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**Transcranial random noise stimulation and
perceptual learning as tools for investigating and
promoting neural plasticity in vision**

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Abstract

Transcranial random noise stimulation (tRNS) is a recent neuro-modulation technique whose effects at both behavioural and neural level are still debated. In the first experiment the well-known phenomenon of motion aftereffect (MAE) was exploited in order to investigate the effects of high- versus low-frequency tRNS on motion adaptation and recovery. 36 Participants were asked to evaluate the MAE duration following the exposure of a circular rotating and expanding grating for 30 seconds, while being stimulated with either Sham or tRNS across different blocks. Different groups were administered with either high- or low-frequency tRNS. Stimulation sites were bilateral V5/MT, early visual areas or frontal areas. Results demonstrated that, whereas no effects on MAE duration were produced by stimulation of early visual areas or frontal areas, high-frequency tRNS over area V5/MT caused a significant decrease in MAE duration whereas low-frequency tRNS (over the same area) caused a significant corresponding increase in MAE duration. These data indicate that high- versus low-frequency tRNS has opposite effects on the unbalance, created by adaptation, between neurons tuned to opposite motion directions, and thus on neuronal excitability.

Following repeated practice on a visual task, perceptual learning (PL) produces a long lasting improvement of visual functions such as an increase of visual acuity (VA) and contrast sensitivity (CS) both in participants with amblyopia and refractive defects. This improvement has been observed with contrast detection tasks in the presence of lateral masking (contrast detection of a central Gabor stimulus flanked by two high contrast Gabors), known to bring

about an increase of lateral interactions between detectors in early cortical pathways. Improvement has also been revealed in the absence of flankers in healthy individuals and those with amblyopia. In the second experiment, a single Gabor PL regime (in the absence of lateral masking) was investigated in a group of participants with mild myopia. This study seeks to understand whether a perceptual training regime really needs to be based on lateral interactions in cases where poor vision is not due to cortical dysfunctions, such as in myopia. 10 participants with mild myopia (max -2D) were recruited. The participants carried out an 8-week behavioural training using a single Gabor PL paradigm, completing a total of 24 sessions. Results indicate that training using a single Gabor protocol results in a VA improvement of 0.16 logMAR. The present study supports the idea that, in the absence of cortical deficits, such as in myopia, some sort of compensatory mechanism can take place at the cortical level by means of PL, resulting in more effective processing of the received blurred input. However, with respect to training based on lateral masking, here we found that improvement of visual functions was smaller and limited to VA. This might suggest that trainings based on lateral masking, able to modify the strength of facilitatory and inhibitory lateral interactions, could be more effective for an optimal recovery of blurred vision.

It has recently been suggested how PL can be boosted by concurrent high-frequency tRNS (hf-tRNS). It has also been shown how PL can generalize and produce an improvement of visual functions in participants with mild refractive defects. By using three different groups of participants, with 10 participants in each group (single-blind study), the third experiment tested the efficacy of a

short (8 sessions) single Gabor contrast-detection training with concurrent hf-tRNS in comparison with the same training combined with Sham stimulation or hf-tRNS with no concurrent training, in improving VA and CS of individuals with uncorrected mild myopia. Results show that a short training with a contrast detection task is able to improve VA and CS only if coupled with hf-tRNS, whereas no effect on VA and marginal effects on CS are seen with the sole administration of hf-tRNS. The results support the idea that, by boosting the rate of PL via the modulation of neuronal plasticity, hf-tRNS can be successfully used to reduce the duration of perceptual trainings while, at the same time, increasing their efficacy in producing PL and generalization to improved VA and CS in individuals with uncorrected mild myopia.

A final experiment extended the aforementioned results onto patients with a cortical visual deficit. Amblyopia is a visual disorder due to an abnormal pattern of functional connectivity of the visual cortex and characterized by several visual deficits of spatial vision including impairments of VA and of the contrast sensitivity function (CSF). Despite being a developmental disorder caused by reduced visual stimulation during early life (critical period), several studies have shown that extensive visual perceptual training can improve VA and CS in people with amblyopia even in adulthood. In this study, a much shorter perceptual training regime was assessed with respect to the standard PL trainings, in association with hf-tRNS in comparison to the perceptual training combined with Sham stimulation, whether it was able to improve visual functions in a group of adult participants with amblyopia. Results demonstrated that, in comparison with previous studies where a large number sessions with a

similar training regime were used, here just eight sessions of training in contrast detection under lateral masking conditions combined with hf-tRNS, were able to substantially improve VA and CS in adults with amblyopia.

In conclusion, this thesis investigates the use and efficacy of tRNS with and without PL on visual cortical excitability and plasticity, in the context of visual functioning.

Riassunto

La stimolazione transcranica a rumore casuale (transcranial random noise stimulation - tRNS) è una tecnica neuromodulatoria recente i cui effetti a livello comportamentale e neurale sono ancora dibattuti. Con il primo esperimento è stato utilizzato l'effetto postumo di movimento, denominato altresì motion aftereffect (MAE), per indagare gli effetti della tRNS ad alta e a bassa frequenza sull'adattamento al movimento e sul suo recupero. A trentasei partecipanti è stato chiesto di valutare la durata del MAE evocato dalla visione di un reticolo con movimento di rotazione ed espansione per 20 secondi, contemporaneamente alla tRNS o ad una stimolazione fittizia (Sham), somministrate in diversi blocchi. A gruppi di partecipanti diversi è stata somministrata la tRNS ad alta o a bassa frequenza. I siti di stimolazione potevano essere l'area V5/MT bilateralmente, le corteccie visive precoci o le aree frontali. I risultati hanno mostrato che, mentre non è stata trovata nessuna variazione con la stimolazione delle aree visive precoci o delle aree frontali, la tRNS ad alta frequenza sull'area V5/MT ha determinato una riduzione significativa della durata del MAE mentre la tRNS a bassa frequenza (sulla stessa area V5/MT) ha provocato un corrispondente incremento della durata del MAE. Questi dati indicano che la tRNS ad alta e a bassa frequenza hanno effetti opposti sullo squilibrio, creato dall'adattamento, tra neuroni che rispondono a direzioni di movimento opposte, e quindi effetti opposti sull'eccitabilità neuronale. Questi dati indicano che la tRNS ad alta e a bassa frequenza ha effetti opposti sullo squilibrio, creato dall'adattamento, tra neuroni che rispondono a direzioni di movimento opposte, e quindi effetti opposti sull'eccitabilità neuronale.

Attraverso un training ripetuto con un determinato compito visivo, l'apprendimento percettivo (perceptual learning - PL) produce un miglioramento duraturo di funzioni visive quali un incremento dell'acuità visiva (AV) e della sensibilità al contrasto (SC) in partecipanti con ambliopia o con difetti refrattivi. Tale miglioramento è stato osservato attraverso l'utilizzo di un training di detezione di contrasto in presenza di flankers (mascheramento laterale), che permette di ottenere un potenziamento delle interazioni laterali tra detettori ai primi livelli di elaborazione visiva corticale. Un simile miglioramento è stato osservato anche in assenza di flankers, sia in partecipanti sani che in partecipanti con ambliopia. Nel secondo studio è stato investigato l'effetto di un training con Gabor singoli (in assenza quindi di mascheramento laterale) in un gruppo di partecipanti con miopia lieve. Con questo studio si è cercato di capire se, per ottenere un miglioramento delle funzioni visive, un training percettivo debba essere necessariamente basato sulle interazioni laterali nel caso in cui una visione sfocata sia dovuta a una disfunzione non corticale come la miopia. 10 partecipanti con miopia lieve (sino a -2D) hanno partecipato ad un training comportamentale di 8 settimane (per un totale di 24 sessioni) utilizzando un compito di detezione di contrasto di Gabor singoli. I risultati mostrano un miglioramento in AV, in assenza di correzione ottica, di 0.16 LogMAR, suggerendo che, pur in assenza di deficit corticali, un meccanismo di compensazione possa aver luogo a livello corticale attraverso il PL, ottenendo perciò un'elaborazione più efficace dall'immagine sfocata in ingresso. Tuttavia, rispetto al training basato sul mascheramento laterale, in questo studio abbiamo trovato un miglioramento delle funzioni visive più contenuto e limitato alla AV.

Questo può suggerire come il training basato sul mascheramento laterale, capace di modificare la forza delle interazioni laterali facilitatorie e inibitorie, possa essere più efficace per un recupero ottimale della visione sfocata.

E' stato suggerito di recente come il PL possa essere potenziato dalla contemporanea somministrazione di tRNS ad alta frequenza. D'altro canto, è stato anche mostrato come il PL possa generalizzare e causare un miglioramento delle funzioni visive in partecipanti con difetti refrattivi lievi. Utilizzando tre diversi gruppi di partecipanti con 10 partecipanti per gruppo (disegno sperimentale in cieco), con il terzo esperimento si è voluto testare l'efficacia di un breve (8 sessioni) training di detezione di contrasto con Gabor singoli, con contemporanea somministrazione di tRNS ad alta frequenza, confrontata con lo stesso training con contemporanea somministrazione di stimolazione fittizia (Sham), e con tRNS ad alta frequenza in assenza di training comportamentale, nel miglioramento di AV e SC di partecipanti con miopia lieve non corretta. I risultati mostrano che un breve training di detezione di contrasto è in grado di migliorare AV e SC solo se unito a contemporanea tRNS ad alta frequenza, mentre nessun sostanziale miglioramento è stato osservato con la sola somministrazione della tRNS. Questi risultati supportano l'idea che, potenziando la velocità del PL attraverso la modulazione della plasticità neurale, la tRNS ad alta frequenza può essere utilizzata con successo per ridurre la durata dei training percettivi, aumentando allo stesso tempo l'efficacia nel produrre PL e generalizzazione (miglioramento di AV e SC) in individui con miopia lieve non corretta.

Un ultimo esperimento ha permesso di estendere i summenzionati risultati su pazienti con deficit visivo di natura corticale. L'ambliopia è un disturbo visivo dovuto ad un pattern di connettività funzionale anormale della corteccia visiva, caratterizzato da diversi deficit in visione spaziale tra cui in AV e in SC. Pur essendo un disturbo dello sviluppo causato da stimolazione visiva ridotta o alterata durante l'infanzia (periodo critico), diversi studi hanno mostrato come training percettivi visivi possano migliorare AV e SC in individui con ambliopia anche in età adulta. In questo studio, è stata valutata l'efficacia di un training percettivo molto più breve rispetto alle durate standard (associato alla tRNS ad alta frequenza rispetto allo stesso training unito a stimolazione Sham), nel miglioramento delle funzioni visive di un gruppo di partecipanti adulti con ambliopia. I risultati hanno mostrato che 8 sessioni di training di detezione di contrasto con mascheramento laterale, unito a tRNS ad alta frequenza, permettono un sostanziale miglioramento di AV e SC in partecipanti adulti con amblyopia.

In conclusione, in questo elaborato si è voluto testare l'efficacia della tRNS con e senza PL sull'eccitabilità e la plasticità della corteccia visiva, nel contesto dei meccanismi delle funzioni visive.

Dedication

I dedicate this thesis to my beloved husband and soul mate, Federico.

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Glossary of Abbreviations

2AFC - Two-alternative forced choice

2IFC - Two-interval forced choice

CPD - Cycles per Degree

CS - Contrast sensitivity

CSF - Contrast sensitivity function

CRF - Cell's receptive field

EEG - Electroencephalography

ERP - Event Related Potential

fMRI - Functional Magnetic Resonance Imaging

HF-TRNS - High-frequency transcranial Random Noise Stimulation

LF-TRNS - Low-frequency transcranial Random Noise Stimulation

LGN - Lateral geniculate nucleus

LTP - Long Term Potentiation

LTD - Long Term Depression

IT - Inferotemporal cortex

MAE - Motion aftereffect

MST – Medial superior temporal area

MT – Medio-temporal area

NMDA – N-methyl -D- Aspartate

NIBS – Non-invasive Brain Stimulation

PET – Positron Emission Tomography

PL – Perceptual Learning

rTMS – Repetitive Transcranial Magnetic Stimulation

RF – Receptive Field

SNR – Signal to Noise Ratio

STS – Superior Temporal Sulcus

tACS – Transcranial Alternating Current Stimulation

tDCS – Transcranial Direct Current Stimulation

TES – Transcranial Electrical Stimulation

TMS – Transcranial Magnetic Stimulation

tRNS – Transcranial Random Noise Stimulation

UCCS – Uncorrected Contrast Sensitivity

UCVA – Uncorrected Visual Acuity

V1 - Primary Visual Cortex

VA - Visual Acuity

VIP - Ventral Intraparietal Area

CHAPTER 1 Introduction and aims of the thesis

“Any man could, if he were so inclined, be the sculptor of his own brain”. Known by many as the father of modern Neuroscience, Santiago Ramón y Cajal eloquently revealed the limitless power of our brains to adapt and to learn. This restorative power of the brain, referred to as neural plasticity, is the foundation of all learning and recovery. Discovering and understanding ways to enhance brain plasticity is the ultimate scope and future of neuroscience.

The visual cortex is one of the most appropriate areas in which to explore the extents of neural plasticity, as it is relatively easy to manipulate its sensory input and assess the neural correlates of these manipulations. Furthermore, millions of people around the world suffer from vision loss, some of which, such as adult amblyopia, remains untreatable in adulthood. For example, estimates of amblyopia prevalence vary between 1.19% (Chia, Dirani, Chan, Gazzard, et al., 2010) and 5% (Lai, Hsu, Wang, Chang, & Wu, 2009) depending on criterion of VA, age group, and region. Vision therapy is not new to the field of visual neuroscience, although techniques and protocols are still being refined in order to offer the best chance for improvement in untreatable conditions such as amblyopia. One such therapy is known as Perceptual Learning (PL) in which the participant undergoes repeated practice on a visual task, most commonly using sine-wave gratings known as Gabor stimuli under the conditions of lateral masking (Polat & Sagi 1994). PL is said to boost visual plasticity through Hebbian-like mechanisms, which in turn enhances visual processing. Despite being effective in boosting neural plasticity in visual deficits such as amblyopia

and myopia, functional improvements tend to depend on lengthy protocols, which makes it an impractical method while increasing the risk of non-compliance to the therapy.

