0mA over 30 s at the end of the stimulation session. During sham stimulation, the 30 s ramp-up was immediately followed by the ramp-down out [33]. Our between subjects design and use of participants entirely naïve to non-invasive brain stimulation ensured that participants remained masked to their treatment allocation. Our application of tRNS conformed to tDCS safety guidelines [35,325].

After the final tRNS sessions, participants were asked to rate the following sensations on a four-level scale (none, mild, moderate and severe): headache, neck pain, scalp pain, tingling, itching, burning sensation, sleepiness, trouble concentrating and acute mood change. Participants were also asked to rate whether any reported sensations were due to tES by selecting from the following options: no, remote chance, probable, definitely (**Table 3**).

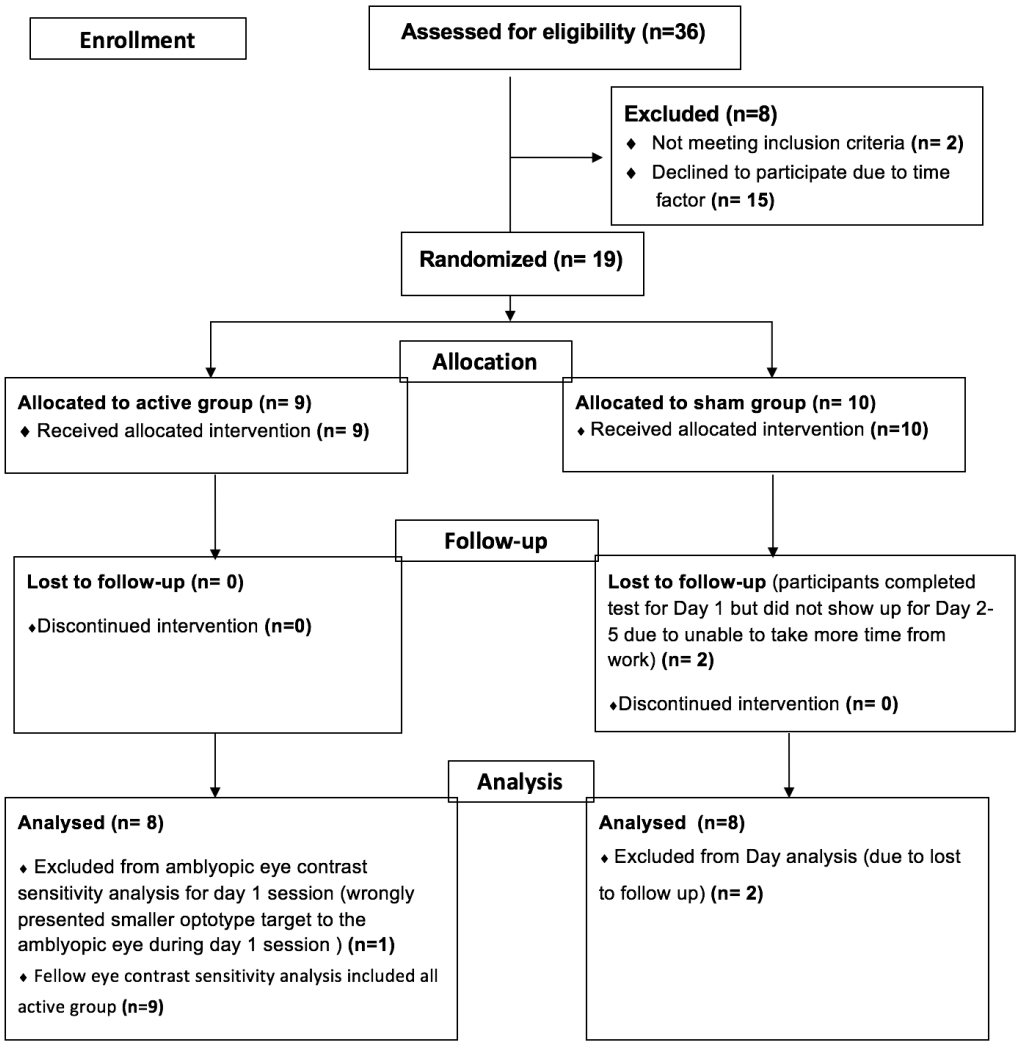


Figure 15: CONSORT flow diagram for the study

5.2.4 Visual Function Measurements

Monocular contrast sensitivity and visual acuity (both crowded and uncrowded) were measured for each eye before, during, 5 min post, and 30 min post stimulation on each stimulation day (**Figure 16**). All measurements were made using Landolt-C optotypes presented using the Freiburg Vision Test ('FrACT') [350,351] software package on a

MacBook Pro (Version 10.13.6, 13-inch, 2.7 GHz, 2560 x 1600). Gamma correction was conducted using a Spyder photometer and the FrACT software provided 10 bits of contrast resolution The Landolt-C optotype was presented at 8 possible orientations and viewed from 3 m in a dark room. Participants identified the gap orientation using button presses. Trials were self-paced with a maximum display time of 30 s. A Bayesian adaptive ("Best PEST") algorithm controlled optotype size for the crowded and uncrowded visual acuity threshold measurements and optotype contrast for the contrast sensitivity threshold measurement. Each threshold measurement lasted approximately 3 mins. For the measurements made during stimulation, threshold measurement started 5 minutes into the stimulation. Landolt-C gap width was fixed at 30 arcmin for the non-amblyopic eye and 100 arcmin for the amblyopic eye during the contrast sensitivity measures. These parameters were based on pilot observations in individuals with moderate and severe amblyopia who could not resolve the 30 arcmin stimuli. Crowded optotypes were surrounded by a circle. Both the fellow eye and amblyopic eye were tested monocularly with the fellow eye tested first within each block. Uncrowded visual acuity was tested first within each block followed by crowded visual acuity and contrast sensitivity.

