UNIVERSITÀ DEGLI STUDI DI TRENTO

Doctoral School in Cognitive and Brain Sciences
Center for Neuroscience and Cognitive Systems@UniTn, Istituto Italiano di Tecnologia

Studies of Cortical Plasticity in the Normal and the Diseased Brain

Advisor
Dott.ssa Lorella Battelli

Doctoral Candidate
Florian Herpich

Doctoral School in Cognitive and Brain Sciences
XXIX cycle

December 2016

UNIVERSITÀ DEGLI STUDI
DI TRENTO
CIMeC - Center for Mind/Brain Sciences

Table of Contents

Abstract

Despite the large amount of work that has been conducted since Donald's Hebb work and described in his famous dissertation "*The Organization of Behavior"* (Hebb, 1949), understanding the experience-dependent mechanisms of plasticity within the primary visual cortex (V1) remains a major priority. Although plasticity effects are strongest during the critical period (early development), studies on cortical plasticity from the last two decades have clearly demonstrated that the human brain is plastic and amenable to changes throughout life. Perceptual Learning (PL) is one of the most commonly used procedure to promote visual improvement in neurotypicals and recovery of functions in a variety of disorders. However, a common feature to most of the training protocols is that they require long time and a high number of sessions to show effective improvement. Recently, non-invasive brain stimulation (NIBS) techniques, specifically transcranial random noise stimulation (tRNS) and anodal tDCS, have been used to modulate activity within the visual cortex to enhance perceptual learning. However, the mechanisms of action and the long-term effects on learning are still unknown.

The questions this thesis work will address are the following: (1) can neuromodulatory techniques be used to boost visual perceptual learning in neurotypicals, which technique is the most effective and what are the long term effects on learning? (2) what are the potential underlying physiological mechanisms modulating cortical excitability of the visual cortex? and (3) contingent upon results from (1), can NIBS be used over early, peri-lesional visual areas during visual training to induce recovery of visuo-perceptual abilities in chronic partial cortical blindness (CB)?

I used tRNS coupled with visuo-perceptual training protocols to promote fast and sustained perceptual learning in neurotypicals. I then provide evidence that tRNS can increase cortical excitability of the visual cortex, measured by priming early visual areas with tRNS, before measuring phosphene threshold with single pulse TMS. Lastly, I provide preliminary evidence of the effectiveness of tRNS in promoting recovery of visual field deficits in partial cortical blindness.

Introduction

Brain plasticity, from the Greek word “Plastos” meaning molded, refers to the ability of the brain to potentially modify its neuronal structural connections and functioning in response to the sensory inputs coming from the environment. Already back in the nineteenth century, Donald Hebb in his famous dissertation “*The Organization of Behavior”,* wrote about the fascinating idea that information in the brain is stored by modifying the strength of neuronal connections (Hebb, 1949). Hebb described the ways in which synaptic connections vary in the visual cortex after repeated exposure to specific stimuli. The seminal experiments by Wiesel and Hubel (Wiesel & Hubel, 1963), which examined the consequences of temporary monocular deprivation (MD) in kittens, revealed that visual experience indeed modifies neuronal connections early in life.

Traditionally, supported by these studies about the critical period, brain plasticity has been thought to occur only during infancy and early development (Hensch 2005a, Hensch 2005b, Hubel & Wiesel, 1964). However, although it remains true that plasticity effects are strongest during childhood, a large amount of work in the last two decades clearly demonstrated that the adult human brain is plastic (Buonomano et al., 1998; Gilbert & Li, 2012; Li, Piëch, & Gilbert, 2004, Gold & Watanabe, 2010; Seitz & Dinse, 2007, Sale et al., 2011). Studies on adult non-human primates have shown that following binocular lesions of regions of the retina, V1 neurons receiving information from the lesioned areas start to shift their receptive fields (Abe et al., 2015; Gilbert & Wiesel, 1992) by remodeling their lateral connections in the perilesional areas through sprouting and pruning phenomena (Yamahachi et al., 2009).

Clinically, efficient neuroplasticity might be critical in determining optimal recovery after brain injury (e.g. after stroke), in order to regain independence to perform daily life activities (e.g. self-care, eating, dressing). Promoting cortical plasticity has become a hotly debated topic, particularly in the last couple of decades, where new experimental protocols have been proposed “to train” the healthy and diseased brain. Behavioral procedures such as training on visual tasks across multiple sessions have been used to promote recovery of functions in a variety of disorders. Perceptual Learning (PL) is one of the most commonly used procedure, whereby improvements in sensory discrimination is promoted through repeated exposure to the same stimuli that vary in difficulty as the subject learns (Levi et al.,2009; Nitsche et al., 2009; Sagi et al., 2011). Several mechanisms promoting these changes have been proposed as responsible for enhancing the effect of training,

such as attention and reward (Ahissar, 2001, Pascucci et al. 2015; Seitz & Watanabe, 2005, Wright et al., 2010), however a common feature to most of the training protocols is that they require extremely long time and a high number of sessions to show effective improvement.

Recently, noninvasive brain stimulation techniques, namely Transcranial Electrical Stimulation (tES), have been used to investigate and modulate visual perception and to promote perceptual learning. tES techniques are promising tool to boost the effect of training and one technique, in particular, has provided clear evidence to be the most effective in supporting improvements, transcranial random noise stimulation (tRNS). It is a relatively new form of brain stimulation, and studies have already shown how it can promote and sustain perceptual learning (Cappelletti et al., 2013, Fertonani et al., 2011, Camilleri et al., 2014, 2016) besides its clear modulatory effects on the motor cortex (Terney et al., 2008).

The overarching goal of this dissertation is to investigate new approaches to use Non-Invasive Brain Stimulation (NIBS) techniques coupled with training protocols, to promote neuronal plasticity in neurotypicals and in neurological patients. Although NIBS has traditionally been used to study causal brain-behavior relationship, more recent tES techniques offer interesting new approaches to modulate and *improve* behavior, indicating strong potential translational application in clinical practice. However, the mechanisms of action are still unclear.

The aims of this thesis work are the following:

Aim 1. **Prime and measure**. In order to contribute to a better understanding of the neurophysiological effects of different neurostimulation techniques, I will study the effect of combined tES with TMS. I will provide some insights into the neurophysiological effect of different tES techniques on Early Visual Areas (EVA). I will measure the magnitude and time course of tRNS and anodal tDCS on EVA excitability by measuring single pulse TMS-elicited phosphenes immediately after tRNS or tDCS preconditioning.

Aim 2. tES in cognitive training. I will study the effect of different neuromodulatory NIBS techniques upon cognitive training in neurotypicals. The goal is to measure the quality and duration of cortical stimulation coupled with perceptual learning, a traditional way of improving visuoperceptual abilities across multiple sessions. I will try to determine: (a) the

feasibility of the procedure, and (b) to compare the differential effects relative to sham and behavioral training alone.

Aim 3. **tRNS in stroke rehabilitation.** Contingent upon results from Aim 1 and 2, I will test the hypothesis that using NIBS over early, peril-lesional visual areas applied during visual discrimination training, can induce recovery of visuo-perceptual abilities in chronic partial cortical blindness (CB). The results, although preliminary, might recommend novel approaches to treat CB. While there is a substantial body of work for rehabilitation of motor impairments, protocols for CB are almost completely lacking, except visual restoration training, a protocol whose efficacy is controversial for the lack of good empirical evidence.

Chapter 1

In this first introductory chapter I will provide an overview of visual perceptual learning (VPL) studies. I will then briefly review the neuromodulatory effects induced by NIBS, with a particular emphasis on NIBS coupled with VPL. Subsequently, I will outline and shortly describe the techniques used in the experiments to induce and promote learning. While this chapter mostly concentrates on visual training and the different neuromodulatory techniques used in this thesis work, some space is also devoted to the anatomy of the visual system and the deficits deriving from cortical lesions to the early visual areas. However, some of these aspects will also be discussed in chapter four, where a preliminary study on stroke patients is presented.

Perceptual Learning

The adult brain retains a degree of plasticity over the entire life time, and particularly the visual system, the focus of this research thesis, is a classical example of a plastic cortical system. Structural and functional modifications of the neuronal network in the visual cortex relay on a complex interplay between physiological neuromodulatory systems together with continuous sensory experience (Kasamatsu at al., 1985; Kasamatsu et al., 1976; Baroncelli et al., 2010).

Experience continuously modifies the perception of the world around us, and the improvements in visuo-perceptual functions following intensive training are the behavioral evidence reflecting neuronal changes associated with perceptual learning (PL) (Gibson, 1969; Gilbert, 1994; Gilbert et al., 2001; Carmel & Carrasco, 2008). PL has been observed for a wide range of perceptual domains, from very simple sensory discrimination like visual and tactile acuity tasks (Fahle et al., 1995; Fahle & Morgan, 1996; Sathian, 1998; Wright et al., 2010), stimulus orientation discrimination (Dosher et al., 1998 Matthews et al., 1999; Schiltz et al.,1999), motion discrimination (Ball and Sekuler, 1992; Thompson et al., 2013; Watanabe et al., 2001; Matthews et al., 1999), texture discrimination (Ahissar & Hochstein, 1996: Karni & Sagi, 1991) to complex object recognition (Furmanski & Engel, 2000; Fahle et al., 2002; Ashby & Maddox 2011, Op de Beeck & Baker 2010).

Visual Perceptual Learning (VPL), in particular, can be defined as a form of implicit memory that can be sharpened and trained to improve visual discrimination, when

repeatedly exposed to particular types of stimuli (Snowden et al., 2000). This enhanced perceptual acuity resulting from VPL is thought to reflect plasticity of the brain (Sagi, 2011). The notion of neuronal plasticity of the visual cortex is pivotal not only to achieve a better understanding of the human visual system and its adaptive mechanisms, but also to develop treatment protocols for the neurological population. Recent studies have shown that training protocols can significantly strengthen the visual abilities of adult subjects with amblyopia or other forms of abnormal vision, indicating potential translation of basic research into real-world application (Ooi et al., 2013; Polat et al., 2004; Polat et al., 2009).

Classically, the effects of VPL have been considered retinotopic specific, with perceptual improvements found for spatially-localized targets only (Ahissar & Hochstein, 1997, Saarinen & Levi, 1995, Watanabe et al., 2002), however more recent research has focused on the possibility of generalization of VPL to both untrained locations and across different features (Seitz & Dinse, 2007; Seitz & Watanabe, 2005; Xiao et al., 2008; Zhang et al., 2010; Wang et al., 2012). This raises the question of how the entire visual cortex might change in response to perceptual learning, and several mechanisms have been proposed as playing a pivotal role in either promoting or preventing learning.

The Visual System

We are a visually oriented species and the loss of vision has a detrimental impact on humans. Most of our impressions, emotions and memories are based on sight. How we see forms or perceive depth, distinguish color or recognize faces, enjoy a landscape or perceive movements are all complex perceptual operations carried out by our visual system which is able to create a thee-dimensional perception of the world from the two-dimensional images projected onto the retina. In humans, and in most of the mammals, all photic information’s are transduced in the rods and cones (photoreceptors) of the retina to be then conveyed to the brain by way of the axons of the output cells, called ganglion cells. This axons, together with the bipolar cells from the optic nerve, project to three major subcortical targets, the pretectum of the midbrain, the superior colliculus (SC) and to the lateral geniculate nucleus (LGN) in the thalamus before reaching the primary visual areas (Brodmann's area 17 or V1, also called striate cortex) (Felleman & Van Essen, 1991). Because throughout most of this course a precise retinotopic arrangement of the fibers is maintained, the striate cortex contains a complete neural map of the retina. Beyond the

striate cortex, also the extrastriate areas, a set of higher-order visual areas, contain a representation of the retina. This precise preservation of the arrangement of the fibers creates the so called retinotopic map.

Half of the visual field of each eye is mapped systematically in the contralateral cerebral hemisphere.

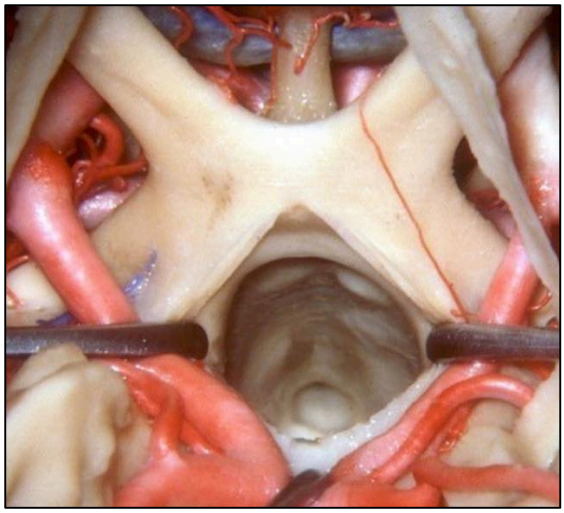

Figure 1.1 Anatomical view of the optic chiasm. Optic Nerves converge in the optic chiasm and from there the optic tracts originate.

In fact, ganglion cells axons travel in the optic nerve to the optic chiasm (Figure 1.1), where they undergo a partial decussation and enter partly the left and partly the right optic tract. In this arrangement, the axons from the left half of each retina (the temporal hemiretina of the left eye and the nasal hemiretina from the right eye) project in the left optic tract, which therefore, carries a complete representation of the right hemifield of vision. On the contrary, fibers originating in the right half of each retina (the nasal hemiretina of the left eye and the temporal hemiretina of the right eye) project to the right optic tract which therefore carries a complete representation of the left hemifield of vision. This partial decussation guarantees that all the information from the contralateral visual filed reaches each optic tract and moreover, because depth perception is based on the comparison of the slightly different views seen by the two eyes, it is necessary to bring together information from the two retinas. Most of the fibers, about 90%, of each optic tracts terminate in the LGN, the thalamic relay nucleus for vision. The fibers originating here, travel trough the internal capsule and corona radiata, curve around the lateral ventricle as the optic radiation before reaching the primary visual cortex in the banks of the calcarine sulcus. This is termed the primary or retino-geniculo-striate pathway and is the major route of visual information between the eye and the cortex. In humans, the LGN, contains six layers of cell bodies separated by intralaminar layers of axons and dendrites

The two most ventral layers of the nucleus contain relatively large cells and are known as the magnocellular layers and their main retinal input is from M ganglion cells. The four dorsal layers instead are known as parvocellular layers and receive input from P ganglion cells. An individual layer in the nucleus receives input from one eye only: fibers from the contralateral nasal hemiretina contact layers 1, 4, and 6; fibers from the ipsilateral temporal hemiretina contact layers 2, 3, and 5. Thus, although one lateral geniculate nucleus carries complete information about the contralateral visual field, the inputs from each eye remain segregated (Figure 1.2). The parvocellular and magnocellular layers in turn project to separate layers of the primary visual cortex.

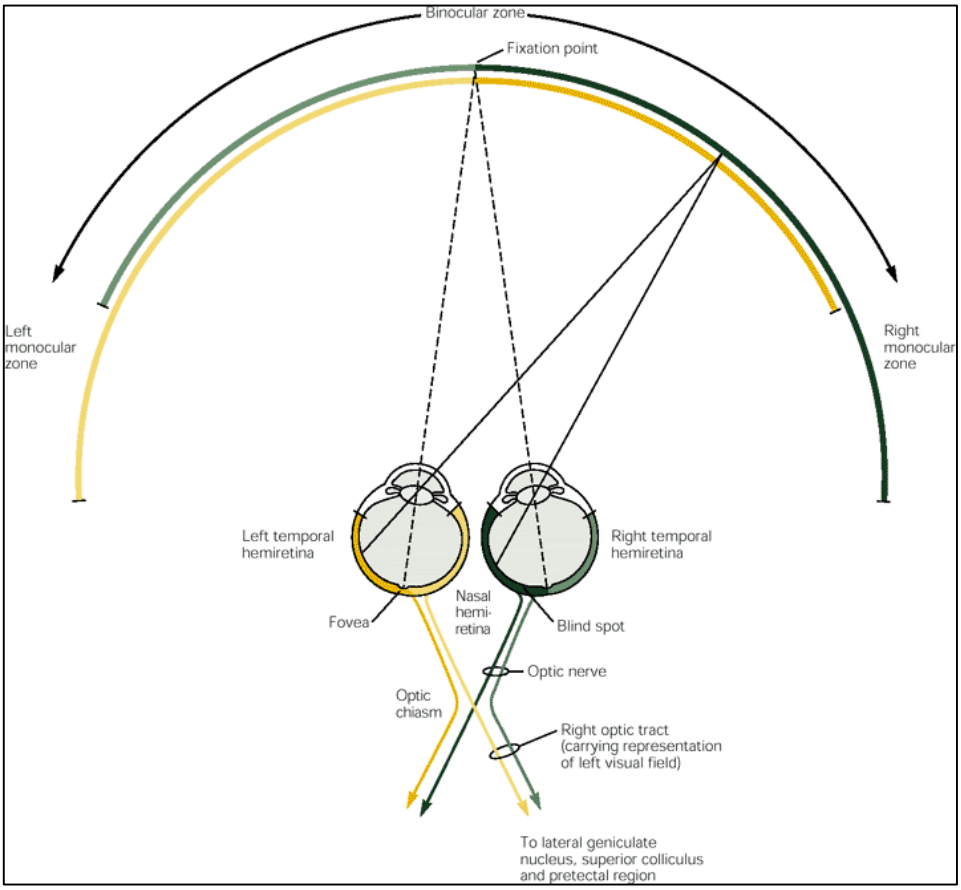

Figure 1.2. Representaion of the visual fields and how the light from the binocular and monocular zones strike the retina. Source: Kandel – Pricniples of Neuroscience.

However, as previously briefly mentioned the LGN is not the only subcortical region that receives inputs from the retina. From the optic chiasm, where the fibers from each eye destined for the one or the other side of the brain, are sorted out, re-bundled and myelinated together in the bilateral optic tracts which project to 2 other major subcortical areas: the Superior Colliculus (SC) and the Pretectum. This targets receive also extensive cortical inputs and control saccadic eye movements (SC) and pupillary reflexes (Pretectum).

Because of this complex and highly organized structure of the primate visual system, damages at different points in the visual pathway result in predictable deficits. The overarching concept is that damages anterior to the optic chiasm affect only the ipsilateral eye, damages at the chiasm result in heteronymous deficits, whereas lesions behind the chiasm cause homonymous deficits. At this point, it is important to mention that visual defects are always named according to the visual field loss and not according to the area of the retina that is involved. Because the retinal image is inverted and reversed, damages to temporal areas cause nasal field defects and damages to the inferior part of the retina result in superior visual field losses (Figure 1.3). Thus, damage to the Early Visual Areas or it post-chiasmatic inputs, results in a loss of conscious vision in the contralateral visual hemifield, termed cortical blindness (CB) (Holmes, 1918; Teuber, 1960; Weiskrantz et al., 1974; Cowey and Stoerig, 1991, 1995). Damage to the primary visual cortex occurs as a result of a stroke in the territory of the posterior cerebral artery in 40 to 90% of the cases (Lawton Smith, 1962; Trobe et al., 1973; Fujino et al., 1986; Zhang et al., 2006a) and is particularly devastating due to the fact that V1 is the primary gateway for visual information to the rest of the higher visual areas. Patients with cortical blindness are impaired in many activities of daily life such as reading, navigating environments or dressing and in personal care.

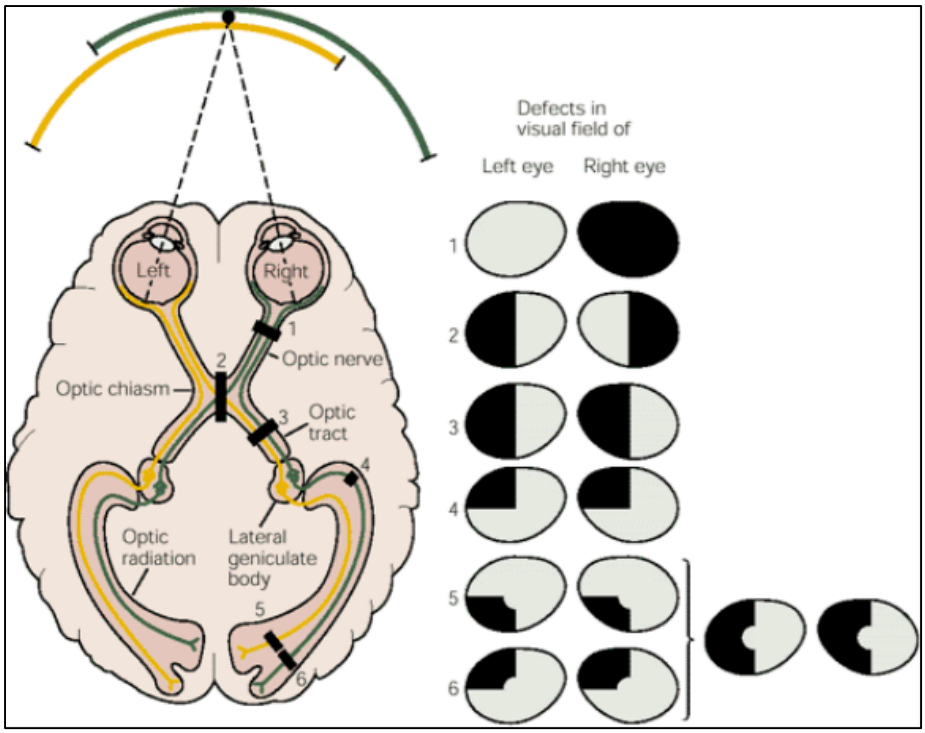

Figure 1.3. Deficits in the visual field produced by a lesion at various points in the visual pathway. The resulting deficits are shown in the visual field map as black areas. 1. Lesion of the right optic nerve results in total loss of vision in the right eye; 2. Lesion of the optic chiasm causes loss of vision in both temporal halves of each visual field (bitemporal heamianopsia); 3. Lesion of the optic tract results in complete loss of vision in the opposite half of the visual field (contralateral hemianopsia); 4. Lesion of the optic radiations in the Meyer's Loop cause loss of vision in the upper quadrant of the opposite half of the visual field of both eyes (quadrantopsia); 5. Partial lesions of the visual cortex lead to partial field deficits on the opposite side, more extensive lesion of the visual cortex, including parts of both banks of the calcarine cortex, would cause a more extensive loss of vision in the contralateral hemifield. Source: Kandel – Principles of Neuroscience.

While V1 damage is usually followed by some amount of spontaneous recovery for homonymous visual field defects, which display 50-60% of probability of spontaneous partial recovery in the first months after the stroke (Zhang et al., 2006b), only little to almost no recovery is observed after 6 moths after the insult (Zhang et al., 2006b). This observed recovery is thought to be due to the resolution of the inflammation and edema around the lesioned area and subsequently to the re-activation of partially-damaged peri-lesional tissue (Sabel, 1997; Poggel et al., 2001).

The exact mechanism underlying visual recovery remains unclear, however several hypotheses have been raised. One possibility is that visual relearning can occur through the so called blindsight pathway (see further discussion in chapter 4, Weiskrantz et al., 1974). Another possibility supported by experimental studies on non-human primates, suggests that residual vision might also be supported by direct projections from the dorso-lateral geniculate nucleus (dLGN) to extrastriate visual cortical areas, bypassing V1 (Hendry and Reid, 2000; Sincich et al., 2004; Cowey and Stoerig, 1989). Strengthening this extra-geniculo-calcarine pathways could account for training-induced perceptual recovery. Whatever the case, these explanations might represent a reasonable substrate for the training-induced visual re-learning and recovery that may be attained in cortically blind subjects.

Mechanisms Underlying Perceptual Learning

The available neurophysiological evidence shows that learning could arise following continuous refinement of localized neuronal populations within low-level visual areas, as V1, V2 and V3 (Adab et al., 2011; Fahle 2002, Xu et al., 2010, Xu et al., 2012a, Yan et al., 2014), but also from better read-out mechanisms of higher-level visual areas like V4, middle-temporal visual areas (MT or V5), V3A (Dosher & Lu, 1998, Liu et al., 2010, Lu et al, 2005; Koyama et al., 2005) or in high level visual areas like the lateral intraparietal cortex and the dorsolateral prefrontal cortex (Shadlen et al., 1996; Heekeren et al., 2004). One hypothesis is that the visual signals encoded by early visual areas are enhanced and fed into higher-order visual areas via two potential mechanisms. Either training selectively increases relevant information carried by neurons in early sensory cortex and thus subsequent readout is facilitated (Yan et al., 2014), or, alternatively, training makes higher-order visual areas better at reading-out the relevant sensory signals by filtering external noise (Dosher & Lu, 1998; Lu et al., 2005; Kahnt et al., 2011; Petrov et al., 2005; Bejjanki

et al., 2011).

Several (high or low-level) mechanisms might play a crucial role in either promoting or preventing learning and, likely these mechanisms operate synergistically via feedforward and feedback connections, where also inter-areal interactions might be influenced by training. For example, using a motion detection task, it has been shown that the increase in activation in human MT+ secondary to the training was concurrent to reduced activity in other extrastriate visual areas (Vaina et al., 1998).

One key mechanism that triggers VPL is boosting stimulus-related cortical activity, normally insufficient to reach a learning threshold, and likely crucial to promote perceptual learning (Seitz & Dinse, 2007) (Figure 1.4). For instance, attention and reinforcement (provided by reward) (Franko et al., 2006) are well studied key factors responsible for boosting the stimulus-related activity, and therefore cause learning. This is for example shown by task-relevant (Fahle et al., 2002; Fine & Jacobs, 2002; Gilbert & Li, 2012; Gold & Watanabe, 2010; Lu et al., 2011; Sagi et al., 2011; Xu et al. 2010; 2012a; 2012b), task-irrelevant (Ahissar, 2001; Nishina et al., 2007; Paffen et al., 2008; Seitz & Watanabe, 2005; Seitz et al., 2005a; Seitz et al., 2006; Tsushima et al., 2008; Watanabe et al., 2001; Watanabe et al., 2002; Wright et al., 2010), and stimulus-reward contingency paradigms (Pascucci, Mastropasqua, & Turatto, 2015; Seitz & Watanabe, 2005).

Presumably, human brain has evolved to optimize behavior in a multisensory environment, however, only recently, perceptual learning studies have focused on training in multisensory modality, showing that, for example, visual and concurrent auditory stimuli can lead to faster and higher rate of improvement than in subjects trained in one modality only (Seitz et al., 2006; Kim et al., 2008).

It is widely accepted that synaptic plasticity underlies learning and, the activity-dependent plasticity resulting from persistent changes in synaptic connections are termed long-term potentiation (LTP) and long-term depression (LTD). LTP, as already described in the early 1970's by Donald Hebb, and its complementary LTD, are long-lasting and input-specific changes that can be induced at one set of synapses or on a cell without affecting other synapses. LTP was originally observed *in vivo* in the hippocampus of anaesthetized rabbits (Bliss et al., 1973) and it has been shown to be N-methyl-D-aspartame (NMDA) dependent (Coan & Collingridge, 1987; Chaieb et al., 2015).

LTP-like effects have been studied extensively as an important physiological mechanism underlying synaptic plasticity, both in animals and humans, in the hippocampus (Bliss &

Lomo, 1973; Beck et al., 2000), the somatosensory cortex (Fox, 2002), temporal lobe (Chen et al., 1996) and in visual cortex (Komatsu et al.,1981; Norman et al., 2007; Elvsåshagen et al., 2012). Interestingly, when the critical period is shifted by binocular deprivation, the occurrence of LTP, that normally only occurs within the critical post-native period, shifts with it, so that the two are always co-occurring. Moreover, the total absence of visual experience of dark-reared animals, extends the critical period far beyond its normal limits (Kirkwood et al., 1995). The first empirical demonstration of LTP was provided by Bliss and Lomo (1973). They induced a brief high-frequency electrical stimulation in the perforant pathway of anesthetized rabbits and they recorded from the dentate gyrus. They discovered an increase of excitatory post-synaptic potentials (EPSPs) over baseline response that lasted up to 10 hours.

Similarly, it has been shown that LTP and LTD can be induced by different patterns of electrical stimulation also in the visual system. While a high-frequency stimulation induces LTP-like mechanisms, low-frequency stimulation protocols promote LTD-like mechanisms (Bliss & Lomo, 1973; Bliss & Collingridge, 1993; Gonzalez et al., 2014; Collingridge et al., 2010). Electrical stimulation techniques have been extensively used as tools to induce LTP and LTD, and animal studies confirmed that when low frequency (1Hz) trains of electrical stimulation are delivered, LTD can be induced in cortical pathways (Kemp et al., 2004).

To mimic similar effects in humans, neuromodulatory techniques like tDCS, have been used to induce localized cortical sustained responses similar to LTP- and LTD-like effects (Ranieri et al., 2012), in vitro and in vivo. NIBS techniques have been used in humans to achieve similar LTP effects, and potential explanations for the effects have been related to glutamatergic mechanisms for most tES techniques (Stefan et al., 2004; Nitsche et al., 2003; Nitsche et al., 2004; Liebetanz et al., 2002) and voltage-gated sodium channels dependent for tRNS stimulation (Chaieb et al., 2015). Interestingly, weak current stimulations can induce the release of brain-derived neurotrophic factor (BDNF), a substance that promotes NMDA—dependent LTP (Figurov et al., 1996; Fritsch et al., 2010). Taken together these studies suggest long-lasting plasticity might be promoted devising specific training protocols and, potentially, noninvasive stimulation techniques in humans.

While it is important to determine what are the neurophysiological mechanisms underlying perceptual learning, recent research has also shown that there are limits. Despite the

proven effectiveness of PL, the length of the training (many weeks or months of up to four or five times weekly) to obtain significant changes in sensory functions could be a limit to the applicability of VPL (Camilleri et al., 2014; Polat et al., 2004: Tan et al., 2008: Huxlin et al., 2009).

The aim of the first experimental work in this thesis is to show that NIBS can promote and boost training protocols, likely increasing stimulus-related cortical activity, speed up and enhance the effect of training (Cappelletti et al., 2013; Fertonani et al., 2011; Mulquiney et al., 2011; Snowball et al., 2013) and potentially mimicking LTP-like effects in humans.