Contemporary research has shown that it is possible to increase human neuroplasticity in adulthood by using non-invasive brain stimulation (NIBS) techniques such as Transcranial Direct Current Stimulation (tDCS), Transcranial Alternating Current Stimulation (tACS) and Transcranial Ransom Noise Stimulation (tRNS). These refined techniques are still in the early stages of being fully understood, yet many studies have provided evidence of their effectiveness in enhancing neural excitability and improving cognitive and behavioural outcomes. tRNS is the newest of the NIBS family and so far is proving to be very promising as an adjunct to cognitive and behavioural interventions. This idea is still in the very early stages of research, and much needs to be done in order to fully grasp the underlying mechanisms by which this improvement is being achieved and in creating the right protocols for different conditions.

The research presented in this thesis aimed at evaluating the therapeutic potential of tRNS combined with existing vision training techniques in the improvement of visual functioning. Furthermore the results contemplate on the underlying neural mechanisms of its action on visual perception and visual motion discrimination. Overall, this work contributes to our understanding of the human visual system while offering new insights into the combined approach of tRNS and visual PL in the recovery and treatment of visual functioning. Additionally, the findings speculate on the underlying mechanisms

by which low and high frequency random noise stimulation differently modulate neural excitability in the visual cortex.

Chapter 2, the literature review, provides the theoretical background related to important themes that will surface throughout the thesis. These include an overview of the visual system, current trends on vision therapy, specifically PL, and non-invasive brain stimulation techniques (NIBS).

The subsequent chapters will present data from four different experiments conducted throughout the PhD, all with the scope of understanding the effects of PL and/or tRNS on neuro-modulation and plasticity of the visual system. Chapter 3 presents data from the first experiment conducted, which sought to investigate the neuromodulatory effects of low and high frequency random noise stimulation on a well-known visual phenomenon known as the Motion After Effect (MAE). The findings of this experiment highlighted a distinct effect of low and high frequency RNS on visual neurons, specifically, motion direction discrimination neurons in area MT.

Chapter 4 discusses the findings from a second experiment carried out in order to evaluate the effectiveness of a single-Gabor protocol of PL in comparison to the more commonly used lateral masking technique. The findings of this study suggest that the lateral masking paradigm seems to be more effective in improving VA and CS in participants with mild myopia.

The following chapter brings together the two different techniques, PL and tRNS. The results of the combined application of tRNS and PL in mild myopia are discussed in Chapter 5. This technique was further extended to patients with a cortical visual deficit, namely, amblyopia, which is explored in

Chapter 6. The results of these two experiments demonstrate the superior outcome of visual functioning (namely, VA and CS), when existing PL techniques are administered in conjunction with online tRNS.

This thesis provides insight into the understanding and the practical application of random noise stimulation in boosting visual neural plasticity. A series of experiments show for the first time that tRNS of the visual cortex combined with PL is an effective therapeutic approach for the recovery of visual functions in myopia and amblyopia in human adults and contemplates on the underlying mechanisms of action.

CHAPTER 2 Literature Review

2.1 Understanding human visual perception

2.1.1 *From retina to cortex: feedback and feed-forward connectivity*

Seeing is an indispensable, natural notion. Yet how do we process the complex images that our brains receive in order to make sense of our complex surroundings? The following section focuses on the human visual system and the elaboration of visual stimuli in order to bring about visual perception. Visual perception begins from the human eye, weighing approximately 7 grams and measuring around 25mm in diameter (Pugh, 1988). The anterior section of the eye, made up of the cornea, lens and iris, contains the eye's optical system, whereas the posterior section contains its neural structures. The optical features of the eye permit light to enter and reach the retina, the first neural tissue involved in the processing of visual stimuli. The retina is the innermost layer containing light-sensitive photoreceptors and associated neural tissue. Before reaching the retina, light is focalized by the cornea and the lens, pass first through the aqueous humor. Due to the dispersion or reflection of the light by the eye's optical structures, only about half the amount of light penetrates the eye and reaches the photosensitive retinal surface (Ferwerda, 1998). The retina contains photoreceptor cells referred to as rods and cones; rods are highly sensitive to light and thus detect stimuli of low light intensity, giving us the ability to see in scotopic (low) levels of illumination. Cones on the other hand, are less sensitive to light yet have the capacity to distinguish colour and permit vision in high (photopic) light conditions. The central area of retina near the optical axis is called the fovea, and is where vision is at its sharpest. The fovea

corresponds to the center of gaze that we direct toward the objects of our interest. The density of photoreceptors, bipolar cells, and ganglion cells are highest in this area, in order to produce the finest image.

The spatially localized groups of photoreceptors, which serve a particular ganglion cell, make up what is called the cell's receptive field; which are the basic components of higher visual functions. These receptive fields are made up of bipolar circular cells which have ON and OFF regions that respond to light differently depending on their response to glutamate released by photoreceptors (Famiglietti & Kolb, 1976). ON bipolar cells have Gprotein-coupled receptors that respond to glutamate by *hyperpolarizing* the cell. OFF bipolar cells, on the other hand, contain glutamate-gated channels that lead to a classical *depolarizing* excitatory postsynaptic potential (EPSP) following the influx of sodium (Na⁺). Simply put, when light falls onto the ON area of the receptive field (RF), the cell's response increases, whereas when it falls on the OFF region, it decreases. Thus, when this area of retina is stimulated by light, the cell's membrane potential is altered, leading to a specific 'visual code' which is then passed on. Each bipolar cell making up the receptive field is divided into the field's circular center and surround, each responding to light in an opposite manner. For example, if illumination of the center leads to a depolarization of the cell, then illumination of the surround area will cause an opposing hyperpolarization and vice versa. Therefore, it can be said that these cells have antagonistic *center-surround receptive fields*. The spatial properties of receptive fields will be targeted in more detail when discussing cortical visual processing in area V1.

All sensory information must finally reach the cortex to be processed and perceived. The axons of retinal ganglion cells are bundled into optic nerves, which give rise to action potentials, allowing visual information to be distributed to several brain structures that perform different functions. The vast majority of these optic tract fibers terminate on neurons in the lateral geniculate nucleus (LGN), located in the dorsal part of the thalamus. The vast majority of visual information passes through the LGN, which is the first synaptic relay in the pathway that serves visual perception. From there, visual information ascends to the cerebral cortex at the occipital poles, and then to other brain areas through feed-forward and feedback connections (Gilbert & Li, 2013).

Like the retina, the LGN is a laminated structure, with six layers of cells, divided in two main groups: the magnocellular layers (layers 1 and 2) and the parvocellular layers (layers 3 to 6). The former receives inputs from the P_{α} ganglion retinal cells whereas the parvocellular layers receive input from the P_{β} cells (Perry, Oehler, & Cowey, 1984; Leventhal, Rodieck & Dreher 1981). The magnocellular layers are made up of large cells that receive input mainly from the peripheral retina - containing no colour opponent, large receptive fields, leading to low acuity. Cells in these layers are colour insensitive but fast responding and have high temporal resolution, making them useful for visual motion processing. Cells in the parvocellular layers, on the other hand, have small bodies that receive input mainly from the foveal region of the retina where colour opponent cells with small receptive fields are found. These cells are slow responding colour sensitive and useful for resolving fine details - high spatial acuity (Ferwerda, 1998). The receptive fields of LGN neurons are almost

indistinguishable to those of the ganglion cells in the retina.

The LGN's main projection target is the primary visual cortex (V1), also known as the striate cortex and Brodmann area 17. Here, visual information is further refined and processed according to its orientation and spatial frequency; different cells in V1 are sensitive to colour, contrast, shape and even motion. Thus, the rudimentary processing that occurs through the RFs at the LGN is further specialized in the primary visual cortex (Ferwerda, 1998). The striate cortex has a columnar organization: The distinct columns are strongly related to differences in the RFs of the neurons (Callaway, 1998). The different projections of magnocellular and parvocellular cells from the LGN project to layers 2 and 3 of V1, packed into columns of blobs and interblobs. The blobs correspond to clusters of color-selective neurons and project in a specific way to extrastriate areas (Livingstone & Hubel, 1982) and interblob areas (Lund & Booth, 1975). Because they contain cells rich in color selectivity and poor with orientation selectivity, the blobs are specialized to provide information about surfaces rather than edges. In fact, blob cells are wavelength sensitive and monocular and lack orientation and direction selectivity. Neurons in interblob areas on the other hand are binocular, orientation or direction selective. They contain both simple cells and complex cells and are wavelength insensitive (Lu & Roe, 2008).

Beyond this complex inter-connectivity in area V1, referred as parallel processing, which will be further discussed in the following section, exist also connections between other visual areas, resulting in what is known as serial processing. This occurs in the successive connections between cortical areas such as connections running from the back of the brain forward and vice versa (feed-

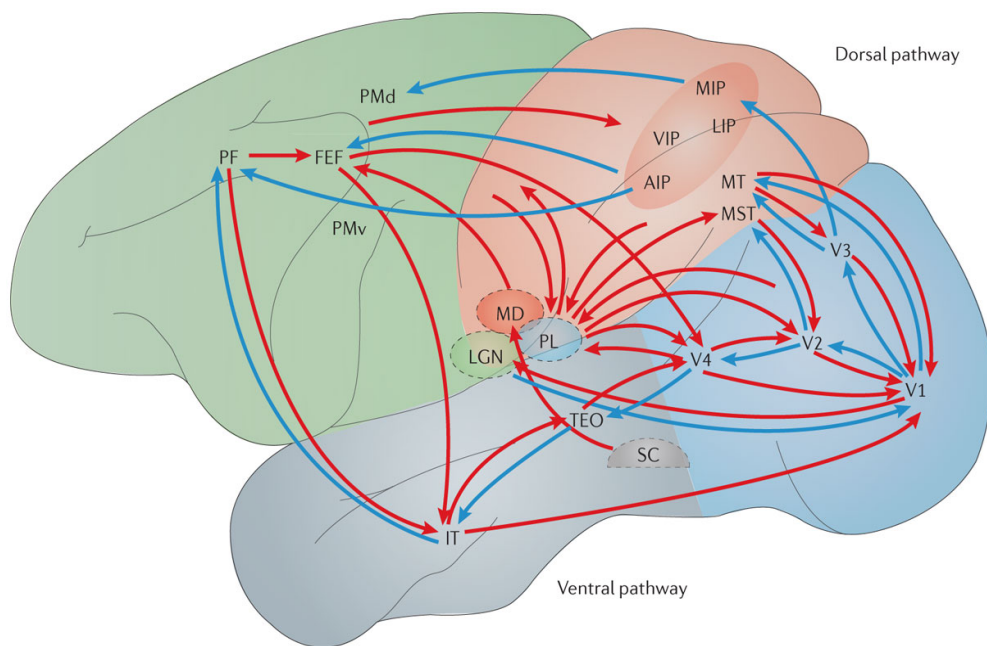
back and feed-forward connections) (Gilbert & Li, 2013). Thus, within the cortex, there is a gradual divergence to successive visual extra striatal areas that lead to the ventral and dorsal streams. The dorsal stream generally serves the analysis of visual motion and the visual control of action. Conversely, the ventral stream is presumed to be involved in the perception of the visual world and the recognition of objects. In general, the dorsal stream seems to be an extension of the V1 magnocellular pathway whereas the ventral stream an extension of V1 parvo-interblob and blob pathways. However, both extrastriate pathways received some input from all parts of V1 (Courtney & Ungerleiger, 1997).

Extrastriate area V2 of the cortex is selective for orientation and also motion direction, albeit a small percentage of neurons in this area (between 8% to 16%) are direction sensitive (Zeki, 1978). Functionally specific cells in V1 communicate with cells of the same specificity in V2. These pathways are not entirely segregated, however, for there is some mixing of information between different visual properties. Area V3 on the other hand is more specialized for motion direction selectivity (at least 40% of cells) (Fellman & Van Essen, 1987). Both these areas relay information to V4, which is considered specialized in colour analysis, as well as orientation and motion perception (Desimone & Schein, 1987). Areas V2 and V3 are well connected to the medio-temporal area (MT) also referred to as area V5, among other motion-sensitive areas such as medial superior temporal area (MST), the superior temporal sulcus (STS) and to the ventral intraparietal area (VIP).

Area MT forms part of the dorsal stream and is responsible for complex motion processing and perception. Neurons in area MT have much larger

receptive fields compared with those in the striate cortex that respond to stimulus movement in a range of directions. It is most notable for the fact that almost all the cells are direction-selective (Dubner & Zeki, 1971), unlike areas earlier in the dorsal stream, or anywhere in the ventral stream. Some studies propose that the direction selectivity characteristics of MT neurons may be inherited from a population of V1 cells which are themselves directionally selective and project to MT. It is still an ongoing investigation as to whether or not neurons in MT are the only protagonists of motion direction interpretation (Movshon & Newsome, 1984; Rodman, Gross & Albright, 1989; Huk, Ress & Heeger, 2001). These motion direction selective neurons are often investigated using the MAE phenomenon. The MAE occurs when prolonged viewing of motion in one direction makes subsequently viewed stationary (or flickering) stimuli appear to move in the opposite direction. This well known illusion is said to be due to a shift in balance of direction-sensitive neurons in area MT, more specifically, an imbalance in the post-adaptation responsiveness of different subpopulations of direction-selective neurons (Hogendoorn & Verstraten, 2013; Anstis, Verstraten & Mather, 1998; Mather, Pavan, Campana & Casco, 2008). Functional MRI studies (Taylor et al., 2000) PET studies (Hautzel et al., 2001), as well as brain stimulation experiments (e.g. Kar & Krekelberg, 2014; Antal, Varga, Nitsche, Chadaide, et al., 2004b; Theoret, Kobayashi, Ganis, Di Capua, et al., 2002) have all implicated a strong role of area MT in the MAE. This phenomenon and its contribution to our understanding of vision motion perception will be discussed in greater detail in Chapter 3.