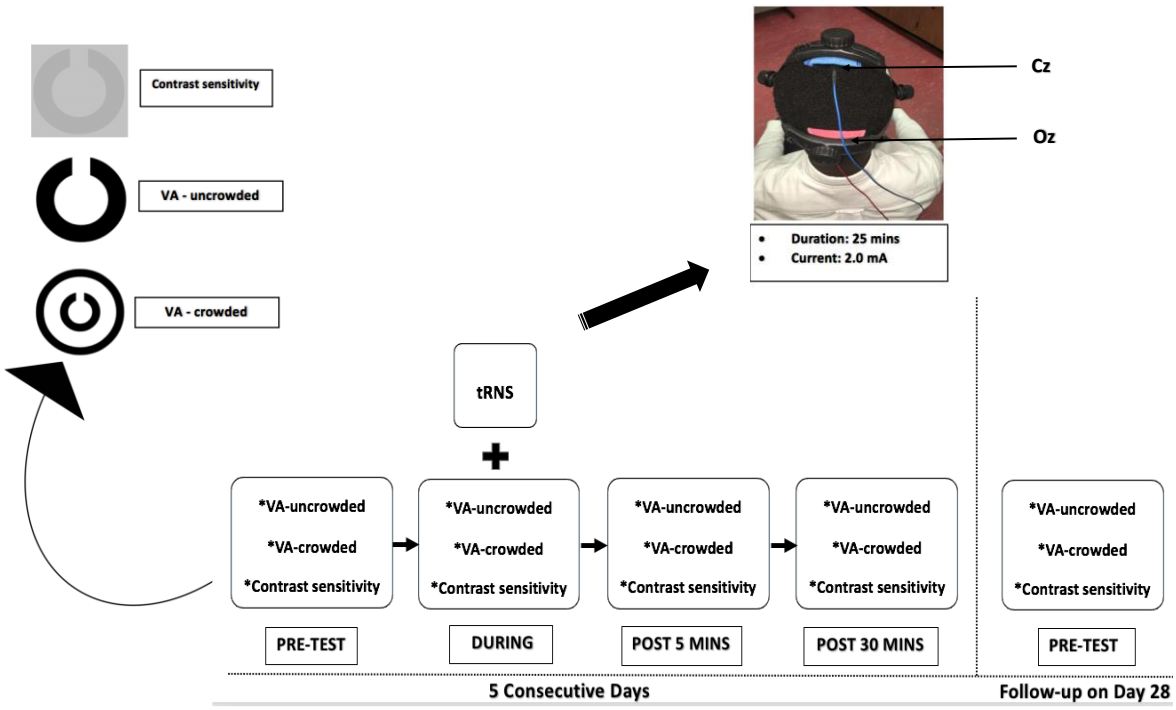


Figure 16: Testing and stimulation protocol. Each measurement was recorded before stimulation (pre-test), during stimulation, 5 min after stimulation (Post 5 mins) and 30 min after stimulation (Post 30 mins) for 5 consecutive days (middle column) Baseline (pre-test) measurements were recorded again for each eye 28 days (Day 28) after the last stimulation session. Stimulation was delivered for 25 mins at 2.0mA (right column). Active and reference electrodes were placed at Oz and Cz respectively. VA = visual acuity.

5.3 Data Analysis

Statistical analyses were performed in R (R Core Team, 2020) [352] using the Bootstrap-Coupled Estimation package [353]. Visual acuities were recorded in logMAR units. Contrast sensitivity was recorded in log units. To test for tRNS effects within the 5 stimulation sessions, a mixed-effects analysis of variance (ANOVA) with a between-subjects factor of

Group (active vs sham), a within-subjects factor of Day (day 1-5), and a within-subjects factor of Time (baseline, during, post 5 and post 30 mins) was conducted for each measurement type for each eye separately. Planned pairwise comparisons (least significance difference test) between baseline and all other timepoints were examined for each day. In addition, to assess whether tRNS had cumulative or long-term effects on visual function, a mixed ANOVA with factors of Group (active vs sham) and Baseline (baseline day 1, baseline day 2, baseline day 3, baseline day 4, baseline day 5, baseline day 28) was conducted for each outcome measure for each eye. All ANOVA analyses reported passed Levene's test for equality of variances ($p>.05$) and test of sphericity ($p>.05$).

Pairwise comparisons were conducted using the effect size Hedge's g by bootstrap estimation (5000 bootstrap samples with replacement), with the 95% confidence interval around the g being bias-corrected and accelerated [353]. The permutation P values reported are calculated with 5000 reshuffles of the baseline and test labels performed for each permutation, with the P-value indicating the likelihood of observing the mean difference, if the null hypothesis of zero difference is true, at an a of .05. Between group differences in the strength of any sensations induced by tRNS were assessed using the chi-squared test.

Table 3: Subjective experiences reported by participants after the day 5 active or sham tRNS session.

	Mild	Moderate	Chi-Square (X^2)
Headache			
Active (N=9)	1	4	0.060

Sham (N=8)	2	0	
Neck pain			0.466
Active (N=9)	2	0	
Sham (N=8)	1	0	
Scalp pain			0.279
Active (N=9)	1	0	
Sham (N=8)	0	0	
Tingling sensation			0.460
Active (N=9)	4	3	
Sham (N=8)	6	1	
Itching			0.277
Active (N=9)	4	2	
Sham (N=8)	5	0	
Burning sensation			0.074
Active (N=9)	2	3	
Sham (N=8)	1	0	
Sleepiness			0.751
Active (N=9)	2	1	
Sham (N=8)	3	2	
Trouble concentrating			0.330
Active (N=9)	0	0	
Sham (N=8)	1	0	
Acute mood change			0.330
Active (N=9)	0	0	
Sham (N=8)	1	0	
Others	Jaw stiffness	Neck tension	0.289
Active (N=9)	1	1	

Sham (N=8)	0	0		
Any of the symptoms related to tES	Remote	Probable	Definite	0.073
Active (N=9)	0	3	6	
Sham (N=8)	2	0	6	

5.4 Results

Nineteen healthy adults with amblyopia were recruited. Two participants in the sham group withdrew from the study after day 1 due to the time commitment required and were excluded from the analysis (**Figure 15**). Clinical details of the remaining seventeen participants are provided in (**Table 2**). A technical error prevented an accurate amblyopic eye contrast sensitivity measurement on day 1 for one participant in the active group. This participant was excluded from the amblyopic eye contrast sensitivity analysis only (**Figure 15**). There was no statistically significant group difference in age for the 17 participants that completed the study (Active group mean age = 38.7 yrs., SD = 13.6; Sham group mean age = 45.8 yrs., SD = 15.4; $t_{15} = 1.0$, $p = 0.3$). There were no adverse effects of tRNS, and there were no statistically significant between-group differences in the range or severity of subjective sensations reported (**Table 3**).

5.4.1 Contrast Sensitivity

For the amblyopic eyes (**Figure 17**- upper panel), there was a significant interaction between Group and Time, $F_{3,42} = 3.584$, $p = .022$, $\eta_p^2 = .216$. No other omnibus main effects or interactions were significant. Planned pairwise comparisons between baseline and all other timepoints were examined for the active and sham groups for each day. During day 1, the

active group exhibited a significant improvement in contrast sensitivity from baseline for all post-test measurements (during: g =.272 [.195, .597], p = 0.01; post 5 min: g =.236 [.039, .726], p = .035; and post 30 min: g = .438 [.052, 1.207], p = 0.034: **Figure 18**). No significant differences between baseline and any post-test were found for days 2-5. No significant differences between baseline and any post-test were found within the sham group for any day.

For the fellow eyes (**Figure 18**- lower panel), there was a significant interaction between Group and Time, $F_{3,45} = 3.303$, p = .029, $\eta_p^2 = .191$. No other omnibus main effects or interactions were significant. During day 1, the active group exhibited a significant improvement in contrast sensitivity from baseline for the post 5 min (g = .639 [.127, 1.248], p = 0.033) and post 30 min (g = .846 [.199, 1.661], p = 0.018) measurements. No significant differences between baseline and any post-test were found for days 2-5. No significant differences between baseline and any post-test were found within the sham group for any day.