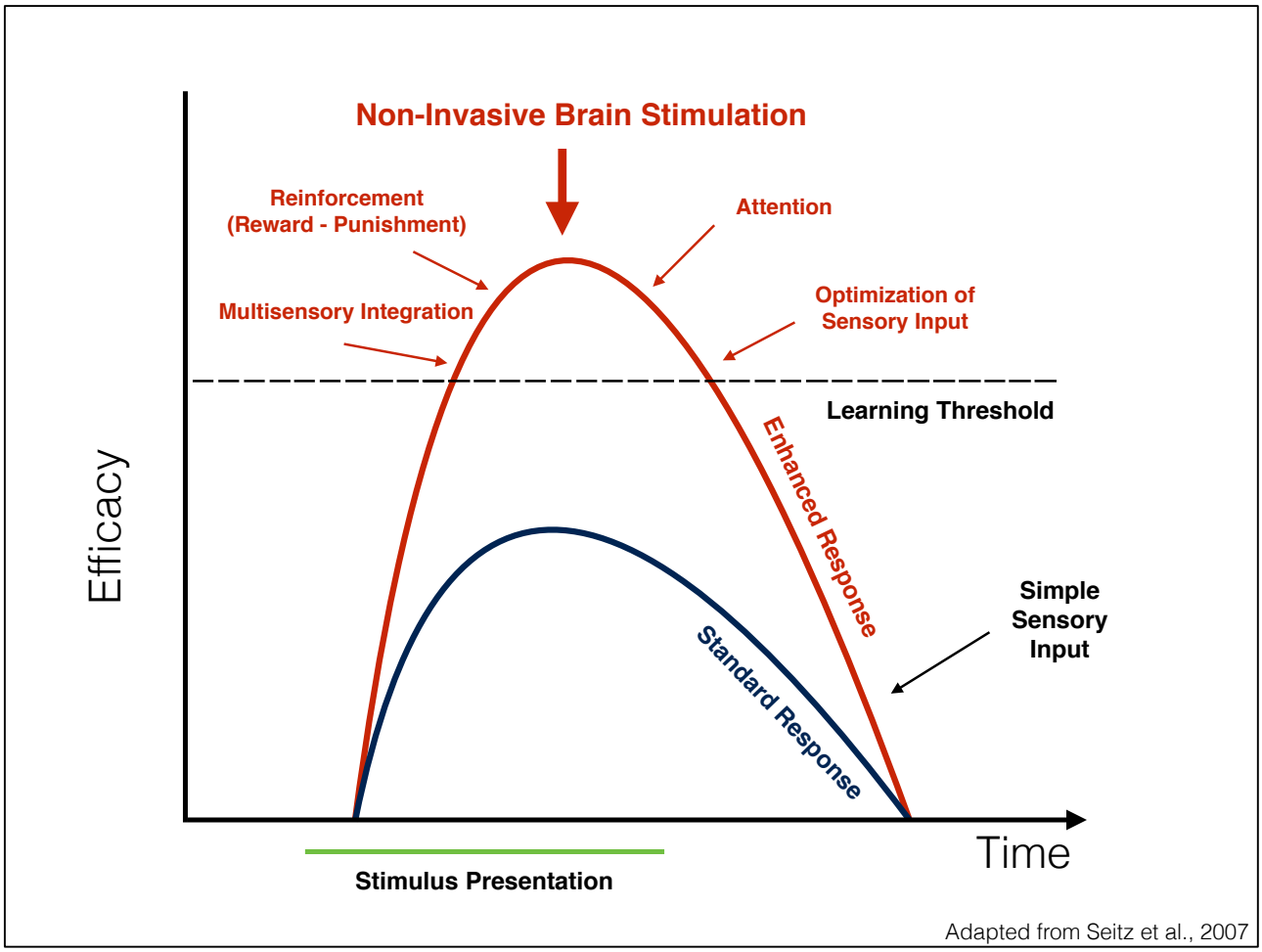

Figure 1.4. Illustration of the possible factors that trigger Perceptual Learning (PL). When a sensory input is insufficient to reach a learning threshold, different factors, like attention, reinforcement or multi-sensory integration might play a role in driving the neuronal response to pass the learning threshold and therefore promoting PL.

Non-Invasive Brain Stimulation (NIBS) Techniques

The main focus of this thesis is to determine whether NIBS can effectively enhance cognitive functions, and if the effect can substantially outlast the training period. While we still don't know what are the best conditions that promote improvement, several potential explanations have been hypothesized on why brain stimulation enhances the effects of training. One interesting idea is that when an appropriate amount of noise is injected into a biological non-linear system, like the brain is, it can enhance the detectability of a signal, a phenomena known as stochastic resonance (SR) (Moss, Ward, & Sannita, 2004). Recent studies have shown that this could be the case when using direct current stimulation, particularly tRNS, the NIBS techniques I used in my studies.

The basic idea is that cortical activity could be enhanced with ad-hoc stimulation protocols, where controlled oscillating frequencies, duration and intensity parameters are used to induce synaptic plasticity resulting in effective perceptual improvements. For example, neurophysiological studies have shown that intracortical microstimulation (ICMS), which is a local application of high-frequency weak electrical stimulation, can induce changes in the size of receptive fields of the visual cortex (Godde, Leonhardt, Cords, & Dinse, 2002). In recent years neurohpysiological studies have also shown that stimulation can induce intracerebral current flows, sufficiently large to be effective in altering neuronal activity (Schoen et al., 2008), and behaviourally measurable with psychophysical paradigms in humans (Antal, Nitsche, & Paulus, 2001; Fertonani, Pirulli, & Miniussi, 2011). Initially, studies using brain stimulations techniques focused mainly on treating psychiatric disorders, but in the last few decades tES showed to reliably modulate cortical excitability also in the healthy population (Antal et al., 2001; Nitsche et al., 2003; Nitsche, Nitsche, Paulus, & Paulus, 2000; Priori, 2003; Terney, Chaieb, Moliadze, Antal, & Paulus, 2008).

Non-Invasive Brain Stimulation - History

The idea of electrical currents application to modify brain functioning is not new, and already in the 18th century Luigi Galvani and Alessandro Volta began to experiment with electrical stimulation on a frog sciatic nerve-gastrocnemius muscle preparation, showing how it could be made to twitch following an electrical impulse (Galvani et al., 1973). Soon after this first evidence showing how electricity can modulate body responses, Volta created the first electrical battery that produced artificial, controllable, constant direct current. It was Galvani's nephew, Giovanni Aldini, who employed voltaic cells to extend his

uncle's invention and showed how a weak transcranial direct-current could ameliorate depressions-like symptoms (Aldini, 1974). This was the first pioneer clinical application of direct current, paving the way for the use of tES as clinical and research tool. Research with low-intensity DC was progressively abandoned in the '30s and replaced with Electroconvulsive Therapy (ECT). Only by the end of the 50s, low intensity direct-current stimulation regained some popularity with the first-study of "cranial electrotherapy stimulation" published by Anan'ev and colleagues (1957). Later, an influential paper was published showing that direct current stimulation could slow down or boost memory retention in rats, in a polarity dependent manner (Albert, 1966), showing the neuromodulatory properties of tES. tES can modulate the membrane potential of neurons and affect the spontaneous firing rate, but it cannot induce direct discharge of resting neurons.

The goal of the following section is to summarize and provide an introduction to the different neuromodulation and neurostimulation techniques used in this study. I will then provide a short description and I will briefly review the available and relevant work in the field. This sections are not intended as comprehensive review of the tES literature, excellent reviews are already available (Paulus et al., 2011).

Transcranial Direct Current Stimulation (tDCS)

tDCS consists of the application of a weak constant current (DC) delivered via two saline soaked electrodes, an anode and a cathode, held in place on the scalp of the subject by a cap and a nonconductive rubber montage. The current is typically applied via rubber electrodes that vary in size from 25 to 35 cm^2. tDCS is usually applied for 5 and up to 30 mins with intensities varying between 1-2mA.

With tDCS the active electrode is positioned above the cortical region of interest, whereas the return electrode is usually placed over an inactive or task-irrelevant region or extra-cephalic. Although low current intensity (0.5mA to 2mA) and the shunting effect of the scalp, studies showed that the current that enters the brain is sufficient to modulate the membrane permeability and therefore the neuronal potential (Datta et al., 2009; Miranda et al., 2006; Salvador et al., 2010).

tDCS is thought to interfere with the spontaneous neural activity by influencing the level of excitability and firing rate of the neurons by depolarizing or hyperpolarizing the permeability of the neuronal membrane. Therefore, like pharmacological neuromodulators,

tDCS induces a shift of the spontaneous firing rate of the neurons that are near their action potential threshold, but do not induce direct neuronal firing (Fritsch et al., 2010).

In general, anodal stimulation exerts excitatory, while cathodal inhibitory effects, however this is not always the case when tDCS is used in studies not involving the motor cortex (Datta et al., 2011; Radman et al., 2009; Reato et al., 2013).

Although there is a general consensus that DC stimulation modulates the corticospinal excitability, it is controversial if the changes are due to direct alteration of the membrane, synaptic transmission or any other molecular effects. The observed long-term effects seem to be mediated by the activation of N-methyl-d-aspartate (NMDA) channels, as demonstrated by several works where the effect of tDCS stimulation could be prolonged or inverted by administering NMDA agonist or antagonists (Chaieb et al., 2015; Liebetanz, Nitsche, Tergau, & Paulus, 2002; Nitsche et al., 2003; Nitsche et al., 2004).

Although several studies have shown that tDCS can improve perceptual and cognitive abilities (Fertonani et al., 2010; Monti et al., 2008; Pisoni et al., 2012; Floel et al., 2008), memory (Andrews et al., 2011; Berryhill et al., 2010;Fregni et al., 2005; Javadi et al., 2013) and attention (Antal et al., 2004; Bolognini et al., 2010; Roe et al., 2016; Fregni et al. 2005), recent meta-analysis have raised concerns about the effectiveness of tDCS, arguing that a single-session tDCS generates little or no reliable effects (Horvath et al., 2015).

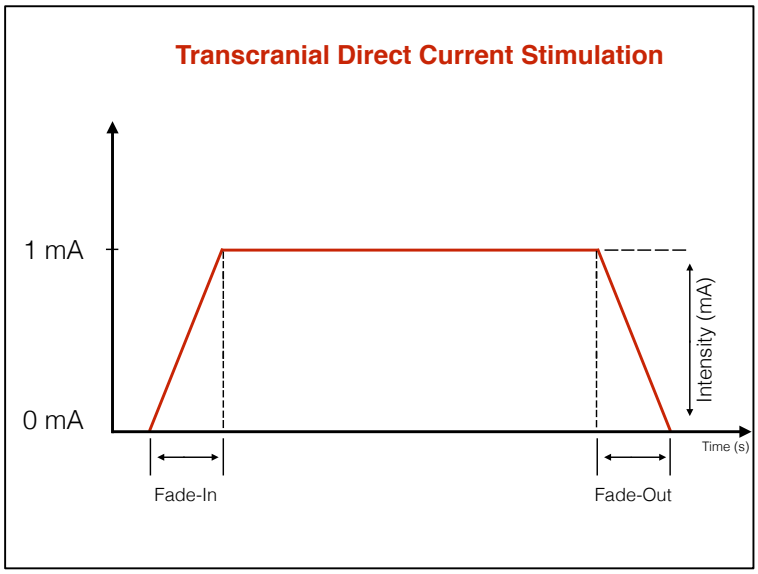

Figure 1.5. In tDCS the current delivered is direct and monopolar. At the beginning and at the end of the stimulation, the current is gradually increased/decreased (Fade-In/Fade-Out Phases). From single neurons registrations, we know that the application of a current can either depolarize, following anodal stimulation, or hyper-polarize, following cathodal stimulation, the neuronal membrane potential (Gartside, 1968).

Transcranial Random Noise Stimulation (tRNS)

tRNS is a very recently developed direct current stimulation technique. It is characterized by the delivery of alternating current at randomly oscillating frequencies in the range of 0.1 Hz up to 640 Hz, typically isolated to either low (0.1 to 100 Hz) or high frequency (101 to 640 Hz) bandwidths, for an extended period of time (on average 20 minutes). tRNS is believed to interfere with, and therefore modulate, ongoing cortical oscillations in the brain, resulting in increased cortical excitability and behavioral changes. For example, tRNS decreases motor thresholds (Terney et al., 2008), modulates visual perception (van der Groen and Wenderoth, 2016), and promotes perceptual learning when delivered over the visual (Camilleri et al., 2016, 2014; Fertonani et al., 2011), the parietal and the dorsolateral prefrontal cortex of healthy young and elderly subjects (Cappelletti et al., 2013; Dormal et al., 2016; Lecce et al., 2015; Snowball et al., 2013).

The delivery of current during tRNS is not as focal, thus it will modulate the neuronal activity not only of those neurons directly under the electrodes, but also the entire network (Fertonani et al., 2011; Schoen and Fromherz, 2008). tRNS potentially injects random activity in the system, which the brain interprets as noise and uses it for information processing (the so called stochastic resonance phenomenon; Moss et al., 2004; van der Groen and Wenderoth, 2016). The injected noise will primarily affect the neurons of a network that are near their firing threshold, therefore the neurons that are functional for the execution of a specific cognitive process, but not neurons that are far below their discharge threshold (Miniussi and Ruzzoli, 2013). Whereas other forms of tES increase cortical excitability in normal subjects (Antal et al., 2014; Ding et al., 2016) and can enhance behavioral training (Richmond et al., 2014), only tRNS has been shown to exert enhancing effects that can last up to 16 weeks post-training, a strong marker of long term potentiation of cognitive functions.

Terney and colleagues showed for the first time the potential effect of tRNS on cortical excitability (Terney et al., 2008). They showed that 10 minutes of a weak random electrical stimulation in the high-frequency band (101 - 640 Hz) targeting the motor cortex (M1) was sufficient to significantly alter the cortical excitability up to 60 minutes after the end of the stimulation. Specifically, they delivered 10 minutes of stimulation over the primary motor cortex, and induced an excitability increase (higher amplitude of the motor evoked potentials – MEP) up to 20-50% relative to baseline, as revealed by single and paired-pulse TMS. A following study extended this results by demonstrating that even

shorter duration stimulation protocols of 5 minutes of tRNS can already induce significant after-effects on corticospinal excitability (Chaieb et al., 2011). To conclude, tRNS seems to be the ideal tool to potentiate cortical plasticity (Grenier et al., 2001), particularly when used on the visual cortex.

tRNS and Visual Perceptual Learning

tRNS has been used for visual perceptual studies only in recent years (Camilleri et al., 2014, Campana et al., 2014; Fertonani et al., 2011; Pirulli et al., 2013). In particular, Fertonani and colleagues demonstrated that high frequency tRNS can significantly improve learning on an orientation discrimination task when compared to tDCS, sham, and low-frequency tRNS (Fertonani et al., 2011). The authors went further and in a follow-up study replicated this facilitatory effect when the stimulation was delivered online (while the subjects were training on the task).

Following studies showed that subjects with mild uncorrected myopia obtained a significant improvement in visual acuity and contrast sensitivity when the training was coupled with hf-tRNS (Campana et al., 2016). Interestingly, tRNS concurrent with a 2-weeks training protocol, yield the same outcome on uncorrected visual acuity, and a better outcome on uncorrected contrast sensitivity, as a two months training protocol only (Camilleri et al., 2014).

These aforementioned works suggest that hf-tRNS exerts its optimal effects when applied online (i.e. during the task), and the effect is weaker when applied offline (i.e. before the task). The beneficial effect of tRNS on visual perceptual learning makes it the ideal protocol to further study cortical plasticity and its future applicability.

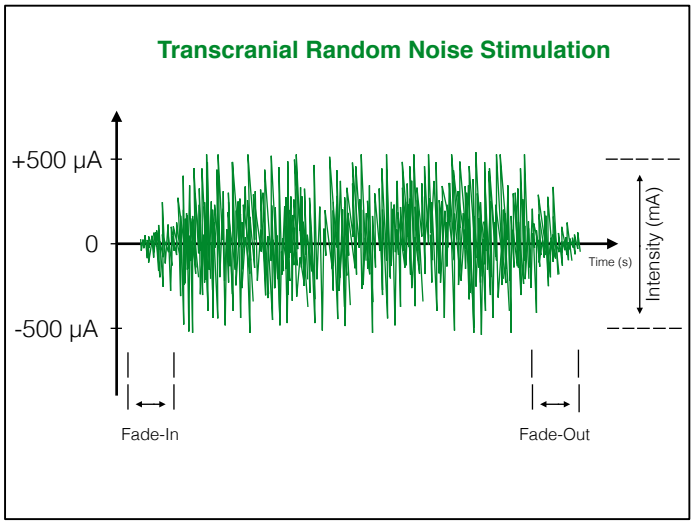

Figure 1.6. tRNS involves the application of alternating currents in a range of frequencies, typically between 0 and 100Hz for Low-Frequency tRNS (Lf-tRNS), between 101 and 640Hz for High-Frequency tRNS (Hf-tRNS) or in the full spectrum band between 0 and 640 Hz (tRNS). Due to its oscillatory nature, rather than direct current, it has been proposed that tRNS is polarity-independent, hence no longer sensitive to the current flow direction. tRNS likely exerts it effects thought repeated random sub threshold stimulation and temporal summation of neuronal activity due to stimuli presented in close sequence resulting in prolonged depolarization and long term potentiation-like phenomena (Fertonani et al., 2011; Terney et al., 2008; Pirulli et al., 2013).

Chapter 2: Prime and measure

Transcranial Random Noise Stimulation Modulates Cortical Excitability of the Visual Cortex

Abstract

tRNS can induce long term increases of corticospinal excitability (Terney et al., 2008), when used to prime the motor cortex before measuring motor evoked potentials. The effect is sustained and long lasting. Moreover, tRNS has been used to improve cognitive functions such as attention and mathematical skills, potentially suggesting an interesting effect upon cortical plasticity. However, it is unclear whether tRNS over visuo-parietal areas causes changes in excitability that are similar to what has been found in the motor cortex. In the present study, the aim was to investigate whether priming the visual cortex with tRNS leads to increases in excitability similar to the motor cortex, as measured with visual phosphenes. In Experiment 1, to quantify the magnitude of cortical excitability changes, we measured phosphene threshold using an objective staircase method. Single-pulse TMS was used to elicit phosphenes before, immediately after, and every 10 minutes up to 1 hour after the end of 20min tRNS or Sham. In Experiment 2, subjects underwent the same procedure, but were tested up to 2 hours post-tRNS. Results showed that phosphenes' threshold was significantly reduced up to 90 minutes' post stimulation relative to sham, hence demonstrating that tRNS can increase the excitability of the visual cortex, and the effect is sustained and long lasting.

Introduction

The traditional tES techniques, such as cathodal or anodal tDCS, can modulate cortical excitability of the motor cortex (M1) in humans (Baudewig et al., 2001; Nitsche et al., 2007; Nitsche et al., 2000; Nitsche et al., 2003). In order to study the mechanisms underlying tDCS, Nitsche and colleagues applied 5 Minutes tDCS over the motor cortex of healthy subjects, and motor evoked potentials (MEP) were measured with TMS, before and after stimulation. The results showed that MEPs amplitude increased 40% when anodal stimulation was applied over M1, and this increase lasted for 10 minutes after the end of the stimulation (Nitsche et al., 2000).

Following this work, Antal and colleagues demonstrated that the neuronal excitability of the visual cortex can be modulated in a similar polarity-dependent manner. Subjects were primed with 10 minutes anodal tDCS and phosphene thresholds (PT) were measured before, immediately after, 10 and 20 minutes after the offset of the stimulation, using short-trains of 5Hz rTMS. Results showed a significant reduction of PT threshold immediately after anodal stimulation (Antal et al., 2003b).

Other subsequent studies have shown that tDCS can modulate the amplitude of visual evoked potentials (VEP's) (Antal, Kincses, Nitsche, Bartfai, & Paulus, 2004), alter the perception of phosphenes (Antal et al., 2003a, 2003b), affect motion detection (Antal et al., 2004) and reduce the duration of the motion after-effect (Antal et al., 2004).

More recently tDCS has also been used to study higher cognitive functions such attention (Gladwin et al., 2012), working memory (Berryhill, Wencil, Branch Coslett, & Olson, 2010; Fregni et al., 2005), long term memory (Rroji et al., 2015), learning (Reis et al., 2009), and also as a rehabilitation tool for patients with brain lesions (Fiori et al., 2011; Jo et al., 2009; Kang et al., 2009).

However, recent systematic reviews have raised doubts about the effectiveness of tDCS, arguing that single-session tDCS generates little to no reliable effects beyond MEP amplitudes changes (Horvath, Forte, & Carter, 2015). The authors suggest that it is crucial to further investigate the effects of tES, and especially to address the question of how it affects other areas of the cortex, besides M1. In the majority of published tES studies, the effects found on M1 are used as a model system to design stimulation protocols for other areas. However, it is well known that different areas have different anatomical characteristics, from skull morphology to axons orientation, hence leading to different

current flow distribution in the brain and to potentially different behavioral outcomes (Datta, Baker, Bikson, & Fridriksson, 2011; Radman, Ramos, Brumberg, & Bikson, 2009).

In fact, in recent years, tDCS studies have revealed the complexity of the technique and the non-linearity of the induced effects (Batsikadze et al., 2013; Fricke et al., 2011; Moliadze et al., 2012; Pirulli et al., 2014). Diverging effects have been reported also in the domain of visual perceptual learning (VPL) where studies showed functional improvements also following cathodal tDCS, classically considered an inhibitory technique (Berryhill et al., 2010; Dockery et al., 2009; Elmer et al., 2009; Williams et al., 2010). More recently, Peters and colleagues showed that anodal tDCS can block consolidation of learning in a contrast detection task (Peters, Thompson, Merabet, Wu, & Shams, 2013). Taken together the above results show that tDCS stimulation effects are not always straightforward and easy to interpret, and that applying the simplistic idea of anodal stimulation resulting in excitation and cathodal in inhibition, does not always lead to the expected neurophysiological or behavioral results.

The most recently developed technique among tES methods is tRNS. It is thought to interfere with noise processing and ongoing neuronal oscillations in the brain, and therefore modulate neuronal excitability. Terney and colleagues (2008) showed that 10 minutes of weak random electrical stimulation within the high-frequency band (101 - 640 Hz) targeting the motor cortex (M1) was sufficient to significantly alter cortical excitability up to 60 minutes after the end of the stimulation. Specifically, they showed that 10min tRNS over the primary motor cortex, induced an excitability increase up to 20-50%, as subsequently measured in MEPs' amplitudes, as revealed by single and paired-pulse TMS. A following study extended these results by demonstrating that even shorter duration stimulation protocols (5 minutes tRNS) can induce significant after-effects on the corticospinal excitability (Chaieb, Paulus, & Antal, 2011).

Since motor learning is always associated with enhancement of M1 corticospinal excitability (Pascual-Leone et al. 1999; Muellbacher et al., 2002; Sczesny-Kaiser et al., 2016), and evidence from recent studies suggest a link between tES induced corticospinal excitability and skill learning (Boggio et al., 2006; Galea & Celnik, 2009; Reis et al., 2009), it is crucial to investigate further the relationship between these two parameters.

The work presented in the previous chapter showed that VPL coupled with online tRNS boosted learning of complex visual motion and crucially, the improvements peaked earlier than in all other conditions, and it was sustained in time. However, it is still unclear how

different types of tES influence the activity of different cortical areas, and whether stimulation with tRNS over other brain areas, besides M1, cause similar changes in cortical excitability.

Here, to investigate the modulatory effect of tES, we focused on the influence of tRNS and anodal tDCS (a-tDCS) on cortical excitability of the primary visual cortex of healthy adults.

To quantify the effect of tRNS and a-tDCS, we measured phosphenes threshold at different time intervals, after we primed the visual cortex with either techniques, in separate experiments. In the first Experiment, 12 participants received High-Frequency tRNS (Hf-tRNS) for 20 minutes. Phosphene threshold (PT) was measured immediately after and up to 60 minutes after the end of stimulation. In the second Experiment, a group of 8 participants underwent the same procedure with the exception that PT was measured up to 120 minutes' post-stimulation. In Experiment 3 we measured PT of a control group of 12 participants after 20 minutes of anodal tDCS.

Materials and Methods

Baseline Phosphene Threshold

Participants were sitting in a semi-darkened room, positioned on a chin-rest forehead combination bar to stabilize their head while blindfolded. They were allowed to adapt to darkness for at least 2 minutes. During a first preliminary session a manual estimation of the resting motor threshold (RMT) was registered. RMT was defined as the minimal output of the stimulator that induced a visible movements of the fingers of the relaxed hand in at least three out of five times. Once resting motor threshold was recorded, a baseline estimation of the PT was registered. Subjects were instructed to keep fixation on an imaginary central fixation cross, directly in front of them and report the presence or absence of a phosphene. Initially the TMS coil was positioned with the handle pointing leftward parallel to the ground with the center placed 2 cm above the inion and 2 cm to the left/right based on the 10-20 electroencephalogram standard measures. We started the stimulation with an intensity equal to the RMT previously obtained for each subject, and we increased it in step of 1% while moving the coil in steps of 0.5 cm. Once the TMS pulse evoked a bright reliable phosphene, the spot was marked on a swimming cap worn by the subject. After an interval of 5 minutes, the participant was stimulated again on the marked spot, and if this stimulation induced a reliable phosphene, the point was marked on the cap.

Once the hotspot was identified, the REPT (Rapid Estimation of Phosphene Threshold, (Abrahamyan et al., 2011) procedure was used to determine individual PT more systematically. Participants were instructed to respond to the presence or absence of the phosphene by pressing the left or right “shift” key on the computer keyboard after the automatically triggered single-pulse TMS stimulation (with at least 3 seconds interval between each stimulation). REPT is a procedure that employs Bayesian adaptive staircase protocol for estimating psychophysical thresholds which showed to be more accurate, reliable and faster relative to other procedure (Modified Binary Search Algorithm, MOBS, Tyrrell & Owens, 1988) (see Abrahamyan et al., 2011). For each participant at least 2 REPT were collected during the preliminary session to assess for PT stability.

Phosphene Detection Task

Based on the coordinates form the preliminary session, participants were stimulated with single-pulse TMS over the same position and at the machine output intensity used during the 2 preliminary REPT sessions. Once the stimulation site was determined, subjects underwent 2 REPT sessions prior to the onset of the stimulation, to determine their baseline. Subsequently, participants sat quietly on a chair while being stimulated with Hf-tRNS or sham, depending on the session, for 20 minutes. After the end of the stimulation 7 REPT measurements were recorded every 10 minutes immediately after and up to one-hour post stimulation.

In Experiment 2, the experimental procedure remained the same as in Experiment 1 except that REPT were recorded every 10 minutes up to one hour, every 30 minutes from 60 minutes after the end of the stimulation, and up to 2 hours after stimulation. For Experiment 3 the same experimental procedure as for experiment 1 was used except that subjects received a-tDCS (instead of tRNS).

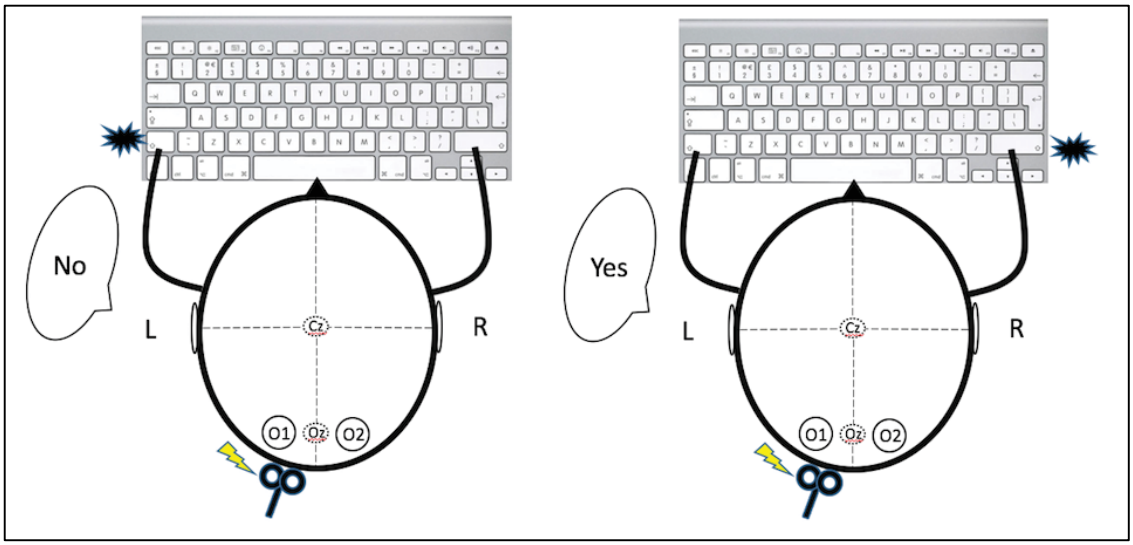

Figure 2.1. Subjects were asked to report whether they perceived a Phosphene immediately after he delivery of a single pulse TMS. Subjects were seated in a semi-darkened room with their head positioned on a chin-forehead-rest combination bar to stabilize and minimize head movements while being stimulated. Subjects were allowed to adapt to darkness and were tested while blindfolded. Phosphene Threshold (PT) was measured using REPT method.

Data Analysis:

For each subject two baseline measurements were obtained on separate days. A first baseline was registered during the preliminary session, and it was obtained by averaging the two REPT sessions. For REPT, the estimated PT corresponds to the position parameter of a Weibull function fitted to the proportion of the phosphene responses which, after a correction to account for lapses on 4% of the trails, converge to a value corresponding to 60% accuracy (see Abrahamyan et al., 2011).

The second baseline was obtained on a separate day, prior to the onset of the stimulation, and it was calculated as the average of the 2 REPT based on the stimulator's output intensity. To have an indication of how stable the phosphene threshold was at baseline, we calculated the percent difference between the 2 baselines as follows: ((REPTBaseline2 - REPTBaseline1) - REPTBaseline1)*100.

To estimate the variation of PT after stimulation, each REPT measurement was compared to the first baseline threshold collected before the onset of the stimulation on the same day, calculated as follows: ((REPTPostX - REPTPre1) - REPTPre1)*100.

The normality of sampling distribution was addressed using Shapiro-Wilk test. Equality of variance was tested using Levene's test. The effect sizes are reported as the partial Eta square *(ηp^2)* values and Cohen's D. The alpha level was $\alpha = 0.05$ and Multiple comparisons were corrected using Bonferroni method.

Experiment 1

Participants

A total of 18 healthy participants gave written informed consent to participate in the study (Mean Age = 22.9, Females = 11). All subjects were right-handed, neurologically healthy and completed a safety questionnaire screening for TMS (Keel et al., 2000). We recruited 18 subjects, but only 12 were able to reliably detect phosphenes and were therefore included in the study. The order of stimulation was randomized, with at least 48 hours between stimulation condition (sham or active) to prevent carry over effects.

Experimental Procedure

The experiment was conducted using a within-subjects design. On day 1, individual baseline measurements of PT was recorded. On a separate day, PT baseline were recorded again for each subject. On the same day subjects received 20 minutes of either Hf-tRNS or sham stimulation delivered offline (subject at rest). After the end of the stimulation the electrodes were removed and PTs were recorded every 10 and up to 60 minutes after the end of the stimulation. On a separate day, subjects underwent the same experimental procedure but with reversed stimulation protocols. Subjects were asked to describe the shape, color, and position of phosphenes after every REPT session.

Stimulation Protocol

High-frequency tRNS (Hf-tRNS) was delivered by a battery driven stimulator *(DC-Stimulator-Plus, NeuroConn GmbH, Ilmenau, Germany)* through a pair of 35 cm^2 saline-soaked rubber electrodes. The electrodes were placed over the cortical target area identified following the 10-20 electroencephalogram standard system and subjects wore a Lycra swimmer's cap on which the hotspots were marked. The electrodes were positioned over early visual areas bilaterally identified as O1/PO7 and O2/PO, and they were kept in position with an elastic band.

Hf-tRNS was applied for 20 minutes with an intensity of 1mA and with 0mA offset at frequencies of alternating current between 101 and 640 Hz. Stimulation started and ended with a fade-in/fade-out ramping of 15 seconds. Sham stimulation consisted of the fade/in-fade/out ramp of 5 seconds, after which stimulation was shut down. Subjects were blinded to the stimulation condition assigned.