These inter-cortical projections are thought to provide a means whereby higher centers in a pathway can influence lower ones, although, to date, the role of feed-forward modulation from higher-order areas on early visual processing is still largely unknown. A recent review by Gilbert and Li (2013), has discussed the dynamic and inter-connected nature of the receptive fields in V1, pointing out that early-stage cortical neurons are subject to top-down influences.



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Figure 1: Taken from Gilbert and Li (2013): Figure displaying the dense inter-connectivity between visual cortical areas and frontal higher-order areas. Blue arrows indicate feed-forward connections whereas red arrows indicate feedback connections.

In fact, alongside all feed-forward pathways, exist feedback pathways that project higher-order information to earlier cortical areas. This top-down signal

conveys a rich amount of information that contributes to the interpretation of the visual scene and enables the visual system to build a stable representation of the images it receives (Gilbert & Li, 2013). The importance of these top-down influences on visual perception will be discussed in further detail in the forthcoming chapters.

2.1.2 Structural and functional properties of V1 cortical receptive fields

In this section, particular focus will be given to understanding the characteristics and functional properties of cortical receptive fields, which result in a person's CS and VA.

The RF organization first evident in the retina, is similarly present throughout the visual system (Hubel & Wiesel, 1962, 1968), becoming more and more specialized along the visual pathway. It is assumed that visual neurons in the striate cortex have the so-called classical receptive field (CRF), which is the visual space whereby the presentation of an appropriate stimulus leads to a modification of the neuron's firing rate. Thus, the CRF of any sensory neuron is the spatial domain of visual processing where stimulation either excites or inhibits the neuron. The specificity and distinction of the CRFs are determined by their size and orientation and are consequently sensitive to different spatial frequencies (De Valois, Yund & Hepler, 1982). In other words, different visual information is conveyed at different spatial scales and is thus processed by specific CRFs: i.e. as the spatial frequency of images received increases, the CRF responding to these images becomes smaller. Thus, early neural processing of vision contains largely overlapping CRFs of different sizes (specifically tuned to

different spatial frequencies), which analyze incoming information conveyed at different spatial dimensions/scales.

David Hubel and Torsten Wiesel in 1959 were the first to discover orientation selectivity, the first emergent property that is identified in the CRFs of V1 cortical neurons. Unlike RFs in the LGN, those in V1 are elongated and parallel instead of circular and concentric, but with similar ON- or OFF- center regions flanked on one or both sides by an antagonistic surround. Additionally, cells with similar orientation preferences are grouped into columns and selectively respond to lines of particular orientations. They discovered that many neurons in V1 respond best to an elongated bar of light moving across their receptive fields. However the orientation of the bar was crucial in determining the firing response of the cell. The greatest response was given to a bar with a particular orientation; bars perpendicular to the orientation of the RF overall elicited weaker responses (figure 2) (Hubel & Wiesel, 1959).

Direction selectivity is another unique feature of V1 CRFs. A subset of V1 clustered neurons respond selectively to a particular direction of motion; i.e. they respond when a bar of light (as displayed in figure 2) at the optimal orientation moves perpendicular to the orientation in one direction but not in the opposing direction.

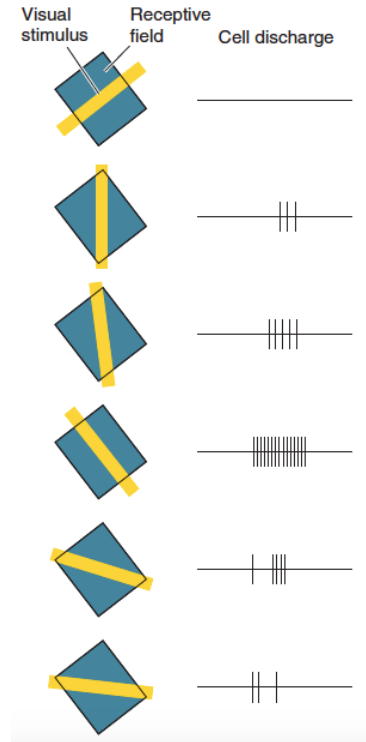


Figure 2: orientation selectivity of V1 neurons: elongated light bars of diverse orientations elicit very different firing responses of the visual neuron in V1. The optimal orientation for this neuron is 45° counterclockwise from vertical (taken from Bear, Connors & Paradiso, 2007).

The cortical CRFs in the striate cortex are divided into simple and complex cells. Hubel and Wiesel identified the simple cells by their separate excitatory and inhibitory regions (the clear segregation of ON and OFF regions); the pattern of summation within the distinct excitatory and inhibitory parts; antagonism between excitatory and inhibitory regions; and finally, the difficulty in predicting their responses to stationary or moving spots of various shapes from a map of the excitatory and inhibitory areas (Hubel & Wiesel, 1962). While simple cells have relatively similar CRF structures, complex CRFs were identified by their diversity: cells with CRFs are absent of clear ON and OFF patterns of responses. Thus, any cortical neuron that did not have a simple cell characteristic was

labeled a complex cell. It is important to point out that Hubel and Wiesel's pioneering work on classifying the CRF's of V1 was conducted on cats' visual cortex using microelectrodes. Nonetheless, diverse models of early vision postulate that V1 CRFs are heavily interconnected both physically and functionally, in a hierarchical, parallel and recurrent pattern (Tao, Shelley, Shapley & McLaughlin, 2001; Martin, 2002; Troyer, Krukowski, Priebe & Miller, 1998; Chance, Nelson & Abbott, 1999).

More recently, renewed interest in the topic has led to new techniques and increasing evidence that challenge the concept of the classic V1 CRF. These studies have pointed out that it may not be the best model for defining the region that can influence the single unit's response. Rather, a more complex relationship exists between the CRFs of V1 cortical neurons. It has been shown that stimuli located outside the CRF of a neuron can influence the response of the given neuron to stimuli located within its CRF (Kapadia, Ito, Gilbert, and Westheimer, 1995). Electrophysiological studies carried out on visual areas of cats and monkeys by Hubel and Wiesel (1958, 1963), gave evidence to support the existence of inhibitory zones surrounding the central area of a CRF, located along the axis for which the unit is selective and on the flanks. These areas are usually defined as "end zones" and "side-bands", respectively and their discovery contributed to the idea of the receptive field, with modulation brought about by its surrounding field. This center surround modulation has been more recently reported in studies using different stimuli (Jones, Grieve, Wang & Sillito, 2001), showing that the area to which the unit is sensitive, comprising both its CRF and the surrounding region that capable of producing modulation, is 2-5 times the

dimension of the field itself (Maffei & Fiorentini, 1976). Furthermore, modulations are found to be stronger for surrounding stimuli that have the same orientation (Knierim & Van Essen, 1992; Levitt & Lund, 1997; Sillito, Grieve, Jones, Cudeiro, & Davis, 1995) and spatial frequency (DeAngelis et al., 1994; Walker et al., 1999) as that of the central stimulus.

This high selectivity and dense cortical lateral interactions among neuron's receptive fields govern the so-called facilitatory or inhibitory nature of a cell's modulation. For example, a stimulus located in the surroundings of the CRF can produce inhibitory modulation for a high-contrast central stimulus, and facilitation when the contrast of the central stimulus is reduced to that unit's threshold (Mizobe, Polat, Pettet & Kasamatsu, 2001; Polat, Mizobe, Pettet, Kasamatsu & Norcia, 1998). Therefore, the center-surround characteristic of V1 units, orientation and direction specific, are intricately connected with units outside their own CRF, creating an interplay of horizontal inhibitory and facilitatory connections and interactions. These long-range connections are mainly located in layers 2 and 3 of the striate cortex and extend from pyramidal cells reaching a length of several millimeters long (Gilbert & Wiesel, 1983; Martin & Whitteridge, 1984; Angelucci, Levitt, Walton, Hupe, Bullier & Lund, 2002; Sincish & Blasdel, 2001). Pyramidal cells tend to connect units with similar orientation selectivity, specifically, cells whose receptive fields are topographically aligned along an axis of collinearity for distances over 700 μm (Schmidt, Goebel, Lowel, & Singer, 1997; Bosking, Zhang, Schofield, & Fitzpatrick, 1997; Chisum, Mooser, & Fitzpatrick, 2003).

Understanding the structure and function of V1's CRFs is imperative when developing psychophysical visual experiments to explore visual perception. In related experiments, sine-wave gratings are frequently used to probe the capabilities of the visual system, such as CS. In these stimuli, the spatial frequency is expressed as the number of cycles per degree of visual angle. These gratings also differ from one another in amplitude or contrast (the magnitude of difference in intensity between light and dark stripes), and orientation.

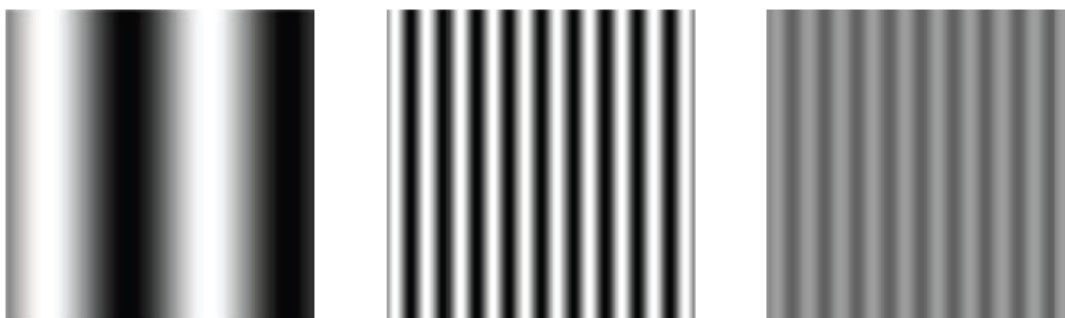


Figure 3: Example of sign wave gratings of different spatial frequencies or contrast.

In such experiments, participants view a display in which the intensity varies about the mean as a sinusoidal function of space (Figure 3). The inverse of the contrast threshold of the grating stimulus, defined as the peak-to-peak amplitude of the sinusoid divided by the sum or the mean (respectively known as Michelson or Weber contrast), plotted against the spatial frequency gives the CSF: a measure of sensitivity of the visual system to different scales/spatial frequencies (Figure 4) (Richman, Spaeth & Wirostko, 2013).

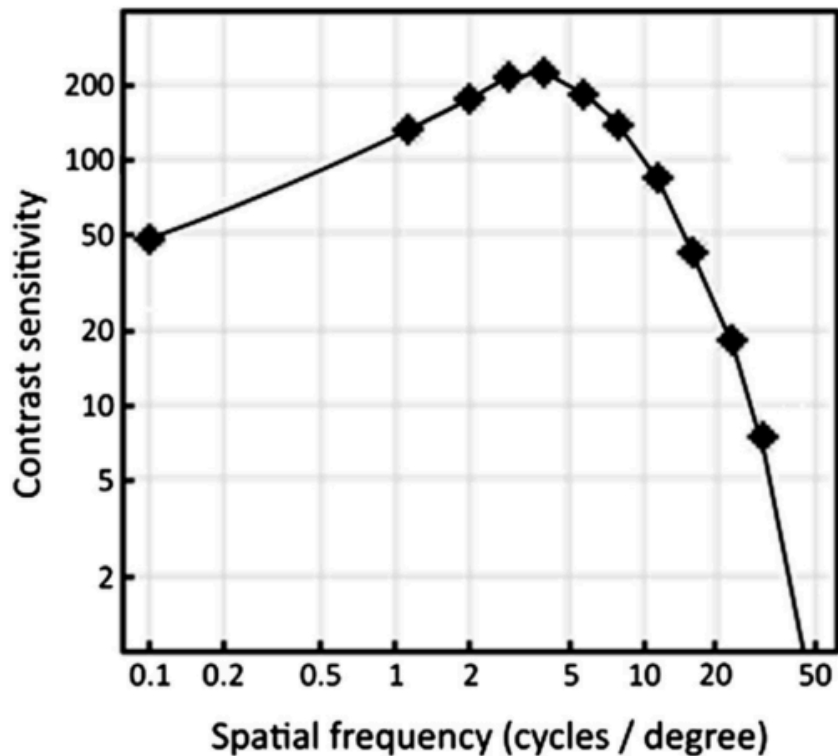


Figure 4: A typical CSF of human participants using gratings of different low to high spatial frequencies. The eye's ability to discriminate between lines of similar contrast is highest at the middle spatial frequencies (3 to 6 cycles per degree [cpd]) and compromised at the low and high frequencies (taken from Richman, Spaeth & Wirostko, 2013).