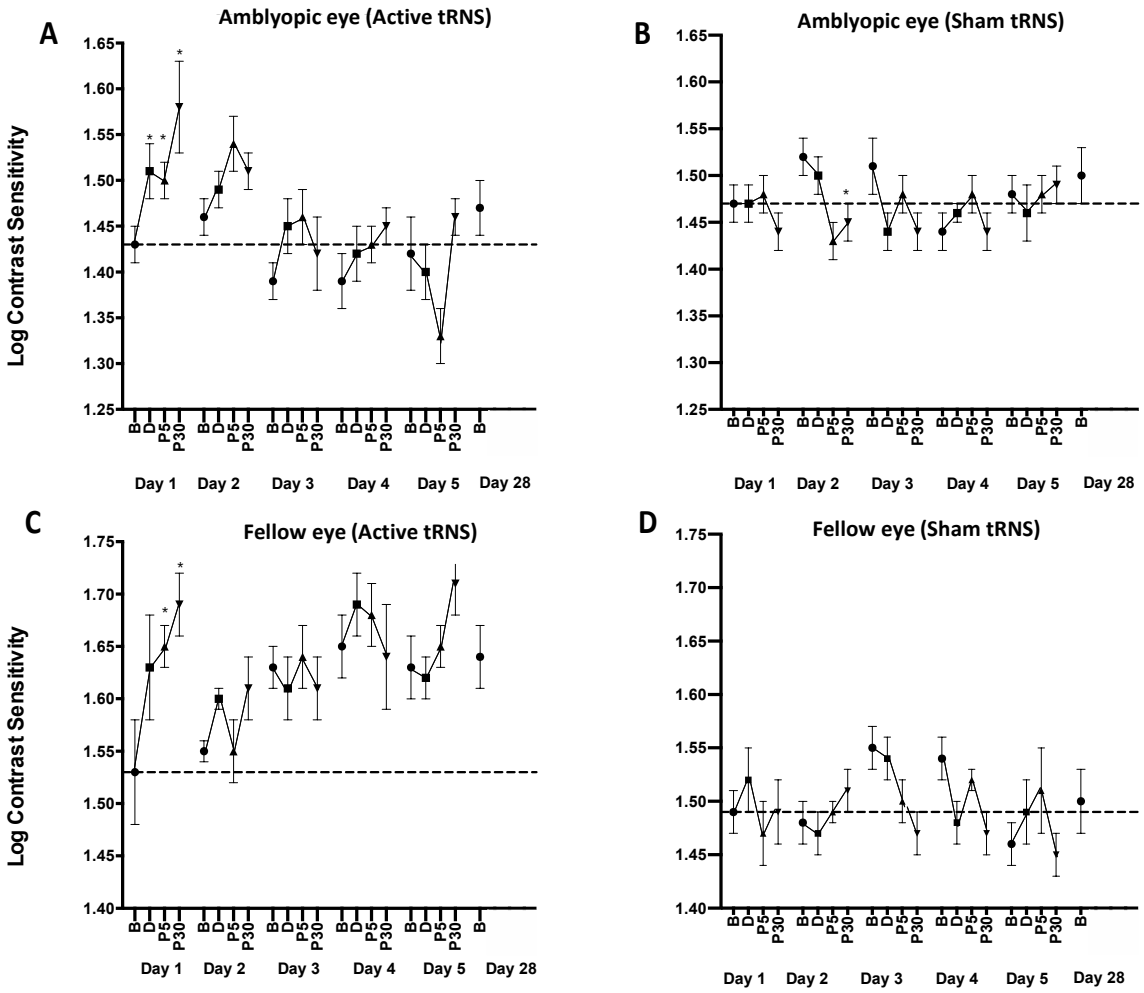


Figure 17: The effects of tRNS on contrast sensitivity during each daily session and at the day 28 follow-up visit. Data are shown separately for the amblyopic (top row) and fellow (bottom row) eyes and for the active (left column) and control (right column) groups at baseline (B) and during (D), 5 min (P5) and 30 min (P30) post tRNS. *Statistically significant difference from baseline ($p < 0.05$). Error bars show within-subject standard error of the mean (SEM). The dashed horizontal lines represent the mean before-stimulation threshold on day 1. Larger y-axis values indicate better contrast sensitivity.