At the end of each stimulation session, participants were asked to fill out a questionnaire about potential discomforts experienced during stimulation. None of the participants reported any unpleasant sensation or side effect due to the stimulation, neither during nor after the end of the session. None of the participants experienced phosphenes induced by the stimulation.

TMS pulses were delivered by a Magstim Rapid 2 Stimulator (Magstim Co., UK) using a 70-mm figure-of-eight coil. To aid brain-site localization the coil was positioned over the previously marked hotspot where phosphenes were reliably reported.

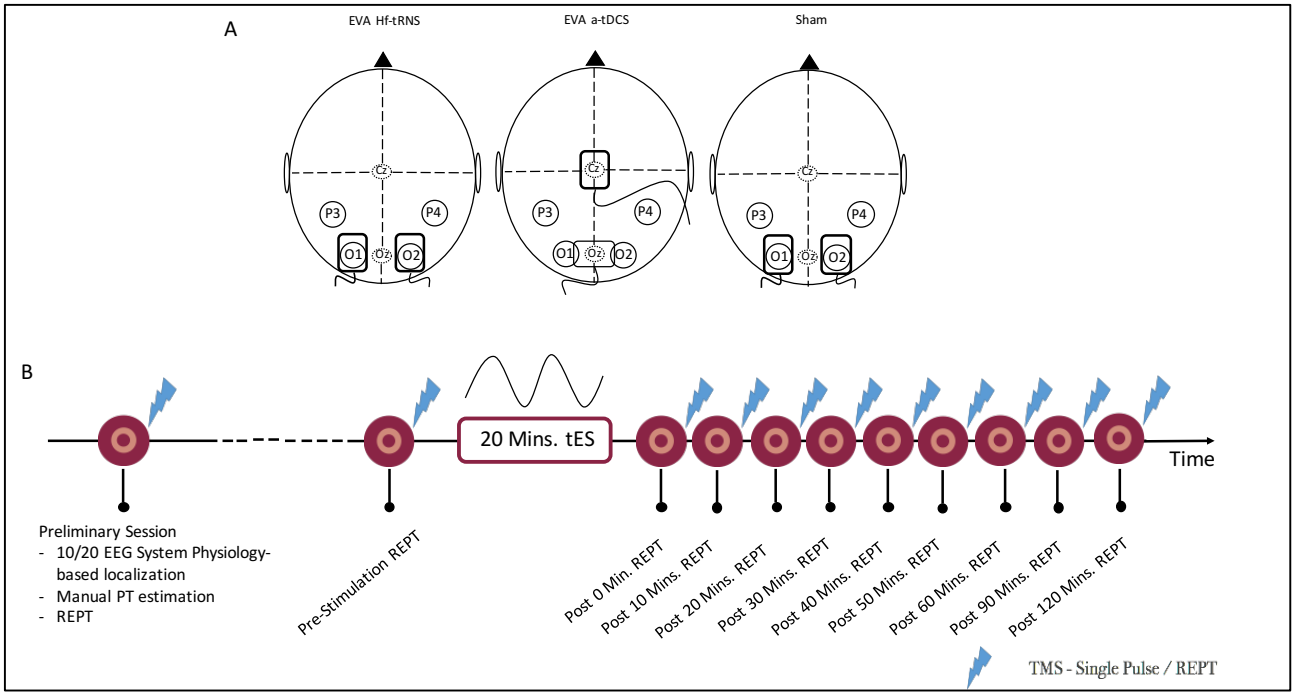

Figure 2.2. A. Participants received either Hf-tRNS (Experiment 1 and Experiment 2) or a-tDCS (Experiment 3) and Sham stimulation (Experiment 1 and Experiment 3) over the early visual areas stimulation. B. During a preliminary Session, starting with the TMS coil positioned with the handle pointing leftward parallel to the ground with the center placed 2 cm above inion and 2 cm to the left/right based on the 10-20 electroencephalogram standard measures, we looked for the location where a TMS pulse evoked a bright reliable phosphene. Once the hotspot was marked, manual Phosphene Threshold (PT) was determined. Subsequently at least 2 REPT were collected to asses how stable PTs are. On a separate day (Pre-Stimulation), 2 more REPT were collected. Subjects sat on a chair at rest while being stimulated with Hf-tRNS or Sham stimulation for 20 minutes. After the end of the stimulation 7 REPT measurements were recorded every 10 minutes up to one hour after the end of the stimulation (Experiment 1 and Experiment 3) and every 30 min minutes up to 120 minutes after the end of the stimulation (Experiment 2).

Results

Figure 2.3 reports the average results for all subjects across the two conditions. Hf-tRNS significantly lowered PT threshold for an extended period of time. We first measured the difference between the two pre-stimulation baseline thresholds by calculating the percent

difference and then performed a one-sample t-test. We found that tRNS (M = .22, SD = 6.11, t_{11} = .12, p = .91) and sham (M = 1.63, SD =8.5, t_{11} = .67, p = .52) did not differ significantly.

Repeated-measure General Linear Model (GLM) {Condition (tRNS vs Sham) X Time (baseline, at time 0, 10, 20, 30, 40, 50, 60 minutes after the end of the stimulation)} revealed a main effect of stimulation ($F_{1,11}$ = 24.23, p = .00046, ηp^2 = .688).

Mauchly's test indicated that the assumption of sphericity had been violated for the main effect of time, X^2 (20) = 34.38, p = .031, therefore degrees of freedom were corrected using Greenhouse–Geisser estimates of sphericity (equal to .49 for the main effect of time). There was no significant effect of time ($F_{2.95,\ 32.46}$ = 1.93, p = .144), and no interaction ([Condition X Time] F (6, 66) = .94, p = .47).

Post-hoc analysis indicated that PT significantly decreased immediately after the end of stimulation (t_{22} = -5.002, p < .001, *r* = 0.73, d = 1.893) and up to 60 minutes' post offset (t_{22} = -4.7521, p < .001, r = 0.694, d = 1.554).

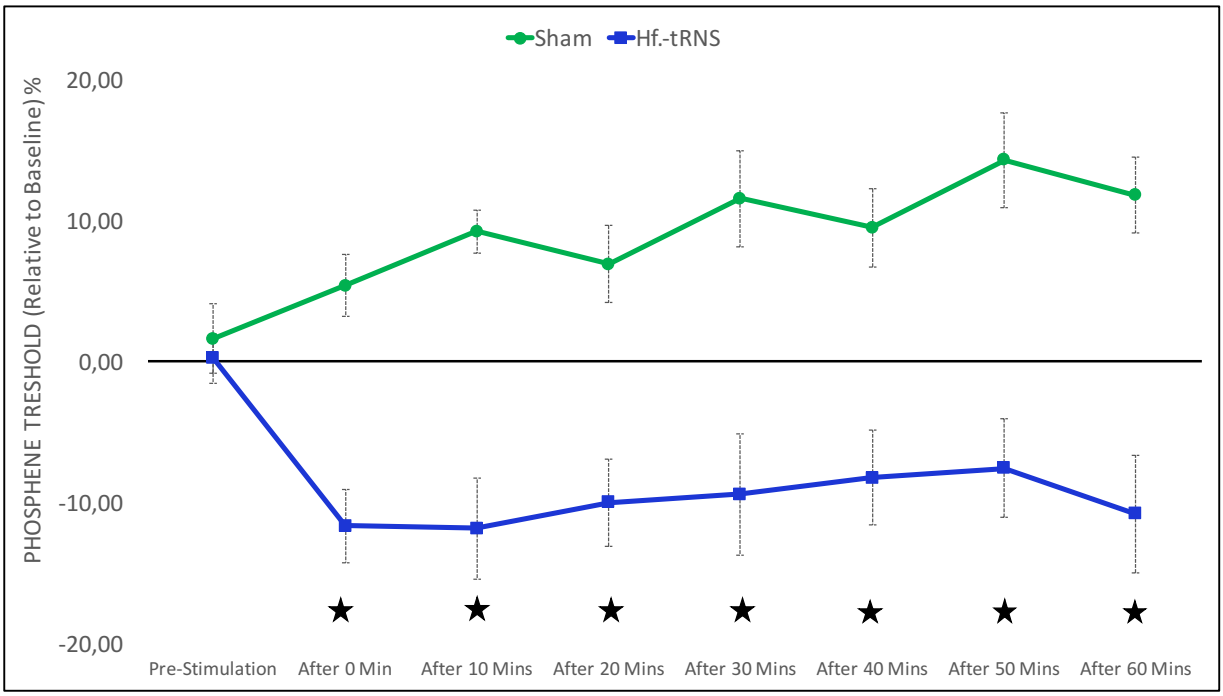

Figure 2.3. Results showing the effect of 20 minutes of Hf-tRNS on cortical excitability. The timecourse shows a significant decrease in Phosphene Threshold, indicating early visual areas excitability changes, immediately after and up to 60 minutes after the end of Hf-tRNS (blue line) compared to Sham stimulation (green line). The single time points show the mean group PT. Error bars are represented as SEM. Asterisks indicate significant difference of PT between the two groups.

Discussion

Results of Experiment 1 indicate that a single session of 20 minutes Hf-tRNS can increase the excitability of the primary visual areas, as shown by a significant decrease in PT that lasts up to 1 hour after the end of the stimulation. On the contrary, PT increased over time following sham stimulation. Although this opposite trend begs further questions, it must be noted that the difference in magnitude between PT in the two conditions following tRNS and sham remains stable and constant across all intervals we measured. This might indicate that over time, there could be a physiological decrease of cortical excitability that affects both conditions (active and sham) in the same manner.

We could speculate that this decrease of cortical excitability could be due to fatigue. Recent works have shown that the production of endocrine markers (melatonin and cortisol) due to wakefulness and tiredness strongly influences cortical excitability (Huber et al., 2013; Ly et al., 2016; Yildirim et al., 2013). Interestingly, the effect of stimulation was still highly significant at 60 minutes post stimulation. We therefore ran a second experiment, to closely study the time course and decay of the effect.

Experiment 2

Participants

A different group of 11 healthy naïve participants gave written informed consent to participate to this study (Mean Age = 20.1, Females = 7). All subjects were right-handed, neurologically healthy and completed a safety questionnaire screening for TMS (Keel et al., 2000). Subjects were interviewed about their state of health and were not taking any medication at the time of the experiment. We recruited 11 subjects, of which only 8 were able to reliably detect phosphenes and were therefore included in the study.

Experimental Procedure

The experimental tRNS procedure was the same as in Experiment 1, except that PTs were also recorded every 30 minutes between 60 and 120 minutes after the end of stimulation.

Stimulation Protocol

The stimulation protocol was exactly the same as in Experiment 1 (Figure 2.2).

Results

We performed the same analysis as we did for Experiment 1 to control for any significant difference between the two pre-stimulation baseline thresholds. The statistical analysis confirmed no significant difference between the two baseline PT (M = .17, SD = 4.82, t_7 = .102, p = .92).

One-sample t-test showed a significant decreased PT for all the time intervals we tested (M = -10.06, SD = 7.26, t_7 =.-3.92, p = .006, r = .83) up to 60 minutes after the end of stimulation (M = -6.02, SD = 5.75, t_7 = -2.96, p = .021, r = .075). Hence we replicated the results from Experiment 1. Moreover, after 90 minutes PT went back to baseline levels, as indicated by the statistical analysis (M = 1.08, SD = 4.59, t_7 = .67, p = .53, r = 0.25).

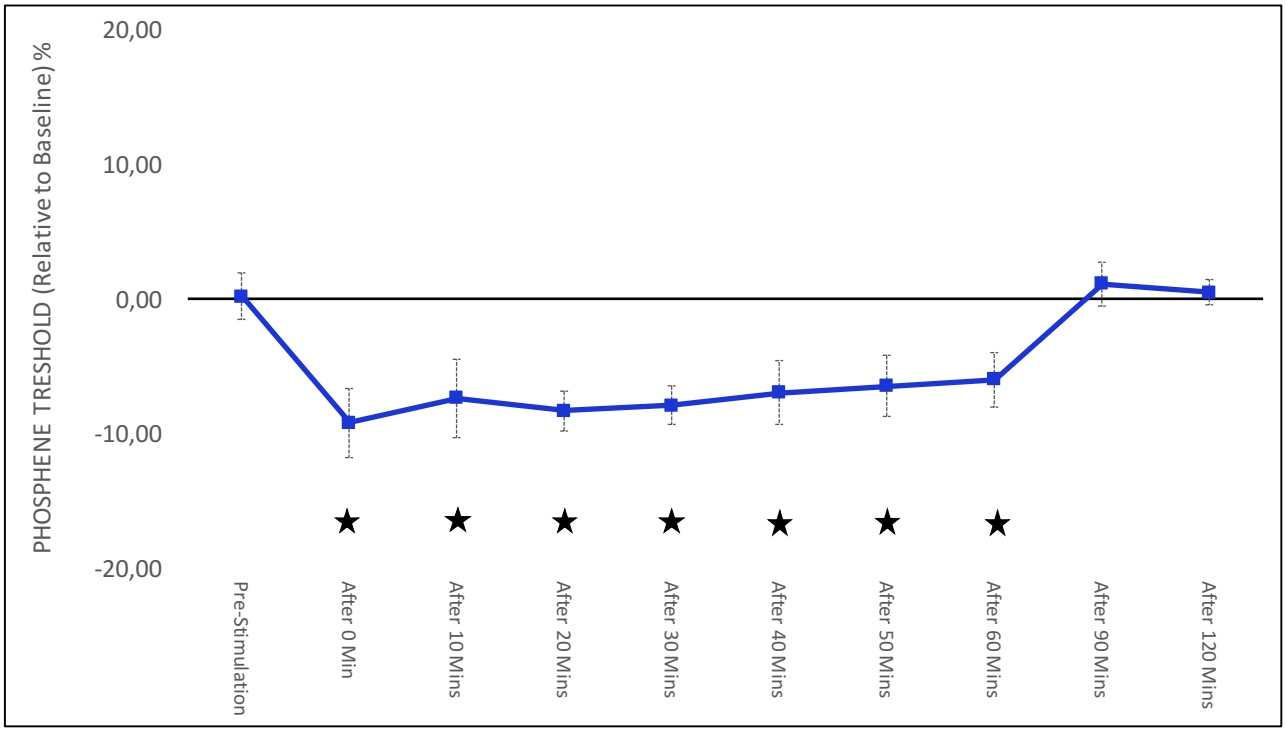

Figure 2.4. Results showing the effect of 20 minutes of Hf-tRNS on cortical excitability. The timecourse shows a significant decrease in PT immediately after and up to 120 minutes after the end of f Hf-tRNS. The single time points show the mean group PT. Error bars are represented as SEM. Asterisks indicate significant difference of PT from baseline (0 on the y-axis).

Discussion

The aim of this experiment was to address the question of how long the effects of Hf-tRNS lasts. In Experiment 2 we replicated the results found in Experiment 1 with a different group of subjects. Moreover, results showed that 90 minutes after the end of the stimulation, the PT went back to baseline, likely indicating a rebalancing of the cortical excitability following tRNS.

Experiment 3

Participants

A total of 22 healthy naïve participants gave written informed consent to participate in the study (Mean Age = 20.9, Females = 6). All subjects were right-handed, neurologically healthy and completed a safety questionnaire screening for TMS (Keel et al., 2000). We recruited 22 subjects, of which 12 were able to detect phosphenes and were therefore included in the study.

Experimental Procedure

The experimental procedure was the same as in experiment 1 (Figure 2.2) except for the stimulation procedure described in the next section.

Stimulation Protocol

Anodal-tDCS (a-tDCS) was delivered by a battery driven stimulator *(DC-Stimulator-Plus, NeuroConn GmbH, Ilmenau, Germany)* through a pair of 35 cm^2 saline-soaked rubber electrodes. The electrodes were placed over the target area identified following the 10-20 EEG standard system. The hotspots were marked on a Lycra swimmer's cap worn by the subjects. The anode was placed on the occipital region over Oz, whereas the cathode (that served as a reference electrode) was placed over the Vertex (Cz). Electrodes were kept in position with an elastic band.

Stimulation was applied for 20 minutes with an intensity of 1mA and started and ended with a fade-in/fade-out ramping of 15 seconds. Sham stimulation consisted of the fade/in-fade/out ramp for 5 seconds, after which stimulation was shut down. Subjects were blinded to the stimulation condition assigned. At the end of each stimulation session, participants were asked to fill out a side effects' questionnaire. None of the participants reported any

side effects, and none of the participants experienced phosphenes induced by the stimulation.

Results

Data reported in Figure 2.5 clearly show that using a-tDCS to prime the cortex did not alter excitability when compared to sham control in our experimental paradigm. As in the previous experiments we first compared the two baseline PT for the two conditions (active stimulation and sham), and we subsequently performed a one-sample t-test to control for any difference from zero for the performance at each time point. Statistical analysis showed no significant difference between baselines for the two conditions a-tDCS (M = .65, SD = 4.79, t_{11} = .47, p = .65) and sham (M = 1.31, SD = 6,37, t_{11} = .715, p= .49).

Repeated-measures General Linear Model (GLM) [Condition (tDCS vs Sham) X Time (each time interval we measured: baseline, 0, 10, 20, 30, 40, 50, 60)] revealed no significant interaction ([Condition x Time] $F_{8,88}$ = .870, p = .545) and no significant effect of stimulation ($F_{1,11}$ = .033, p = .859). However, there was a significant effect of time ($F_{8,88}$ = 3.35, p = .002, ηp^2 = ,233).

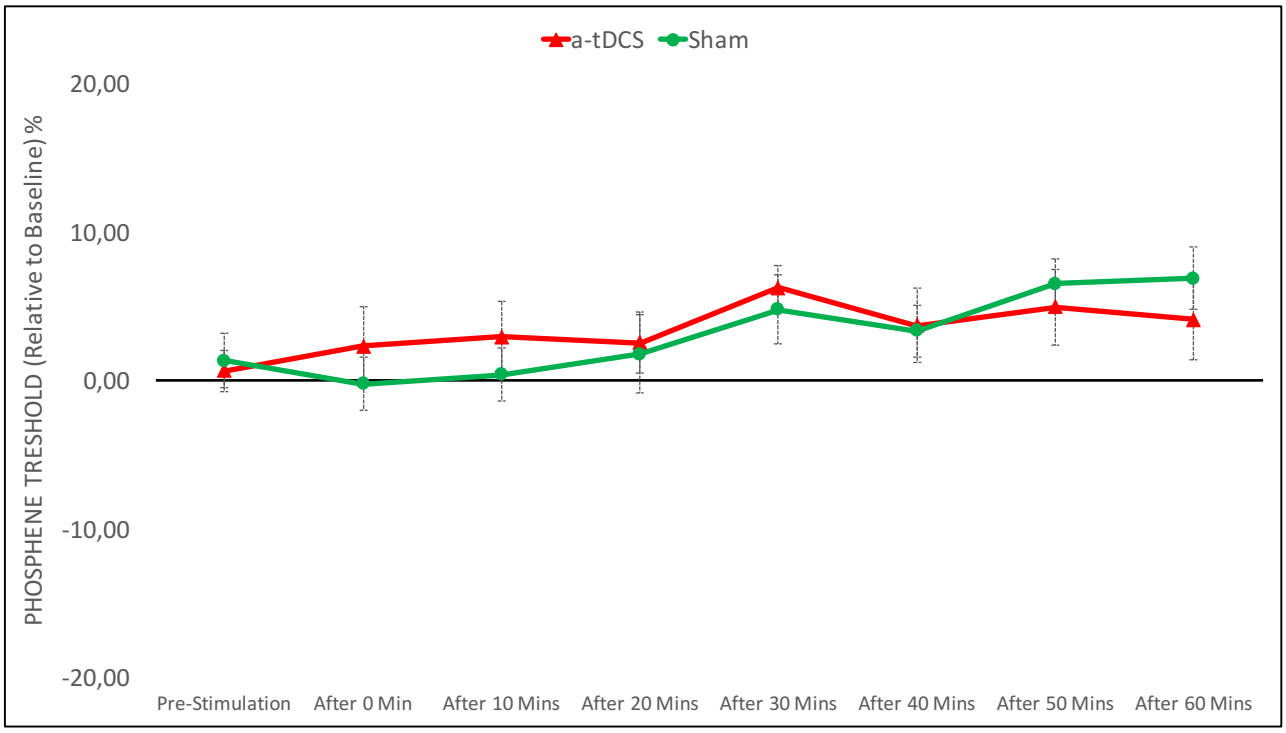

Figure 2.5. Effects of 20 minutes a-tDCS on cortical excitability. The timecourse shows no significant decrease of PT after a-tDCS (green line) compared to Sham (red line). The single time points show the mean group PT. Error bars are represented as SEM.

Discussion

Data from this last experiment show that priming the early visual cortex with a-tDCS did not exert a significant change in cortical excitability relative to sham, as measured with phosphenes' report. There was however a significant effect of time, indicating that the cortical response likely changes during the hour post stimulation. This might be in part attributable to the level of wakefulness, as well as circadian rhythms associated with significant changes in the state of the cortical circuits during the day (Gobbo & Falciati, 2014; Huber et al., 2013; Ly et al., 2016; Yildirim et al., 2013). Additionally, in our experiment, participants were repeatedly stimulated every 10 minutes, and this might have caused some cumulative effect and potential neuronal habituation that might have lowered sensitivity and increased PT (Pell, Roth, & Zangen, 2011). Whatever the case, the effect was the same both for the active and the sham condition, indicating that it did not depend on a-tDCS.

General Discussion

The primary aim of this work was to investigate whether priming the visual cortex with tRNS leads to cortical excitability changes similar to those described in the motor cortex (Terney et al., 2008). We found a significant decrease in PT measured with TMS, after we primed the visual cortex with 20min tRNS. The effect lasts up to 60 minutes' post offset and goes back to baseline after 90 minutes. Hence, we demonstrate that tRNS applied within high-frequency range enhances cortical excitability for a prolonged interval, and this might indicate sensitivity of the cortex to plastic changes.

Previous works demonstrated that short tDCS stimulation protocols (10 minutes) induce alteration of cortical excitability of the visual cortex, with anodal stimulation resulting in increased excitability and cathodal stimulation resulting in significant decrease of the corticospinal excitability, but, unlike the motor cortex, the after-effects after stimulation of the visual cortex were much shorter (lasting 10' minutes post offset) (Antal et al., 2003a). It was therefore suggested that the primary visual cortex may be less amenable to neuroplastic changes than the motor cortex. However, here we demonstrate that early visual cortex excitability can be modulated by tRNS and the after-effects follow a very similar time progression as seen for the primary motor cortex. Notably, as previously mentioned, Terney and colleagues (2008) found that Hf-tRNS over the primary motor cortex induced excitability changes lasting for 60 minutes and returning to baseline

between 1 hour and 90 minutes, a result closely matching what we found in the visual cortex.

A possible explanation, for the different effects seen on cortical excitability of early visual areas following anodal-tDCS could be found in the length of the stimulation protocol used. Whereas, a 10 min tDCS leads to a short lived increase in cortical excitability (Antal et al., 2003a), here we show that 20 minutes of a-tDCS does not exert any effect on PT. One interesting suggestion is that with a-tDCS, following an initial facilitation, prolonged stimulation protocols could lead the neuronal membrane response to adapt to the constant flowing current in which the neurons are embedded, resulting in homeostatic effects of the ion neuronal channels (Camilleri et al., 2016; Fertonani et al., 2011). We speculate, therefore, that when long stimulation protocols are applied, like in our case, the neuronal population adapts to the a-tDCS stimulation, likely driving neurons to return to their baseline "resting" state.

tRNS might be the ideal tool to induce facilitatory effects compared to tDCS, as demonstrated in recent studies, where tRNS significantly increased performance in tests of mathematical and attention skills (Cappelletti et al., 2013; Cappelletti et al., 2015), on visual perceptual learning (Fertonani et al., 2011; Herpich et al., 2015; Pirulli et al., 2013) and in visual functions in the clinical population (Camilleri, Pavan, Ghin, Battaglini, & Campana, 2014).

One might speculate that a possible physiological explanation of the effects of tRNS, might be that stimulation with a random frequency pattern exerts a temporal succession of depolarization and hyperpolarization of neuronal populations, this being the outcome of repeated subthreshold stimulation. This, in turn, might prevent homeostatic adaptation of neurons (Fertonani et al., 2011).

An intriguing hypothesis is also that the effect of tRNS might induce stochastic resonance (Moss et al., 2004). Noise is generally considered a factor that limits the capacity of a system to process information, but nonlinear systems, like the brain is, can efficiently use noise to enhance weak input signals and determine synchronization of different neuronal populations. In fact, it has been shown that the detection of subthreshold visual stimuli can be enhanced when an optimal level of noise is added to the visual cortex. When tRNS is delivered to the neuronal network of the human cortex, it has important signal-enhancing effect on subthreshold, but not supra-threshold, stimuli (van der Groen & Wenderoth, 2016). In summary tRNS appears to be an effective technique to induce long lasting modifications closely associated with learning (Boggio et al., 2006; Galea & Celnik, 2009;

Muellbacher et al., 2002; Pascual-Leone et al., 1999; Ragert, Franzkowiak, Schwenkreis, Tegenthoff, & Dinse, 2008; Reis et al., 2009; Sczesny-Kaiser et al., 2016; Tegenthoff et al., 2005).

Here we showed that tRNS has the ability to enhance cortical excitability of the visual cortex, suggesting that this type of tES could support learning via synaptic potentiation. This might have translational potential in clinical application such as in neurorehabilitation. tES are already being used in rehabilitation protocols for motor impairments, however it is still unclear whether the same protocols can be applied to improve cognitive and sensory functions such as perception and vision. In particular, data on the use of tES on the visual cortex are scant and controversial. tRNS might be a promising alternative, with a clear and more sustained effect in time. It can exert facilitatory effects faster and more efficiently than other stimulation protocols in a variety of cognitive and perceptual domains on healthy population (Camilleri et al., 2016, 2014; Cappelletti et al., 2013; Cappelletti et al., 2015; Fertonani et al., 2011; Herpich et al., 2015; Pirulli et al., 2013). However, very little is known about the neurophysiological effects, and our results might contribute to the understand them.

Although we did not test other behavioral outcome in the present protocol, we tentatively suggest that an increased state of excitation of the visual cortex could help explain the behavioral improvements previously reported in several different cognitive domains (Cappelletti et al., 2013; Cappelletti et al., 2015; Fertonani et al., 2011; Herpich et al., 2015; Pirulli et al., 2013).

Chapter 3: tES in cognitive training

Non-invasive brain stimulation protocols differentially impact visual perceptual learning of global motion

Abstract

Recent psychophysical studies have demonstrated that visuo-perceptual functions can improve over multiple training sessions, both in healthy adults (Sagi, 2011) and in hemianopic stroke patients (Das, Tadin, & Huxlin, 2014). To date, rehabilitative therapies for hemianopic patients have shown significant improvements *only* after many weeks of daily training. Recent studies using transcranial direct current stimulation (tDCS) have shown enhancement of visual performance in normal subjects. Notably, when current is applied in a random noise mode (tRNS), effects are seen earlier and are longer lasting. Here, we asked whether tDCS or tRNS can be used to boost visual perceptual learning of global direction discrimination, thus providing a proof-of-concept for the potential use of this approach in pathological populations.

We tested 45 healthy, visually-intact subjects, aged 19-26 (7 males) who were randomly assigned to 4 training groups: “anodal tDCS”, high frequency “Hf-tRNS”, ‘high Frequency Parietal tRNS “sham” and “no-stimulation”. All subjects were trained to discriminate the left or right global motion direction of random-dot stimuli for 10 days (one session/day). Before and after training, we measured the subjects’ direction range and motion signal thresholds. Brain stimulation was delivered concurrently with the training task. On average, all subjects improved over the two-weeks training period. However, the hf-tRNS group exhibited the largest improvements.

Introduction

Improvements in sensory discrimination after intensive training are the behavioral evidence of neuronal changes associated with perceptual learning (Seitz et al., 2007), and this enhanced perceptual performance is thought to reflect brain plasticity (Sagi, 2011). The question of how the visual cortex changes in response to visual perceptual learning (VPL) is still open and debated, as several mechanisms might play a crucial role in either promoting or preventing learning. While neurophysiological studies have shown that perceptual learning selectively modifies the signal strength of neurons responding to relevant stimulus features, while concurrently suppressing the activity of task irrelevant information (Yan et al., 2014), psychophysical studies have led to the hypothesis that boosting stimulus-related cortical activity might be one of the mechanisms promoting perceptual learning (Seitz & Dinse, 2007). However, it has been recently demonstrated that there are some limitations to the applicability of PL in humans, in particular the length of the training (many weeks and months of daily training) make it less suitable to be used with the clinical population. Interestingly, new scientific evidence shows that non-invasive brain stimulation, coupled with training protocols, is particularly promising for boosting stimulus-related cortical activity, and ultimately speeding up and enhancing the effects of training (Cappelletti et al., 2013; Fertonani et al., 2011; Mulquiney et al., 2011; Snowball et al., 2013).

One way of measuring the effect of NIBS upon behavior in humans is to use psychophysical testing (Antal, Nitsche, & Paulus, 2001; Fertonani, Pirulli, & Miniussi, 2011) and, interestingly, a relatively recent alternating current stimulation technique, transcranial random noise stimulation (tRNS, Terney et al., 2008), has proven effective in facilitating and improving behavior when concurrently coupled with VPL (Fertonani et al., 2011). Some recent studies have also shown that a combination of tRNS and cognitive training results in significantly bigger and longer lasting improvements (up to 16 weeks) when compared to cognitive training alone, in both, young and elderly population. These studies also indicate that tRNS may be a useful tool for cognitive enhancement and rehabilitation of special population (Cappelletti et al., 2013; Cappelletti et al., 2015).

Several studies have ascertained that many visual abilities can improve with training (Levi et al., 2015), however some studies failed to show improvement with practice, posing the question of what are the best conditions that favor improvement and, mostly, what cortical areas play a direct role in promoting improvement. This is not trivial, as for instance in stroke patients affected by vision loss after V1 damage, it would be of paramount

importance to determine precise cortical targets to promote visual field recovery with noninvasive brain stimulation (for a review see Melnick et al., 2015). Training on global motion discrimination is a typical paradigm used in perceptual learning. Its efficacy in promoting recovery of visual field loss, and transfer to other visual functions, has been demonstrated both in healthy subjects (Levi et al., 2015; Wang et al., 2016) as well as in neurological patients (Huxlin et al., 2009; Das et al., 2014). Therefore, we chose motion coherence training coupled with brain stimulation to contrast the efficacy of tRNS and tDCS administered during visual training at boosting and speeding up visual learning. Some recent controversial studies have conducted meta-analysis of data collected with tDCS protocols and they showed no cognitive effects after one session of tDCS (Horvath et al., 2015). However, the effect of multiple stimulation sessions upon learning bears some interesting and still unanswered questions that would help assess the applicability of noninvasive stimulation to facilitate cortical plasticity.