Simply put, the CS gives an indication on how well people can distinguish between bright and dim parts of an image, and thus the ability to differentiate between different shades of grey, for different sizes of the stimulus. The combined neural response of different cells' RFs, which are specifically tuned for location, orientation and spatial frequency, determine the CSF (Polat, 2009). CS is also a result of the signal to noise ratio (SNR) of this combined neuronal activity. The SNR determines the relationship between the neuronal responses and how well we see (perception) (Geisler & Albrecht, 1997). Our visual performance depends on how efficiently our brain reduces this signal to noise ratio by

averaging out noisy activity of single cells (Polat & Sagi, 1994). In humans, at intermediate spatial frequencies the peak CSF is elicited (e.g. 2-3 cycles/degree visual angle) whereas at the high cut-off spatial frequencies (the smallest grating lines that can be distinguished), VA functioning is represented (Leguire et al., 2011). In sum, the CSF overall captures a broader range of visual functioning, which includes sensitivity to multiple spatial frequencies, the finest of these defines VA.

CS is typically measured psychophysically with contrast detection experiments. An example of these experiments is demonstrated in Polat and Sagi's pioneering work (Polat & Sagi, 1993). They implemented a threshold detection task with lateral masking displays in order to investigate spatial interactions between visual channels selective for spatial locations (Figure 5). Presenting a Gabor patch (a sinusoidal grating in a gaussian envelope) in the fovea, flanked by two high-contrast Gabor patches (masks/flankers) located at the same lateral distance respect to the target, the authors measured contrast thresholds for different target-to-flankers distances. This made it possible to investigate the interactions between neighboring channels. Gabor stimuli are often used in psychophysical experiments because they optimally stimulate the receptive field of simple cells in the primary visual cortex. Their results indicated two regions in which contrast thresholds were modulated, one inhibitory and one excitatory, along the target-to-masks separations, indicated as λ (the wavelength of the Gabor stimulus). Up to a distance of 1.5λ of separation the interaction resulted in higher contrast threshold, indicating an inhibitory interaction between cells. Starting at $1.5-2 \lambda$ there is an area of threshold

reduction, reaching a maximal elevation at 3λ and then smoothly coming back to the normal threshold (absence of interaction) around 12λ (Polat & Sagi, 1993).

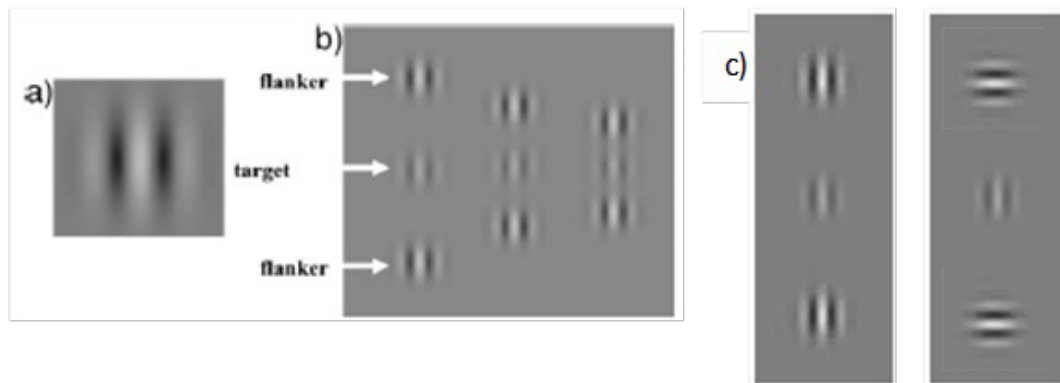


Figure 5: An example of the Gabor stimuli used by Polat and Sagi (1993) to investigate lateral interactions which are only observed when collinear, iso-oriented flankers are used. a) a single Gabor patch; b) a typical configuration with target and flankers, located at different target-flanker separations. c) collinear and orthogonal target-flanker conditions.

It is also well established that repeated practice on these tasks results in an improvement of CS at the trained as well as a transfer on to untrained spatial frequencies and VA (for a detailed review see Sagi, 2011). This is known as PL and will be discussed in great detail in section 2.3.

2.1.3 Cortical and optical deficits of visual perception: deficits in visual acuity and contrast sensitivity.

Whether something is altered physically in the eye, or disrupted at some point along the complex and intricate visual pathways, the resulting outcome is a compromised visual system, usually reflected by a decrement in VA and CS. VA is another measure, like CS, which defines our ability to see, and is actually the

most common clinical measurement of visual functioning. VA is the standardized recording of a person's ability to distinguish a black symbol on a white background, from a fixed distance, while modifying the size of the symbol to alter the level of difficulty, by doing so determining the smallest letters (or line gratings) that can be identified by the individual. A standard way of measuring VA is through vision charts such as the Landolt C. It consists of a C-shaped ring containing a gap, this gap can be at eight different positions (left, right, bottom, top and the 45° positions in between) and the task of the participant is to decipher where the gap is, sitting at a certain fixed distance from the chart. The size of the C and its gap are reduced until the subject makes a specified rate of errors. The minimum perceivable angle of the gap is taken as measure of the VA (see figure 6).

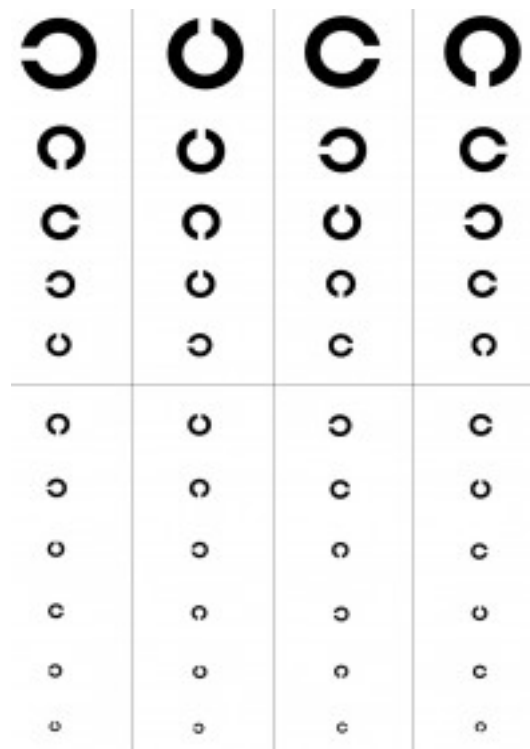


Figure 6: An example of a Landolt C chart used to measure VA. The figure shows different angles of resolution and different orientations.

When disruptions in visual perception occurs at the ocular level, altering the shape of the cornea or its distance from the retina, refractive defects as in the case of myopia or hypermetropia, come about. Alternatively, when there is a deficit or under-development somewhere along the cortical pathway, a condition known as amblyopia occurs. Myopia, also known as shortsightedness, is the state of refraction in which parallel rays of light are focused in front of the retina of a resting eye (Curtin, 1985). It is an optical defect in emmetropization and is not considered a developmental difficulty, unlike amblyopia. The neuronal connectivity has developed normally in childhood and is capable of processing images efficiently; however, the visual input is limited by an optical de-focus. In individuals with Myopia, visibility of high spatial frequencies is perceived as low contrast even when their physical contrast is high, resulting in degraded vision (Tan & Fong, 2008). Thus, a resulting decrement in VA and CS that is not cortically based, are the typical features of myopia.

Among other side effects such as a difficulty in binocular vision, know as stereopsis, amblyopia is another condition resulting in a compromised VA and CS. Amblyopia is a developmental abnormality resulting from the abnormal binocular visual experience during the “sensitive period” early in life. Despite some early disagreements (Ikeda & Wright, 1974) and the existing evidence for the involvement of subcortical visual centers (Hess, Thompson, Gole, & Mullen, 2009, 2010), the primary “dysfunctional” site of amblyopia is thought to be located within the primary visual cortex (Wiesel & Hubel, 1963). Amblyopia originates in the early phases of postnatal visual development and derives from an abnormal visual input during this period. It is characterised by an imbalance

between the excitation and inhibition within the primary visual cortex whereby the neuronal population driven by the amblyopic eye is chronically suppressed by the neurons responding to the fellow fixing eye, possibly via GABAergic inhibitory circuits (Farivar, Thompson, Mansouri, & Hess, 2011; Li et al., 2011; Sengpiel & Blakemore, 1996; Hess, Mansouri & Thompson, 2010). The main causes of amblyopia include an imbalance in refractive error between the two eyes (anisometropia), eye axes misalignment (strabismus), or a physical obstruction in the optical system of the eye such as cataracts (form deprivation). Therefore, depending on the etiology of the condition, three main types of amblyopia can be distinguished: refractive (anisometropic) amblyopia, strabismic amblyopia, and form-deprivation amblyopia.

As a result of an abnormal pattern of functional connectivity of the visual cortex, impaired CS is one of the basic characteristics of amblyopia. In general, the amblyopic CSF is characterised by a shift of the cut-off (the highest visible spatial frequency) towards lower ones (Levi & Harwerth, 1977; Thomas, 1978; Volkens, Hagemans, Vanderwildt, & Schmitz, 1987), or band-specific increases in threshold CS (Bradley & Freeman, 1981; Campos, Prampolini, & Gulli, 1984; Hess & Howell, 1977; Levi & Harwerth, 1977). These CSF deficits have been attributed more so to the severity of amblyopia, rather than the cause. Whereas in relatively mild amblyopia high spatial frequency deficits were observed, severe amblyopia has been associated with an overall decrease in the CSF (Bradley & Freeman, 1981; Campos, et al., 1984; Thomas, 1978). The loss of vision in amblyopia is said to result from abnormal interactions of the neuronal network within the primary visual cortex, particularly of orientation-selective neurons (Polat et al., 2004). In

fact, both psychophysical (Polat, Sagi, & Norcia, 1997) and electrophysiological studies (Levi & Manny, 1980) report abnormal interactions in amblyopic patients, with an increased range of inhibition and reduced facilitation at certain distances (4λ) compared to healthy controls. This compromised lateral inhibition in amblyopia patients leads to a great increase in visual crowding in the central visual, which is pronounced in normal peripheral vision and is also present in the central field of strabismic amblyopes (Bonneh, Sagi, & Polat, 2004; Elliott & Firth, 2009; Levi & Klein, 1982). Crowding occurs when the distance between nearby objects is too small, leading to impaired object or stimulus identification (Chung, Li & Levi, 2012), in normal sighted individuals, crowding is more evident in the periphery (Doron, Spierer & Polat, 2015; Lev & Polat, 2015). The underlying mechanism of crowding remains unclear, although, it is said to be cortical in nature (for a review see Levi, 2008) and likely due to lateral inhibition (Levi, Hariharan, & Klein, 2002). A study demonstrating the effects of crowding on VA in the amblyopic, and healthy eye both in central vision and in the periphery show that flanks for grating and Vernier acuity similarly affected anisometric amblyopic eyes, whereas in strabismic eyes, flanking had a more pronounced effect on Vernier acuity (Levi & Klein, 1985). Interestingly, the fellow fixing eyes of strabismic amblyopes showed a larger spatial interference in Vernier acuity relative to controls and the fellow fixing eyes of the anisometric amblyopes. These findings support the idea of “central deficits” within the strabismic visual cortex, which affect visual performance in both eyes.

Attempts to improve VA and quality of vision in general have led to advances in visual outcome assessments, imaging as well as surgical techniques. However, even if the perfect method to correct the optics of the eye did exist, our vision would still be ultimately determined by the retina-brain collaboration. With regards to current available interventions to improve VA and CS in myopia, the most commonly implemented techniques are invasive such as refractive surgery (i.e. surgery that corrects refractive errors such as myopia, astigmatism, and hyperopia). However, in the case of amblyopia, despite any effort many patients make in undertaking refractive surgery so as to correct any existing ocular error, due to the underlying cortical deficits, individuals with amblyopia remain with an overall reduced visual perception and a somewhat 'permanent' reduction of VA and CS (Paciuc, 2005). This has raised many concerns and interest over the years and has inspired many scientists to come up with non-invasive methods to target the cortical deficits in amblyopia. Recently, these non-invasive behavioural methods have also been applied to myopia, in the hope of enhancing the feedback connections in order to compensate for the blurred image received thanks to the optical defocus of the eye (Durrrie & Mc Minn, 2007; Tan & Fong, 2008; Camilleri et al., 2014a). This method has been termed perceptual learning (PL) and will be the main focus of the following two sections.

2.2 Visual perceptual learning

2.2.1 Perceptual learning and cortical plasticity

Brain plasticity is not a stranger to the field of neuroscience. As early as the 1890s it has been referred to as “the possession of a structure weak enough to yield to an influence, but strong enough not to yield all at once” (William James, 1890). The father of Psychology William James, also stated that “our nervous system grows to the modes in which it has been exercised”. This is the foundation of PL whereby exercising the brain makes it possible for an individual to improve his sensory functions. This section aims to encapsulate the literature that has thus far been carried out with regards to the understanding and improvement of visual functioning through PL.

Recanzone and colleagues were one of the first who demonstrated that improvement on a tactile frequency discrimination task following practice was correlated with the extent of expansion of the cortical map that represented the trained skin area (Recanzone, Merzenich, Jenkins, Grajski & Dinse, 1992). This study provided the first indication of low-level cortical processing in PL. Since then, the depth of adult plasticity has been observed in all sensory systems, including those responsible for early visual processing. PL is a form of implicit learning, where encoding and retrieval do not require conscious awareness. It is the unconscious acquisition of improved visual ability through practice of a simple discrimination and detection task (Durrie & McMinn, 2007). The visual system allows for this process to occur due to its highly plastic nature and thus its ability to adapt itself and respond to changes in the environment, which is a vital requirement. As described earlier, human vision is composed of a

hierarchically refined processing system. It starts from photoreceptors in the retina and extends through several stages of spatial integration in the cortex, each forming receptive fields of increasing complexity resulting in more refined sensory discrimination. Cortical mechanisms of PL clearly outline the importance of the early stages of stimulus processing, which occur mainly in area V1. The learning of more complex stimulus features requires a mechanism that is context dependent and likely involves higher order cortical areas which are responsible for processing these complex features (Gilbert, Sigman & Crist 2001). For example object recognition is thought to be encoded in later cortical areas, namely the inferotemporal cortex (IT) (Tanaka & Taylor, 1991).