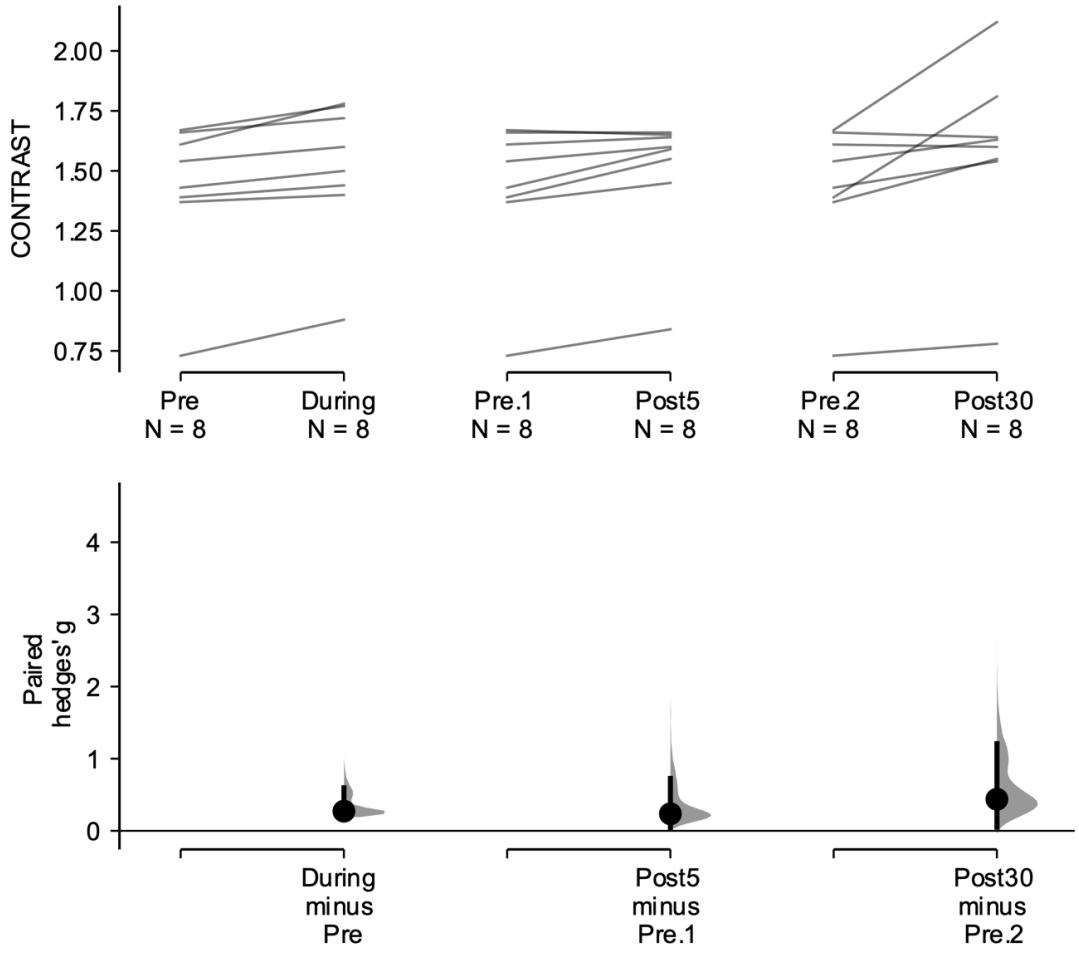


Figure 18: Paired Hedges' g for three comparisons (during stimulation, Post 5 mins, Post 30 mins) to pre-test contrast sensitivity are shown using a Cumming estimation plot. Raw contrast threshold data for each participant are plotted on the upper axes; each paired set of observations is connected by a line. On the lower axes, paired Hedges's *g* is plotted as a bootstrap sampling distribution. Hedge's *g* value is depicted as dots; 95% confidence intervals are indicated by the ends of the vertical error bars.

5.4.2 Uncrowded Visual Acuity

For the amblyopic eyes (**Figure 19**- upper panel), there was a significant interaction between Group and Time, $F_{3,45} = 3.325$; $p = .029$, $\eta_p^2 = .192$). No other omnibus main effects or interactions were significant. During day 1, the active group exhibited a significant improvement in uncrowded visual acuity from baseline for all post-test measurements (during: g =.224 [.084, .575], p=.010; post 5: g = .281 [.009, .640], p= .05; post 30: g = .307 [.118, .795], p = .003). During days 2 and 3, the active group exhibited a significant difference between baseline and only the post 5 min measurement (day 2: g = .231 [.091, .383], p = .015, day 3: g = .126 [.003, .304], p = .038). No significant differences between baseline and any post-test were found during days 4 and 5. No significant difference between baseline and any post-test was found within the sham group for any day. By chance, there was a substantial difference in baseline uncrowded Landolt-C visual acuity between the active and sham group (compare the dashed lines in **Figure 19**- upper panel).

For the fellow eyes (**Figure 19**- lower panel), there was a significant interaction between Group and Time, $F_{3,45} = 3.504$; $p = .023$, $\eta_p^2 = .200$. No other omnibus main effects or interactions were significant. During day 1, the active group exhibited a significant improvement in contrast sensitivity from baseline for the post 5 (g = .817 [.164, 1.75], p = 0.035) and post 30 (g = .774 [.199, 1.54], p = 0.02) min measurements. No significant differences between baseline and any post-test were found for days 2-5. No significant differences between baseline and any post-test were found within the sham group for any day.

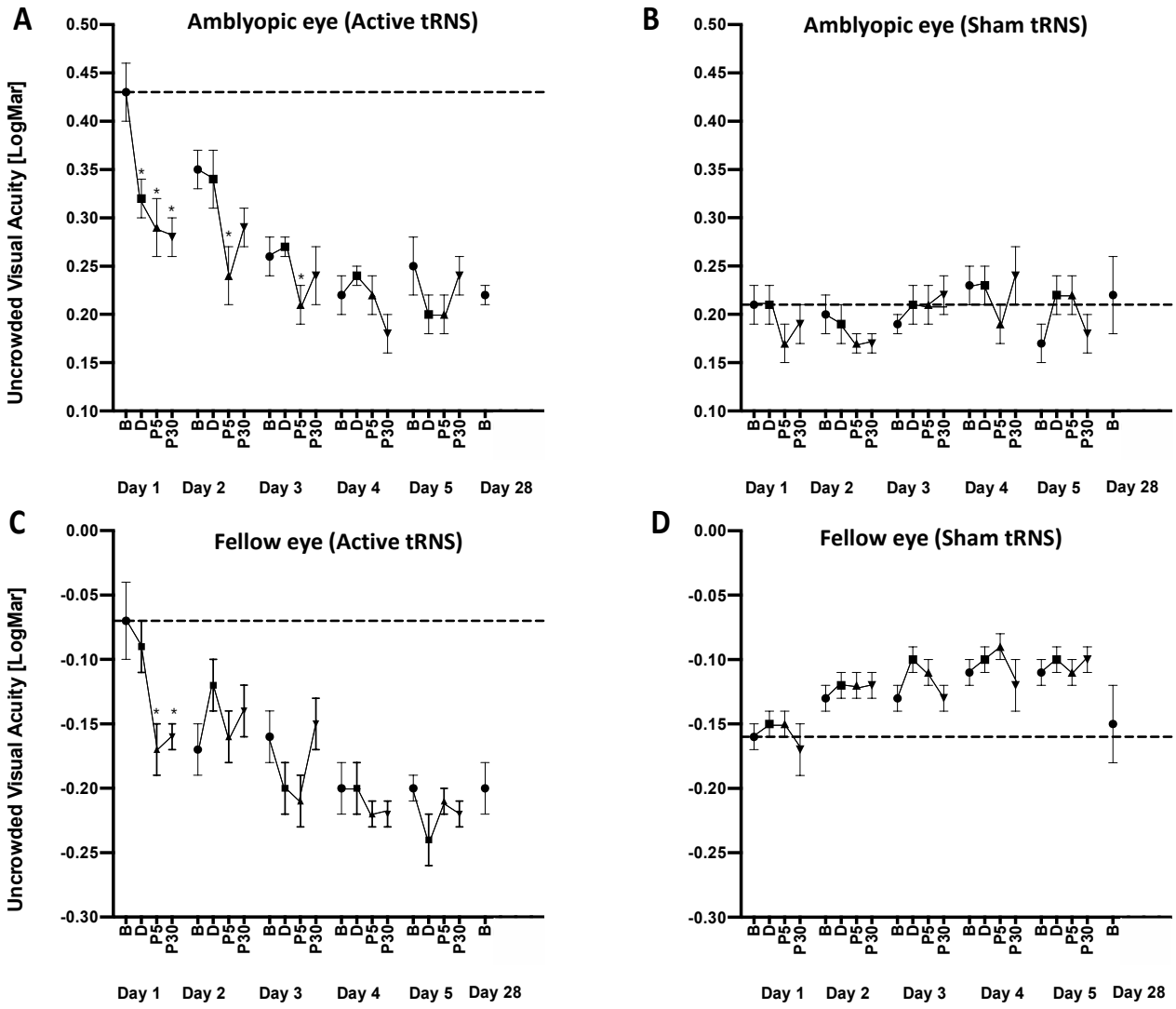


Figure 19: The effects of tRNS on uncrowded visual acuity during each daily session and at the day 28 follow-up visit. Data are shown as in Figure 33. Lower (smaller/more negative) y-axis values indicate better uncrowded visual acuity.