On the other hand, tRNS has proven to enhance cortical excitability in the motor cortex (Terney et al., 2008) and, as suggested from results in chapter 2, to modulate neuronal excitability of the primary visual cortex. Moreover, recent studies have shown that tRNS can induce improvements of cognitive functions when delivered over the visual cortex (Camilleri et al., 2014; 2016; Campana et al., 2014; Pirulli et al., 2013), however it is not known whether the same effect can be measured using global motion concurrent with tRNS across multiple sessions. It has been suggested that stimulation-related improvements might exert from the state of the neurons at the time of stimulation (Silvanto et al., 2008), and that adding noise to the cortex might enhance sensory detection, in particular when the stimuli are presented at threshold, embedded in noise (Abrahamyan et al., 2015). This is the so called stochastic resonance phenomenon, whereby random noise enhances the detectability of a weak signal in a nonlinear system (van der Groen and Wenderoth, 2016). Therefore, we hypothesized that tRNS could potentially improve detection performance when coupled with global motion training, an optimized stimulus whose detectability can be manipulated by psychophysically manipulating the signal-to-noise ratio. We wanted to see whether tRNS can shorten the training to gain significant improvement and, importantly, we questioned whether the effect can persist months after the end of the training, indicating long term plasticity. Finally, we contrasted the effect of tRNS with anodal-tDCS and we expected tRNS to lead to better performance.

Materials and Methods

Participants

A total of forty-five subjects participated in the experiment (mean age: 19.9 years old; range: 19-36; 32 females and 13 males). All subjects were right-handed, neurologically normal, with normal or corrected-to-normal vision and gave written informed consent prior to the beginning of the study approved by the Ethical Committee of the University of Trento. All subjects underwent 10 days of training, preceded by a pre-training test to measure baseline performance and a post-training follow-up at six months. During this follow-up session, participants repeated the behavioral baseline tests.

Participants were randomly assigned to one of five groups (9 subjects for each group). Three received active online stimulation: high frequency tRNS over early visual areas (EVA Hf-tRNS), Hf-tRNS over parietal cortex (Hf-tRNS Parietal) and anodal tDCS over EVA (EVA a-tDCS). One group received sham stimulation over the early visual areas (EVA Sham), and a fifth group performed the behavioral task only without stimulation (Behaviour).

Experimental Set-Up

During each session participants were positioned on a chinrest-forehead bar combination to stabilize their heads and placed so that their eyes were 57 cm viewing distance from a 24-inch computer monitor (BenQ 120Hz). Real-time eye fixation was enforced by an Eye Tracking System (EyeLink 1000 Plus – SR Research Ltd., Canada) that monitored the pupil center and corneal reflection of the left eye. All testing, training and visual field perimetry procedures were done monocularly with the left eye, while the right eye was occluded with an eye patch.

Apparatus

All experimental sessions took place in the same room under same light and noise condition and with the same apparatus. Visual stimuli were generated on a MacBook Pro running software based on the Psychophysics Toolbox (*Brainard,1997; Pelli, 1997)* in Matlab (*MathWorks*). Stimuli were presented on a linearized SensEye 3 LED 24 Inch (*BenQ*) monitor with a refresh rate of 120 Hz. The monitor was luminance-calibrated with gamma =1 with a professional monitor calibrator (Datacolor Spyder 5). Eye fixation was controlled in real time using an EyeLink 1000 Plus Eye Tracking System (*SR Research*

Ltd., Canada) whose infrared camera monitored the pupil center and corneal reflection of the left eye. Limits were set so that if the participant's eye moved > 1.5 ° in any direction away from the fixation spot, three loud tones sounded and the currently displayed trial was aborted and excluded form the final analysis. The fixation window for this task was set at 3x3° in size.

Every subjects visual field was tested with an automated visual field perimetry *(Optopol PTS 1000 Visual Field, Canon)* to verify their field of vision and ensure they did not exhibit overt deficits prior to the onset of the visual training. Only after confirming the absence of any visual field deficit subjects were included in the current study.
tDCS and tRNS were delivered using a battery-driven stimulator *(DC-Stimulator-Plus, NeuroConn GmbH, Ilmenau, Germany)* through a pair of saline-soaked conductive rubber electrodes (35cm^2).

Psychophysical Stimuli and tasks

Global Motion Direction Discrimination (GMD). We measured direction range thresholds for left-right motion discrimination of stimuli that contained a limited percentage of signal dots (Newsome & Pare, 1988). Once a motion signal level was selected for each participant, the task used a quest adaptive procedure (Watson, 1983) to estimate the broadest distribution of dot directions for which the subject could correctly discriminate the global direction of motion. The QUEST criterion was set to converge when 82% of accuracy was reached. The random dot stimuli were presented within a Gaussian aperture 5° in diameter at a density of 2.6 dots/deg^2. Each dot had a diameter of 0.06° and moved at a speed of 10°/s with a lifetime of 250ms. Stimulus duration was 500ms. The signal-to-noise ratio of the coherent left/right moving dots and the randomly noisy moving dots, was calibrated for each subject individually during a first preliminary baseline session. For all but 3 subjects, the random dot stimuli contained 40% of coherent motion signal. Three subjects were trained with a stimulus containing 30% coherent motion and 70% noise dots, to allow enough room for improvement. The signal-to-noise ratio of the stimulus was chosen based on preliminary testing aimed to identify a motion signal level that allowed participants to perform just above change (50% correct). Each participant started training with direction ranges set to 0°.

Participants were asked to fixate on a central cross for 1000ms immediately followed by a tone signaling the appearance of the stimulus that was presented for 500ms. The stimulus

was centered at [-5, 5] deg for all subjects in order to fall into the periphery of the visual field (Das et al., 2014; Huxlin et al., 2009). Once the stimulus disappeared, participants had to indicate the perceived global direction of motion by pressing the left or right arrow keys on the keyboard. The two motion directions (leftward and rightward) were randomized across trials. Acoustic feedback was provided, indicating whether the participant gave a correct (high tone) or an incorrect (low tone) response. When participants broke fixation by more than 1.5° of visual angle in any direction, three tones sounded an alarm and the trial was aborted.

During training stimuli were presented monocularly to the left eye for 10 days (one/day from Monday to Friday, for 2 weeks) while they received either active, sham or no stimulation. Subjects run 350 trials/day for a total of 3500 trials at the end of the 2 weeks of training. The total duration of the daily training session for each group was set to last 20 min.

Stimulation Protocol

Each subject was randomly assigned to one of the 5 stimulation groups. The electrodes were bilaterally placed over the target areas identified following the 10-20 electroencephalogram reference system. To target the brain hotspot to stimulate, participants wore a Lycra swimmer's cap on which the points were marked.

For group 1 (EVA hf-tRNS) the electrodes were positioned over the early visual areas, corresponding to the EEG system electrode positions O1/PO7 and O2/PO8, for the left and right hemisphere, respectively. Whereas electrodes were positioned over P3 and P4 for group 2 (Parietal hf-tRNS). For the a-tDCS (group 3) the anode was positioned over Oz and the cathode over Cz (Nitsche et al., 2008; Antal & Paulus, 2008*).* For the Sham group we used the same bilateral montage as for group 1.

The intensity of stimulation was set to 1.0 mA, and was delivered for 20 min with a fade in/out period of 20 seconds. For the a-tDCS group the polarity of the active electrode was anodal. For the tRNS condition, the random noise stimulation was applied with a 0 mA offset at frequencies of alternating current ranging from 101 to 640 Hz (hf-tRNS). For the Sham stimulation group, the stimulation was shut down after 20 s. At the end of each session, we asked all subjects to fill out a questionnaire about potential discomfort or any unusual sensation they experienced during the stimulation. Only minor side-effects were reported by the tDCS group (2 subjects reported slight itching under the electrode, 1

subject reported a slight subjective temperature increase under the electrode), whereas none of the tRNS group participants reported any sensation of being stimulated.

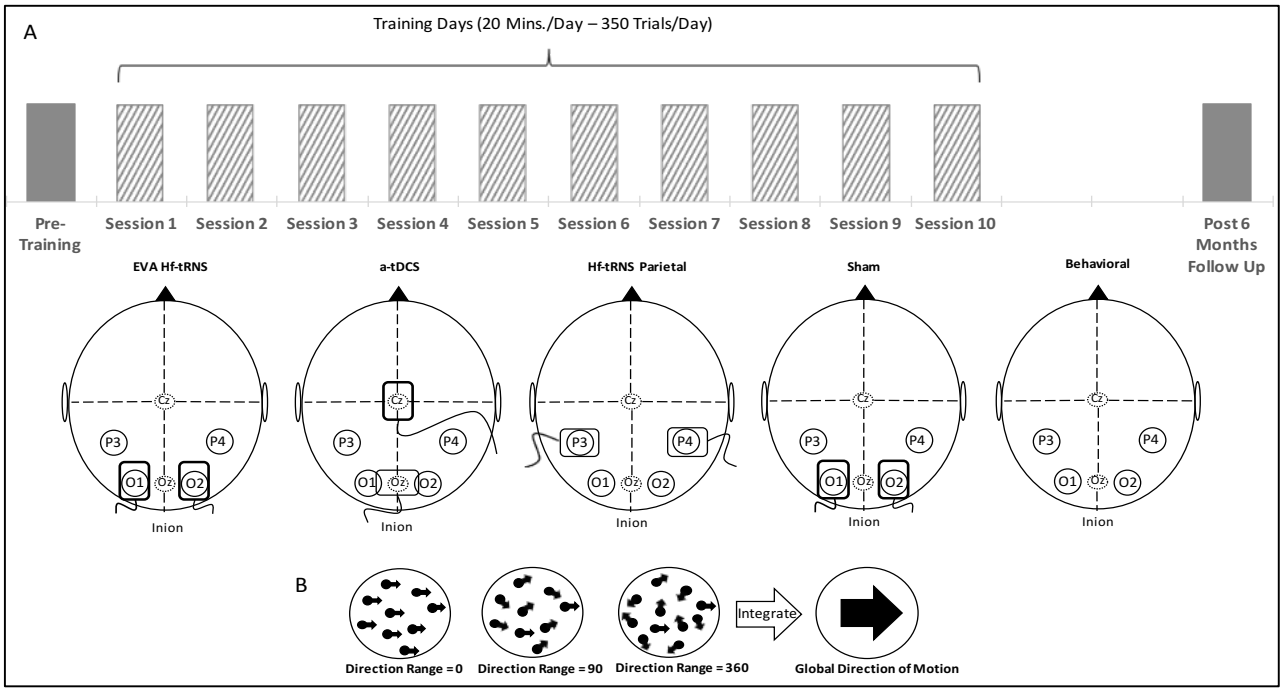

Figure 3.1. Experimental procedure and Stimulation protocol used. A. Participants were divided into 5 different groups. For EVA-Hf-tNRS Group the electrodes were positioned over the early visual areas, corresponding to the EEG system electrode positions O1/PO7 and O2/PO8. For the EVA a-tDCS Group the anode was positioned Oz and the cathode over Cz (Nitsche et al., 2008; Antal & Paulus, 2008). For the Hf-tRNS Parietal control group, electrodes were positioned over P3 and P4, whereas for the Sham group the same bilateral montage as in EVA Hf-tRNS group was used. The intensity of the stimulation was set to 1 mA, and was delivered for 20 minutes. For the anodal tDCS the polarity of the active electrode was anodal. For the tRNS condition, the random noise stimulation was applied with a 0 mA offset at frequencies of alternating current ranging from 101 to 640 Hz (hf-tRNS). B, before the training (Pre-Training) all participants were tested on the Global Motion Direction Discrimination (GMD) task to determine baseline performance. Subsequently, subjects underwent 10 days of training on the GMD task while receiving online stimulation. Subjects were tested again on the GMD task 6 months after the end of the stimulation (Follow Up), to test for long-term effects of the training protocol. B. Global Motion Direction Discrimination Task (GDM) used.

Data Analysis

Shapiro-Wilk test was used to control for the normality of data distribution. Data Sphericity was addressed using Maulchy's test, and Greenhouse-Geisser correction was used in case of non-sphericity of the data. Levene's test was used to address the assumption of equality of variances. P-values were considered significant for values of < .05. For multiple comparisons, we used Fisher's (LSD) method to test our specific a priori hypothesis (I.e. to compare different stimulation condition at a given time point). The effect sizes are reported as the partial Eta-squared (ηp^2) values. Correction for multiple comparisons was performed with the Benajmini & Hochberg, FDR method (FDR $p < 0.05$) (Benjamini & Hochberg, 1995).

Results

Impact of different brain stimulation protocols on the magnitude of global motion learning

Results showed that all subjects from all groups benefited from two-weeks training, however direction integration thresholds for the group that underwent tRNS over EVA improved faster and of higher magnitude relative to all other conditions. All subjects performed similarly at baseline on day one (One-Way ANOVA, $F_{4,\ 40} = 1.19$, $p = .33$), however the rate of improvement was different across conditions.

A repeated measures ANOVA, using *Stimulation (EVA Hf-tRNS vs. EVA a-tDCS vs. Parietal Hf-tRNS vs. Sham vs. Behavioral)* as a between-subject factor and *Session (Training Session 1 to Training Session 10*) as a within-subjects factor showed a significant Session*Stimulation interaction ($F_{14.98,\ 149.77} = 2.908$, $p < .001$, $\eta p^2 = .265$). Maulchy's test indicated that the assumption of sphericity had been violated (X^2 (44) = 165.63, $p < .001$), therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon = .42$). We subsequently conducted multiple independent t-test comparisons which revealed that EVA Hf-tRNS was significantly different from all the other conditions: EVA a-tDCS ($t_{16} = - 5.66$, $p = .000035$), Parietal Hf-tRNS ($t_{16} = 3.41$, $p = .004$), Behavioral ($t_{16} = 3.25$, $p = .005$), and from Sham condition ($t_{16} = 2,15$, $p = .047$).

We also performed planned comparisons comparing performance in the Sham condition vs Behavioral, and we found no significant difference in magnitude of learning (when comparing direction integration thresholds at day one and day 10), ($t_{16} = 1.13$, $p = .275$).

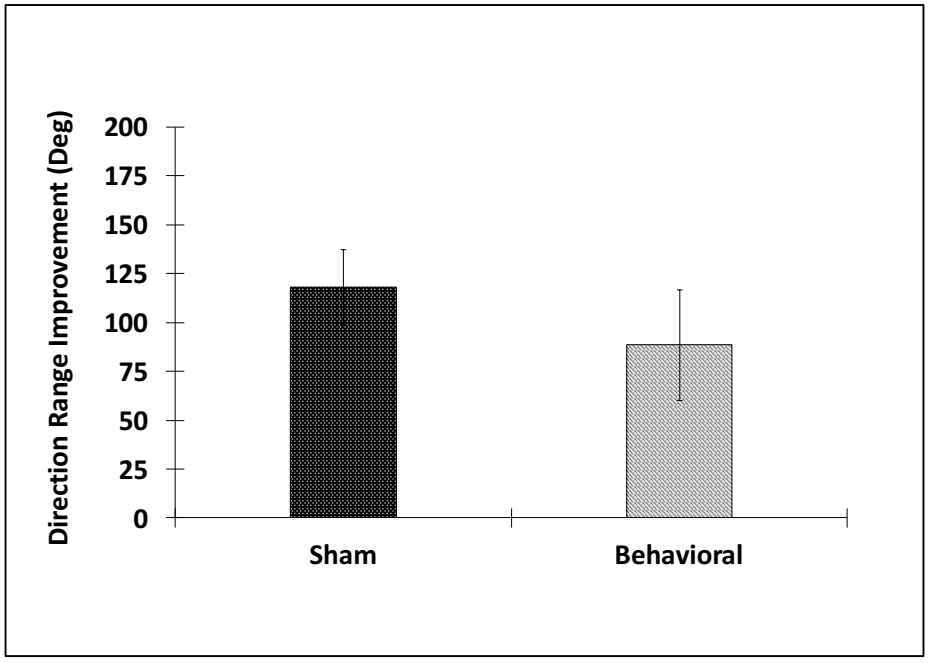

Figure 3.2. Histogram illustrating mean performance improvements on the GMD task presented as Direction Range Thresholds after 10 days of training for the Sham group vs. Behavioral training group. There was no significant difference between the Sham and the Behavioral group following 10 days of training. All data in the histogram are expressed as means and error bars are represented as ±SEM. (Deg., Degree).

We next compared the Sham condition to EVA a-tDCS and found a significant difference, indicating that the a-tDCS group showed the lowest overall improvement, (t_{16} = -2.809, p =.016).

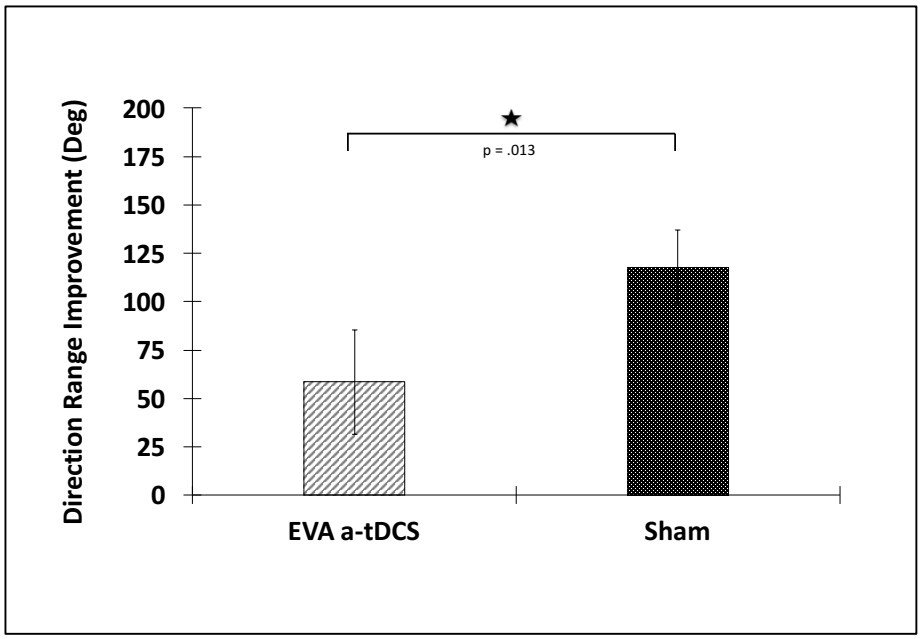

Figure 3.3 Histogram illustrating mean performance improvements on the GMD task presented as Direction Range Thresholds after 10 days of training for the Early Visual Areas a-tDCS (EVA a-tDCS) vs. Sham group. Performance was significantly different between the groups following 10 days of training. All data in the histogram are expressed as means and error bars are represented as ±SEM. (Deg., Degree).

Finally, Figure 3.4 reports the comparison between the average performance for the EVA Hf-tRNS versus Parietal Hf-tRNS and Sham. These data clearly indicate that only the EVA Hf-tRNS stimulation condition exerted a significant improvement relative to Sham.

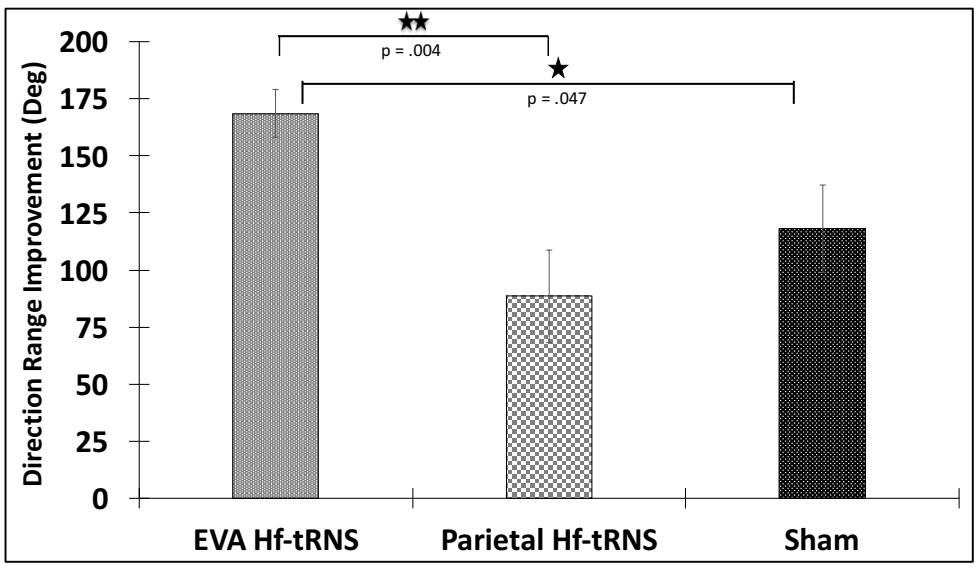

Figure 3.4. Histogram illustrating mean performance improvements on the Global Motion Discrimination task (GMD) presented as Direction Range Thresholds after 10 days of training for the Early Visual Areas tRNS (EVA Hf-tRNS) vs. Parietal Hf-tRNS vs. Sham group. Significant difference emerged for EVA Hf-tRNS vs. Parietal Hf-tRNS and for EVA Hf-tRNS vs. Sham following 10 days of training. All data in the histogram are expressed as means and error bars are represented as ±SEM. (Deg., Degree).

Impact of different brain stimulation protocols on the rate of global motion learning

We next looked at the rate of learning across the two weeks of training. To analyze the rate of improvement across sessions as a function of stimulation coupled with training, we performed a linear regression analysis for each subject's performance. To conform to assumption of normality and homoscedasticity of variance, Beta values were first Box-Cox (Box and Cox, 1964) power transformed (lambda = 2.6102). Specifically, Beta is the coefficient measuring the steepness of the regression line, therefore indicating how fast each group of subjects improved on the GMD task over the 2 weeks' period. We subsequently ran a one-way ANOVA with Stimulation *(EVA Hf-tRNS vs. EVA a-tDCS vs. Parietal Hf-tRNS vs. Sham vs. Behavioral)* as between factor. Results showed a statistically significant difference in the Beta between the different groups, ($F_{4, 40}$ =2.93, p = .03, $\eta 2$ = 0.226) therefore confirming a difference in the slope of the growing curve between the 5 different groups. Post-hoc independent t-test (Fisher's LSD) showed that EVA Hf-tRNS Beta coefficients were significantly different from the Parietal Hf-tRNS condition (p = .03), moreover the slopes of the a-tDCS vs. the Sham group were significantly different (p = .026). These results indicated that subjects assigned to the EVA Hf-tRNS active stimulation condition improved faster than the Parietal control active stimulation group. Finally, subjects in the anodal active stimulation group were those showing the lowest improvement.

Figure 3.5a. Regression lines represent the rate of learning across the two weeks of training. Beta coefficients, which measure the steepness of the regression line indicating therefore how fast each group of subjects improved on the GMD

task over the 10 days period. The analysis showed no significant difference for the Beta coefficients for Sham and Behavioral condition. All data points are expressed as means and error bars are represented as ±SEM. (Deg., Degree).

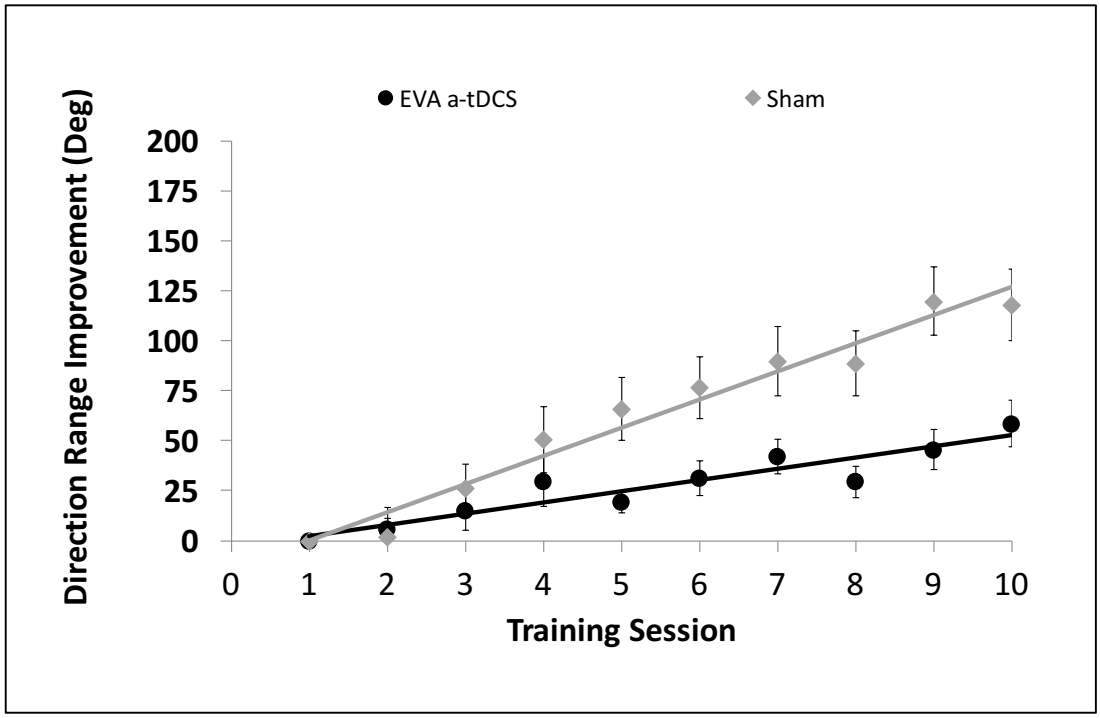

Figure 3.5b. Regression lines represent the rate of learning across the two weeks of training. Beta coefficients, which measure the steepness of the regression line indicating therefore how fast each group of subjects improved on the GMD task over the 10 days period. The analysis showed a significant difference for the Beta coefficients for EVA a-tDCS and Sham condition (p = .026). All data points are expressed as means and error bars are represented as ±SEM. (Deg., Degree).

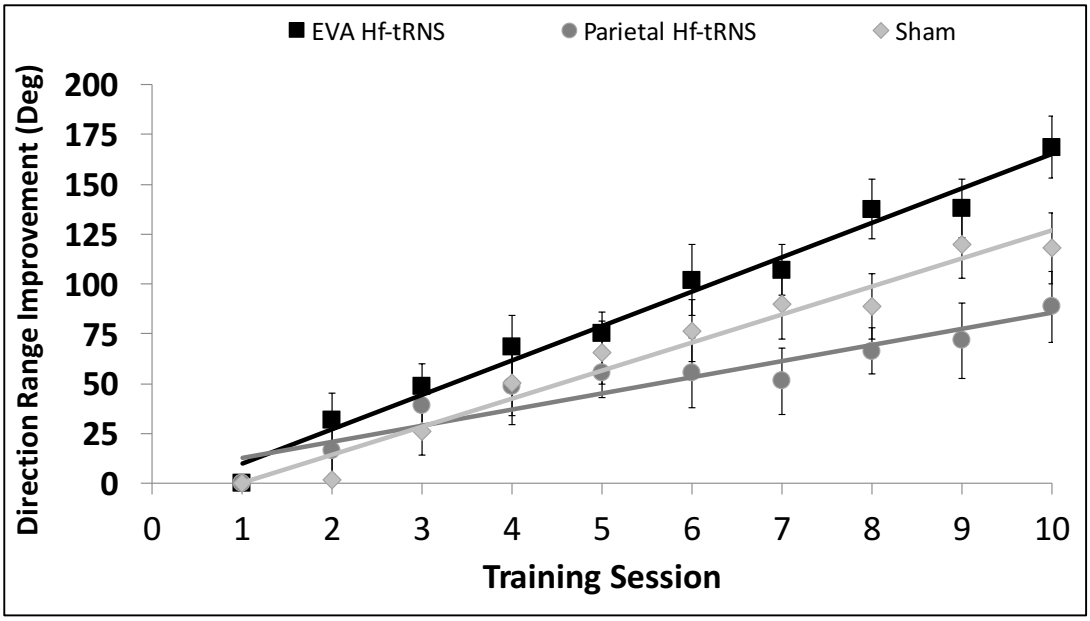

Figure 3.5c. Regression lines represent the rate of learning across the two weeks of training. Beta coefficients, which measure the steepness of the regression line indicating therefore how fast each group of subjects improved on the GMD task over the 10 days' period. The analysis showed a significant difference for the Beta coefficients for EVA Hf-tRNS and

Parietal Hf-tRNS condition (p = .03). All data points are expressed as means and error bars are represented as ±SEM. (Deg., Degree).

Since EVA-Hf-tRNS administered during training appeared to cause faster learning, we analyzed at what time-point EVA-Hf-tRNS started to differ from all other groups. We hence conducted pairwise comparisons as independent t-tests. Results showed that on Day 8 EVA Hf-tRNS group's performance differed significantly from all other conditions: EVA a-tDCS ($t_{12.2}$ = -6.39, p = .000032), Parietal Hf-tRNS (t_{16} = 3.74, p = .002), Behavioral (t_{16} = 2.9, p = .011) and Sham condition (t_{16} = 2.21, p = .042).

Persistence of stimulation effects

We next asked whether the magnitude of learning that we found immediately at the end of the training sessions persisted across time. We therefore tested a subgroup of subjects (n = 36) 6 months after the end of stimulation, and compared their thresholds with those attained at the end of the 2 weeks of training. We ran a repeated measure ANOVA using *Stimulation (EVA Hf-tRNS vs. EVA a-tDCS vs. Parietal Hf-tRNS vs. Sham vs. Behavioral)* as between-subject and *Session (Post-Training vs. 6 Months Follow-Up*) as within-subjects factor. The analysis revealed no significant difference between the post-training threshold and the threshold at 6 months' follow-up test ($F_{4, 31}$ = 1.046, p = .4), showing that the improvement measured immediately at the end of the training was still present after 6 months, hence indicating consolidation of learning. We next conducted repeated measure t-tests between subjects' average performance on Day 1 (pre-training) compared to 6-months post stimulation to determine whether performance remained stable for all conditions we tested. These analyses indicated a significant difference in performance for the EVA Hf-tRNS group (t_7 = -8.87, p=.00005), and the same was true for the Sham (t_5 = -5.94, p=.002), Parietal Hf-tRNS (t_6 = -5.18, p=.002), Behavior (t_7 = -4.687, p=.002) and the a-tDCS group (t_6 = -3.68, p=.010).