A task involving repeated exposure to the same visual stimulus and training conditions leads to an improved performance on the task. Discussions have focused on whether PL is specific for the trained task (Ahissar, Laiwand, Kozminsky & Hochstein, 1998) or whether it can transfer on to other tasks and different stimulus attributes. Numerous studies give evidence for the specificity of PL; it is understood to be specific to the trained visual field (Fahle, Edelman & Poggio, 1995; Fiorentini & Berardi, 1981), to stimulus attributes such as orientation and spatial frequency (Ramachandran & Braddick, 1973; Sigman & Gilbert, 2000), and also to the trained eye (Karni & Sagi, 1991 & Fahle, 2005). For example, in one of the first studies on PL, Fiorentini and Berardi (1981) investigated the effects of training on the discrimination of briefly flashed gratings. In all tasks involving discrimination of complex luminance gratings they reported that a percentage of correct responses increases progressively with repetition of the task up to 100–200 trials and then levels off, showing long term

effects up to days or even weeks after the training. Additionally, they found that improvement in the task is specific to the spatial frequency trained and to the orientation of the grating. Another study which measured regional cerebral blood flow using 3D Positron Emission Tomography (PET) before and after training on a visual orientation discrimination task, demonstrated that following training there was a decrease in brain activity in the striate and extrastriate visual cortex, more specifically in the right calcarine sulcus, the left lingual gyrus, the left middle occipital, and the right inferior occipital gyrus (Schiltz, Bodart, Dubois, Dejardin, et al., 1999). Their findings also support the hypothesis that in adult humans, learning induced changes might occur at early levels of visual processing (Schiltz et al., 1999). In fact, considering that at early cortical sites exist neurons with receptive fields functionally specialised for simple stimulus parameters like orientation and spatial frequency (Van Essen, Anderson & Felleman, 1992), these findings have been taken as evidence that learning begins at, but is not necessarily confined to, the earlier stages of visual processing, such as in area V1.

Nonetheless there has been continuous debate on the neural mechanisms and locations involved in PL, whether or not learning is always as specific as is being portrayed and finally, whether the modest changes seen in early visual neurons are sufficient to account for the large behavioral improvements observed in psychophysical experiments. Numerous studies have demonstrated a total transfer of learning from one trained location to another and one orientation to another (Schoups, Vogels & Orban, 1995, Liu & Weinshall, 2000; Xiao, Zhang, Wang, Klein, et al., 2010; McGovern, Webb & Peirce, 2012; Camilleri et al., 2014a).

It has been proposed that the degree of transfer depends on the difficulty of the task as well as the task-relevant stimulus attributes (Ahissar & Hochstein, 1997). In their *Nature* article, Ahissar and co-worker explain how learning begins with the practice of easy conditions, which subsequently guide the learning of hard ones. Under easy, characteristically simple conditions, learning is able to generalise across orientations and even retinal location, reflecting the spatial generalization and simple feature-invariance of higher visual areas. Whereas, under conditions of elevated task difficulty, learning tends to be more specific with respect to both orientation and position, reflecting the fine spatial retinotopy and simple feature-dependency of lower areas. Taken together, improvement begins at higher generalizing levels, which, in turn, direct harder-condition learning to the subdomain of their lower-level inputs. Moreover, Jeter and coworkers gave evidence for task precision, rather than task difficulty, as determining the level of transfer in PL (Jeter, Doshier, Petrov, & Lu, 2009). Specifically, they demonstrated that training conditions for high (and not low) precision transfer tasks account for the improvements seen. Other studies have shown that also the length/duration of training has an impact on the degree of generalization. For example, in visual PL, transfer is more likely to occur following just a few training sessions (Jeter, Doshier, Liu, & Lu, 2010) and following a few trials (Aberg, Tartaglia, & Herzog, 2009). It has been proposed that this pattern of transfer may come about through the mechanisms of long-term potentiation (Aberg & Herzog, 2012). McGovern and colleagues conclude that for PL to transfer from one trained task to another there should be an overlap, in part, of their underlying neural processing, and finally, the trainings'

complexity/ task difficulty level is an important contributing factor (McGovern et al., 2012). Keeping in mind the conditions and circumstances under which visual PL is transferable has significant implications for setting up correct training protocols and stimulus parameters.

Another goal of PL, like any learning quest, is to make the task at hand increasingly automatic and effortless in order that the higher cortical functions (top-down control) will be released from the task. Liberating this higher-level control of neural activity is the essence of plasticity and thus improved functioning. This is why repeated, effort-full practice on a visual task leads not only to an improvement of the task at hand but also to an automatization of that task. Top down influences such as selective attention on improving visual functioning is currently well recognised as being part and parcel of the complex visual PL process (Gilbert & Li, 2013; Freeman, Driver, Sagi & Zhaoping, 2003; Ito, Westheimer & Gilbert, 1998). Psychophysical studies point out the importance of selective attention on both perception as a whole and on PL (Crist, Li & Gilbert, 2001; Shiu & Pashler, 1992; Ahissar & Hochstein, 1993). A participant's ability to attend to discreet changes of specific stimulus attributes used in the task, determines his performance and thus his progress on the task. Attention is also essential for feedback mechanisms that support neural plasticity. Strong effects of feedback have been reported, resulting in faster and more extensive improvement in performance (Herzog & Fahle, 1997). Consistent feedback is also necessary to augment and maintain participant's motivation on the task and thus lead to better training. Moreover, while attention itself is subject to improving and PL can be enhanced through top down influences, PL

ultimately leads to a reduction of attentional control from the task at hand and results in, to some extent, an automatization of the perceptual task, due to cortical plasticity (Sigman & Gilbert, 2000). Thus for optimum results, PL should be carried out consistently, in a task specific fashion and under top down attentional control.

A recent review highlights the complexity of PL and the cortical demands of different perceptual tasks (Gilbert & Li, 2013). This paper drives us away from the classic bottom-up approach of PL and goes a step further by pointing out that early cortical processes are subject to top-down influences. Top-down control is often associated with spatial attention, often characterized in terms of gain control, which is the enhancement of neural responses, as well as the suppression of responses external to the focus of attention (Motter, 1993; Chen et al., 2008). Spatial attention allows us to select task-relevant stimuli and to analyse specific parts of the visual field. In fact, in their review Gilbert and colleague point out how the most notable effects of top-down influences are exercised on contextual characteristics, those same characteristics that are said to be processed primarily in early visual areas. The goal of these top-down influences is to modify receptive field properties and play a part in the selection of information carried by neurons. As a consequence, vision, and PL, can be thought of as an active, dynamic process, requiring expectation or hypothesis testing in order to evaluate and interpret the visual scene. These findings imply that PL extends beyond the early visual cortices and points towards a complex inter-connectivity of the visual system and the crucial role for higher visual cortical areas that occupy top down control on PL.

2.2.2 *Perceptual learning in the context of neuronal lateral interactions*

PL has been extensively studied in a lateral masking context, as described earlier (Polat & Sagi, 1993, 1994). Adini and colleagues (Adini, Sagi & Tsodyks, 2002; Adini, Wilkonsky, Haspel, Tsodyks & Sagi, 2004) found that contrast discrimination of a Gabor stimulus can improve with practice only if flanked by pairs of similar high contrast Gabor stimuli. The mechanism underlying improvement of contrast detection with flankers has been attributed to an increase of the range of facilitation between collinear elements resulting from a cascade of local connections between detectors based on Hebbian synaptic mechanisms (Polat 1999; Polat & Sagi 1994). In the 1994 study, Polat and his colleague demonstrated that PL, using a lateral masking paradigm, results in an increase in the cortical spatial range of lateral interactions. In this experiment, participants were trained to detect a Gabor target that was flanked by two high-contrast Gabor masks, where the distance (λ) of the flankers to the target varied along the time course of the training. The interaction range before and after extensive training (40 sessions) on a threshold detection task was measured. A two-alternative temporal forced-choice paradigm was implemented in this study where each trial contained two stimuli presented successively, only one of which presented the target Gabor stimulus and both containing the flanked stimuli. The participants' role was to identify which of the two stimuli presented contained the target stimulus. They measured the interaction range before and after extensive training on a threshold detection task. Results showed that the target threshold was facilitated by flanker presence at distances up to six times the target period. However, practice had the effect of increasing the facilitation

range by at least a factor of three. Thus they demonstrated that PL, using a lateral masking paradigm results in an increase in the cortical spatial range of lateral interactions (Polat & Sagi, 1994a; 1994b). More specifically, these longer-range facilitatory interactions were mainly found for target and flankers arranged in a collinear fashion, i.e. in the direction defined by the target Gabor's orientation: training with diagonally oriented stimuli and flanker presented in horizontal configurations did not show any learning effect. Furthermore, in 1997, Adini and colleagues applied a PL paradigm on a lateral masking configuration in which the target was surrounded by multiple flankers located at 2λ . They found a decrease in the range and efficacy of these inhibitory modulations (Adini, Sagi and Tsodyks, 1997). Interestingly, practice decreased lateral inhibition and moreover increased facilitation of target detection by neighboring flankers, possibly as a result of reduced inhibitory modulations from flankers to target.

One study has gone a step further and identified the neural underpinnings of neuronal lateral interactions by recording Event Related Potentials (ERPs) together with psychophysical measures for targets flanked by collinear or orthogonal gratings in a perifoveal contrast discrimination task (Khoe, Freeman, Woldorff & Mangun, 2004). The behavioral measures in this study showed that performance improved in the context of collinear versus orthogonal flankers. Taking a look at the event related potential (ERP) data, a short-latency difference in polarity (increased positivity) was observed between 90 and 140ms at the occipital-midline electrodes, for central targets with collinear (but not with orthogonal) flanker configurations. Longer-latency differences (between 245 and 295ms and 300 and 350ms) were observed at lateral occipital sites (consistent

with activity in extrastriate visual cortex). These ERP effects were correlated with improved contrast discrimination for central targets presented with collinear flanks. These results indicating a preference towards collinearly aligned flankers have also been demonstrated in other studies using single-unit recordings, which show that the neuronal response to a central stimulus in V1 is enhanced by the presence of collinear flankers positioned outside the cell's CRF (Chen, Kasamatsu, Polat & Norcia, 2001; Polat, Mizobe, Pettet, Kasamatsu & Norcia, 1998).

2.2.3 The application of perceptual learning in the improvement of visual functions

Our ability to see contours and distinguish objects is dependent upon the integrity of the eyes in effectively processing light resulting in clear images. It is also dependent upon efficient neural processes responsible for processing and integrating neural information. Numerous studies have investigated the effects of PL on VA and CS in individuals with amblyopia (Polat, Ma-Naim, Belkin & Sagi, 2004; Huang, Zhou & Lu, 2008) and also a few in those with refractive defects (Camilleri et al., 2014a; Polat et al., 2009, 2013; Tan & Fong, 2008; Durrie & McMinn, 2007). Nowadays, following much work on PL in healthy participants, the protocols have been refined and developed in to a more controlled, participant-specific training, which leads to positive long-term outcomes in VA and CS.

Different PL paradigms have been implemented on adults with amblyopia that have provided insight into the underlying neural processes of improving CS.

Studies have provided evidence for improved visual functioning through the induction of LTP following PL of a Vernier acuity (hyperacuity) task (Levi & Polat, 1996; Levi, Polat & Hu, 1997). These controlled studies have shown that repetitive practice leads to significant improvement in the trained task in the amblyopic eye. Their task consisted of a standard Vernier acuity paradigm where two dark, short lines were presented one above the other with an offset between the two lines (4 arc min long and 0.9 arc min wide, at the viewing distance of 4m). The participants had to determine the relative position of the upper line with respect to the lower one (whether it is appearing to the left or to the right). The results of these studies have shown that PL on Vernier tasks improved not only vernier performance, but also improvement in (standard) VA, in the amblyopic eye, suggesting cortical plasticity in adults with amblyopia (Levi et al., 1997). In addition, Levi and Polat (1996) found that improvement in performance on a visual task was both orientation and task specific; this result may reflect that training is targeting specific orientation tuned neurons (Saarinen & Levi, 1995). Their results also indicated a partial transfer of learning to the untrained non-amblyopic eye and this transfer was significant for the trained orientation but not for the untrained orientation (Levi & Polat, 1996). Inter-ocular transfer following PL in patients with amblyopia, is compatible with learning occurring at an early stage of cortical processing, possibly at the striate cortex or beyond where binocular interactions are reported (Horton, 2006; Hubel & Wiesel, 1968).

Other more recent studies have tried to determine the relationship between VA and improvements in contrast detection/CS. These studies have also reported visual plasticity in adults with amblyopia (Chung, Li & Levi, 2006;

Fronius, Cirina, Kuhli, Cordey, & Ohrloff, 2006; Levi, 2005; Li & Levi, 2004; Polat et al., 2004, Zhou, Huang, Xu, Tao, Qiu, Li et al., 2006). Some of the aforementioned studies along with others have reported transfer between training on contrast detection and improvement in VA tasks (e.g. Polat, 2008; Zhou et al., 2006). In the latter study, 23 adult anisometric amblyopes were recruited and divided into three groups, each receiving different training regimens. Group I trained on grating detection in the amblyopic eye near each individual's pre-training cut-off spatial frequency (the spatial frequency where contrast detection threshold was equal to 0.5 Michelson contrast), group II received training of repeated CSF measurements (so at varying spatial frequencies) in the amblyopic eye and group III did not receive any training. The training condition of the CSF was carried out using single Gabors at the centre of the screen which were viewed monocularly in the fovea at a distance of 2.28 m in a dimly lit room. Results of this study indicated that training improved VA and CSF in the amblyopic eyes of all the participants in groups I and II (the largest improvements were seen in group I), whereas no significant improvement in performance was observed in group III. Long-term retention of improved VA was observed in a few of the cases tested for up to 1 year post training. This was another valuable study that indicated that the adult amblyopic brain might still be capable of plasticity and recovery of function.