5.4.3 Crowded Visual Acuity

For the amblyopic eyes (**Figure 20**- upper panel), there were no significant main effects or interactions (all $p > 0.05$). No significant changes from baseline were observed for any day for any group. As for uncrowded visual acuity, there was a substantial difference in baseline performance between the two groups that occurred by chance during randomization.

For the fellow eyes (**Figure 20**- lower panel), there was a significant interaction between Group and Time, $F_{3,45} = 5.733$; $p = .002$, $\eta_p^2 = .291$. No other omnibus main effects or interactions were significant. During day 1, the active group exhibited a significant improvement in crowded acuity from baseline for the post 5 ($g = .404$ [.083, .9], $p = 0.05$) and post 30 min ($g = .457$ [.09, .913], $p = 0.039$) measurements. During days 3 and 5, the active group exhibited a significant improvement in crowded acuity from baseline to post 5 min ($g = .389$ [.065, 1.06], $p = .007$) and post 30 min ($g = .721$ [.047, 1.4], $p = .044$) respectively. No significant differences between baseline and any post-test were found for days 2 and 4. No significant differences between baseline and any post-test were found for the sham group.

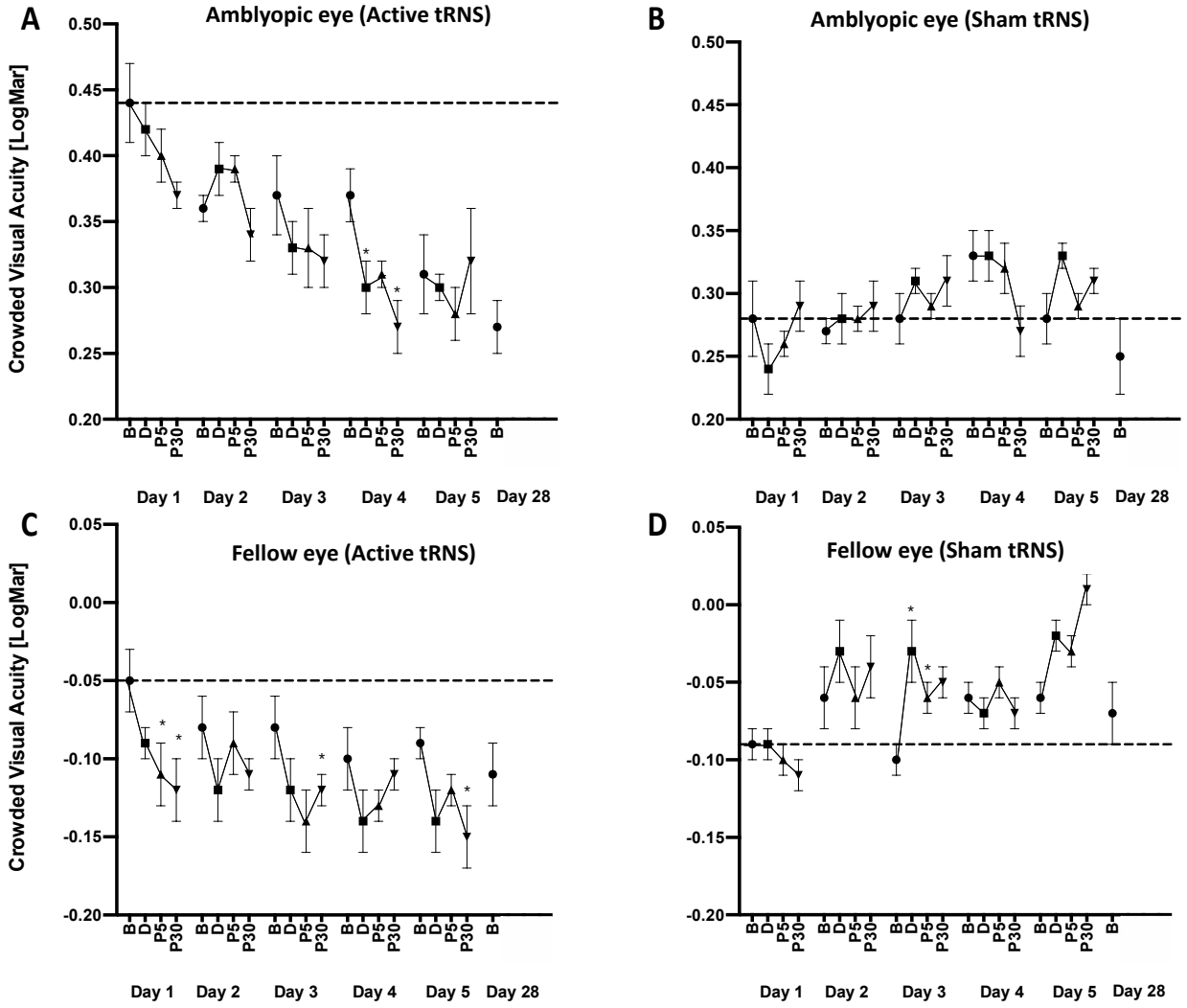


Figure 20: The effects of tRNS on crowded visual acuity during each daily session and at the day 28 follow-up visit. Data are shown as in Figure 33. Lower (smaller/more negative) y-axis values indicate better crowded visual acuity.

5.4.4 Cumulative and long-term effects of tRNS

For the amblyopic eyes, there was a significant interaction between Group and Baseline for uncrowded visual acuity ($F_{5,65} = 3.372$; $p = .009$, $\eta_p^2 = .206$). Pairwise comparisons for the

active group revealed a significant difference between the day 1 baseline and the day 3 (g = .372 [.163, .771], p = 0.011), day 4 (g = .461 [.243, .93], p < 0.003), day 5 (g = .369 [.065, .809], p = 0.034), and day 28 (g = .454 [.219, 1.03], p = 0.003) baselines. However, no pairwise comparisons were significant for the sham group. There were no significant interactions for amblyopic eye contrast sensitivity or crowded visual acuity measurements or any of the fellow eye measurements.

5.5 Discussion

Our results partially supported our experimental hypothesis that five daily sessions of visual cortex tRNS would improve amblyopic eye contrast sensitivity as well as crowded and uncrowded visual acuity in adult patients. We observed tRNS-induced improvements in contrast sensitivity and uncrowded visual acuity relative to the sham group for both amblyopic and fellow eyes. Crowded visual acuity improved for the fellow but not the amblyopic eyes. Across all outcome measures, pairwise comparisons revealed that acute tRNS effects were statistically significant on day 1 but became non-significant for later sessions. Only amblyopic eye uncrowded visual acuity exhibited a lasting effect of tRNS at follow-up. Our discussion will focus primarily on the results for contrast sensitivity because initial baseline performance was matched between the groups. There were pronounced between-group baseline differences for amblyopic eye uncrowded and crowded visual acuity that occurred by chance during the randomization procedure (randomization occurred before baseline measures were conducted). The difference in baseline performance for the acuity outcome measures make it difficult to properly segregate tRNS effects from task learning

effects. One reason for these baseline differences might be a difference in the proportion of patients with anisometropic amblyopia in active (78%) and sham (50%) groups. However, a much larger scale study will be required to determine whether amblyopia subtype influences the response to visual cortex tRNS.