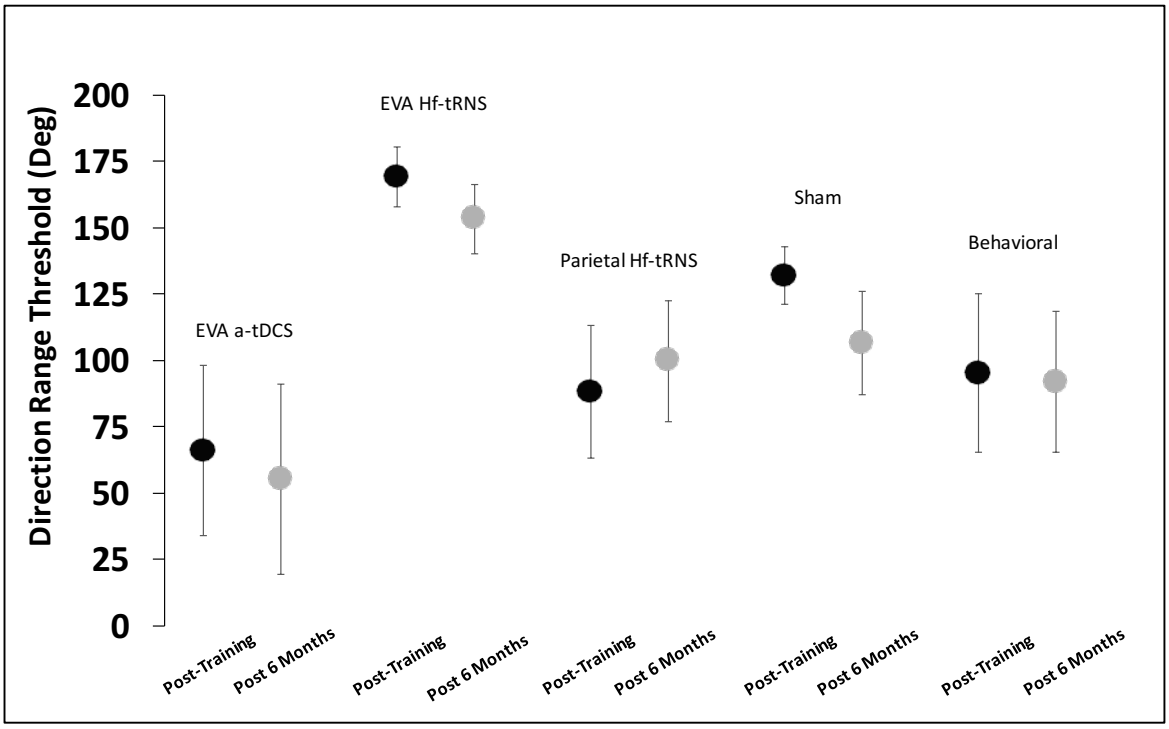

Figure 3.6. Plot illustrating the persistence of the stimulation effects represented as mean Direction Range Threshold (DR). The black circles indicate the DR reached immediately after the end of the 10 days of training, whereas the grey circles indicate the DR of the Follow Up session measured after 6 months after the end of the stimulation. The analysis revealed no significant difference between the post-training threshold and the threshold at 6 months Follow Up test. This results indicate that the improvement reached immediately after the training was still persistent after 6 months, indicating consolidation of learning. (Deg., Degree).

Discussion

In the present study we asked whether brain stimulation can be used to boost and speed up visual perceptual learning, for conditions where it would normally take multiple learning sessions across months. We further compared different neuromodulation techniques to determine the most effective in promoting learning. Importantly, we wanted to test if the effect of training coupled with a stimulation protocol could exert long lasting plasticity.

Results show that Hf-tRNS applied bilaterally to the early visual cortex speeds-up and boosts the effect on visual perceptual learning. Specifically, we found that on average all subjects improved over two-weeks of training, however we observed a significant improvement of global motion direction discrimination in subjects who received stimulation to the occipital lobe with Hf-tRNS (average 168.56° improvement in direction range thresholds). Improvement for the control Hf-tRNS condition over the parietal lobe was much smaller (improvement average 88.52°) and did not differ from the other active stimulation condition a-tDCS (average 58.5°). Moreover, performance did not differ for the two “non-active” stimulation groups, sham and behavior (average improvement of 117.89°

and 88.42°, respectively). Crucially, this improvement was persistent and still significant six months after the end of the training, indicating consolidation of learning.

Although all groups improved in global motion discrimination, improvement was faster and larger only when tRNS was applied over the early visual cortex, indicating the direct involvement of EVA in VPL and the potential of tRNS as a way to boost cortical excitability and improve performance.

Various aspects might contribute to facilitate perceptual learning, however to promote learning, the sensory signal should be increased to boost the stimulus related activity that is normally insufficient to reach a learning threshold (Seitz & Dinse, 2007). Despite well known factors that can trigger learning, like attention (Ahissar, 2001) and reinforcement (Pascucci, Mastropasqua, & Turatto, 2015; Seitz & Watanabe, 2005), we suggest here that tRNS coupled with a psychophysical training protocol can boost the crucial target-related cortical activity, and therefore speed up and enhance the effects of learning. One might ask whether stimulation alone could have exerted the same effect, however recent work has shown that this is not the case, only behavioral training coupled with tRNS stimulation can boost the rate and intensity of perceptual learning (Camilleri et al., 2016).

There might be several reasons why brain stimulation enhances the effect of training, and one of the leading theory suggests that stimulation adds noise to a non-linear system (as the brain is), hence improving the detectability of the signal. This, in turn, boosts the stimulus-related activity that promotes perceptual learning (Moss et al., 2004). Interestingly, a recent work by van der Groen and Wenderoth (2016) demonstrated that tRNS over the visual cortex has the same enhancement effect as adding optimal noise to a visual stimulus to increase the detection accuracy of a sub-threshold visual stimulus, which alone, would not surpass the critical threshold levels to produce a behavioral response. Cortical activity could therefore be enhanced with ad-hoc stimulation protocols, and this can induce synaptic plasticity resulting in effective perceptual improvements.

Interestingly, in our study a-tDCS over the visual cortex did not facilitate learning, and the result is consistent with recent studies showing that anodal tDCS impairs overnight consolidation of visual learning (Peters et al., 2013). Overall, the a-tDCS data might even suggest that this type of stimulation could potentially inhibit learning.

Moreover, in chapter 2 we showed that tRNS can enhance cortical excitability of the primary visual cortex with effects lasting up to 1 hour after one single session of 20 minutes of stimulation. On the contrary, a-tdcs stimulation delivered over the visual cortex,

did not exert any shift in cortical excitability. Although in the present study we did not directly correlate the behavioral outcomes with the physiological results, we tentatively suggest that an increased state of excitation of the visual cortex could help explain the behavioral improvements found here in visuo-perceptual learning coupled with tRNS. We suggest that shifts in cortical excitability might also be responsible for behavioral results previously found in other studies (Cappelletti et al., 2013; Cappelletti et al., 2015; Fertonani et al., 2011; Herpich et al., 2015; Pirulli et al., 2013). Taken together, our behavioral results might be explained by the state of the cortical excitability following the different stimulation protocols we used. While tRNS results in behavioral improvement, and likely significant shifts in cortical excitability (chapter 2), no behavioral facilitation nor neuronal modulation (chapter 2) was evident following a-tdcs.

An important point is to understand whether the plastic changes induced by stimulation outlast the stimulation time. The issue of consolidation and stabilization of learning is not trivial as many VPL studies have failed to see long lasting effects and/or transfer of learning to other tasks (Ball & Sekuler, 1987; Seitz, Nanez, Holloway, Tsushima, & Watanabe, 2006). Crucially, we demonstrated here that our training protocol coupled with 10 days of Hf-tRNS induced the biggest enhancement on performance and, even more interestingly, long lasting effects, up to 6 months from the end of the training.

Finally, we provided a proof-of-concept for the potential use of this technique on the pathological population, particularly for those patients who can benefit from visual training, but only after intensive training that requires many months (up to 18 or more) of daily practice (Huxlin et al., 2009; Das, Tadin, Huxlin, 2014). tRNS might be the ideal tool to promote faster and stronger recovery. This point will be discussed in details in Chapter four.

Chapter 4: tRNS in stroke rehabilitation

tRNS to promote recovery in cortical blindness: a preliminary study

Abstract

Cortical blindness is a severe clinical outcome after stroke affecting the primary visual areas. Most commonly, it affects portions of the visual fields (quadrantopsia) or the entire hemifield. The deficit can be profoundly invalidating as the subject is no longer able to function normally during every day activities. When the blind portions of the visual field are systematically tested using forced choice procedures with psychophysical tasks, evidence of some residual vision has been found, namely called blindsight. Potentially these patients can undergo treatment to help them recover some portions of the blind visual field. However, this can only be done through very long visual tranining, that can take up to 16 months to have measurable significant changes. Based on our previous results, in this last chapter we took advantage of the potential of tRNS to boost perceptual learning performance, and we applied it to support recovery in blindsight patients. We tested four patients and we randomly assigned them to active or sham stimulation (two patients for each condition). They underwent two weeks of VPL training coupled with Hf-tRNS or sham. Results indicate that the active stimulation group achieved a significantly better performance relative to sham. These preliminary data strongly indicate a potential clinical application of our experimental procedure.

Introduction

Damage to primary visual cortex (V1) can cause partial or complete loss of conscious vision called cortical blindness (CB) (Holmes, 1918; Huber, 1992; Leopold, 2012; Zhang et al., 2006). Stroke involving the posterior or middle cerebral arteries is responsible for the great majority of cases. The incidence of CB in the general population is remarkably high (Geddes et al., 1996; Pollock et al., 2011; Pollock et al., 2012; Gilhotra et al., 2002). According to WHO estimates, the number of stroke in Europe is likely to increase form 1.1 million per year in 2000 to more the 1.5 million per year in 2025 (Truelsen et al., 2006), of which 27% to 57% exhibit damage to V1 or its afferents (Pollock et al., 2011; Pollock et al., 2012). Usually, spontaneous recovery is limited within the first few weeks after the insult (Zhang et al., 2006), with almost no spontaneous improvements reported after 6 months from the brain damage (Kolmel, 1991; Zhang et al., 2006). Contrary to well-established physical therapies to treat motor deficits following strokes to the motor cortex, no established validated clinical therapies exist for the restoration of visual field deficits (reviewed by Melnick et al., 2015).

The reason for this lack of rehabilitation strategies to treat CB patients, is the assumption that chronically damaged visual system is incapable of functional recovery (Horton, 2005; Reinhard et al., 2005). However, CB subjects can retain residual visual processing abilities in their blind fields, a well known phenomena described for the first time by Weiskrantz and colleagues in 1974 and called “blindsight” (Sanders et al., 1974; Weiskrantz et al., 1974). Interestingly, blindsight subjects can perform above chance when forced to discriminate or detect visual stimuli within their blind fields (Cowey, 2010; Stoerig & Cowey, 1997). In recent years, some research groups have devised innovative psychophysical techniques to encourage recovery of portions of the blind field (Das et al., 2014; Huxlin et al., 2009; Raninen et al., 2007; Sahraie et al., 2006, 2010; Sahraie et al., 2008), and others have used visual restoration therapy, the latter with inconsistent results (Plow et al., 2011). Recently, intensive visual perceptual learning training has been used to try to recover portions of the defective visual field. In particular, visual training with forced choice discrimination tasks, using stimuli presented repeatedly in a gaze-contingent manner on the border of the scotoma, showed to improve simple and complex visual motion processing in the blind field of adults with damage to V1 (Cavanaugh et al., 2015; Huxlin et al., 2009). This visual restitution approach which requires subjects not only to detect, but also to make a judgment about the nature of the stimuli presented in their blind field, builds

upon work conducted on animals (Huxlin & Pasternak, 2004) and humans (Cavanaugh et al., 2015; Das et al., 2014; Huxlin et al., 2009), showing how gaze contingent training can promote recovery of motion direction and static orientation discrimination at trained blind locations. Huxlin and colleagues reported not only relearning of coarse (left/right) global motion discrimination, but also a decrease in the size of the scotoma of patients, as confirmed by Humphrey automated perimetry (Huxlin et al., 2009). Intensive training promoted recovery of patients' ability to discriminate global direction of motion, however many months (≥ 16 months) of daily training were required to see significant improvements.

In the mature visual cortex, damage to the brain shifts the excitatory-inhibitory balance in the residual surviving visual circuitry toward excessive inhibition which has been proposed as a responsible mechanism limiting plasticity and recovery (for a review see Spolidoro, Sale, Berardi, & Maffei, 2009). Sale and colleagues, in fact, demonstrated pharmacologically how reduction of intra-cortical inhibition reactivated Ocular Dominance (OC) plasticity in response to monocular deprivation (MD) in adult rats (Sale et al., 2010). Following this first direct demonstration, several recent papers showed the fundamental role of the inhibitory/excitatory system in modulating plasticity in the brain (He et al., 2007). When emerging excessive intra-cortical inhibition cannot be counteracted, restoration of plasticity is prevented (Spolidoro et al., 2009).

It appears, from the above described studies, that an excessive intra-cortical inhibition might be what limits recovery, providing a possible explanation why training is so effortful and only partially successful. One possibility to promote recovery, is to either decrease intra-cortical inhibition or to enhance the strength of the feed-forward signals. Different several methods have been proposed to do so: pharmacologically (Vetencourt et al., 2008; Pizzorusso, 2002), through visual deprivation (He et al., 2006, 2007), enriched environments (Fedorka & Mousseau, 2004; van Praag et al., 2000) or through noninvasive brain stimulation (Miniussi, Harris, & Ruzzoli, 2013). Drugs like acetylcholine or serotonin, which decrease the breakdown of neurotransmitters, seem to increase the plasticity in the visual cortex by boosting the stimulus-related feed-forward signals relative to inhibitory connections (Vetencourt et al., 2008; Rokem & Silver, 2010; Silver et al., 2008). However, pharmacological interventions and their associated side-effects are often not well tolerated.

The work presented in the previous chapters has provided strong indication that tRNS

could be the ideal technique to promote recovery also in patients. We have seen how it can enhance and speed up visual perceptual learning in healthy adults (Camilleri et al., 2016; Cappelletti et al., 2013; Cappelletti et al., 2015; Fertonani et al., 2011; Mulquiney et al., 2011; Snowball et al., 2013) and enhance cortical excitability.

Experiments from chapters two and three showed that tRNS applied in the high frequency range (101-640Hz), enhances training-induced visual perceptual learning, and likely cortical excitability, to a larger degree and more reliably than tDCS. Crucially, this improvement persisted months after the end of the training indicating long term plasticity (Herpich et al., 2015). In addition, tRNS, showed not only to be more effective in boosting stimulus-related cortical activity (Camilleri et al., 2016, 2014; Campana et al., 2014; Cappelletti et al., 2013; Fertonani et al., 2011; Herpich et al., 2015), but also showed to have no side effects and to be well tolerated. Crucially, subjects cannot determine whether they are undergoing active stimulation or sham, making it easier to conduct a blind study.

An interesting hypothesis is that in partially blind patients extrastriate visual areas remain mostly intact (Baseler et al., 1999; Papanikolaou et al., 2014), however they might have lost a significant portion of their feed-forward input, due to the lesion to V1. Therefore, we hypothesize that tRNS could potentially improve the responses of spared neuronal populations to weak inputs, through selectively boosting feed-forward signals associated with the training stimulus or, by decreasing plasticity-limiting inhibitory processes. If so, we expect, based also on recent data showing enhancement of cognitive training in healthy adults (Camilleri et al., 2016, 2014; Campana et al., 2014; Cappelletti et al., 2013; Cappelletti et al., 2015; Fertonani & Miniussi, 2016; Pirulli et al., 2013), a potentiation of the training-induced visual improvements in chronic CB subjects. In this preliminary study we tested a small group of CB patients who received either tRNS or Sham stimulation daily for 10 days. The results are promising and indicate a good potential for the technique to promote recovery.

Materials and Methods

Case histories

We tested 4 adult patients, in the chronic phase post stroke (> 6 Months post onset). They were all left with damage to the early visual areas, causing homonymous visual defects, confirmed by neurological examination and automated visual perimetry. They all suffered

from stroke involving the territory of the posterior cerebral artery as confirmed by radiological examinations (CT or MRI). None had history or evidence of degenerative or psychiatric disorders. All participants were right handed, with normal or corrected-to-normal visual acuity. All had a stable, homonymous visual defect. None exhibited other forms of visual deficits or visual neglect, as determined by neurological examination.

Patient PD, a 58 years old woman, suffered an ischemic stroke in April 2012. Neuroradiological examinations showed a large ischemic lesion involving right fronto-parieto-occipital regions following occlusion of the internal carotid artery (Figure 4.1).

Patient CA, a 76 years old man, suffered an ischemic stroke in June 2015. Neuroradiological examinations showed a right posterior capsule-thalamic and occipital lobe lesion following a stroke with cardio-embolic genesis (Figure 4.1).

Patient AP, a 69 years old man, suffered an ischemic stroke in July 2014. Neuroradiological examinations showed a lesion of the left occipital lobe (involving cuneus, precuneus and the cingulate gyrus) and the posterior capsule-thalamic area of the homonymous hemisphere (Figure 4.1).

Patient PA, a 72 years old man, suffered from an ischemic stroke in January 2016. Neuroradiological examination showed a lesion of the left occipital lobe and the internal capsule due to an ischemia of the posterior cerebral artery (Figure 4.1).

All patients showed homonymous visual defects as confirmed by the visual field perimetry we performed before the inclusion to the study. They all gave written informed consent before participating in the study, according to the ethical standards of the Declaration of Helsinki. The study was approved by the ethical committee of the University of Trento and the ethical committee for clinical experimentation of the “Azienda Provinciale per i Servizi Sanitari” (APSS). Patients were recruited at the Center for Neurocognitive Rehabilitation (CeRiN) affiliated to the University of Trento and the Rehabilitation Hospital “Villa Rosa”, in Pergine, Italy.

All subjects underwent 10 days of training, preceded by a pre-training test to measure baseline performance. Automated visual field perimetry was performed during pre-training, at the end of the first week of training and at the end of the 10 days of training.
Participants were randomly assigned to one of two groups. Two patients received active online Hf-tRNS over early visual areas (EVA Hf-tRNS), whereas the control group received

a Sham stimulation with electrodes placed over the early visual areas (EVA Sham).

Patient	Date of Birth	Visual Defect	Lesion	Time Post Onset (Months)
P.D.	16.06.1957	Lateral homonymous hemianopia	Right fronto-parieto-occipital lobe	36
A.P.	07.10.1947	Lateral homonymous hemianopia	Left occipital lobe and left posterior capsule-thalamic area	24
P.A.	25.09.1944	Lateral homonymous quadrantopsia	Left occipital lobe and internal capsule	9
C.A.	29.03.1940	Lateral homonymous quadrantopsia	Right posterior capsule-thalamic and occipital lobe	15

Table 1. Demographic and lesion data. All patients suffered from a cerebrovascular stroke resulting in occipital lobe lesions. Visual defects described in table were assessed with an automated visual field perimetry. The last column indicates the time from onset at which we tested the patients.

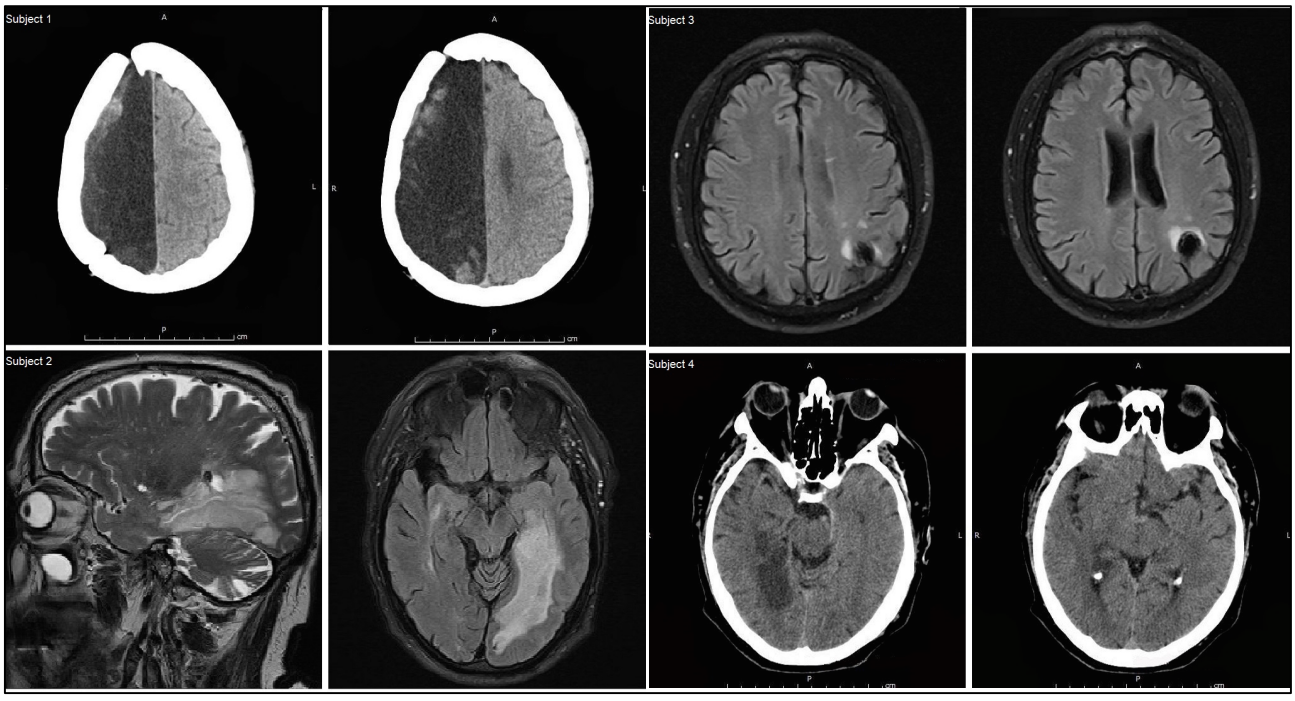

Figure 4.1. Neuroradiological images of the 4 patients that participated in this study (2 for each patient). Top (from left): P.D., P.A., bottom (from left): A.P., C.A. All tested patients sustained stroke-induced damage of early visual areas or the optic radiations. As for neuroradiological convention left is right and right is left in each picture.

Experimental Set-Up

During each session participants were positioned on a chinrest-forehead bar combination to stabilize their heads and placed so that their eyes were 57 cm viewing distance from a 24-inch computer monitor (BenQ 120Hz). Real-time eye fixation was enforced by an Eye Tracking System (EyeLink 1000 Plus – SR Research Ltd., Canada) that monitored the pupil center and corneal reflection of the left eye. All testing, training and visual field perimetry procedures were done monocularly with the left eye, while the right eye was occluded with an eye patch.

Apparatus

Visual stimuli were generated on a MacBook Pro running software based on the Psychophysics Toolbox (Brainard, 1997; Pelli, 1997) in Matlab (*MathWorks*). Stimuli were presented on a linearized SensEye 3 LED 24 Inch (*BenQ*) monitor with a refresh rate of 120 Hz. The monitor was luminance-calibrated with gamma =1 with a professional monitor calibrator (Datacolor Spyder 5). Eye fixation was controlled in real time using an EyeLink 1000 Plus Eye Tracking System (*SR Research Ltd., Canada*) whose infrared camera monitored the pupil center and corneal reflection of the left eye. Limits were set so that if the participant's eye moved > 1.5 ° in any direction away from the fixation spot, three loud tones sounded and the currently displayed trial was aborted and excluded form the final analysis. The fixation window for this task was set at 3x3° in size.

Every subjects' visual field was tested with an automated visual field perimetry *(Optopol PTS 1000 Visual Field, Canon)*.
tRNS was delivered using a battery-driven stimulator *(DC-Stimulator-Plus, NeuroConn GmbH, Ilmenau, Germany)* through a pair of saline-soaked conductive rubber electrodes ($35cm^2$).

Baseline Measure of Visual Performance

Perimetric Visual Field Test: Every patient underwent automated visual field perimetry before the onset of the retraining. This allowed us to exactly map the extent of each subject's visual deficit and allowed us to verify its stability compared with previous examinations. The visual field perimetry was performed again by the same researcher who performed the pre training tests, at the end of the first week and at the end of the 10 days of training.

Psychophysical Global Motion Direction Discrimination abilities

After automated visual filed perimetry, for each subject we measured direction range thresholds for left-right motion discrimination of stimuli that contained a limited percentage of signal dots (Newsome & Pare, 1988). This allowed us to psychophysically map motion sensitivity of each participant. Before each testing session, the EyeLink Eye Tracking system was calibrated by requiring the subjects to maintain fixation on a target stimulus that was moved on either side of the screen along the vertical and horizontal axis of the computer monitor in front of them. Once fixation was calibrated, subjects were asked to perform direction discrimination and detection task centrally and at different visual field locations to map their threshold for global motion discrimination abilities.

To measure global motion discrimination abilities of each subject, a QUEST staircase procedure was used. The QUEST criterion was set to converge when 82% of accuracy was reached.

Global Motion Direction Discrimination (GMD) Task

Subjects were asked to perform left-right direction of motion discrimination of random-dot moving stimuli. To map the motion sensitivity of each participant, they were asked to performed 100 trials of the task at different locations, with each trail initiated by fixation of a small fixation circle. Participants were asked to fixate on a central cross for 1000ms immediately followed by a tone signaling the appearance of the stimulus that was presented for 500ms. Once the stimulus disappeared, participants had to indicate the perceived global direction of motion by pressing the left or right arrow keys on the keyboard. The two motion directions (leftward and rightward) were randomized across trials. Acoustic feedback was provided, indicating whether the participant gave a correct (high tone) or an incorrect (low tone) response. When participants broke fixation by more than 1.5° of visual angle in any direction, three tones sounded an alarm and the trial was aborted.

The random dot stimuli were presented within a Gaussian aperture 5° in diameter at a density of 2.6 dots/deg^2. Each dot had a diameter of 0.06° and moved at a speed of 10°/s with a lifetime of 250ms. Stimulus duration was 500ms.

Visual Retraining

After completing baseline testing, visual training protocol began. The chin-forehead-rest system allowed the subject's eye to be positioned at 57cm from the monitor where the stimuli were presented. During the training, stimuli were presented monocularly to the left eye of the participants for 10 days (one/day from Monday to Friday, for 2 weeks) while they received either active or sham stimulation. The total duration of the daily training session for each group was set to last 20 min. The eccentricity of presentation of the stimuli at which each subjects performed the training was determined during pre-training session. Initially the stimuli were presented at locations close to the border of the blind visual field, moving progressively deeper into the blind field as global motion direction discrimination dropped above change. The first location were subjects performed below change was used as initial training eccentricity.

For each patients we also measured global motion discrimination abilities in at least 2 other locations in the healthy visual field as a control, to ensure subjects were able to correctly perform the task in their normally sighted visual hemifield.

Stimulation Protocol

Each patient was randomly assigned to one of the 2 stimulation groups. The electrodes were bilaterally placed over the target areas identified following the 10-20 electroencephalogram reference system. Patients wore a Lycra swimmer's cap on which the target stimulation hotspots were marked.

For group 1 (EVA hf-tRNS) the electrodes were positioned over the early visual areas, corresponding to the EEG system electrode positions O1/PO7 and O2/PO8, for the left and right hemisphere, respectively. The same bilateral montage was used for the Sham group.

The intensity of stimulation was set to 1.0 mA, and was delivered for 20 min with a fade in/out period of 20 seconds. For the tRNS condition, the random noise stimulation was applied with a 0 mA offset at frequencies of alternating current ranging from 101 to 640 Hz (hf-tRNS). The same parameters were used for the Sham condition, except that the stimulator was programmed so that the current was shut down after 20 sec of ramping-up. At the end of each session, we asked all subjects to fill out a side effects questionnaire. None of the participants reported any sensation of being stimulated.

Results

Results are preliminary as we tested two patients per group. Therefore we only performed exploratory analysis. We performed linear regressions to test if the type of stimulation that the participants received significantly predicts subject's behavioral outcome on the visual restoration training. A significant regression equation was found, as confirmed with a Pearson's correlation coefficient, for the patients who received Hf-tRNS stimulation ($F_{1,8}$ = 12.05, p = .008, β =.775). The results of the regression indicated that Stimulation as a predictor explained 60.1% of the variance in the case of Hf-tRNS (R^2 =.601).

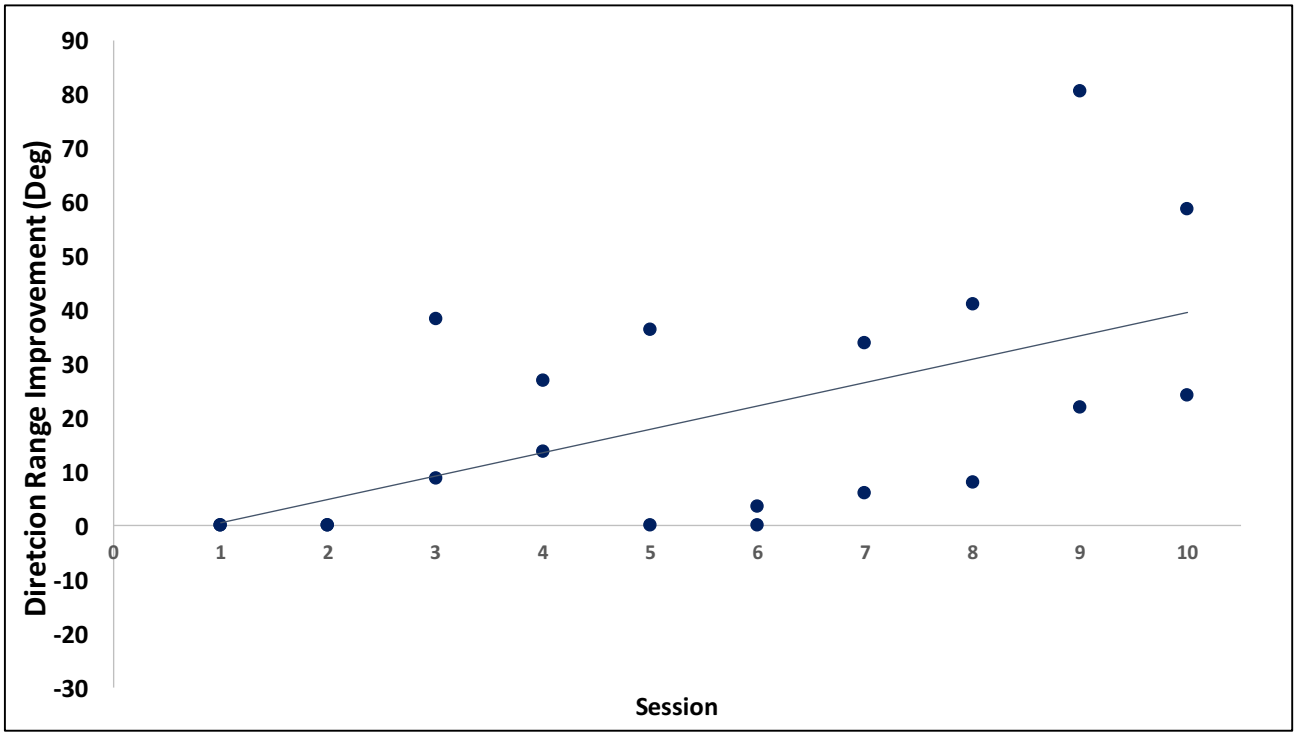

Figure 4.2. Regression analysis revealed a significant positive interaction between the visual restoration training and performance on Global Motion Discrimination for the Hf-tRNS patients (r =.775, p = .008, R^2 = .601, β = ,775).

No significant regression equation emerged, as confirmed with a Pearson's correlation coefficient, for the group of patients who received Sham stimulation ($F_{1,7}$ =.43, p =.53, β =.240). The results of the regression indicated that Stimulation as a predictor explained the 5.7% of the variance in the case of Sham stimulation (R^2 =.057).