Another study by Polat and colleagues, investigated the effects of a two alternative forced choice lateral masking paradigm on the CSF of participants with amblyopia (Polat et al., 2004). The stimuli used for the training were local gray-level gratings (Gabor stimulus) with spatial frequencies of 1.5–12 cycles per

degree (cpd). During the sessions, the spatial frequency and orientation of the stimuli were changed, starting the training with lower spatial frequencies and moving progressively to the higher ones, using four orientations at each spatial frequency. The sessions were designed on an individual basis by using an automated computerized decision-maker algorithm. Each training session was made up of 10–15 blocks (with a total of ~28 sessions) with different target-flanker separations, whilst the spatial frequency and orientation were kept constant. A second control group underwent a similar Gabor stimulus contrast detection training in the absence of flankers and their starting spatial frequency was lower than that which was used for the first group (therefore these participants always achieved a perfect performance). In this control group, the attributes of the stimuli remained unchanged between and within the training sessions. The results of this study give evidence for poor facilitation in amblyopic patients and an increased range of lateral inhibition in the higher spatial frequencies compared to normal participants. This inhibitory effect is a reflection of the well-known crowding phenomenon typical of amblyopia (Levi & Klein, 1985; Hussain et al., 2012). The results of the second, control group (using lower spatial frequencies) demonstrated a close-to-normal facilitation, which is in line with the well-known normal vision of amblyopic individuals with stimuli of low spatial-frequencies (Ciuffreda et al., 1991). Training results for the treatment group demonstrate a significant improvement in CS at all spatial frequencies, with the high spatial-frequency range improving to reach normal values. Furthermore, the lateral-inhibitory effects demonstrated at baseline were significantly reduced following the training. This practice-induced reduction of

cortical inhibition was linked to the improvement in VA. Thus, lower level training of the visual cortex of adults with amblyopia using lateral masking techniques result in large improvements in VA and CS which are maintained over time (Polat et al., 2004). This and other studies using lateral-masking paradigms point to plasticity of spatial interactions in adults following repetitive training on a target-flanker task.

Thus, it has been well established that CS outcome at low levels can be increased by a factor of 2 after controlling for the Gabor stimulus parameters during training, in healthy adults (Adini, Wilkonsky, Haspel, Tsodyks & Sagi, 2004) as well as those with amblyopia (Huang et al., 2008; Chung et al., 2006; Polat et al., 2004). The neural underpinnings of this improvement have been explained by an increase in the range of excitatory interactions (Polat & Sagi, 1994) and a reduction in the short-range inhibition (Polat et al., 2004; Zenger & Sagi, 1996). Lateral masking training experiments postulate that learning induces an increase in the spatial range of lateral interactions (Polat & Sagi, 1994). Despite positive outcomes on CS and VA using single Gabor PL paradigms, PL under the conditions of lateral masking, (as highlighted in the review by Levi & Li, 2009), seems to be the most effective procedure.

In comparison to the exhaustive studies carried out on PL in healthy participants and patients with amblyopia, studies on PL with refractive defects are fewer and far between. A widespread technique already mentioned which is used in PL is lateral masking. These techniques have also been applied to refractive defects such as myopia and presbyopia (Polat, Schor, Tong, Zomet, et al., 2012; Durrie & McMinn, 2007; Tan & Fong, 2008). In a prospective non-

controlled clinical study by Tan and Fong (2008), 20 adults with low myopia (within the range of -0.5 diopter (D) to -1.5 D in the worst eye and with astigmatism not exceeding 0.5 D in either eye) were recruited to evaluate the efficacy and safety of PL with lateral masking in improving uncorrected visual acuity (UCVA) and uncorrected contrast sensitivity (UCCS). Training sessions involved the detection of a low-contrast central Gabor stimulus flanked by two high-contrast Gabors, with a two interval forced choice (2IFC) task. Spatial arrangement, global and local orientation, target-flankers separation, exposure time and spatial frequency were all varied between blocks. Training sessions lasted for approximately 30 minutes and were carried out 2 to 3 times per week. Following every 5 training sessions, the UCVA and UCCS were tested to monitor the subject's progress. The number of training sessions were subject specific and continued until no further visual improvement was observed. Following training, the participants carried out a post training evaluation to establish the extent of UCVA and UCCS improvement. In this study the maximum improvement was reported to be subject dependent, typically achieved in approximately 20 to 30 sessions over a course of 3 months. Results indicated that following training, the mean UCVA had improved to a value of 0.08 logMAR (95% CI, 0.12-0.40), leading to an overall mean improvement of 2.1 logMAR lines. The eyes with worse UCVA at baseline had greater improvement than eyes with better baseline UCVA. Furthermore, sixteen of the participants trained carried out a 6 month follow up, it was shown that ninety percent of the visual improvement in UCVA was maintained from 0.30 logMAR before treatment to 0.08 logMAR immediately after treatment and 0.10 logMAR after 6 months (Tan

& Fong, 2008). This clinically significant improvement in VA was also corroborated with a significant improvement in mean CSF post-treatment over a range of spatial frequencies, this improvement was maintained at a 12 month follow up visit.

Durrie and his colleague carried out a similar study in 2007 investigating the effectiveness of NVT on a group of individuals with refractive defects (mild myopia and presbyopia). In their study, the improvement in UCVA and unaided CSF among the low myopic and early presbyopia groups was found to be significant. They reported a mean improvement of 2.2 logMar lines in distance UCVA for patients with low myopia and 2.2 logMar lines in unaided near VA for those with early presbyopia (Durrie & McMinn, 2007). Another study investigating the effects of a lateral masking PL paradigm on individuals with presbyopia demonstrated that training under certain conditions can improve VA and CS, and in some cases, result in performance levels similar to the younger-aged control group (Polat et al., 2012). Moreover, their results showed that training improved supra-threshold contrast discrimination and reading speed for small letters. Having controlled for ocular characteristics before and after training, this study was the first to establish that the improvements found are not the result of improved optical performance of the eye (accommodation, pupil size or depth of focus) (Polat et al., 2012).

Another very recent study that sought to understand whether or not, through PL, the brain is able to compensate for the refractive defocus, is the work by Yan and colleagues (2015). 23 myopic participants were either trained monocularly in the non-dominant eye, on a two-interval forced choice single

Gabor grating detection task near their individual cut-off spatial frequencies (experimental group) or, in the case of the control group, repeated VA and CSF measurements were taken, separated by about 10 days. Results demonstrate that the monocular training resulted in significantly improved CS (by 3.6 dB) and VA (by 5.1 dB) in the trained eye, as well as a 2.3 dB and 4.0 dB improvement in the untrained eye. These improvements were seen for a wide range of spatial frequencies and not just for the trained spatial frequency. For the control group, there was no significant change in CS or VA in neither the non- dominant or dominant eye. The authors attribute these improvements to a neural origin, since no changes in optical characteristics were reported, this is in line with the earlier study on PL and presbyopia (Polat et al., 2012). These findings support growing evidence that neural plasticity is retained in the adult brain and can compensate for ocular visual deficiencies. As with all learning situations, the outcome will vary according to the time and effort invested by the participant as well as according to the intrinsic limits of each individual. Thus, the final outcome of the training is influenced by a number of stimuli characteristics, as well as the variability of the individuals' effort and motivation.

Although behavioural techniques such as PL are constantly being refined and improved to cater for each individuals' needs, the use of a complementary technique known to induce neuroplasticity is on the rise. The following section will focus on such technique, known as Transcranial Electrical Stimulation (TES), with a specific focus on tRNS.

2.3 Non-invasive electrical brain stimulation and visual neural plasticity

Brain electrical stimulation is not a new concept to science and from as early as 1755 Charles Le Roy attempted to treat blindness by stimulating the optic nerve and visual cortex through the invasive administration of electrical signals (Antal, Paulus & Nitsche, 2011). However, despite several attempts, the patient's blindness remained untreated. The use of implanted electrodes to treat neurological defects was gradually replaced with non-invasive stimulation techniques such as TES developed by Merton and Morton in 1980. They demonstrated that electrical stimulation over the occipital cortex of an intact skull resulted in phosphene perception. The limitations and dangers of the earlier techniques led to the development of more refined techniques such as non-invasive Transcranial Magnetic Stimulation (TMS) and TES. This section will focus on TES applications in the context of vision.



Figure 7: Non-invasive brain electrical stimulation device.

Recent advancements in the field have highlighted the potential benefits of non-invasive brain stimulation (NIBS) techniques. These techniques allow for external modulation of neural activation and inhibition in the human brain. TES is non invasive and if used correctly should not lead to any aversive effects, it is relatively cheap and can be implemented in various contexts as an adjunct to existing techniques, which although are effective in isolation, might not be offering the most optimal treatment to patients. Recent advancements in brain imaging and brain stimulation tools have brought along developments in the clinical application of these tools extending beyond diagnostic means. The application of TES as a potential tool in neuro-rehabilitation is a relatively young concept. Yet many studies are seeking to understand the mechanisms by which different TES techniques can complement an existing behavioural training (e.g. Yun, Chun & Kim, 2015, Dhaliwal, Meek & Modirrousta, 2015; Krause & Kadosh, 2013). In general, TES can be delivered as direct current (tDCS), alternating current (tACS), or random noise (tRNS) at low or high frequencies.

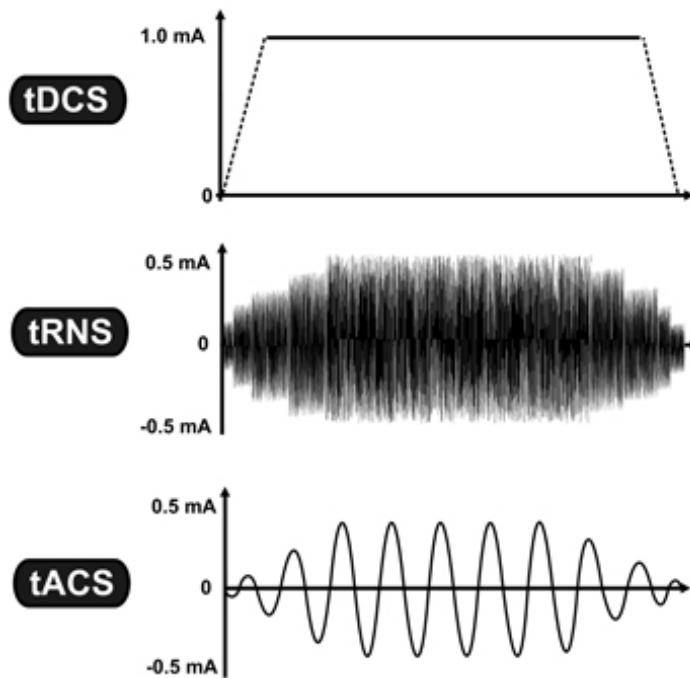


Figure 8: Transcranial electrical stimulation is based on the application of low-intensity electrical current, which can be administered as direct, or alternating. tDCS uses a fixed, direct current intensity, while tRNS and tACS use oscillating current at random and fixed frequencies respectively. The vertical axis represents the current intensity in milliamp (mA), while the horizontal axis illustrates the time-course.

tDCS is a widely used tool that is used to induce and investigate neural excitability. The effects of tDCS in human participants were first explored in the primary motor cortex (M1) (Nitsche & Paulus, 2000). It was demonstrated that direct current TES induces prolonged polarity-dependent cortical excitability alterations. Despite the different cyto-architecture of the cortices, neuronal membrane properties and different spatial orientations of neurons, similarly to M1, the visual cortex can undergo spontaneous and induced neuroplastic changes, leading to both short- and long-term alterations of synaptic strength and neural excitability (Sherman & Spear, 1982; Creutzfeldt, Fromm & Kapp,

1962). The first study to explore the effects of tDCS on visual perception, found that a short-lasting (7-minute) stimulation of the primary visual cortex (V1) on contrast perception resulted in cortical and behavioural changes. They demonstrated that cathodal tDCS diminished the excitability and reduced contrast perception, whereas anodal tDCS did not result in any significant cortical or behavioural modulations (Antal, Nitsche, & Paulus, 2001). The differences between the results reported in these studies might be attributed to the differences between the stimulation protocols as well as task parameters. In fact, depending on whether it is cathodal or anodal stimulation that is administered, tDCS modulates cortical excitability in this polarity dependant fashion; generally, anodal stimulation is said to increase neural activity whereas cathodal stimulation, reduces or inhibits neural activity (Nitsche, Cohen, Wassermann, Priori et al., 2008). Over the years, various tDCS parameters have been explored such as stimulation intensity and duration. In fact, in a later study, longer anodal tDCS duration (15 min) of V1 showed improved CS of central visual areas (Kraft, Kehrner, Hagendorf & Brandt, 2011). The effectiveness of tDCS over visual areas has also been demonstrated through the measurement of phosphene thresholds (PTs) using TMS. TMS pulses delivered to early visual areas can elicit visual sensations, known as phosphenes (Meyer, Diehl, Steinmetz, Britton, & Benecke, 1991). The average TMS intensity required to evoke these phosphenes is defined as the PT. The PT is stable within participants across time, and therefore is used as a representation of visual cortex excitability (Boroojerdi, Prager, Muellbacher & Cohen, 2000). Using short trains of 5-Hz rTMS delivered over V1, Antal and colleagues induced phosphenes and modulated their

intensity using tDCS (Antal, Kincses, Nitsche & Paulus, 2003). Interestingly, they found that cathodal stimulation over V1 significantly increased PTs, and attributed this result to diminished cortical excitability in V1. Anodal stimulation on the other hand resulted in the opposite effect, probably due to stimulation-induced cortical hyper-excitability.