5.5.1 tRNS-induced improvements in contrast sensitivity

Our observation that visual cortex tRNS improved amblyopic eye contrast sensitivity is consistent with a growing literature reporting improved contrast sensitivity, visual acuity, stereopsis, and an enhanced cortical response to amblyopic eye inputs following non-invasive visual cortex stimulation in adults with amblyopia [8,10,20–22,89,90]. A number of potential mechanisms have been proposed for tRNS effects. These include stochastic resonance and changes in the resting membrane potential [4,28]. Stochastic resonance refers to an improvement in signal to noise ratio when a certain amount of noise (in this case neural noise induced by tRNS) is added to non-linear systems [348]. A number of psychophysical studies have provided compelling evidence that stochastic resonance occurs during visual cortex tRNS [108,309,354,355]. It is possible that the during-stimulation improvements we observed on day 1 for amblyopic eye contrast sensitivity were due to stochastic resonance. However, tRNS aftereffects (i.e., effects that outlast the duration of stimulation) cannot easily be explained by stochastic resonance.

Terney et al.,2008 proposed that increased motor cortex excitability following tRNS is related to the activity of sodium channels within the neural membrane [4]. Specifically, they

proposed that tRNS may cause repetitive membrane depolarization that is sufficient to repeatedly open sodium channels but sub-threshold for generating an action potential. These synchronized local depolarizations were further hypothesized to induce lasting long-term potentiation-like effects at the level of individual neurons. However, a subsequent study found that pharmacological manipulation of NMDA receptors had no effect on tRNS aftereffects, whereas the GABA agonist lorazepam and carbamazepine, a sodium channel blocker, attenuated tRNS aftereffects [171]. These results are not consistent with a mechanism related to long-term potentiation but do support the involvement of sodium channels. Alternative mechanisms for the effects of electrical stimulation have also been proposed including regional increases in cortical blood flow [208], modified brain connectivity [241] and changes in neurotransmitter concentration [168,169]. The precise underlying mechanism for the tRNS aftereffects we observed remains to be determined.

A previous study [36] reporting improved amblyopic eye contrast sensitivity following both excitatory and inhibitory visual cortex rTMS proposed a mechanism linked to cortical homeostasis. According to this hypothesis, excitatory stimulation has a more pronounced effect on weakly activated/suppressed neural populations whereas inhibitory stimulation has a greater effect on strongly activated populations. Therefore, both excitatory and inhibitory stimulation are capable of restoring a level of homeostasis to the amblyopic visual cortex by reducing the difference in activation between amblyopic eye dominated neurons (weak activation/suppression) and fellow eye dominated neurons (strong activation) [21]. This, in turn, reduces suppression and/or the relative attenuation of amblyopic-eye-driven neural

activity. It is plausible that the excitatory tRNS we employed in this study acts through a homeostatic mechanism.

We also observed improved fellow eye contrast sensitivity in the tRNS group relative to the sham group. Non-invasive visual cortex stimulation studies have reported varying fellow eye effects. Studies using inhibitory stimulation protocols (1 Hz rTMS and continuous theta burst stimulation; cTBS) have reported reduced fellow eye contrast sensitivity [35, 36] whereas those using excitatory protocols (anodal tDCS and tRNS) [22,108], including the present study, observed improvements. This pattern of results is consistent with the homeostasis hypothesis which predicts relatively impaired fellow eye function following inhibitory stimulation and does not rule out improved fellow eye function following excitatory stimulation. This is because excitatory effects may still occur within neuronal populations dominated by the fellow eye, just to a lesser extent than those dominated by the amblyopic eye.

5.5.2 Successive and cumulative tRNS effects on contrast sensitivity

A day by day analysis of the contrast sensitivity data revealed that tRNS effects were pronounced for both eyes on day 1. However, the within-session tRNS effects waned across sessions, becoming non-significant by day 2 for both eyes. This reduction in within-session tRNS effects was accompanied by stable session to session baseline performance indicating the absence of a cumulative tRNS effect on contrast sensitivity. The waning of within-session effects is consistent with Clavagnier et al's [40] study of repeated cTBS sessions in

amblyopia, however cTBS did induce cumulative effects that improved baseline performance across sessions. One possible explanation for the waning of within-session tRNS effects and the absence of a cumulative effect on contrast sensitivity relates to stimulation intensity. The relationship between tRNS intensity and visual function improvement during stimulation is an "inverted U", whereby stimulation that is weaker or stronger than an optimum level has limited effects [28,308]. Lasting changes in cortical excitability induced by prior sessions of tRNS might shift the optimal stimulation intensity towards lower levels, causing a waning of tRNS effects across sessions if stimulation intensity remains constant. If this is the case, tapering stimulation intensity across sessions would be a possible solution.

Another possible explanation for an effect on day 1 and not subsequent days is a placebo effect. Although this cannot be completely ruled out, the use of a single masked, between subjects design combined with automated collection of outcome measures was intended to minimize this source of bias. In addition, participant reported sensations did not differ significantly between the two groups suggesting the adequate masking was preserved throughout the study.

5.5.3 tRNS effects of crowded and uncrowded visual acuity

It is not possible to draw strong conclusions relating to the amblyopic eye datasets for crowded and uncrowded visual acuity because there were large between-group differences in baseline performance that occurred by chance. However, baseline group differences were minimal for the fellow eye datasets and the results followed those for contrast sensitivity very

closely; significant differences between groups that were characterized by within-session improvements early in the experiment and a gradual waning of tRNS effects. This suggests that transient tRNS effects occur for a range of visual functions and that long lasting effects may occur for uncrowded visual acuity.

5.6 Study limitations

The primary limitation of this study is the relatively small sample size. However, there is no indication in our data that the lack of long-term effects of visual cortex tRNS on amblyopic eye contrast sensitivity is due to insufficient statistical power. We had sufficient power to detect an effect of tRNS on day 1 and this effect waned across subsequent sessions. The small sample size did preclude the use of stratification for amblyopia subtype and baseline clinical characteristics within our randomization procedure and this likely contributed to the between group differences in baseline amblyopic eye visual acuity that are present in our data.

5.7 Conclusions

tRNS can induce short-term contrast sensitivity improvements in adult amblyopic eyes, however sessions of tRNS with a fixed set of stimulation parameters do not lead to enhanced or long-lasting effects. In agreement with previous non-invasive brain stimulation studies, these results demonstrate considerable short-term plasticity within the visual cortex of human adults with amblyopia and identify new pathways for future research such as the modification of stimulation parameters across sessions to maximize cumulative stimulation effects and the exploration of specific rather than random stimulation frequency bands.