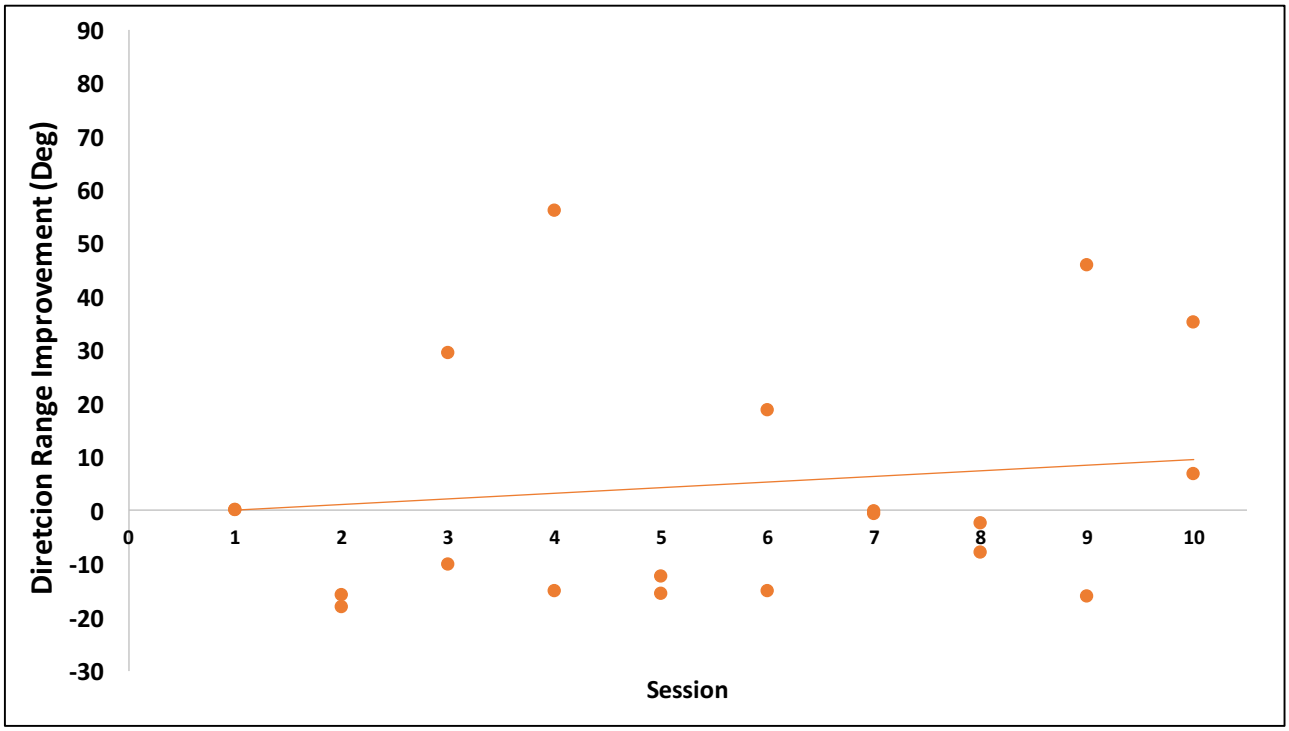

Figure 4.3. Regression analysis revealed that the interaction between visual restoration training and performance on Global Motion Discrimination was not significant for the sham patients (R = .231, p = .520, R^2 = .057, β = -,231).

Subsequently, a one sample t-test was performed to determine the overall behavioral improvements of the patients who received Hf-tRNS stimulation following 10 days of training. Behavioral improvements scores were normally distributed, as assessed by Shapiro-Wilk's test (p >.05) and there were no outliers in the data, as assessed by inspection of a boxplot with Cook's distance method. Mean improvement (M = 20.05, SD = 16.99) was statistically significant for the Hf-tRNS group (t_9 = 3.73, p =.005, d = 1.18) after 10 days of training compared to their performance prior to the onset of the training.

A one sample t-test was performed to determine the overall behavioral improvements of the patients who received Sham stimulation following 10 days of training. Behavioral improvements scores were normally distributed, as assessed by Shapiro-Wilk's test (p >.05). One data point that was identified as an outlier, as assessed by inspection of a boxplot with Cook's distance method, was removed form the analysis. Mean improvement (M = 5.43, SD =11.95) was not statistically significant for the sham group (t_8 = 1,36, p =.21, d = .45) after 10 days of training compared to their performance prior to the onset of the training.

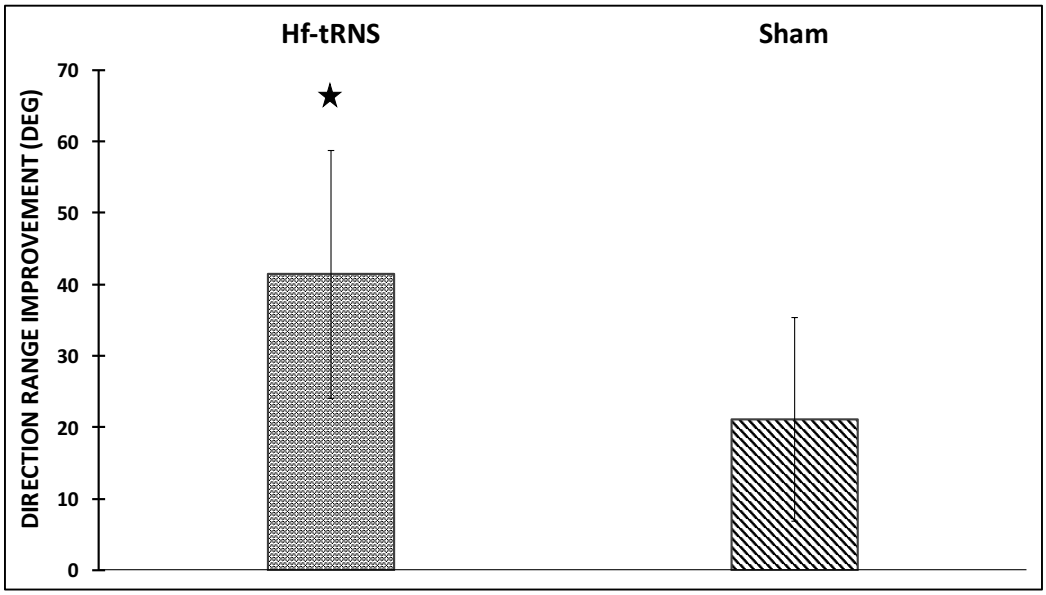

Figure 4.4. A two-tailed, one sample t-test revealed that mean improvement, following 10 days of training, was statistically significant for the Hf-tRNS patients when compared to their performance at baseline prior to the onset of the training (t(9) = 3.73, p =.005, d = 1.18). Improvement for the Sham stimulated group was not significant relative to baseline (t(8) = 1,36, p =.21, d = .45).

A qualitative clinical-diagnostic analysis of the automated visual field perimetry performed before and after the end of the training, showed a trend toward an expansion of the visual field for the subjects who received active Hf-tRNS stimulation (Figure 4.5), whereas only minor improvements was evident for the Sham stimulation group (Figure 4.6). Pre and post-training maps of luminance sensitivity were performed always by the same person and under exactly the same conditions of light. The obtained maps show a shifting of the blind border after 10 days of daily training, potentially suggesting that Hf-tRNS may be able to significantly speed up the process of recovery of visual field functions compared to sham condition.

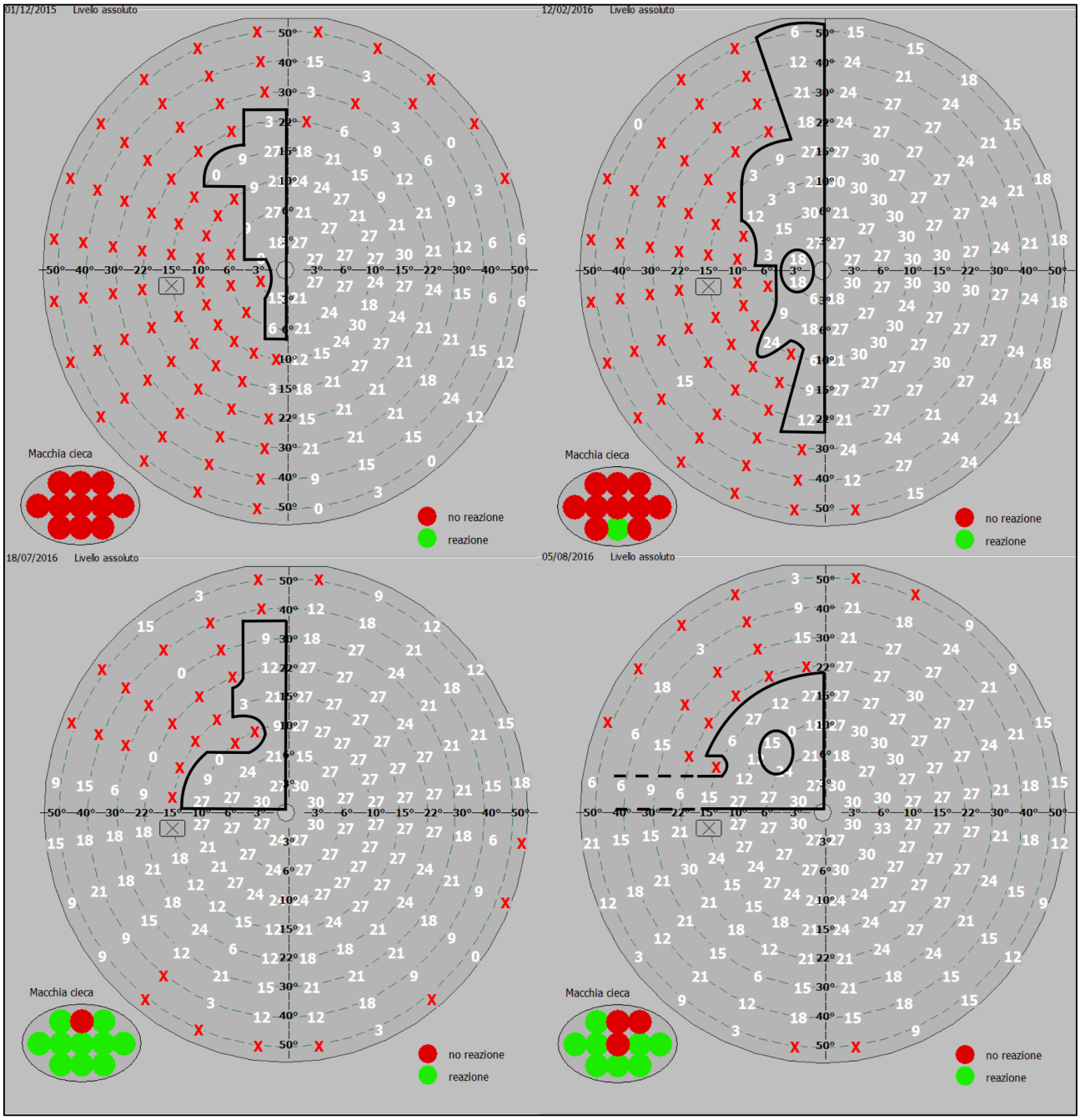

Figure 4.5. Shift of the visual field defects for the two active HF-tRNS stimulated patients after 10 days of visual perceptual training. The visual maps on the left show the size of the scotoma prior to the training, whereas the visual maps on the right shows the expansion of the visual field after 10 days of training. The solid black lines outline the expansion of the visual field after 2 weeks of daily training. The black circle indicates the topographical location of the stimuli used during training. Enlargement of the visual field is particularly evident in the area overlapping the retrained location and in the immediate surroundings.

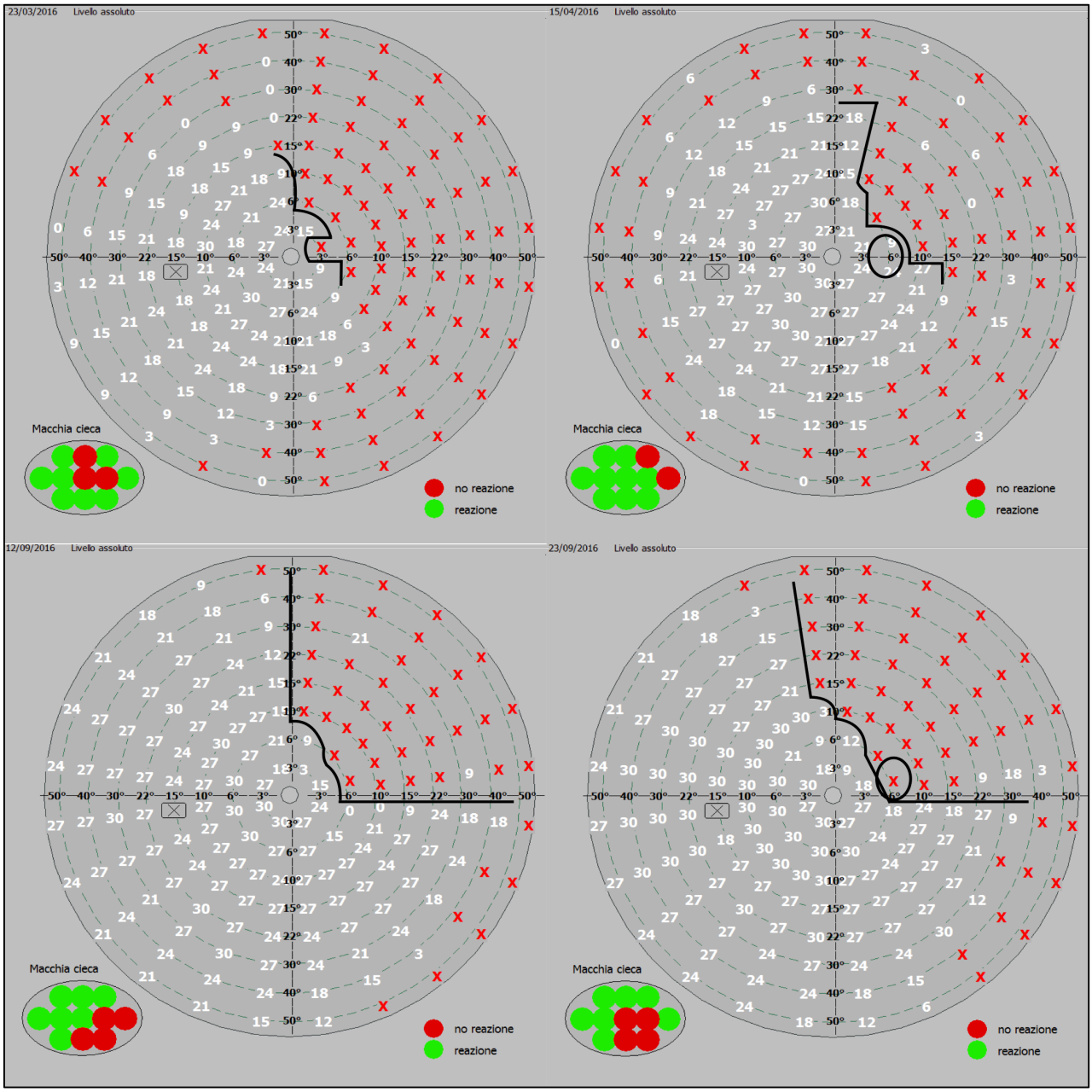

Figure 4.6. Shift of the visual field defects for the two Sham stimulated patients after 10 days of visual restoration training. Only minimal expansions of the visual field emerged in the area overlapping the retrained location for one subject (top), whereas no difference in the scotoma was evident for the second subject (bottom).

Discussion

Here we tested the hypothesis that bilateral brain stimulation of peri-lesional early visual cortex applied during visual discrimination training in partially blind subjects, could attain significantly greater and faster recovery of visuo-perceptual abilities than sham stimulation coupled with training. These preliminary results indicate that 20 minutes of Hf-tRNS stimulation applied for 10 consecutive days over the primary visual areas led to significant improvements of patients' abilities to discriminate visual motion, and, concurrently the

visual field deficit was reduced. These results are relevant and provide a further demonstration that tRNS can be used to potentiate cognitive training, also in the pathological population. The empirical work presented in chapter three, on healthy participants, already indicated that tRNS coupled with visual perceptual learning training can boost improvement. The present results on partial cortical blind patients further validate the procedure as potential tool for the clinical setting. These results are also in agreement with the current proposed hypothesis about the mechanism of action of tRNS (Fertonani et al., 2011; Pirulli et al., 2013; van der Groen & Wenderoth, 2016). One hypothesis is that stroke patients with damage to V1 retain residual motion detection abilities (Cavanaugh et al., 2015; Das et al., 2014; Huxlin et al., 2009) (Azzopardi & Cowey, 2001; Huxlin et al., 2009), and tRNS might play a pivotal role is promoting recovery of functions that otherwise would remain subthreshold. Previous elegant work on cortically blind patients has used the exact same psychophysical procedure we used with our patients. However reliable improvement in visual motion processing in the perimetrically blind field of V1 became significant only after many months (>16) of daily training (Huxlin et al., 2009), and, although results were variable among subjects, none of the patients they tested improved after only two weeks of training. Here we show that when gaze-contingent training is coupled with Hf-tRNS stimulation, partial cortically blind patients experience bigger improvements and faster recovery of visual motion processing abilities compared to sham trained subjects, after only 10 days of daily training, suggesting a potential boosting effect of the stimulation. Furthermore, these data confirm that the damaged adult visual system is amenable to changes and it is able to relearn lost visual abilities (Das et al., 2014; Huxlin et al., 2009). Damage to the primary visual cortex causes homonymous contra-lesional loss of vision that, in strong contrast to well-established physical therapies available to treat motor deficits, was considered irreversible until recently, but the available treatments are only experimental and not part of clinical routine yet (Huxlin et al., 2009). While plasticity of spared visual circuits is believed to underlie such recovery, the specific mechanisms are still unclear. However, recent works suggest that tRNS can serve as a pedestal to boost the response of the neuronal system to weak sensory inputs (Miniussi & Ruzzoli, 2013; van der Groen & Wenderoth, 2016) and therefore promote neuroplasticity.

To further quantify the potential effect on visual recovery, we asked whether the training protocol also decreased the size of the visual deficit, hence we measured patients' visual perimetry at baseline and, for comparison, at the end of the training. Automated visual

field perimetries provided a good indication of the localization of the visual defects for each participant. As previously mentioned, global motion discrimination training alone is not able to induce expansion of the visual field in CB patients in only two weeks of training (Das et al., 2014; Huxlin et al., 2009). A preliminary qualitative clinical-diagnostic analysis of the visual fields maps, suggests that the training protocol used here instead is able to induce expansion of the visual field, and therefore a decrease in the size of the scotoma, already after 10 days of training. Since these are only preliminary data, caution is warranted. However, this early work suggest a potential beneficial effect of our training protocol.

The present work generates a series of further questions that require empirical testing, and main points are how long this effect can last and how can we make these changes stable in time. Importantly, it is now really important to determine how much these effects translate into daily functions and this should be tested with standardized clinical testing and further physchophysical test batteries measuring other dynamic and stationary visual stimuli.

References

Abe, H., McManus, J. N. J., Ramalingam, N., Li, W., Marik, S. a., Meyer zum Alten Borgloh, S., & Gilbert, C. D. (2015). Adult Cortical Plasticity Studied with Chronically Implanted Electrode Arrays. *Journal of Neuroscience*, *35*(6), 2778–2790. http://doi.org/10.1523/JNEUROSCI.3579-14.2015

Abrahamyan, A., Clifford, C. W. G., Arabzadeh, E., & Harris, J. A. (2015). Low intensity TMS enhances perception of visual stimuli. *Brain Stimulation*, *8*(6), 1175–1182. http://doi.org/10.1016/j.brs.2015.06.012

Abrahamyan, A., Clifford, C. W. G., Ruzzoli, M., Phillips, D., Arabzadeh, E., & Harris, J. A. (2011). Accurate and Rapid Estimation of Phosphene Thresholds (REPT). *PLoS ONE*, *6*(7), e22342. http://doi.org/10.1371/journal.pone.0022342

Adini, Y., Sagi, D., & Tsodyks, M. (2002). Context-enabled learning in the human visual system. *Nature*, *415*(6873), 790–793. http://doi.org/10.1038/415790a

Ahissar, M. (2001). Perceptual training: a tool for both modifying the brain and exploring it. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(21), 11842–11843. http://doi.org/10.1073/pnas.221461598

Ahissar, M., & Hochstein, S. (1996). Learning pop-out detection: Specificities to stimulus characteristics. *Vision Research*, *36*(21), 3487–3500. http://doi.org/10.1016/0042-6989(96)00036-3

Ahissar, M., & Hochstein, S. (1997). Task difficulty and the specificity of perceptual learning. *Nature*. http://doi.org/10.1038/387401a0

Ahissar, M., & Hochstein, S. (2004). The reverse hierarchy theory of visual perceptual learning. *Trends in Cognitive Sciences*. http://doi.org/10.1016/j.tics.2004.08.011

Albert, D. J. (1966). The Effects of Polarizing Currents on the Consolidation of Learning. *Neuropsychologia*, *4*, 65–77. http://doi.org/10.1016/0028-3932(66)90021-2

Andrews, S. C., Hoy, K. E., Enticott, P. G., Daskalakis, Z. J., & Fitzgerald, P. B. (2011). Improving working memory: The effect of combining cognitive activity and anodal transcranial direct current stimulation to the left dorsolateral prefrontal cortex. *Brain Stimulation*, *4*(2), 84–89. http://doi.org/10.1016/j.brs.2010.06.004

Antal, A., Ambrus, G. G., & Chaieb, L. (2014a). The impact of electrical stimulation techniques on

behavior. *Wiley Interdisciplinary Reviews: Cognitive Science*, *5*(6), 649–659. http://doi.org/10.1002/wcs.1319

Antal, A., Ambrus, G. G., & Chaieb, L. (2014b). Toward unraveling reading-related modulations of tdcs-induced neuroplasticity in the human visual cortex. *Frontiers in Psychology*, *5*(JUN), 20–23. http://doi.org/10.3389/fpsyg.2014.00642

Antal, A., Kincses, T. Z., Nitsche, M. A., Bartfai, O., & Paulus, W. (2004). Excitability changes induced in the human primary visual cortex by transcranial direct current stimulation: direct electrophysiological evidence. *Investigative Ophthalmology & Visual Science*, *45*(2), 702–707. http://doi.org/10.1167/iovs.03-0688

Antal, A., Kincses, T. Z., Nitsche, M. A., & Paulus, W. (2003a). Modulation of moving phosphene thresholds by transcranial direct current stimulation of V1 in human. *Neuropsychologia*, *41*(13), 1802–1807. http://doi.org/10.1016/S0028-3932(03)00181-7

Antal, A., Kincses, T. Z., Nitsche, M. a, & Paulus, W. (2003b). Manipulation of phosphene thresholds by transcranial direct current stimulation in man. *Experimental Brain Research. Experimentelle Hirnforschung. Experimentation Cerebrale*, *150*(3), 375–378. http://doi.org/10.1007/s00221-003-1459-8

Antal, A., Nitsche, M. a., Kincses, T. Z., Kruse, W., Hoffmann, K. P., & Paulus, W. (2004). Facilitation of visuo-motor learning by transcranial direct current stimulation of the motor and extrastriate visual areas in humans. *European Journal of Neuroscience*, *19*(10), 2888–2892. http://doi.org/10.1111/j.1460-9568.2004.03367.x

Antal, A., Nitsche, M. A., & Paulus, W. (2001). External modulation of visual perception in humans. *Neuroreport*, *12*(16), 3553–3555. http://doi.org/10.1097/00001756-200111160-00036

Antal, A., Nitsche, M. a, Kruse, W., Kincses, T. Z., Hoffmann, K.-P., & Paulus, W. (2004). Direct current stimulation over V5 enhances visuomotor coordination by improving motion perception in humans. *Journal of Cognitive Neuroscience*, *16*(4), 521–527. http://doi.org/10.1162/089892904323057263

Antal, A., & Paulus, W. (2008). Transcranial direct current stimulation and visual perception. *Perception*, *37*(3), 367–374. http://doi.org/10.1068/p5872

Antal, A., & Paulus, W. (2013). Transcranial alternating current stimulation (tACS). *Frontiers in Human Neuroscience*, *7*(June), 317. http://doi.org/10.3389/fnhum.2013.00317

Ashby, F. G., & Maddox, W. T. (2011). Human category learning 2.0. *Annals of the New York*

Academy of Sciences. http://doi.org/10.1111/j.1749-6632.2010.05874.x

Azzopardi, P., & Cowey, a. (2001). Motion discrimination in cortically blind patients. *Brain : A Journal of Neurology*, *124*(Pt 1), 30–46. http://doi.org/10.1093/brain/124.1.30

Ball, K., & Sekuler, R. (1987). Direction-specific improvement in motion discrimination. *Vision Research*, *27*(6), 953–965. http://doi.org/10.1016/0042-6989(87)90011-3

Baroncelli, L., Sale, A., Viegi, A., Maya Vetencourt, J. F., De Pasquale, R., Baldini, S., & Maffei, L. (2010). Experience-dependent reactivation of ocular dominance plasticity in the adult visual cortex. *Experimental Neurology*, *226*(1), 100–109. http://doi.org/10.1016/j.expneurol.2010.08.009

Başar, E., Başar-Eroglu, C., Karakaş, S., & Schürmann, M. (2000). Gamma, alpha, delta, and theta oscillations govern cognitive processes. *International Journal of Psychophysiology*, *39*(2–3), 241–248. http://doi.org/10.1016/S0167-8760(00)00145-8

Baseler, H. a, Morland, a B., & Wandell, B. a. (1999). Topographic organization of human visual areas in the absence of input from primary cortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *19*(7), 2619–2627.

Batsikadze, G., Moliadze, V., Paulus, W., Kuo, M.-F., & Nitsche, M. A. (2013). Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans. *The Journal of Physiology*, *591*, 1987–2000. http://doi.org/10.1113/jphysiol.2012.249730

Baudewig, J., Nitsche, M. A., Paulus, W., & Frahm, J. (2001). Preceeding transcranial direct current stimulation modulates BOLD MRI responses to sensorimotor activation in humans. *Neuroimage*, *13*(6), S1127–S1127. http://doi.org/10.1016/S1053-8119(01)92452-8

Bejjanki, V. R., Beck, J. M., Lu, Z.-L., & Pouget, A. (2011). Perceptual learning as improved probabilistic inference in early sensory areas. *Nature Neuroscience*, *14*(5), 642–648. http://doi.org/10.1038/nn.2796

Benjamini, Y., & Hochberg, Y. (1995). Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society B*, *57*(1), 289–300. http://doi.org/10.2307/2346101

Berryhill, M. E., Wencil, E. B., Branch Coslett, H., & Olson, I. R. (2010). A selective working memory impairment after transcranial direct current stimulation to the right parietal lobe. *Neuroscience Letters*, *479*(3), 312–316. http://doi.org/10.1016/j.neulet.2010.05.087

Bliss, T. V. P., & Cooke, S. F. (2011). Long-term potentiation and long-term depression: a clinical perspective. *Clinics (São Paulo, Brazil)*, *66 Suppl 1*(Suppl 1), 3–17. http://doi.org/10.1590/S1807-59322011001300002

Bliss, T. V. P., & Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the preforant path. *Journal of Physiology*, *232*, 331–356. http://doi.org/4727084

Bliss, T. V, & Collingridge, G. L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. *Nature*, *361*(6407), 31–39. http://doi.org/10.1038/361031a0

Bliss, T. V, & Gardner-Medwin, A. R. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. *The Journal of Physiology*, *232*(2), 357–74. http://doi.org/4727084

Boggio, P. S., Castro, L. O., Savagim, E. A., Braite, R., Cruz, V. C., Rocha, R. R., … Fregni, F. (2006). Enhancement of non-dominant hand motor function by anodal transcranial direct current stimulation. *Neuroscience Letters*, *404*(1–2), 232–236. http://doi.org/10.1016/j.neulet.2006.05.051

Bolognini, N., Fregni, F., Casati, C., Olgiati, E., & Vallar, G. (2010). Brain polarization of parietal cortex augments training-induced improvement of visual exploratory and attentional skills. *Brain Research*, *1349*, 76–89. http://doi.org/10.1016/j.brainres.2010.06.053

Box, G. E. P., & Cox, D. R. (1964). An analysis of transformations. *Journal of the Royal Statistical Society. Series B (Methodological*, 211–252. http://doi.org/10.2307/2287791

Brainard, D. H. (1997). The Psychophysics Toolbox. *Spatial Vision*, *10*, 433–436. http://doi.org/10.1163/156856897X00357

Buonomano, D. V, & Merzenich, M. M. (1998). Cortical plasticity: from synapses to maps. *Annual Review of Neuroscience*, *21*(149), 149–186. http://doi.org/10.1146/annurev.neuro.21.1.149

Camilleri, R., Pavan, A., & Campana, G. (2016). The application of online transcranial random noise stimulation and perceptual learning in the improvement of visual functions in mild myopia. *Neuropsychologia*, *89*, 225–231. http://doi.org/10.1016/j.neuropsychologia.2016.06.024

Camilleri, R., Pavan, A., Ghin, F., Battaglini, L., & Campana, G. (2014). Improvement of uncorrected visual acuity and contrast sensitivity with perceptual learning and transcranial random noise stimulation in individuals with mild myopia. *Frontiers in Psychology*, *5*(October),

1–6. http://doi.org/10.3389/fpsyg.2014.01234

Campana, G., Camilleri, R., Pavan, A., Veronese, A., & Giudice, G. Lo. (2014). Improving visual functions in adult amblyopia with combined perceptual training and transcranial random noise stimulation (tRNS): A pilot study. *Frontiers in Psychology*, *5*(DEC), 1–6. http://doi.org/10.3389/fpsyg.2014.01402

Cappelletti, M., Gessaroli, E., Hithersay, R., Mitolo, M., Didino, D., Kanai, R., … Walsh, V. (2013). Transfer of cognitive training across magnitude dimensions achieved with concurrent brain stimulation of the parietal lobe. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *33*(37), 14899–907. http://doi.org/10.1523/JNEUROSCI.1692-13.2013

Cappelletti, X. M., Pikkat, H., Upstill, E., Speekenbrink, X. M., & Walsh, V. (2015). Learning to Integrate versus Inhibiting Information Is Modulated by Age. *The Journal of Neuroscience*, *35*(5), 2213–2225. http://doi.org/10.1523/JNEUROSCI.1018-14.2015

Carmel, D., & Carrasco, M. (2008). Perceptual Learning and Dynamic Changes in Primary Visual Cortex. *Neuron*. http://doi.org/10.1016/j.neuron.2008.03.009

Cattaneo, Z., & Silvanto, J. (2008). Investigating visual motion perception using the transcranial magnetic stimulation-adaptation paradigm. *Neuroreport*, *19*(14), 1423–1427. http://doi.org/10.1097/WNR.0b013e32830e0025

Cavanaugh, M. R., Zhang, R., Melnick, M. D., Das, A., Roberts, M., Tadin, D., … Huxlin, K. R. (2015). Visual recovery in cortical blindness is limited by high internal noise. *Journal of Vision*, *15*(10), 9. http://doi.org/10.1167/15.10.9

Chaieb, L., Antal, A., & Paulus, W. (2015). Transcranial random noise stimulation-induced plasticity is NMDA-receptor independent but sodium-channel blocker and benzodiazepines sensitive. *Frontiers in Neuroscience*, *9*(April), 1–9. http://doi.org/10.3389/fnins.2015.00125