Depending on a number of factors, the effects of stimulation can persist beyond the end of the session – known as the aftereffects of stimulation. In light of this possibility, the technique became a reliable tool to bring about long lasting plastic effects and LTP (Nitsche, et al., 2008; Nitsche, Nitsche, Klein, Tergau, et al., 2003a; Nitsche & Paulus 2000; 2001). It is important to keep in mind however that reliable aftereffects of any stimulation depend upon a number of factors and conditions related to stimulation parameters; these include, the stimulation duration, current intensity, electrode size, current density (Faria, Hallett & Miranda, 2011), the type of current administered (direct, oscillating current), and additional factors related to the current type, for example stimulation frequency in the case of alternating current (Antal et al., 2008). Furthermore, the timing of stimulation is paramount, whether for example it is delivered online (during task performance), or offline (before or after task performance) (Pirulli, Fertonani & Miniussi, 2013), as well as the electrode montage (i.e., position of the electrodes on the scalp) (Bikson, Rahman & Datta, 2012). Other stimulation-independent factors could also influence the aftereffect of stimulation, such as the wakefulness of the participants (Huber, Mäki, Rosanova, Casarotto, et al., 2013), the baseline state of participants receiving the stimulation (for example whether during rest or during behavioral/cognitive performance) (Silvanto, Muggleton & Walsh,

2008), the individual differences in the neuroanatomy of the brain, handedness (Schade, Moliadze, Paulus & Antal, 2012), as well as the chosen experimental paradigm (e.g., motor, visual, cognitive). Furthermore, stimulation aftereffects are dependent upon the functioning of the glutamatergic system and calcium channels (Liebetanz, Nitsche, Tergau & Paulus, 2002; Nitsche et al., 2003b, 2004a, b). It has been demonstrated that in order to achieve reliable aftereffects, stimulation lasting for at least three minutes with the intensity of at least 0.6 mA is required (Nitsche & Paulus, 2000).

NIBS has been shown to induce long-term plastic changes (LTP), mainly through the modulation of calcium at the L-type voltage gated calcium channel (L-VGCC). These L-VGCCs have been referred to as 'molecular switches', were they mediate neuronal metaplasticity induced by endogenous activation (*For a more detailed review see Paulus, 2011*). Mechanisms and long-term effects of tDCS have been modulated by the application of neuro-active drugs (e.g. Liebetanz, Nitsche, Tergau & Paulus, 2002; Nitsche et al., 2004a, b; Nitsche et al., 2003b; Abbruzzese, Michieli, Rupolo, Toffola, et al., 2010). For example, the dose-dependent reversal effects of tDCS by dopamine have been well documented (Kuo, Paulus & Nitsche, 2008; Nitsche, Kuo, Grosch, Bergner, et al., 2009; & Monte-Silva, Liebetanz, Grundey, Paulus, et al., 2010). Many studies of this kind suggest that the mechanisms underlying tDCS are ion-channel dependent, producing, LTP and long term depression (LTD)-like effects by selectively altering neurons and generating excitatory and inhibitory modulations in cortical excitability. For example, pharmacological administration in combination with tDCS has shown that its modulatory effects are largely NMDA receptor

dependent (Liebetanz et al., 2002). Furthermore, tDCS- anodal after-effects have been prolonged when an NMDA-receptor agonist (d-cycloserine and amphetamine) was administered. Likewise, cathodal-tDCS aftereffects have been selectively modulated by low dose dopamine receptor agonist (pergolide) (Nitsche, Jaussi, Liebetanz, Lang et al., 2004a; Nitsche, Grundey, Liebetanz, Lang et al., 2004b; Nitsche, Lampe, Antal, Liebetanz, et al., 2006; Monte-Silva, Kuo, Thirugnanasambandam, Liebetanz, et al., 2009). Conversely, a voltage-gated sodium channel blocker (carbamazepine) and a calcium channel antagonist (flunarizine) obliterated the short-duration aftereffects produced by anodal tDCS, but not by cathodal tDCS (Liebetanz et al., 2002; Nitsche et al., 2003b).

Electrophysiological studies have also given evidence to the effect of tDCS on the visual cortex. The first work exploring these effects was published in 2004 by Antal and colleagues (Antal, Kincses, Nitsche, Bartfai, & Paulus, 2004a). The amplitude and latency of the N70 and P100 visual evoked potentials (VEPs) were measured using both low and high contrast stimuli with tDCS. Using a V1 (active electrode) and Cz (reference) montage of tDCS, polarity significant aftereffects of stimulation were seen only when low-contrast stimuli were used. Conversely, when high-contrast stimuli were presented to the participants, tDCS did not modify VEP amplitudes. Anodal tDCS significantly increased the amplitude of the N70 component, while cathodal stimulation diminished it. Furthermore, cathodal tDCS slightly increased the amplitude of P100, but this was not significant. This study did not demonstrate any latency effects on the VEP components. Another study exploring the underlying electrophysiological components of tDCS used pattern-reversal checkerboard stimuli and a different

electrode montage (reference electrode placed on the anterior or posterior neck-base). Their results showed that anodal tDCS reduced the amplitude of the P100 component, whereas cathodal stimulation significantly increased it (Accornero, Li Voti, La Riccia, & Gregori, 2007). In this study, only when low contrast stimuli were presented, the aftereffects lasted for about 10 minutes with regards to cathodal stimulation and about 3 minutes with anodal tDCS. Interestingly, recent studies have shown that the combination of anodal tDCS applied on the occipital pole, together with visual field rehabilitation appears to enhance visual functional outcomes compared with visual rehabilitation alone (Plow, Obretenova, Halko, Kenkel, et al., 2011; Plow, Obretenova, Fregni, Pascual-Leone, & Merabet, 2012).

tACS is a newer stimulation technique that is able to modulate cortical excitability in a non-invasive manner (Terney, Chaieb, Moliadze, Antal, & Paulus, 2008). tACS is thought to affect the neuronal membrane potential through its oscillatory electrical pattern, applied with specific frequencies. It is said to interact with on-going rhythmic cortical activity during sensory or cognitive processes. Studies on visual perception have shown that tACS of the visual cortex affects phosphene sensations in a frequency-dependent manner (Kanai, Chaieb, Antal, Walsh & Paulus, 2008). Specifically, they demonstrated that phosphene perception was more evident when tACS was applied in the beta frequency range (12.5 and 30 Hz) in an illuminated surrounding, whereas tACS at alpha frequencies (10Hz), improved phosphene perception in a dark environment. A more recent study by Laczó and colleagues applied tACS in the high gamma frequency range (60 Hz) on area V1 and found that it improved

contrast perception, whereas no effects on spatial attention were observed (Laczó, Antal, Niebergall, Treue & Paulus, 2012). Electrophysiological evidence of tACS reveals that tACS over V1 is able to entrain the neuronal oscillatory activity in each individual's alpha frequency range. This kind of stimulation elevated the endogenous alpha power in parieto-central electrodes of the EEG (Zaehle, Rach & Herrmann, 2010).

A type of alternating electric current technique, tRNS is an innovative method of boosting neural excitability through the application of a weak alternating current at random frequencies (0.1–640 Hz). The neuromodulatory effects of tRNS are said to facilitate or inhibit neuronal activity by synchronising or desynchronising it (Ponomarenko, Li, Korotkova, Huston & Haas, 2008; Moss, Ward & Sannita, 2004; Grenier, Timofeev & Steriade, 2001). As with tACS, it is suggested that this kind of stimulation induces LTP-like cortical plasticity via augmenting the activity of sodium channels (Terney et al., 2008). It has so far been shown to be a very effective technique by interacting with ongoing firing rates in the cortex while avoiding the directional sensitivity of standard tDCS (Paulus, 2011). Unlike in tDCS, where the neurons are embedded in a constant electrical field and may result in a homeostatic effect of the ion neural channels after prolonged use, random noise stimulation counteracts this phenomenon due to its fluctuating pattern. In fact, one known disadvantage of continuous use of tDCS for rehabilitative purposes is that it may result in a homeostatic effect of the neural population being stimulated, in that neurons tend to return to their initial 'resting state'. This counteracting effect of tRNS may either be attributed to the repeated opening of sodium channels or to a higher sensitivity of neuronal

networks to field modulation than the average single neuron threshold (Terney et al., 2008).

Unlike tDCS, tRNS has only recently been explored within the visual domain (Camilleri et al, 2014b; Campana et al., 2014; Fertoni et al., 2011; Pirulli et al., 2013). Studies show tRNS as yielding faster and more effective PL as well as transfer to VA and CS in healthy participants, people with mild myopia and amblyopia (Camilleri et al., 2014b; Campana et al., 2014). In particular, a preliminary study showed that applying tRNS with a PL training protocol of 2 weeks (8 sessions) is able to achieve the same functional outcome on UCVA and a better outcome on UCCS as a two-month training protocol (24 sessions) (Camilleri et al., 2014a, b). In another study by Fertoni and colleagues (2011), different brain stimulation protocols and techniques were used to investigate their effectiveness on the performance of an Orientation Discrimination Task (ODT) in one hundred and seven healthy participants. High-frequency tRNS (hf-tRNS, 100–640 Hz), low-frequency tRNS (lf-tRNS, 0.1–100 Hz), anodal-tDCS (a-tDCS), cathodal-tDCS (c-tDCS), and Sham stimulation were applied to the early visual areas of the brain in a group of volunteers while they performed visual task (ODT). The findings revealed that the different stimulation conditions had a distinctive effect on the learning effect seen during task execution and the resulting performance. Results showed that hf-tRNS significantly improved performance accuracy compared with anodal tDCS, cathodal tDCS, Sham, and Cz stimulations (Fertoni et al., 2011). In conclusion their results support the efficacy of hf-tRNS of the visual cortex over other stimulation protocols in improving behavioural performance on a visual discrimination task. The

superior result of tRNS over the visual cortex was explained by a proposed mechanism of action where tRNS is based on repeated subthreshold stimulations which prevents the sensitisation of the system and may potentiate task relevant neural activity (Fertonani et al, 2011; Cash and Yuste, 1998; Miniussi Ruzzoli & Walsh, 2010). The stochastic resonance phenomenon (e.g., Miniussi et al., 2010) is another proposed mechanism of random noise stimulation action. tRNS is by definition a stimulation that induces random noisy activity in the system through its alternating frequency. Nonlinear systems like the brain can use noise to enhance performance through stochastic resonance (see Moss et al., 2004). The presence of neuronal noise might confer to neurons more sensitivity to a given range of weak inputs, i.e., those neurons “randomly activate” that go in the same direction as the signal, thereby rendering the noise in in the signal. In this framework, it is possible to explain facilitatory results in terms of the relationship between noise and signal in the nervous system; i.e. an improved performance could be observed with an optimum level of noise (Antal et al., 2004c; Ruzzoli, Marzi & Miniussi, 2010).

A more recent study explored the underlying mechanisms of tRNS through the application of single pulse TMS and the administration of five pharmacological agents in order to differentiate essential receptors and ion channels which may be involved in the generation of tRNS aftereffects: lorazepam (LOR: GABAA receptor agonist), ropinirol (ROP: dopamine receptor 2/3 agonist), carbamazepine (CBZ: a sodium channel blocker), dextromethorphan (DMO: NMDA receptor antagonist) and D-cycloserine (D-CYC: partial NMDA receptor agonist) (Chaieb, Antal & Paulus, 2015). Their

results propose that unlike the NMDA-receptor dependency of tDCS aftereffects, the aftereffects of random noise stimulation seem to be independent of NMDA receptors and instead are suppressed by benzodiazepines and are sodium channel dependant. Their paper is the first to demonstrate that tDCS and tRNS aftereffects are dependent on different underlying mechanisms.

Therefore, tRNS may optimize the effects of a behavioural training with measurable changes in the brain by modulating neuronal excitability that are involved in LTP (Fritsch et al., 2010; Stagg et al., 2009) which may ultimately lead to neuroplasticity. LTP has been postulated as a likely mechanism underlying these functional long lasting improvements (Nitsche et al., 2009). However, the question still remains as to what is accountable for this accelerated improvement and which treatment protocols are most suitable. These observed neuroplastic changes make random noise brain stimulation an important consideration in neurorehabilitation settings.