Chapter 6: General Discussion

This work investigated tES innovative treatment modality for adults with amblyopia. In particular, the overall objective of this work was to assess whether tES can be used as a potential treatment option for amblyopia in adult humans. Firstly, we conducted a pilot study to identify an effective tES protocol for modulating visual function in healthy adults by comparing anodal-tDCS and full spectrum tRNS with sham stimulation. The rationale of our pilot study was based on comparative studies that demonstrated tRNS induced greater cortical excitability changes than other tES modalities[97,261,262]. However, this pilot study did not find significant effects on contrast sensitivity for either tES protocol among healthy adults. This may be due to the small sample size, inter and intra-individual differences, the stimulation parameters employed or the fact that an optimal contrast sensitivity threshold had already been reached within healthy subjects. With regards to sample size, our pilot study involved a between subjects design with 6 subjects per group. This sample may not have provided sufficient statistical power to detect any significant effects of tDCS or tRNS on visual function. While our study recruited limited subjects, it is consistent with a prior study by Antal et al.[7] that recruited a slightly larger number of subjects (sample size, n = 15) but reported that anodal tDCS did not reveal significant effects on contrast sensitivity in healthy adults. Similarly, Brükner et al.[356] found no significant effect of tDCS on phosphene perception in healthy adults (sample size, n = 32). Therefore, although it is possible that the small sample size may have led to a type II error, compared with similar prior studies in healthy adults, the small sample size may not have been the only contributing factor to the null effect of tES in healthy subjects. In fact, inter and intra-individual variability in subjects

may play an important role in detecting reliable effects of tES protocols. Inter-individual variability has been studied by varying stimulation parameters (current intensity, stimulus duration, electrode size, positions and polarity). Nitsche and Paulus[88] reported that increased tDCS duration and amplitude led to larger and longer lasting effects on cortical excitability. However, recent studies have indicated a more complex relationship between the duration and intensity of stimulation and changes in cortical excitability. For example, a non-linear relationship between current intensity and the stimulation efficacy of tES has been reported whereby changes in stimulation intensity and stimulation modality (Paired Associative Stimulation (PAS25), Anodal transcranial DC stimulation (A-tDCS) and intermittent theta burst stimulation (iTBS)) did not alter the effect of stimulation on MEP amplitude or short intracortical inhibition (SICI)[247]. Specific intra-individual factors such as gender, age, gene phenotype and hormonal levels can also affect the extent of neuroplasticity enhancement induced by NIBS[246,248,357–361]. Alternatively, healthy participants may already have optimal contrast sensitivity for the stimuli we used, hence, tES could not induce an effect beyond the already existing maximum contrast sensitivity threshold within the primary visual cortex. For instance, studies in adults with amblyopia have reported significant effects of tDCS or tRNS in inducing neuroplastic changes revealed in measurable visual function changes[89,108,264]. Although different stimuli were used, these results support the idea that tES protocols can elicit significant contrast sensitivity improvements in amblyopia which is characterized by reduced contrast sensitivity prior to stimulation[22,42,47,227,344].

Experiment II involved an objective assessment of tRNS effects on visual cortex excitability. We chose tRNS as the stimulation protocol for Experiment II because previous tES protocol comparative studies had reported greater efficacy of tRNS effects compared to tDCS and tACS [97,261,262].

We investigated the effects of transcranial random noise stimulation on primary visual cortex excitability in healthy adults using EEG. The aim of the study was to test the hypothesis that active visual cortex tRNS in human adults would induce an acute increase in visual cortex excitability as evidenced by increased VEP amplitude. This was the first time the effects of tRNS of the visual cortex had been measured objectively using modulation of VEP amplitude. Previous studies have used changes in VEP[9,22,90,281,362], auditory evoked potential (AEP)[363], MEP[88,364–366] and somatosensory evoked potential (SEP)[367] amplitudes to investigate the effect of tDCS on cortical excitability changes. tDCS is a polarity-dependent tES modality in which anodal stimulation increases cortical excitability whereas cathodal stimulation decreases cortical excitability. However, previous studies reported opposite effects of tDCS on cortical excitability measured using evoked potentials in different regions of the brain. For instance, studies using MEPs reported that effect of anodal-tDCS depressed cortical excitability[365], but in contrast effects of anodal-tDCS increased[364] cortical excitability, in human primary motor cortex. Also, studies using SEPs and AEPs reported similar trend of increased cortical excitability following anodal-tDCS cortex[363]. In the visual cortex, Antal et al.[9,362] reported contrasting polarizing effects of anodal and cathodal-tDCS for N70 and P100 VEP components, whereby cathodal stimulation

decreased the amplitude of the N70 component whereas anodal stimulation increased the amplitude of the N70 component. Contrarily, the P100 waveform component increased in amplitude with cathodal stimulation whilst anodal stimulation had no effect [9]. In order to address the conflicting findings reported from previous studies that used tDCS, it was essential to use a non-polarity dependent form of tES, in this case tRNS, to investigate NIBS effects on visual cortex excitability.

Our results revealed that visual cortex tRNS induced visual cortex excitability changes in healthy human adults. In particular, we found significant a VEP increase for lateral electrodes, however, other electrodes showed non-significant increase for active tRNS whereas sham tRNS resulted in VEP amplitude decreases post stimulation. The relative decrease in VEP amplitude post tRNS for the sham group could have been due to habituation and/or fatigue effects [181,327]. VEP habituation in control subjects occurs as a result of repeated checkerboard visual stimulation [313,328–330]. Specifically, when control subjects viewed pattern reversing checkerboards repeatedly for 15 mins there was a gradual decrease in VEP amplitudes[313]. We observed habituation of the VEP amplitude in the sham tRNS condition and habituation probably caused an underestimation of the VEP increase induced by active tRNS for both right and left eye conditions. Also, our findings of significant VEP increase at a lateral electrode was expected due to the phenomenon of paradoxical laterization of hemi-field pattern reversal VEPs as reported earlier by Barret et al.[311], whereby higher amplitude visually evoked responses occur at the occipital electrodes placed over the hemisphere ipsilateral to the pattern reversal stimulus. Paradoxical localization occurs because the electric fields underlying of pattern-reversal VEPs are primarily radially

oriented to the generator region which includes the medial surface of the occipital cortex. Thus, electrodes placed over the contralateral scalp are more closely aligned with the axis of this generator region and therefore recording minimal evoked responses [311,312,334]. Our findings are consistent with previous studies [9,22,90,281,362] that used VEPs to investigate the effects of visual cortex transcranial direct current stimulation. Hence, our study supported the hypothesis that tRNS can induce neuroplastic changes in excitability within the occipital cortex of healthy adults.