Chaieb, L., Paulus, W., & Antal, A. (2011). Evaluating aftereffects of short-duration transcranial random noise stimulation on cortical excitability. *Neural Plasticity*, *2011*. http://doi.org/10.1155/2011/105927

Chen, W. R., Lee, S., Kato, K., Spencer, D. D., Shepherd, G. M., & Williamson, a. (1996). Long-term modifications of synaptic efficacy in the human inferior and middle temporal cortex. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(July), 8011–8015. http://doi.org/10.1073/pnas.93.15.8011

Coan, E. J., & Collingridge, G. L. (1987). Characterization of an N-methyl-d-aspartate receptor component of synaptic transmission in rat hippocampal slices. *Neuroscience*, *22*(1), 1–8. http://doi.org/10.1016/0306-4522(87)90192-8

Collingridge, G. L., Peineau, S., Howland, J. G., & Wang, Y. T. (2010). Long-term depression in the CNS. *Nature Reviews. Neuroscience*, *11*(7), 459–473. http://doi.org/10.1038/nrn2867

Collins, J. J., Imhoff, T. T., & Grigg, P. (1996). Noise-enhanced tactile sensation. *Nature*. http://doi.org/10.1038/383770a0

Collins, J. J., Imhoff, T. T., & Grigg, P. (1997). Noise-mediated enhancements and decrements in human tactile sensation. *Physical Review E*, *56*(1), 923–926. http://doi.org/10.1103/PhysRevE.56.923

Cowey, A. (2010). The blindsight saga. *Experimental Brain Research*, *200*(1), 3–24. http://doi.org/10.1007/s00221-009-1914-2

Das, A., & Huxlin, K. R. (2010). New approaches to visual rehabilitation for cortical blindness: outcomes and putative mechanisms. *The Neuroscientist : A Review Journal Bringing Neurobiology, Neurology and Psychiatry*, *16*(4), 374–387. http://doi.org/10.1177/1073858409356112

Das, A., Tadin, D., & Huxlin, K. R. (2014). Beyond Blindsight: Properties of Visual Relearning in Cortically Blind Fields. *Journal of Neuroscience*, *34*(35), 11652–11664. http://doi.org/10.1523/JNEUROSCI.1076-14.2014

Datta, A., Baker, J. M., Bikson, M., & Fridriksson, J. (2011). Individualized model predicts brain current flow during transcranial direct-current stimulation treatment in responsive stroke patient. *Brain Stimulation*, *4*(3), 169–174. http://doi.org/10.1016/j.brs.2010.11.001

Datta, A., Bansal, V., Diaz, J., Patel, J., Reato, D., & Bikson, M. (2009). Gyri-precise head model of transcranial direct current stimulation: Improved spatial focality using a ring electrode versus conventional rectangular pad. *Brain Stimulation*, *2*(4), 201–207. http://doi.org/10.1016/j.brs.2009.03.005

Dill, M., & Fahle, M. (1997). The role of visual field position in pattern-discrimination learning. *Proceedings. Biological Sciences / The Royal Society*, *264*, 1031–1036. http://doi.org/10.1098/rspb.1997.0142

Dockery, C. A., Hueckel-Weng, R., Birbaumer, N., & Plewnia, C. (2009). Enhancement of planning ability by transcranial direct current stimulation. *The Journal of Neuroscience : The Official*

Journal of the Society for Neuroscience, *29*(22), 7271–7. http://doi.org/10.1523/JNEUROSCI.0065-09.2009

Doeltgen, S. H., & Ridding, M. C. (2010). Behavioural exposure and sleep do not modify corticospinal and intracortical excitability in the human motor system. *Clinical Neurophysiology*, *121*(3), 448–452. http://doi.org/10.1016/j.clinph.2009.11.085

Dosher, B. A., Liu, S. H., Blair, N., & Lu, Z. L. (2004). The spatial window of the perceptual template and endogenous attention. In *Vision Research* (Vol. 44, pp. 1257–1271). http://doi.org/10.1016/j.visres.2004.01.011

Dosher, B. a, & Lu, Z. L. (1998). Perceptual learning reflects external noise filtering and internal noise reduction through channel reweighting. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(23), 13988–93. http://doi.org/10.1073/pnas.95.23.13988

Elmer, S., Burkard, M., Renz, B., Meyer, M., & Jancke, L. (2009). Direct current induced short-term modulation of the left dorsolateral prefrontal cortex while learning auditory presented nouns. *Behavioral and Brain Functions : BBF*, *5*, 29. http://doi.org/10.1186/1744-9081-5-29

Elvsshagen, T., Moberget, T., Bøen, E., Boye, B., Englin, N. O. A., Pedersen, P., … Andersson, S. (2012). Evidence for impaired neocortical synaptic plasticity in bipolar II disorder. *Biological Psychiatry*, *71*(1), 68–74. http://doi.org/10.1016/j.biopsych.2011.09.026

Fahle, M. (2002). Perceptual learning: gain without pain? *Nature Neuroscience*, *5*(10), 923–924. http://doi.org/10.1038/nn1002-923

Fahle, M., Edelman, S., & Poggio, T. (1995). Fast perceptual learning in hyperacuity. *Vision Research*, *35*(21), 3003–3013. http://doi.org/10.1016/0042-6989(95)00044-Z

Fahle, M., & Morgan, M. (1996). No transfer of perceptual learning between similar stimuli in the same retinal position. *Current Biology*, *6*(3), 292–297. http://doi.org/10.1016/S0960-9822(02)00479-7

Fedorka, K. M., & Mousseau, T. A. (2004). Naturalistic experience transforms sensory maps in the adult cortex of caged animals. *Nature*, *429*(6987), 65–67. http://doi.org/10.1038/nature02492

Fertonani, A., & Miniussi, C. (2016). Transcranial Electrical Stimulation: What We Know and Do Not Know About Mechanisms. *The Neuroscientist*. http://doi.org/10.1177/1073858416631966

Fertonani, A., Rosini, S., Cotelli, M., Rossini, P. M., & Miniussi, C. (2010). Naming facilitation induced by transcranial direct current stimulation. *Behavioural Brain Research*, *208*(2), 311–

318. http://doi.org/10.1016/j.bbr.2009.10.030

Fertonani, a., Pirulli, C., & Miniussi, C. (2011). Random Noise Stimulation Improves Neuroplasticity in Perceptual Learning. *Journal of Neuroscience*, *31*(43), 15416–15423. http://doi.org/10.1523/JNEUROSCI.2002-11.2011

Figurov, A., Pozzo-Miller, L. D., Olafsson, P., Wang, T., & Lu, B. (1996). Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. *Nature*, *381*(6584), 706–709. http://doi.org/10.1038/381706a0

Fine, I., & Jacobs, R. A. (2002). Comparing perceptual learning tasks: a review. *Journal of Vision*, *2*(2), 190–203. http://doi.org/10:1167/2.2.5

Fiori, V., Coccia, M., Marinelli, C. V, Vecchi, V., Bonifazi, S., Ceravolo, M. G., … Marangolo, P. (2011). Transcranial direct current stimulation improves word retrieval in healthy and nonfluent aphasic subjects. *Journal of Cognitive Neuroscience*, *23*(9), 2309–23. http://doi.org/10.1162/jocn.2010.21579

Flöel, A., Rösser, N., Michka, O., Knecht, S., Breitenstein, C., Flo, A., … Breitenstein, C. (2008). Noninvasive brain stimulation improves language learning. *Journal of Cognitive Neuroscience*, *20*(8), 1415–1422. http://doi.org/10.1162/jocn.2008.20098

Fox, K. (2002). Anatomical pathways and molecular mechanisms for plasticity in the barrel cortex. *Neuroscience*, *111*(4), 799–814. http://doi.org/10.1016/S0306-4522(02)00027-1

Frankó, E., Seitz, A. R., & Vogels, R. (2010). Dissociable neural effects of long-term stimulus-reward pairing in macaque visual cortex. *Journal of Cognitive Neuroscience*, *22*(7), 1425–1439. http://doi.org/10.1162/jocn.2009.21288

Fregni, F., Boggio, P. S., Nitsche, M., Bermpohl, F., Antal, A., Feredoes, E., … Pascual-Leone, A. (2005). Anodal transcranial direct current stimulation of prefrontal cortex enhances working memory. *Experimental Brain Research*, *166*(1), 23–30. http://doi.org/10.1007/s00221-005-2334-6

Fricke, K., Seeber, a a, Thirugnanasambandam, N., Paulus, W., Nitsche, M. a, & Rothwell, J. C. (2011). Time course of the induction of homeostatic plasticity generated by repeated transcranial direct current stimulation of the human motor cortex. *Journal of Neurophysiology*, *105*(3), 1141–1149. http://doi.org/10.1152/jn.00608.2009

Fritsch, B., Reis, J., Martinowich, K., Schambra, H. M., Ji, Y., Cohen, L. G., & Lu, B. (2010). Direct current stimulation promotes BDNF-dependent synaptic plasticity: Potential implications for

motor learning. *Neuron*, *66*(2), 198–204. http://doi.org/10.1016/j.neuron.2010.03.035

Furmanski, C. S., & Engel, S. A. (2000). Perceptual learning in object recognition: Object specificity and size invariance. *Vision Research*, *40*(5), 473–484. http://doi.org/10.1016/S0042-6989(99)00134-0

Furuya, S., Klaus, M., Nitsche, M. A., Paulus, W., & Altenmu, E. (2014). Ceiling Effects Prevent Further Improvement of Transcranial Stimulation in Skilled Musicians. *Journal of Neuroscience*, *34*(41), 13834–13839. http://doi.org/10.1523/JNEUROSCI.1170-14.2014

Galea, J. M., & Celnik, P. (2009). Brain polarization enhances the formation and retention of motor memories. *Journal of Neurophysiology*, *102*(1), 294–301. http://doi.org/10.1152/jn.00184.2009

Geddes, J. M., Fear, J., Tennant, a, Pickering, a, Hillman, M., & Chamberlain, M. a. (1996). Prevalence of self reported stroke in a population in northern England. *Journal of Epidemiology and Community Health*, *50*(2), 140–143. http://doi.org/10.1136/jech.50.2.140

Gibson, E. J. (1969). Principles of perceptual learning and development. *Principles of Perceptual Learning and Development.*, 353. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=psyh&AN=1969-35014-000&lang=fr&site=ehost-live

Gilbert, C. D. (1994). Commentary Early perceptual learning. *Proc Natl Acad Sci U S A*, *91*(February), 1195–1197. http://doi.org/10.1073/pnas.91.4.1195

Gilbert, C. D., & Li, W. (2012). Adult Visual Cortical Plasticity. *Neuron*, *75*(2), 250–264. http://doi.org/10.1016/j.neuron.2012.06.030

Gilbert, C. D., & Wiesel, T. N. (1992). Receptive field dynamics in adult primary visual cortex. *Nature*, *356*(6365), 150–152. http://doi.org/10.1038/356150a0

Gilbert, C., Sigman, M., & Crist, R. (2001). The neural basis of perceptual learning. *Neuron*, *31*, 681–697. http://doi.org/10.1016/S0896-6273(01)00424-X

Gladwin, T. E., den Uyl, T. E., Fregni, F. F., & Wiers, R. W. (2012). Enhancement of selective attention by tDCS: Interaction with interference in a Sternberg task. *Neuroscience Letters*, *512*(1), 33–37. http://doi.org/10.1016/j.neulet.2012.01.056

Gobbo, M., & Falciati, L. (2014). Neuromodulation and cognitive rehabilitation: Addressing the methodological issue of circadian rhythms. *Frontiers in Psychiatry*, *5*(OCT). http://doi.org/10.3389/fpsyt.2014.00150

Godde, B., Leonhardt, R., Cords, S. M., & Dinse, H. R. (2002). Plasticity of orientation preference maps in the visual cortex of adult cats. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(9), 6352–6357. http://doi.org/10.1073/pnas.082407499

Godde, B., Stauffenberg, B., Spengler, F., & Dinse, H. R. (2000). Tactile coactivation-induced changes in spatial discrimination performance. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *20*(4), 1597–604. http://doi.org/tactile, haptic, somatosensory, coactivation; associative pairing; perceptual learning; cortical reorganization; plasticity; Hebbian learning; attention, passive

Gold, J. I., Law, C.-T., Connolly, P., & Bennur, S. (2010). Relationships between the threshold and slope of psychometric and neurometric functions during perceptual learning: implications for neuronal pooling. *Journal of Neurophysiology*, *103*(1), 140–154. http://doi.org/10.1152/jn.00744.2009

Gold, W. (2010). Quick guide Perceptual learning. *Current Biology*, *20*(2), 46–48. http://doi.org/10.1016/j.cub.2009.10.066

Gonzalez, J., Morales, I. S., Villarreal, D. M., & Derrick, B. E. (2014). Low-frequency stimulation induces long-term depression and slow onset long-term potentiation at perforant path-dentate gyrus synapses in vivo. *Journal of Neurophysiology*, *111*(6), 1259–73. http://doi.org/10.1152/jn.00941.2012

Grenier, F., Timofeev, I., & Steriade, M. (2001). Focal synchronization of ripples (80-200 Hz) in neocortex and their neuronal correlates. *Journal of Neurophysiology*, *86*(4), 1884–1898.

He, H.-Y., Hodos, W., & Quinlan, E. M. (2006). Visual deprivation reactivates rapid ocular dominance plasticity in adult visual cortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *26*(11), 2951–2955. http://doi.org/10.1523/JNEUROSCI.5554-05.2006

He, H.-Y., Ray, B., Dennis, K., & Quinlan, E. M. (2007). Experience-dependent recovery of vision following chronic deprivation amblyopia. *Nature Neuroscience*, *10*(9), 1134–1136. http://doi.org/10.1038/nn1965

Hebb, D. O. (1949). The organization of behavior: A Neuropsychological Approach. *The American Journal of Psychology*. http://doi.org/10.2307/1418888

Heekeren, H. R., Marrett, S., Bandettini, P. a, & Ungerleider, L. G. (2004). A general mechanism for perceptual decision-making in the human brain. *Nature*, *431*(7010), 859–862. http://doi.org/10.1038/nature02966

Helfrich, R. F., Schneider, T. R., Rach, S., Trautmann-Lengsfeld, S. A., Engel, A. K., & Herrmann, C. S. (2014). Entrainment of brain oscillations by transcranial alternating current stimulation. *Current Biology*, *24*(3), 333–339. http://doi.org/10.1016/j.cub.2013.12.041

Hensch, T. K. (2005a). Critical Period Mechanisms in Developing Visual Cortex. *Current Topics in Developmental Biology*. http://doi.org/10.1016/S0070-2153(05)69008-4

Hensch, T. K. (2005b). Critical period plasticity in local cortical circuits. *Nature Reviews. Neuroscience*, *6*(11), 877–88. http://doi.org/10.1038/nrn1787

Herpich, F., Melnick, M., Huxlin, K. R., Tadin, D., Agosta, S., & Battelli, L. (2015). Transcranial Random Noise Stimulation Enhances Visual Learning In Healthy Adults. *Journal of Vision*, *15*(12), 40. http://doi.org/10.1167/15.12.40

Herrmann, C. S., Rach, S., Neuling, T., & Strüber, D. (2013). Transcranial alternating current stimulation: a review of the underlying mechanisms and modulation of cognitive processes. *Frontiers in Human Neuroscience*, *7*(June), 279. http://doi.org/10.3389/fnhum.2013.00279

Herrmann, C. S., Strüber, D., Helfrich, R. F., & Engel, A. K. (2016). EEG oscillations: From correlation to causality. *International Journal of Psychophysiology*. http://doi.org/10.1016/j.ijpsycho.2015.02.003

Heynen, a J., Abraham, W. C., & Bear, M. F. (1996). Bidirectional modification of CA1 synapses in the adult hippocampus in vivo. *Nature*. http://doi.org/10.1038/381163a0

Holmes, G. (1918). Disturbances of Vision By Cerebral Lesions. *The British Journal of Ophthalmology*, *2*(7), 353–384. http://doi.org/10.1136/bjo.2.7.353

Horton, J. C. (2005). Disappointing results from Nova Vision's visual restoration therapy. *The British Journal of Ophthalmology*, *89*(1), 1–2. http://doi.org/10.1136/bjo.2004.058214

Horvath, J. C., Forte, J. D., & Carter, O. (2015). Evidence that transcranial direct current stimulation (tDCS) generates little-to-no reliable neurophysiologic effect beyond MEP amplitude modulation in healthy human subjects: A systematic review. *Neuropsychologia*. http://doi.org/10.1016/j.neuropsychologia.2014.11.021

Hubel, D. H., & Wiesel, T. N. (1964). Effects of monocular deprivation in kittens. *Naunyn-Schmiedebergs Archiv F??r Experimentelle Pathologie Und Pharmakologie*, *248*(6), 492–497. http://doi.org/10.1007/BF00348878

Huber, A. (1992). Homonymous hemianopia. *Neuro-Ophthalmology*, *12*(6), 351–366. http://doi.org/10.3109/01658109209036989

Huber, R., Mäki, H., Rosanova, M., Casarotto, S., Canali, P., Casali, A. G., … Massimini, M. (2013). Human cortical excitability increases with time awake. *Cerebral Cortex*, *23*(2), 332–338. http://doi.org/10.1093/cercor/bhs014

Huxlin, K. R. (2008). Perceptual plasticity in damaged adult visual systems. *Vision Research*, *48*(20), 2154–2166. http://doi.org/10.1016/j.visres.2008.05.022

Huxlin, K. R., Martin, T., Kelly, K., Riley, M., Friedman, D. I., Burgin, W. S., & Hayhoe, M. (2009). Perceptual relearning of complex visual motion after V1 damage in humans. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *29*(13), 3981–3991. http://doi.org/10.1523/JNEUROSCI.4882-08.2009

Huxlin, K. R., & Pasternak, T. (2004). Training-induced Recovery of Visual Motion Perception after Extrastriate Cortical Damage in the Adult Cat. *Cerebral Cortex*, *14*(1), 81–90. http://doi.org/10.1093/cercor/bhg106

Javadi, A. H., & Walsh, V. (2012). Transcranial direct current stimulation (tDCS) of the left dorsolateral prefrontal cortex modulates declarative memory. *Brain Stimulation*, *5*(3), 231–241. http://doi.org/10.1016/j.brs.2011.06.007

Jo, J. M., Kim, Y.-H., Ko, M.-H., Ohn, S. H., Joen, B., & Lee, K. H. (2009). Enhancing the working memory of stroke patients using tDCS. *American Journal of Physical Medicine & Rehabilitation / Association of Academic Physiatrists*, *88*(5), 404–9. http://doi.org/10.1097/PHM.0b013e3181a0e4cb

Kahnt, T., Grueschow, M., Speck, O., & Haynes, J.-D. (2011). Perceptual Learning and Decision-Making in Human Medial Frontal Cortex. *Neuron*, *70*(3), 549–559. http://doi.org/http://dx.doi.org/10.1016/j.neuron.2011.02.054

Kanai, R., Paulus, W., & Walsh, V. (2010). Transcranial alternating current stimulation (tACS) modulates cortical excitability as assessed by TMS-induced phosphene thresholds. *Clinical Neurophysiology*, *121*(9), 1551–1554. http://doi.org/10.1016/j.clinph.2010.03.022

Kang, E. K., Baek, M. J., Kim, S., & Paik, N. J. (2009). Non-invasive cortical stimulation improves post-stroke attention decline. *Restorative Neurology and Neuroscience*, *27*(6), 645–650. http://doi.org/10.3233/RNN-2009-0514

Kang, N., Summers, J. J., & Cauraugh, J. H. (2016). Transcranial direct current stimulation facilitates motor learning post-stroke: a systematic review and meta-analysis. *Journal of Neurology, Neurosurgery, and Psychiatry*, *87*(4), 345–55. http://doi.org/10.1136/jnnp-2015-311242

Karni, a, & Sagi, D. (1991). Where practice makes perfect in texture discrimination: evidence for primary visual cortex plasticity. *Proceedings of the National Academy of Sciences of the United States of America*, *88*(11), 4966–4970. http://doi.org/DOI 10.1073/pnas.88.11.4966

Kasamatsu, T., & Pettigrew, J. D. (1976). Depletion of brain catecholamines: failure of ocular dominance shift after monocular occlusion in kittens. *Science (New York, N.Y.)*. http://doi.org/10.1126/science.959850

Kasamatsu, T., & Shirokawa, T. (1985). Involvement of beta-adrenoreceptors in the shift of ocular dominance after monocular deprivation. *Experimental Brain Research*, *59*(3), 507–514. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2993012

Kasamatsu, T., Watabe, K., Heggelund, P., & Schöller, E. (1985). Plasticity in cat visual cortex restored by electrical stimulation of the locus coeruleus. *Neuroscience Research*, *2*(5), 365–386. http://doi.org/10.1016/0168-0102(85)90047-1

Keel, J. C., Smith, M. J., & Wassermann, E. M. (2000). Letter to the Editor. A safety screening questionnaire for transcranial magnetic stimulation. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *112*, 720. http://doi.org/10.3111/13696998.2014.909438

Kemp, A., & Manahan-Vaughan, D. (2004). Hippocampal long-term depression and long-term potentiation encode different aspects of novelty acquisition. *Proceedings of the National Academy of Sciences*, *101*(21), 8192–8197. http://doi.org/10.1073/pnas.0402650101

Kim, R. S., Seitz, A. R., & Shams, L. (2008). Benefits of stimulus congruency for multisensory facilitation of visual learning. *PLoS ONE*, *3*(1). http://doi.org/10.1371/journal.pone.0001532

Kirkwood, A., Lee, H.-K., & Bear, M. F. (1995). Co-regulation of long-term potentiation and experience-dependent synaptic plasticity in visual cortex by age and experience. *Nature*, *375*, 328–331. Retrieved from file://c/My files/RefMan10/RefMan pdfs/7708.pdf

Kitajo, K., Nozaki, D., Ward, L. M., & Yamamoto, Y. (2003). Behavioral stochastic resonance within the human brain. *Physical Review Letters*, *90*(21), 218103. http://doi.org/10.1103/PhysRevLett.90.218103

Kolmel, H. W. (1991). Patterns of recovery from homonymous hemianopia subsequent to infarction in the distribution of the posterior cerebral artery. *Neuro-Ophthalmology*, *11*(1), 33–39. http://doi.org/10.3109/01658109109009640

Komatsu, Y., Toyama, K., Maeda, J., & Sakaguchi, H. (1981). Long-term potentiation investigated

in a slice preparation of striate cortex of young kittens. *Neuroscience Letters*, *26*(3), 269–274. http://doi.org/10.1016/0304-3940(81)90144-0

Koski, L., Schrader, L. M., Wu, A. D., & Stern, J. M. (2005). Normative data on changes in transcranial magnetic stimulation measures over a ten hour period. *Clinical Neurophysiology*, *116*(9), 2099–2109. http://doi.org/10.1016/j.clinph.2005.06.006

Koyama, S., Sasaki, Y., Andersen, G. J., Tootell, R. B. H., Matsuura, M., & Watanabe, T. (2005). Separate processing of different global-motion structures in visual cortex is revealed by fMRI. *Current Biology*, *15*(22), 2027–2032. http://doi.org/10.1016/j.cub.2005.10.069

Lang, N., Rothkegel, H., Reiber, H., Hasan, A., Sueske, E., Tergau, F., … Paulus, W. (2011). Circadian modulation of GABA-mediated cortical inhibition. *Cerebral Cortex*, *21*(10), 2299–2306. http://doi.org/10.1093/cercor/bhr003

Leopold, D. a. (2012). Primary Visual Cortex: Awareness and Blindsight *. *Annual Review of Neuroscience*, *35*(1), 91–109. http://doi.org/10.1146/annurev-neuro-062111-150356

Leopold, D. A. (2012). Primary visual cortex: awareness and blindsight. *Annual Review of Neuroscience*, *35*(301), 91–109. http://doi.org/10.1146/annurev-neuro-062111-150356

Lesmes, L. A., Lu, Z. L., Baek, J., & Albright, T. D. (2010). Bayesian adaptive estimation of the contrast sensitivity function: the quick CSF method. *Journal of Vision*, *10*(3), 17 1-21. http://doi.org/10.1167/10.3.17

Levi, A., Shaked, D., Tadin, D., & Huxlin, K. (2014). Is improved contrast sensitivity a natural consequence of visual training? *Journal of Vision*, *14*(10), 1158–1158. http://doi.org/10.1167/14.10.1158

Li, W. (2016). Perceptual Learning : Use-Dependent Cortical Plasticity, (July), 1–22. http://doi.org/10.1146/annurev-vision-111815-114351

Li, W., Piëch, V., & Gilbert, C. D. (2004). Perceptual learning and top-down influences in primary visual cortex. *Nature Neuroscience*, *7*(6), 651–657. http://doi.org/10.1038/nn1255

Liebetanz, D., Nitsche, M. A., Tergau, F., & Paulus, W. (2002a). Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. *Brain : A Journal of Neurology*, *125*(10), 2238–47. http://doi.org/10.1093/brain/awf238

Liebetanz, D., Nitsche, M. A., Tergau, F., & Paulus, W. (2002b). Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex

excitability. *Brain : A Journal of Neurology*, *125*(Pt 10), 2238–2247. http://doi.org/10.1093/brain/awf238

Liu, J., Lu, Z.-L., & Dosher, B. a. (2010). Augmented Hebbian reweighting: interactions between feedback and training accuracy in perceptual learning. *Journal of Vision*, *10*(10), 29. http://doi.org/10.1167/10.10.29

Lu, Z. L., & Dosher, B. A. (2005). External noise distinguishes mechanisms of attention. *Neurobiology of Attention*, *38*(9), 448–453. http://doi.org/10.1016/B978-012375731-9/50078-1

Lu, Z. L., Hua, T., Huang, C. B., Zhou, Y., & Dosher, B. A. (2011). Visual perceptual learning. *Neurobiol Learn Mem*, *95*(2), 145–151. http://doi.org/S1074-7427(10)00164-4 [pii]\r10.1016/j.nlm.2010.09.010

Ly, J. Q. M., Gaggioni, G., Chellappa, S. L., Papachilleos, S., Brzozowski, A., Borsu, C., … Vandewalle, G. (2016). Circadian regulation of human cortical excitability. *Nat Commun*, *In Press*(May). http://doi.org/10.1038/ncomms11828

Matthews, N., Liu, Z., Geesaman, B. J., & Qian, N. (1999). Perceptual learning on orientation and direction discrimination. *Vision Research*, *39*(22), 3692–3701. http://doi.org/10.1016/S0042-6989(99)00069-3

Maya Vetencourt, J. F., Sale, A., Viegi, A., Baroncelli, L., De Pasquale, R., O'Leary, O. F., … Maffei, L. (2008). The antidepressant fluoxetine restores plasticity in the adult visual cortex. *Science (New York, N.Y.)*, *320*(5874), 385–8. http://doi.org/10.1126/science.1150516

Melnick, M. D., Tadin, D., & Huxlin, K. R. (2015). Relearning to See in Cortical Blindness. *The Neuroscientist*. http://doi.org/10.1177/1073858415621035

Miniussi, C., Harris, J. A., & Ruzzoli, M. (2013). Modelling non-invasive brain stimulation in cognitive neuroscience. *Neuroscience and Biobehavioral Reviews*. http://doi.org/10.1016/j.neubiorev.2013.06.014

Miniussi, C., & Ruzzoli, M. (2013). *Transcranial stimulation and cognition*. *Handbook of Clinical Neurology* (1st ed., Vol. 116). Elsevier B.V. http://doi.org/10.1016/B978-0-444-53497-2.00056-5

Miranda, P. C., Lomarev, M., & Hallett, M. (2006). Modeling the current distribution during transcranial direct current stimulation. *Clinical Neurophysiology*, *117*(7), 1623–1629. http://doi.org/10.1016/j.clinph.2006.04.009

Miyaguchi, S., Onishi, H., Kojima, S., Sugawara, K., Tsubaki, A., Kirimoto, H., … Yamamoto, N.

(2013). Corticomotor excitability induced by anodal transcranial direct current stimulation with and without non-exhaustive movement. *Brain Research*, *1529*, 83–91. http://doi.org/10.1016/j.brainres.2013.07.026

Moliadze, V., Atalay, D., Antal, A., & Paulus, W. (2012). Close to threshold transcranial electrical stimulation preferentially activates inhibitory networks before switching to excitation with higher intensities. *Brain Stimulation*, *5*(4), 505–511. http://doi.org/10.1016/j.brs.2011.11.004

Monti, A., Cogiamanian, F., Marceglia, S., Ferrucci, R., Mameli, F., Mrakic-Sposta, S., … Priori, A. (2008). Improved naming after transcranial direct current stimulation in aphasia. *Journal of Neurology, Neurosurgery & Psychiatry*, *79*(4), 451–453. http://doi.org/10.1136/jnnp.2007.135277

Moss, F., Ward, L. M., & Sannita, W. G. (2004). Stochastic resonance and sensory information processing: A tutorial and review of application. *Clinical Neurophysiology*, *115*(2), 267–281. http://doi.org/10.1016/j.clinph.2003.09.014

Muellbacher, W., Ziemann, U., Wissel, J., Dang, N., Kofler, M., Facchini, S., … Hallett, M. (2002). Early consolidation in human primary motor cortex. *Nature*, *415*(6872), 640–4. http://doi.org/10.1038/nature712

Neuling, T., Rach, S., & Herrmann, C. S. (2013). Orchestrating neuronal networks: sustained after-effects of transcranial alternating current stimulation depend upon brain states. *Frontiers in Human Neuroscience*, *7*(April), 161. http://doi.org/10.3389/fnhum.2013.00161

Newsome, W. T., & Pare, E. B. (1988). A selective impairment of motion perception following lesions of the middle temporal visual area (MT). *The Journal of Neuroscience*, *8*(6), 2201–2211. http://doi.org/http://www.ncbi.nlm.nih.gov/pubmed/3385495

Nishina, S., Seitz, A. R., Kawato, M., & Watanabe, T. (2007). Effect of spatial distance to the task stimulus on task-irrelevant perceptual learning of static Gabors. *Journal of Vision*, *7*(2007), 2.1-10. http://doi.org/10.1167/7.13.2

Nitsche, M. a., Cohen, L. G., Wassermann, E. M., Priori, A., Lang, N., Antal, A., … Pascual-Leone, A. (2008). Transcranial direct current stimulation: State of the art 2008. *Brain Stimulation*, *1*(3), 206–223. http://doi.org/10.1016/j.brs.2008.06.004

Nitsche, M. A., Doemkes, S., Karaköse, T., Antal, A., Liebetanz, D., Lang, N., … Paulus, W. (2007). Shaping the effects of transcranial direct current stimulation of the human motor cortex. *Journal of Neurophysiology*, *97*(4), 3109–17. http://doi.org/10.1152/jn.01312.2006

Nitsche, M. A., Fricke, K., Henschke, U., Schlitterlau, A., Liebetanz, D., Lang, N., … Paulus, W. (2003). Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. *The Journal of Physiology*, *553*(Pt 1), 293–301. http://doi.org/10.1113/jphysiol.2003.049916

Nitsche, M. A., Jaussi, W., Liebetanz, D., Lang, N., Tergau, F., & Paulus, W. (2004). Consolidation of human motor cortical neuroplasticity by D-cycloserine. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *29*(8), 1573–1578. http://doi.org/10.1038/sj.npp.1300517

Nitsche, M. A., Liebetanz, D., Antal, A., Lang, N., Tergau, F., & Paulus, W. (2003). Chapter 27 Modulation of cortical excitability by weak direct current stimulation - technical, safety and functional aspects. *Supplements to Clinical Neurophysiology*, *56*(C), 255–276. http://doi.org/10.1016/S1567-424X(09)70230-2

Nitsche, M. A., Schauenburg, A., Lang, N., Liebetanz, D., Exner, C., Paulus, W., & Tergau, F. (2003). Facilitation of implicit motor learning by weak transcranial direct current stimulation of the primary motor cortex in the human. *Journal of Cognitive Neuroscience*, *15*(4), 619–626. http://doi.org/10.1162/089892903321662994

Nitsche, M. a, Kuo, M.-F., Grosch, J., Bergner, C., Monte-Silva, K., & Paulus, W. (2009). D1-receptor impact on neuroplasticity in humans. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *29*(8), 2648–53. http://doi.org/10.1523/JNEUROSCI.5366-08.2009

Nitsche, M. a, Nitsche, M. a, Paulus, W., & Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *The Journal of Physiology*, *527 Pt 3*, 633–9. http://doi.org/PHY_1055 [pii]

Nitsche, M. a, & Paulus, W. (2001). Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology*, *57*(10), 1899–1901. http://doi.org/10.1212/WNL.57.10.1899

Nitsche, M. a, & Paulus, W. (2011). Transcranial direct current stimulation--update 2011. *Restorative Neurology and Neuroscience*, *29*(6), 463–92. http://doi.org/10.3233/RNN-2011-0618

Normann, C., Schmitz, D., Fürmaier, A., Döing, C., & Bach, M. (2007). Long-Term Plasticity of Visually Evoked Potentials in Humans is Altered in Major Depression. *Biological Psychiatry*, *62*(5), 373–380. http://doi.org/10.1016/j.biopsych.2006.10.006

Ooi, T. L., Su, Y. R., Natale, D. M., & He, Z. J. (2013). A push-pull treatment for strengthening the "lazy eye" in amblyopia. *Current Biology*. http://doi.org/10.1016/j.cub.2013.03.004

Op de Beeck, H. P., & Baker, C. I. (2010). The neural basis of visual object learning. *Trends in Cognitive Sciences*. http://doi.org/10.1016/j.tics.2009.11.002

Paffen, C. L. E., Verstraten, F. a J., & Vidnyánszky, Z. (2008). Attention-based perceptual learning increases binocular rivalry suppression of irrelevant visual features. *Journal of Vision*, *8*(2008), 25.1-11. http://doi.org/10.1167/8.4.25

Papanikolaou, A., Keliris, G. A., Papageorgiou, T. D., Shao, Y., Krapp, E., Papageorgiou, E., … Smirnakis, S. M. (2014). Population receptive field analysis of the primary visual cortex complements perimetry in patients with homonymous visual field defects. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(16), E1656-65. http://doi.org/10.1073/pnas.1317074111

Pascual-Leone, a, Tarazona, F., Keenan, J., Tormos, J. M., Hamilton, R., & Catala, M. D. (1999). Transcranial magnetic stimulation and neuroplasticity. *Neuropsychologia*, *37*(2), 207–17. http://doi.org/S0028393298000955 [pii]

Pascucci, D., Mastropasqua, T., & Turatto, M. (2015). Monetary Reward Modulates Task-Irrelevant Perceptual Learning for Invisible Stimuli. *Plos One*, *10*(5), e0124009. http://doi.org/10.1371/journal.pone.0124009

Paul G. Mulquiney a, Kate E. Hoya,⇑, Zafiris J. Daskalakis b, P. B. F. (2011). Improving working memory: The effect of combining cognitive activity and anodal transcranial direct current stimulation to the left dorsolateral prefrontal cortex. *Brain Stimulation*, *4*(2), 84–89. http://doi.org/10.1016/j.brs.2010.06.004

Pell, G. S., Roth, Y., & Zangen, A. (2011). Modulation of cortical excitability induced by repetitive transcranial magnetic stimulation: Influence of timing and geometrical parameters and underlying mechanisms. *Progress in Neurobiology*, *93*(1), 59–98. http://doi.org/10.1016/j.pneurobio.2010.10.003

Pelli, D. G. (1997). The VideoToolbox software for visual psychophysics: transforming numbers into movies. *Spatial Vision*. http://doi.org/10.1163/156856897X00366

Peters, M. a K., Thompson, B., Merabet, L. B., Wu, A. D., & Shams, L. (2013). Anodal tDCS to V1 blocks visual perceptual learning consolidation. *Neuropsychologia*, *51*(7), 1234–1239. http://doi.org/10.1016/j.neuropsychologia.2013.03.013

Petrov, A. a, Dosher, B. A., & Lu, Z.-L. (2005). The dynamics of perceptual learning: an incremental reweighting model. *Psychological Review*, *112*(4), 715–743. http://doi.org/10.1037/0033-295X.112.4.715

Pirulli, C., Fertonani, A., & Miniussi, C. (2013). The role of timing in the induction of neuromodulation in perceptual learning by transcranial electric stimulation. *Brain Stimulation*, *6*(4), 683–689. http://doi.org/10.1016/j.brs.2012.12.005

Pirulli, C., Fertonani, A., & Miniussi, C. (2014). Is neural hyperpolarization by cathodal stimulation always detrimental at the behavioral level? *Frontiers in Behavioral Neuroscience*, *8*(JUNE), 226. http://doi.org/10.3389/fnbeh.2014.00226

Pisoni, A., Papagno, C., & Cattaneo, Z. (2012). Neural correlates of the semantic interference effect: New evidence from transcranial direct current stimulation. *Neuroscience*, *223*, 56–67. http://doi.org/10.1016/j.neuroscience.2012.07.046

Pizzorusso, T. (2002). Reactivation of Ocular Dominance Plasticity in the Adult Visual Cortex. *Science*, *298*(5596), 1248–1251. http://doi.org/10.1126/science.1072699

Polat, U., Ma-Naim, T., Belkin, M., & Sagi, D. (2004). Improving vision in adult amblyopia by perceptual learning. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(17), 6692–6697. http://doi.org/10.1073/pnas.0401200101

Polat, U., Ma-Naim, T., & Spierer, A. (2009). Treatment of children with amblyopia by perceptual learning. *Vision Research*, *49*(21), 2599–2603. http://doi.org/10.1016/j.visres.2009.07.008

Pollock, A., Hazelton, C., Henderson Clair, A., Angilley, J., Dhillon, B., Langhorne, P., … Shahani, U. (2011). Interventions for visual field defects in patients with stroke. *Cochrane Database of Systematic Reviews*. http://doi.org/10.1002/14651858.CD008388.pub2

Pollock, a., Hazelton, C., Henderson, C. a., Angilley, J., Dhillon, B., Langhorne, P., … Shahani, U. (2012). Interventions for Visual Field Defects in Patients With Stroke. *Stroke*, *43*(4), e37–e38. http://doi.org/10.1161/STROKEAHA.111.639815

Priori, A. (2003). Brain polarization in humans: A reappraisal of an old tool for prolonged non-invasive modulation of brain excitability. *Clinical Neurophysiology*, *114*(4), 589–595. http://doi.org/10.1016/S1388-2457(02)00437-6

Priori, A., Berardelli, A., Rona, S., Accornero, N., & Manfredi, M. (1998). Polarization of the human motor cortex through the scalp. *Neuroreport*, *9*(July), 2257–2260. http://doi.org/10.1097/00001756-199807130-00020

Radman, T., Ramos, R. L., Brumberg, J. C., & Bikson, M. (2009). Role of cortical cell type and morphology in subthreshold and suprathreshold uniform electric field stimulation in vitro. *Brain Stimulation*, *2*(4). http://doi.org/10.1016/j.brs.2009.03.007

Ragert, P., Franzkowiak, S., Schwenkreis, P., Tegenthoff, M., & Dinse, H. R. (2008). Improvement of tactile perception and enhancement of cortical excitability through intermittent theta burst rTMS over human primary somatosensory cortex. *Experimental Brain Research*, *184*(1), 1–11. http://doi.org/10.1007/s00221-007-1073-2

Rahman, A., Reato, D., Arlotti, M., Gasca, F., Datta, A., Parra, L. C., & Bikson, M. (2013). Cellular effects of acute direct current stimulation: somatic and synaptic terminal effects. *The Journal of Physiology*, *591*(Pt 10), 2563–78. http://doi.org/10.1113/jphysiol.2012.247171

Ranieri, F., Podda, M. V, Riccardi, E., Frisullo, G., Dileone, M., Profice, P., … Grassi, C. (2012). Modulation of LTP at rat hippocampal CA3-CA1 synapses by direct current stimulation. *Journal of Neurophysiology*, *107*(7), 1868–80. http://doi.org/10.1152/jn.00319.2011

Raninen, a, Vanni, S., Hyvärinen, L., & Näsänen, R. (2007). Temporal sensitivity in a hemianopic visual field can be improved by long-term training using flicker stimulation. *Journal of Neurology, Neurosurgery, and Psychiatry*, *78*(1), 66–73. http://doi.org/10.1136/jnnp.2006.099366

Reato, D., Gasca, F., Datta, A., Bikson, M., Marshall, L., & Parra, L. C. (2013). Transcranial Electrical Stimulation Accelerates Human Sleep Homeostasis. *PLoS Computational Biology*, *9*(2). http://doi.org/10.1371/journal.pcbi.1002898

Reato, D., Rahman, A., Bikson, M., & Parra, L. C. (2013). Effects of weak transcranial alternating current stimulation on brain activity-a review of known mechanisms from animal studies. *Frontiers in Human Neuroscience*, *7*(October), 687. http://doi.org/10.3389/fnhum.2013.00687

Reinhard, J., Schreiber, a, Schiefer, U., Kasten, E., Sabel, B. a, Kenkel, S., … Trauzettel-Klosinski, S. (2005). Does visual restitution training change absolute homonymous visual field defects? A fundus controlled study. *The British Journal of Ophthalmology*, *89*(1), 30–35. http://doi.org/10.1136/bjo.2003.040543

Reis, J., & Fritsch, B. (2011). Modulation of motor performance and motor learning by transcranial direct current stimulation. *Current Opinion in Neurology*, *24*(6), 590–596. http://doi.org/10.1097/WCO.0b013e32834c3db0

Reis, J., Schambra, H. M., Cohen, L. G., Buch, E. R., Fritsch, B., Zarahn, E., … Krakauer, J. W. (2009). Noninvasive cortical stimulation enhances motor skill acquisition over multiple days

through an effect on consolidation. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(5), 1590–5. http://doi.org/10.1073/pnas.0805413106

Riani, M., & Simonotto, E. (1994). Stochastic resonance in the perceptual interpretation of ambiguous figures: A neural network model. *Physical Review Letters*, *72*(19), 3120–3123. http://doi.org/10.1103/PhysRevLett.72.3120

Roe, J. M., Nesheim, M., Mathiesen, N. C., Moberget, T., Alnæs, D., & Sneve, M. H. (2016). The effects of tDCS upon sustained visual attention are dependent on cognitive load. *Neuropsychologia*, *80*, 1–8. http://doi.org/10.1016/j.neuropsychologia.2015.11.005

Rokem, A., & Silver, M. A. (2010). Cholinergic enhancement augments magnitude and specificity of visual perceptual learning in healthy humans. *Current Biology*, *20*(19), 1723–1728. http://doi.org/10.1016/j.cub.2010.08.027

Rosenthal, O., & Humphreys, G. W. (2010). Perceptual organization without perception. The subliminal learning of global contour. *Psychological Science*, *21*(12), 1751–8. http://doi.org/10.1177/0956797610389188

Rroji, O., Van Kuyck, K., Nuttin, B., & Wenderoth, N. (2015). Anodal tDCS over the primary motor cortex facilitates long-term memory formation reflecting use-dependent plasticity. *PLoS ONE*, *10*(5). http://doi.org/10.1371/journal.pone.0127270

Saarinen, J., & Levi, D. M. (1995). Perceptual learning in vernier acuity: What is learned? *Vision Research*, *35*(4), 519–527. http://doi.org/10.1016/0042-6989(94)00141-8

Sagi, D. (2011). Perceptual learning in Vision Research. *Vision Research*, *51*(13), 1552–1566. http://doi.org/10.1016/j.visres.2010.10.019

Sahraie, A., MacLeod, M. J., Trevethan, C. T., Robson, S. E., Olson, J. A., Callaghan, P., & Yip, B. (2010). Improved detection following Neuro-Eye Therapy in patients with post-geniculate brain damage. *Experimental Brain Research*, *206*(1), 25–34. http://doi.org/10.1007/s00221-010-2395-z

Sahraie, A., Trevethan, C. T., & MacLeod, M. J. (2008). Temporal properties of spatial channel of processing in hemianopia. *Neuropsychologia*, *46*(3), 879–885. http://doi.org/10.1016/j.neuropsychologia.2007.11.008

Sahraie, A., Trevethan, C. T., MacLeod, M. J., Murray, A. D., Olson, J. A., & Weiskrantz, L. (2006). Increased sensitivity after repeated stimulation of residual spatial channels in blindsight. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(40),

14971–14976. http://doi.org/10.1073/pnas.0607073103

Sale, A., Berardi, N., Spolidoro, M., Baroncelli, L., & Maffei, L. (2010). GABAergic inhibition in visual cortical plasticity. *Frontiers in Cellular Neuroscience*, *4*(March), 10. http://doi.org/10.3389/fncel.2010.00010

Sale, A., De Pasquale, R., Bonaccorsi, J., Pietra, G., Olivieri, D., Berardi, N., & Maffei, L. (2011). Visual perceptual learning induces long-term potentiation in the visual cortex. *Neuroscience*, *172*, 219–225. http://doi.org/10.1016/j.neuroscience.2010.10.078

Salvador, R., Mekonnen, A., Ruffini, G., & Miranda, P. C. (2010). Modeling the electric field induced in a high resolution realistic head model during transcranial current stimulation. *Conference Proceedings : ... Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Conference*, *2010*, 2073–2076. http://doi.org/10.1109/IEMBS.2010.5626315

Sanders, M. D., Warrington, E., Marshall, J., & Wieskrantz, L. (1974). "BLINDSIGHT": VISION IN A FIELD DEFECT. *The Lancet*, *303*(7860), 707–708. http://doi.org/10.1016/S0140-6736(74)92907-9

Sathian, K., & Zangaladze, A. (1998). Perceptual learning in tactile hyperacuity: Complete intermanual transfer but limited retention. *Experimental Brain Research*, *118*(1), 131–134. http://doi.org/10.1007/s002210050263

Schiltz, C., Bodart, J. M., Dubois, S., Dejardin, S., Michel, C., Roucoux, a, … Orban, G. a. (1999). Neuronal mechanisms of perceptual learning: changes in human brain activity with training in orientation discrimination. *NeuroImage*, *9*, 46–62. http://doi.org/10.1006/nimg.1998.0394

Schoen, I., & Fromherz, P. (2008). Extracellular stimulation of mammalian neurons through repetitive activation of Na+ channels by weak capacitive currents on a silicon chip. *Journal of Neurophysiology*, *100*(1), 346–357. http://doi.org/10.1152/jn.90287.2008

Sczesny-Kaiser, M., Beckhaus, K., Dinse, H. R., Schwenkreis, P., Tegenthoff, M., & Höffken, O. (2016). Repetitive Transcranial Direct Current Stimulation Induced Excitability Changes of Primary Visual Cortex and Visual Learning Effects—A Pilot Study. *Frontiers in Behavioral Neuroscience*, *10*(June), 1–11. http://doi.org/10.3389/fnbeh.2016.00116

Seitz, A. R., & Dinse, H. R. (2007). A common framework for perceptual learning. *Current Opinion in Neurobiology*, *17*(2), 148–153. http://doi.org/10.1016/j.conb.2007.02.004

Seitz, A. R., Kim, R., & Shams, L. (2006). Sound Facilitates Visual Learning. *Current Biology*,

16(14), 1422–1427. http://doi.org/10.1016/j.cub.2006.05.048

Seitz, A. R., Nanez, J. E., Holloway, S., Tsushima, Y., & Watanabe, T. (2006). Two cases requiring external reinforcement in perceptual learning. *Journal of Vision*, *6*(9), 966–973. http://doi.org/10.1167/6.9.9

Seitz, A., & Watanabe, T. (2005). A unified model for perceptual learning. *Trends in Cognitive Sciences*, *9*(7), 329–334. http://doi.org/10.1016/j.tics.2005.05.010

Shadlen, M. N., & Newsome, W. T. (1996). Motion perception: seeing and deciding. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(January), 628–633. http://doi.org/10.1073/pnas.93.2.628

Silvanto, J., Muggleton, N., & Walsh, V. (2008). State-dependency in brain stimulation studies of perception and cognition. *Trends in Cognitive Sciences*, *12*(12), 447–454. http://doi.org/10.1016/j.tics.2008.09.004

Silver, M. A., Shenhav, A., & D'Esposito, M. (2008). Cholinergic Enhancement Reduces Spatial Spread of Visual Responses in Human Early Visual Cortex. *Neuron*, *60*(5), 904–914. http://doi.org/10.1016/j.neuron.2008.09.038

Simonotto, E., Riani, M., Seife, C., Roberts, M., Twitty, J., & Moss, F. (1997). Visual Perception of Stochastic Resonance. *Physical Review Letters*, *78*(6), 1186–1189. http://doi.org/10.1103/PhysRevLett.78.1186

Singh Gilhotra, J., Mitchell, P., Healey, P. R., Cumming, R. G., & Currie, J. (2002). Homonymous visual field defects and stroke in an older population. *Stroke*, *33*(10), 2417–2420. http://doi.org/10.1161/01.str.0000037647.10414.d2

Snowball, A., Tachtsidis, I., Popescu, T., Thompson, J., Delazer, M., Zamarian, L., … Kadosh, R. C. (2013). Long-Term Enhancement of Brain Function and Cognition Using Cognitive Training and Brain Stimulation. *Current Biology : CB*, *23*(11), 987–992. http://doi.org/10.1016/j.cub.2013.04.045

Snowden, P. T., Davies, I. R. L., & Roling, P. (2000). Perceptual learning of the detection of features in X-ray images: A functional role for improvements in adults' visual sensitivity? *Journal of Experimental Psychology: Human Perception and Performance*, *26*(1), 379–390. http://doi.org/10.1037/0096-1523.26.1.379

Spolidoro, M., Sale, A., Berardi, N., & Maffei, L. (2009). Plasticity in the adult brain: Lessons from the visual system. In *Experimental Brain Research* (Vol. 192, pp. 335–341).

http://doi.org/10.1007/s00221-008-1509-3

Staatsbibliothek, B., & Staatsbibliothek, B. (1816). Aloysi Galvani Abhandlung über die Kräfte der thierischen Elektrizität auf die Bewegung der Muskeln : nebst einigen Schriften der H . H . Valli , Carminati und Volta über eben diesen Gegenstand . Eine Übersezung, 4–5.

Stagg, C. J., Jayaram, G., Pastor, D., Kincses, Z. T., Matthews, P. M., & Johansen-Berg, H. (2011). Polarity and timing-dependent effects of transcranial direct current stimulation in explicit motor learning. *Neuropsychologia*, *49*(5), 800–804. http://doi.org/10.1016/j.neuropsychologia.2011.02.009

Stefan, K., Wycislo, M., & Classen, J. (2004). Modulation of associative human motor cortical plasticity by attention. *Journal of Neurophysiology*, *92*(1), 66–72. http://doi.org/10.1152/jn.00383.2003

Stoerig, P., & Cowey, A. (1997). Blindsight in man and monkey. *Brain*, *120*(3), 535–559. http://doi.org/10.1093/brain/120.3.535

Tamm, A. S., Lagerquist, O., Ley, A. L., & Collins, D. F. (2009). Chronotype influences diurnal variations in the excitability of the human motor cortex and the ability to generate torque during a maximum voluntary contraction. *Journal of Biological Rhythms*, *24*(3), 211–224. http://doi.org/10.1177/0748730409334135

Tan, D. T. H., & Fong, A. (2008). Efficacy of neural vision therapy to enhance contrast sensitivity function and visual acuity in low myopia. *Journal of Cataract and Refractive Surgery*, *34*(4), 570–577. http://doi.org/10.1016/j.jcrs.2007.11.052

Tegenthoff, M., Ragert, P., Pleger, B., Schwenkreis, P., Förster, A. F., Nicolas, V., & Dinse, H. R. (2005). Improvement of tactile discrimination performance and enlargement of cortical somatosensory maps after 5 Hz rTMS. *PLoS Biology*, *3*(11), 2031–2040. http://doi.org/10.1371/journal.pbio.0030362

Terney, D., Chaieb, L., Moliadze, V., Antal, A., & Paulus, W. (2008). Increasing human brain excitability by transcranial high-frequency random noise stimulation. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *28*(52), 14147–14155. http://doi.org/10.1523/JNEUROSCI.4248-08.2008

Thompson, B., Tjan, B. S., & Liu, Z. (2013). Perceptual Learning of Motion Direction Discrimination with Suppressed and Unsuppressed MT in Humans: An fMRI Study. *PLoS ONE*, *8*(1). http://doi.org/10.1371/journal.pone.0053458

Truelsen, T., Piechowski-Jóźwiak, B., Bonita, R., Mathers, C., Bogousslavsky, J., & Boysen, G. (2006). Stroke incidence and prevalence in Europe: A review of available data. *European Journal of Neurology*. http://doi.org/10.1111/j.1468-1331.2006.01138.x

Tsushima, Y., Seitz, A. R., & Watanabe, T. (2008). Task-irrelevant learning occurs only when the irrelevant feature is weak. *Current Biology*. http://doi.org/10.1016/j.cub.2008.04.029

Tyrrell, R. a., & Owens, D. A. (1988). A rapid technique to assess the resting states of the eyes and other threshold phenomena: The Modified Binary Search (MOBS). *Behavior Research Methods, Instruments, & Computers*, *20*(2), 137–141. http://doi.org/10.3758/BF03203817

Vaina, L. M., Belliveau, J. W., Roziers, E. B. des, & Zeffiro, T. A. (1998). Neural systems underlying learning and representation of global motion. *Proceedings of the National Academy of Sciences*, *95*(21), 12657–12662. http://doi.org/10.1073/pnas.95.21.12657

van der Groen, O., & Wenderoth, N. (2016). Transcranial Random Noise Stimulation of Visual Cortex: Stochastic Resonance Enhances Central Mechanisms of Perception. *Journal of Neuroscience*, *36*(19), 5289–5298. http://doi.org/10.1523/JNEUROSCI.4519-15.2016

van Praag, H., Kempermann, G., & Gage, F. H. (2000). Neural consequences of environmental enrichment. *Nat Rev Neurosci*, *1*(3), 191–198. http://doi.org/10.1038/35044558

Wang, R., Wang, J., Zhang, J.-Y., Xie, X.-Y., Yang, Y.-X., Luo, S.-H., … Li, W. (2016). Perceptual Learning at a Conceptual Level. *The Journal of Neuroscience*, *36*(7), 2238–2246. http://doi.org/10.1523/jneurosci.2732-15.2016

Ward, L. M., Neiman, A., & Moss, F. (2002). Stochastic resonance in psychophysics and in animal behavior. *Biological Cybernetics*, *87*(2), 91–101. http://doi.org/10.1007/s00422-002-0328-z

Watanabe, T., Náñez, J. E., Koyama, S., Mukai, I., Liederman, J., & Sasaki, Y. (2002). Greater plasticity in lower-level than higher-level visual motion processing in a passive perceptual learning task. *Nature Neuroscience*, *5*(10), 1003–1009. http://doi.org/10.1038/nn915

Watanabe, T., Náñez, J. E., & Sasaki, Y. (2001). Perceptual learning without perception. *Nature*, *413*(6858), 844–848. http://doi.org/10.1038/35101601

Watson, A. B., & Pelli, D. G. (1983). QUEST: a Bayesian adaptive psychometric method. *Perception & Psychophysics*, *33*(2), 113–120. http://doi.org/10.3758/BF03202828

Weiskrantz, L., Warrington, E. K., Sanders, M. D., & Marshall, J. (1974). Visual capacity in the hemianopic field following a restricted occipital ablation. *Brain*, *97*(4), 709–728. http://doi.org/10.1093/brain/97.4.709

Wiesel, T. N., & Hubel, D. H. (1963). Effects of Visual Deprivation on Morphology and Physiology of cells in the Cat's Lateral Geniculate Body. *J Neurophysiol*, *26*, 978–993. Retrieved from http://hubel.med.harvard.edu/papers/HubelWiesel1963Jneurophysiol3.pdf

Williams, J. A., Pascual-Leone, A., & Fregni, F. (2010). Interhemispheric modulation induced by cortical stimulation and motor training. *Physical Therapy*, *90*(3), 398–410. http://doi.org/10.2522/ptj.20090075

Wright, B. a, Sabin, A. T., Zhang, Y., Marrone, N., & Fitzgerald, M. B. (2010). Enhancing perceptual learning by combining practice with periods of additional sensory stimulation. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *30*(38), 12868–12877. http://doi.org/10.1523/JNEUROSCI.0487-10.2010

Xiao, L. Q., Zhang, J. Y., Wang, R., Klein, S. a., Levi, D. M., & Yu, C. (2008). Complete Transfer of Perceptual Learning across Retinal Locations Enabled by Double Training. *Current Biology*, *18*(24), 1922–1926. http://doi.org/10.1016/j.cub.2008.10.030

Xu, J. P., He, Z. J., & Ooi, T. L. (2010). Effectively reducing sensory eye dominance with a push-pull perceptual learning protocol. *Current Biology*, *20*(20), 1864–1868. http://doi.org/10.1016/j.cub.2010.09.043

Xu, J. P., He, Z. J., & Ooi, T. L. (2012). Perceptual learning to reduce sensory eye dominance beyond the focus of top-down visual attention. *Vision Research*, *61*, 39–47. http://doi.org/10.1016/j.visres.2011.05.013

Yamahachi, H., Marik, S. A., McManus, J. N. J., Denk, W., & Gilbert, C. D. (2009). Rapid Axonal Sprouting and Pruning Accompany Functional Reorganization in Primary Visual Cortex. *Neuron*, *64*(5), 719–729. http://doi.org/10.1016/j.neuron.2009.11.026

Yan, Y., Rasch, M. J., Chen, M., Xiang, X., Huang, M., Wu, S., & Li, W. (2014). Perceptual training continuously refines neuronal population codes in primary visual cortex, *17*(10). http://doi.org/10.1038/nn.3805

Yildirim, M., Aydin-Abidin, S., Abidin, I., Akca, M., Canpolat, S., & Cansu, A. (2013). Evaluation of the role of chronic daily melatonin administration and pinealectomy on penicillin-induced focal epileptiform activity and spectral analysis of ECoG in rats: An in vivo electrophysiological study. *Neurochemical Research*, *38*(8), 1672–1685. http://doi.org/10.1007/s11064-013-1069-1

Zaehle, T., Rach, S., & Herrmann, C. S. (2010). Transcranial Alternating Current Stimulation Enhances Individual Alpha Activity in Human EEG. *PLoS ONE*, *5*(11).

http://doi.org/10.1371/journal.pone.0013766

Zeng, F. G., Fu, Q. J., & Morse, R. (2000). Human hearing enhanced by noise. *Brain Research*, *869*(1–2), 251–255. http://doi.org/10.1016/S0006-8993(00)02475-6

Zhang, J.-Y., Zhang, G.-L., Xiao, L.-Q., Klein, S. a, Levi, D. M., & Yu, C. (2010). Rule-based learning explains visual perceptual learning and its specificity and transfer. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *30*(37), 12323–12328. http://doi.org/10.1523/JNEUROSCI.0704-10.2010

Zhang, T., Xiao, L. Q., Klein, S. A., Levi, D. M., & Yu, C. (2010). Decoupling location specificity from perceptual learning of orientation discrimination. *Vision Research*, *50*(4), 368–374. http://doi.org/10.1016/j.visres.2009.08.024

Zhang, X., Kedar, S., Lynn, M. J., Newman, N. J., & Biousse, V. (2006). Homonymous hemianopias: Clinical-anatomic correlations in 904 cases. *Neurology*. http://doi.org/10.1212/01.wnl.0000203913.12088.93

Zimerman, M., Nitsch, M., Giraux, P., Gerloff, C., Cohen, L. G., & Hummel, F. C. (2013). Neuroenhancement of the aging brain: Restoring skill acquisition in old subjects. *Annals of Neurology*, *73*(1), 10–15. http://doi.org/10.1002/ana.23761

Zivari Adab, H., & Vogels, R. (2011). Practicing coarse orientation discrimination improves orientation signals in macaque cortical area V4. *Current Biology*, *21*(19), 1661–1666. http://doi.org/10.1016/j.cub.2011.08.037

Abbreviations

ANOVA	Analysis of Variance
BDNF	Brain-derived neurotrophic factor
CNS	Central Nervous System
EEG	Electroencephalography
EPSPs	Excitatory Post-Synaptic Potentials
EVA	Early Visual Areas
ICMS	Intracortical Microstimulations
LTD	Long Term Depression
LTP	Long Term Potentials
M1	Primary Motor Cortex
V1	Primary Visual Cortex
MEP	Motor-evoked potentials
VEP	Visual-evoked potentials
MD	Monocular deprivation
NMDA	N-methyl-D-aspartate
NIBS	Non-Invasive Brain Stimulation Techniques
OD	Ocular Dominance
RMT	Resting Motor Threshold
PT	Phosphenes Threshold
PL	Perceptual Learning
tACS	Transcranial Alternating Current Stimulation
tDCS	Transcranial Direct Current Stimulation
tES	Transcranial Electrical Stimulation

TMS	Transcranial Magnetic Stimulation
tRNS	Transcranial Random Noise Stimulation
VPL	Visual Perceptual Learning

Funding Sources

This work was supported by the Autonomous Province of Trento, Call "Grandi Progetti 2012", project "Characterizing and Improving Brain Mechanisms of Attention – ATTEND – Grant.

Acknowledgments

I would like to heartily thank my advisor Lorella Battelli for her support, mentoring and for showing me how and what it means to do research and to think as a scientist. I am grateful for all the help and advices she gave me during all these years and for allowing me to work in such a great and stimulating scientific environment.

Another heartily thanks go to my committee members Dr. Zaira Cattaneo and Dr. Duje Tadin for their excellent suggestions and feedbacks.

A big thank you to Prof. Krystel Huxlin, Dr. Michael Melnick and Dr. Duje Tadin form the Huxlin Lab at the University of Rochester for the excellent productive collaboration and all the precious help during all these years of work together.

A special thank goes to my oversight Committee, Prof. Wieske van Zoest and Prof. Olivier Collignon, who wisely advice, supported and helped me during all these years of work at CiMeC.

I also wish to thank the Final Exam Committee Members Prof. Cattaneo Zaira, Prof. Lerch Jason and Prof. Pourtois Gilles.

Finally, I wish to heartily thank my family, and especially my Mum, for giving me this opportunity, for all the support and for always believing in me.