Lastly, building on our VEP findings, we tested the hypothesis that five daily sessions of visual cortex tRNS would lead to improved amblyopic eye contrast sensitivity, crowded and uncrowded amblyopic eye visual acuity in adult patients. Our aim was to replicate findings from other studies that demonstrated acute effects of single session visual cortex tDCS on modulation of contrast sensitivity in amblyopic eyes[21,22] and expand on these earlier findings to assess the possibility of long lasting effects with multiple stimulation sessions. Our day 1 session revealed reliable acute effects whereby tRNS improved contrast sensitivity and visual acuity in both amblyopic and fellow eyes. Studies from animal and humans showed that an improvement in visual function in amblyopia is associated with an enhancement in visual cortex neuroplasticity[10,62,368]. In agreement with previous non-invasive brain stimulation studies[21,22], these results demonstrate considerable short-term plasticity within the visual cortex of human adults with amblyopia. Therefore, our day 1 session findings support the evidence that NIBS can be used as a potential tool to promote neuroplasticity in adults with amblyopia. We also aimed to assess the possibility of long-

lasting and sustained effects of tRNS on visual function in the amblyopic eye following repeated sessions. Our findings showed that tRNS did not induce sustained or long-lasting effects on visual function improvements in adult with amblyopia following repeated sessions of tRNS. This may be due a number of reasons including the choice of stimulation intensity, heterogenicity of amblyopic patients and type of stimuli used. Van der Groen et al.[28] and Pavan et al.[308] showed limited effects induced by stimulation intensity that is weaker or stronger than an optimal stimulation level. We applied the stimulation intensity to the visual cortex across each day which might have shifted the optimal stimulation intensity towards lower levels, causing a waning of tRNS effects across sessions. The possible option to avoid limited effects could have been tapering stimulation intensity across daily sessions.

Also, we recruited a heterogeneous sample of participants with amblyopia including different types of amblyopia which may have contributed to unreliable effects of tRNS across each day. Recruitment of patients was challenging and therefore a homogenous sample was not possible. Different forms of visual functions in amblyopia are affected differently by the type of amblyopia[42,369]. For instance, strabismic amblyopia greatly affects binocular vision, specifically stereoacuity, but less affects contrast sensitivity and monocular spatial resolution, whereas anisometropic amblyopia greatly affects fixational stability and contrast sensitivity but less affects binocular vision, in particular stereoacuity and binocular motion sensitivity[54,227,342,369]. These differential deficits may result from differential effects of strabismic and anisometropic amblyopia at the lateral geniculate nucleus (LGN), whereby parvocellular and magnocellular pathways are affected differently. The parvocellular pathway is mainly responsible for processing low contrast, high spatial frequency and low

temporal frequency stimuli, and vice versa for magnocellular pathway[370]. Therefore, effects of amblyopia on LGN, for example the parvocellular pathway, result in reduced ability to process luminance contrast sensitivity for stimuli of high spatial frequencies and low temporal frequencies.

We used Landolt-C optotype stimuli to assess the effects of tRNS. Our choice of stimuli may have affected the outcome of the long-lasting effects across daily sessions. Previous studies have used grating stimuli or gabor patch stimuli as the outcome measure to assess NIBS effects. Studies that used grating stimuli tested the subjects with either low or high spatial frequency. However, gabor patches or Landolt C optotype are composed of a mixture of low to high spatial frequencies[351,371], capable of detecting different levels of contrast sensitivity deficits in amblyopia. We selected Landolt-C stimuli in all the experiments because it is used as a clinical outcome measure in the optometric clinic. Therefore, we reasoned that the use of these stimuli would allow us to translate tES protocols from research to clinical settings in the longer term.

We have demonstrated the tRNS can induce short-term visual function and VEP improvements in adults with amblyopia and healthy adults. However, repeated sessions of tRNS with a fixed set of stimulation parameters, a heterogeneous amblyopia group and a relatively small sample size do not lead to enhanced or long-lasting effects. In agreement with previous non-invasive brain stimulation studies, these results reveal substantial acute neuroplasticity within the visual cortex of human adults with amblyopia.

This work has identified a number of limitations that may have hindered the induction of significant long-lasting effects of visual cortex tRNS across several days. These limitations include, but not limited to, a fixed stimulation protocol, heterogeneous amblyopia subjects, sample size, stimulation of primary visual cortex and task stimulus. Future research should employ alternative NIBS protocols, perhaps with varying stimulation intensities and duration, to assess long-lasting or sustained effects. Also, we suggest that future studies should incorporate the combination of NIBS protocols with perceptual or dichoptic training. To reduce inter-individual variability, recruiting homogeneous amblyopia subjects especially focusing on recruiting subjects with same type of amblyopia, similar magnitude of visual function deficits and subjects who had received similar prior treatment such as patching, may contribute to assessing long-lasting NIBS effects. Recruitment of adults with amblyopia is a major challenge, however, we suggest the future studies should pay attention to sample size. A larger sample in a randomized controlled clinical trial would provide more statistical power to avoid a type II error. Lastly, our EEG study showed that visual cortex tRNS resulted in significant aftereffects on cortical lateral electrodes, not at the locations with the largest amplitudes. Therefore, we suggest that future research should evaluate the combined impact of NIBS across channels using source localization techniques to increase power. As amblyopia affects multiple levels of visual processing, the location of the NIBS stimulation could be systematically altered to explore stimulation of not only to the primary but also to extrastriate visual cortices.

EEG could be used as a short- and long-term outcome measure in future studies with amblyopic participants. VEPs from amblyopic eyes are markedly diminished and delayed and they improve in line with improvements in visual function with treatment in children. Thus, any normalization of VEPs induced by NIBS is likely to reflect an impact on cortical plasticity as well as visual function in the participants with amblyopic. A well-controlled study design that incorporated our suggestions may elicit greater and reliable long-lasting or sustained neuroplastic effects within the visual cortex of adults with amblyopia.

6.1 Strengths and Limitations

This work investigated the efficacy of NIBS as a treatment regimen for adults with amblyopia. We showed that visual cortex tRNS induced short-term neuroplastic effects within the visual cortex as evident through enhancement of visual function in amblyopia and increased occipital VEP amplitude in healthy controls. The main limitation of this work was the relatively small sample size in each study. Recruiting for non-invasive brain stimulation studies is a major challenge and small sample sizes are prevalent in the literature. In the future, larger scale, multi-site studies will be required to increase statistical power and assess clinical efficacy. Future work may also involve different stimulation parameters (frequency range, current intensity, stimulation duration etc.) and/or combination of brain stimulation and perceptual learning techniques to enhance treatment effects.

6.2 Conclusion and future Directions

It is important investigate the effects of non-invasive brain stimulation on the visual cortex to understand the mechanisms that underly the modulation of cortical neurons and to enhance visual function in amblyopia. Our findings buttress the existing reports on the efficacy of NIBS in modulating visual cortex neuroplasticity in amblyopia. In agreement with previous non-invasive brain stimulation studies, our results demonstrate considerable short-term plasticity within the visual cortex of human adults with amblyopia. Future research should incorporate the modification of stimulation parameters, session frequencies and combinations of NIBS with additional therapies to increase the efficacy of NIBS in amblyopia treatment.

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Appendix A: Supplementary figures of averaged waveforms generated when the pattern reversal VEP stimulus was presented to the cortical hemisphere that received tRNS for the right eye viewing condition.