CHAPTER 3 Opposite effects of high- and low- frequency transcranial random noise stimulation probed with visual motion adaptation

3.1 Introduction

After observing a rotating stimulus (adapting stimulus) for about 20-30 seconds, the successive presentation of a static or flickering stimulus (test stimulus) will appear to move in the opposite direction. This is a powerful visual illusion known as the MAE (Sutherland, 1961). The MAE is said to come about due to a shift in the balance of opposing direction selective neurons, specifically, a relative suppression of activity corresponding to the adapting direction, together with enhancement of the activity coding for the direction of illusory motion (Hogendoorn & Verstraten, 2013). On the presentation of a static or flickering stimulus following an adapted stimulus, the adapted neurons would respond less strongly than their oppositely tuned counterparts. Consequently, the balance of activity between the two opposing directions favors the unadapted direction, leading to an 'activation shift' and as a result, perceived illusory motion. Other computational models of illusory motion perception suggest that it is this relative shift of activity of neural populations with different direction tuning that generates the MAE (e.g. Anstis, Verstraten, and Mather, 1998; Mather and Harris, 1998; Mather et al., 2008; Simoncelli and Heeger, 1998; Sutherland, 1961). Depending on the test stimulus used, different types of MAE, involving different neural populations

tuned to either low or high temporal frequencies, have been identified: static and dynamic MAE, the latter arising upon the presentation of a flickering test stimulus (Mather, Pavan, Campana, and Casco, 2008). Motion direction selective neurons are strongly implicated in eliciting this effect, and although direction specific neurons are located along most areas of visual processing, the medial temporal area (MT+) is said to be strongly involved in generating the MAE (Toothel, Reppas, Dale, Look, et al., 1995). Single cell recordings on monkeys (Petersen, Baker & Allman 1985), transcranial direct current stimulation (Antal, Varga, Nitsche, Chadaide et al., 2004), alternating current stimulation at 10 Hz (Kal & Krekelberg, 2014) as well as repetitive transcranial magnetic stimulation in humans (Stewart et al., 1999) have all shown a specific involvement of area MT/V5 during the MAE. Interestingly, these studies found a significant reduction in the MAE duration when stimulation was focused over area MT whereas no effects were seen when other visual areas, or frontal areas were stimulated (Antal et al., 2004d; Kal & Krekelberg, 2014; Stewart et al., 1999). However, Campana and colleagues (2013) found that both the static and dynamic MAE rely upon the activity of the same low- and intermediate visual areas involved in visual motion processing, including area V5/MT (Campana, Maniglia & Pavan, 2013). It is important to point out that this study investigated this effect using simple translational motion stimuli rather than complex motion stimuli, which cannot be processed by low-level visual areas (Morrone, Tosetti, Montanaro, Fiorentini, et al., 2000; Wall, Lingnau, Ashida, & Smith, 2008). Theoret and colleagues (2002) found that rTMS disrupted the perception of MAE both

when delivered in the early parts of the storage period (between the adapting stimulus and static stimulus) and when it was applied during the perceptual MAE itself (without a storage period) (Theoret et al., 2002). Culham and colleagues (1999) also reported that MT+ activation increased even when adaptation (moving stimulus) and test phases (static stimulus) were separated by a storage period (Culham, Dukelow, Vilis, Hassard et al., 1999). Furthermore, using fMRI, Hogendoorn and Verstraten (2013) found that BOLD activation was largest at MT during the MAE phase compared to a control condition without MAE (e.g., alternating direction motion).

Scant work has been carried out on the differences between low and high frequency tRNS, however it has been postulated that high frequency tRNS results in neuronal excitation whereas low frequency tRNS in neuronal inhibition (Terney et al., 2008). tRNS has been shown to increase cortical excitability in M1 (Terney et al., 2008) as well as improve VA and CS in both cortical and non-cortical visual defects (amblyopia and myopia respectively) (Campana et al., 2014, Camilleri et al., 2014b). So far the MAE has not been investigated under the conditions of random noise stimulation. The aims of the present experiment were twofold: firstly, which areas are more involved in modulating the static MAE duration (using complex motion stimuli) when adding random noise stimulation? Secondly, how will low and high frequency random noise stimulation differ in their effects on the MAE? It was hypothesized that the perceived MAE duration will be diminished only when tRNS is administered on area MT/V5. Secondly, as was found with cathodal and anodal stimulation (Antal et al., 2004d) both high and low frequency

tRNS will result in a reduction of the MAE. To attempt to answer these questions and explore the mechanisms of random noise stimulation on visual motion perception, three experiments were conducted.

3.2 Methods

3.2.1 Experiment 1

This experiment was carried out in order to investigate specifically the effects of hf-tRNS on MAE duration when stimulating early visual areas compared to bilateral V5/MT.

Participants

Twelve participants with normal or corrected-to-normal vision, who were unaware of the purpose of the study took part in Experiment 1. All participants were screened by means of a structured interview for any condition that may increase the risks associated with the use of TES. All participants gave written informed consent according to the Declaration of Helsinki. The study (consisting of three experiments) was approved by the Local Ethics Committee at the University of Padova, where the data were collected.

Apparatus

Stimuli were generated using Matlab and Psychtoolbox (Brainard, 1997; Pelli, 1997) and displayed on a 22-inch Philips Brilliance 202P4 monitor with a refresh rate of 60Hz and a resolution of 1280 × 1024 pixels. The monitor was luminance-calibrated (gamma-corrected with $\gamma = 1$). Participants sat in a dark

room at a viewing distance from the monitor of 54 cm. Each pixel subtended 1.7' (0.028 deg). Viewing was binocular. They were instructed to fixate the centre of the screen and underwent practice blocks to familiarize them with the stimuli and task.

Stimuli and Perceptual Task

Adapting and test stimuli consisted of a checkerboard pattern composed by a radial grating rotating clockwise or counter-clockwise (16 cycles, 2.5 Hz, 0.5 Michelson contrast) superimposed on a concentric grating expanding or contracting at 2.5 Hz. The concentric grating had 4 cycles and a contrast of 0.5. Therefore, the resulting contrast was 1 (Michelson contrast). Adapting and test patterns had the same spatial contrast. The resulting temporal frequency was 5 Hz. Stimuli were viewed throughout a circular annulus with an outer radius of 5.5 deg and an inner radius of 1 deg. A white fixation point (diameter 0.38 deg) was placed at the center of the stimuli (Figure 8).

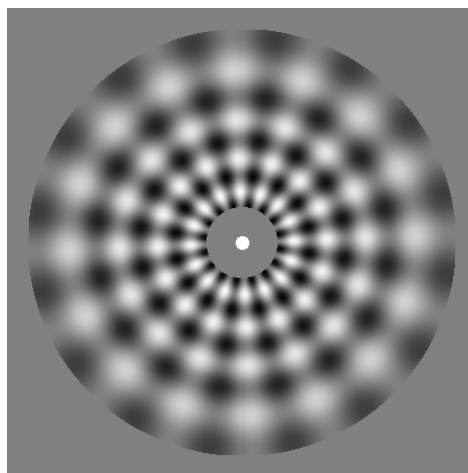


Figure 8. Representation of the stimulus used in the Experiments.

The adapting pattern was presented at the center of the screen and observers had to maintain their fixation on the white fixation point. The adapting stimulus was presented for 20 s. After the adaptation period, we presented a stationary version of the adapting pattern (test stimulus) and observers judged both the direction of the illusory motion and when it stopped by pressing one of two designated keys on a standard Italian keyboard. In particular, observers had to press the “Right Arrow” key when the illusory clockwise motion stopped and the “Left Arrow” key when the illusory counter-clockwise motion stopped.

The motion direction of the adapting pattern was randomized on a trial basis with the constraint that the same adapting direction could not be repeated for more than two consecutive trials. Observers were adapted to two clockwise directions: clockwise outward (superimposing a clockwise radial pattern and an expanding circular pattern) and clockwise inward (superimposing a clockwise radial pattern and a contracting circular pattern), and to two counter-clockwise directions: counter-clockwise outward (superimposing a counter-clockwise radial pattern and an expanding circular pattern) and counter-clockwise inward (superimposing a counter-clockwise radial pattern and a contracting circular pattern).

During the adapting phase of each trial, observers carried out a secondary task at fixation. For this secondary task, a similar procedure was used to that reported by Hogendoorn and Verstraten (2013). Between one and four times, the size of the central fixation point became smaller (from 0.38 deg to 0.09 deg) for just one frame (~17 ms). The task of the observer was to detect and count these changes during the adapting phase of each trial. During the inter-trial interval,

observers verbally reported the number of fixational changes. We did not provide feedback on this secondary task. The purpose of this task was to aid fixation and keep attention engaged (Castelo-Branco, Kozak, Formisano, Teixeira, Xavier, & Goebel, 2009; Hogendoorn & Verstraten, 2013; Huk, Ress, & Heeger, 2001). To allow recover from adaptation the, inter-trial interval was 10 s (Figure 9). There were 24 trials in total (i.e., 6 trials per each adapting direction).

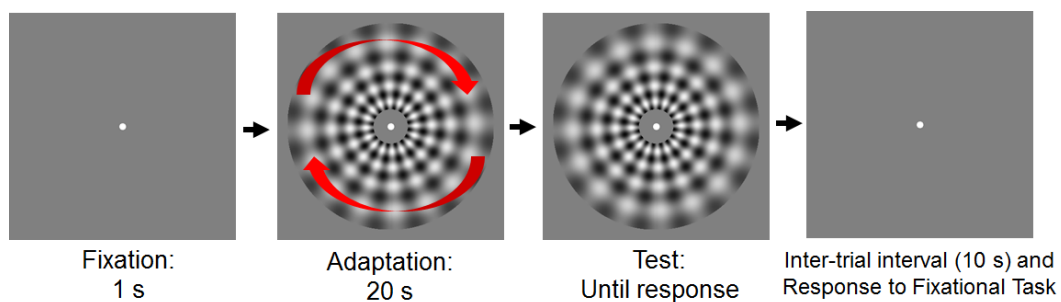


Figure 9. Schematic representation of the perceptual task used in the Experiments. Only clockwise motion is represented.

Procedure

On two different days, with at least 3 days interval in between, participants underwent two different stimulation conditions: hf-tRNS of the occipital lobe (early visual areas), or bilateral hf-tRNS of area V5/MT respectively. The order of site of stimulation condition was counterbalanced across participants. In each day, 2 blocks of 24 trials each (with a pause between the first and the second block) were administered: the first one with Sham stimulation, the second one with hf-tRNS. The order of Sham vs. hf-tRNS stimulation could not be counterbalanced across participants: administering the real brain stimulation on the first block could have resulted in a modulation of

cortical excitability that could have extended to the second block with Sham stimulation, deeming it impossible to distinguish between the effects of real vs. Sham stimulation.

Transcranial electrical stimulation

Electrical stimulation was delivered using a battery-driven stimulator (BrainSTIM, EMS) through a pair of saline-soaked sponge electrodes. Impedance was always kept below 5 Kohm. The hf-tRNS consisted of an alternating current (1.5 mA intensity with no offset) applied at random frequencies ranging from 100 to 640 Hz. The stimulation started ~4 mins before the beginning of the second block and lasted for the whole duration of the block (approximately 17-18 min). Current intensity was linearly increased up to 1.5 mA during the first 30 s and was then kept constant until the end of the block. All electrodes had an area of 25 cm². For the stimulation of early visual areas, one electrode was placed at 3 cm above the inion, whereas the other was centred on Cz. For the bilateral stimulation of V5/MT, the two electrodes were placed at a site located 3 cm above the inion and 5 cm anteriorly on the left and on the right, respectively. In this way, in both conditions we were able to stimulate the targeted areas of both hemispheres. Current density (0.0094 A/m²) was well below the safety limits (Poreisz, Boros, Antal & Paulus, 2007). Sham stimulation was delivered by linearly increasing current intensity for 30 s up to 1.5 mA, and decreasing it during the successive 30 s up to 0 mA, just before the beginning of a block. The electrodes were kept in place with bandages. Electrode montage was performed

before the beginning of the first block and kept unaltered until the end of the second block.

3.2.2 Experiment 2

In order to control for any effect of block repetition as well as to further explore the specificity of the site of tRNS in MAE disruption, this second experiment was carried out. Twelve participants with normal or corrected-to-normal vision, who were unaware of the purpose of the study, took part in the second experiment. As in Experiment 1, all participants were screened with a structured interview and gave written informed consent according to the Declaration of Helsinki. Apparatus, stimuli and parameters of transcranial electrical stimulation were the same as in Experiment 1.

Procedure

On two different days, with at least 3 days interval in between, participants underwent two different conditions: high frequency random noise stimulation of the frontal lobe bilaterally vs. Sham stimulation, or Sham stimulation for two consecutive blocks with electrodes positioned bilaterally over V5/MT. On each day, 2 blocks of 24 trials each (with a pause between the first and the second block) were administered. On one day electrodes were positioned over F7 and F8, according to the 10-20 EEG system; during the first block Sham stimulation was administered, whereas in the second block, concurrent hf-tRNS was given. On the other day electrodes were positioned over V5/MT and Sham

stimulation was delivered on both the first and the second block. The order of site of stimulation condition was counterbalanced across participants.

3.2.3 *Experiment 3*

A final experiment was carried out in order to explore the effects of lf-tRNS on the MAE duration. A third group of twelve participants with normal or corrected-to-normal vision, who were unaware of the purpose of the study took part in Experiment 3. As in the previous experiments, all participants were screened with a structured interview and gave written informed consent according to the Declaration of Helsinki. Apparatus, stimuli and task, parameters of TES and experimental procedure were the same as in Experiment 1, except for the fact that low frequency random noise stimulation (frequencies ranging from 0.1 to 100 Hz) was used instead of hf-tRNS.

3.3 Results

3.3.1 *Experiment 1*

For each participant the mean MAE duration for each block of 24 trials was computed. A test of normality (Kolmogorov-Smirnov) on the mean duration of the sample of 12 participants, separately for each condition, was significant at least on one condition. For this reason, we decided to use non-parametric tests (Wilcoxon signed-rank test) to compare the relevant conditions.

Figure 10 shows the mean MAE duration as a function of stimulation (Sham vs. hf-tRNS) and targeted areas (early visual areas vs. V5/MT). When hf-tRNS was applied over the occipital pole (early visual areas), duration of the

MAE did not significantly differ with respect to when Sham stimulation was applied ($z = -0.863$, $p > .05$, $r = -.17$). On the contrary, when stimulation was applied over the V5/MT, the hf-tRNS condition yielded significantly shorter durations (~ 1.5 s shorter, on average) with respect to the Sham condition ($z = -2.667$, $p < .01$, $r = -.54$).

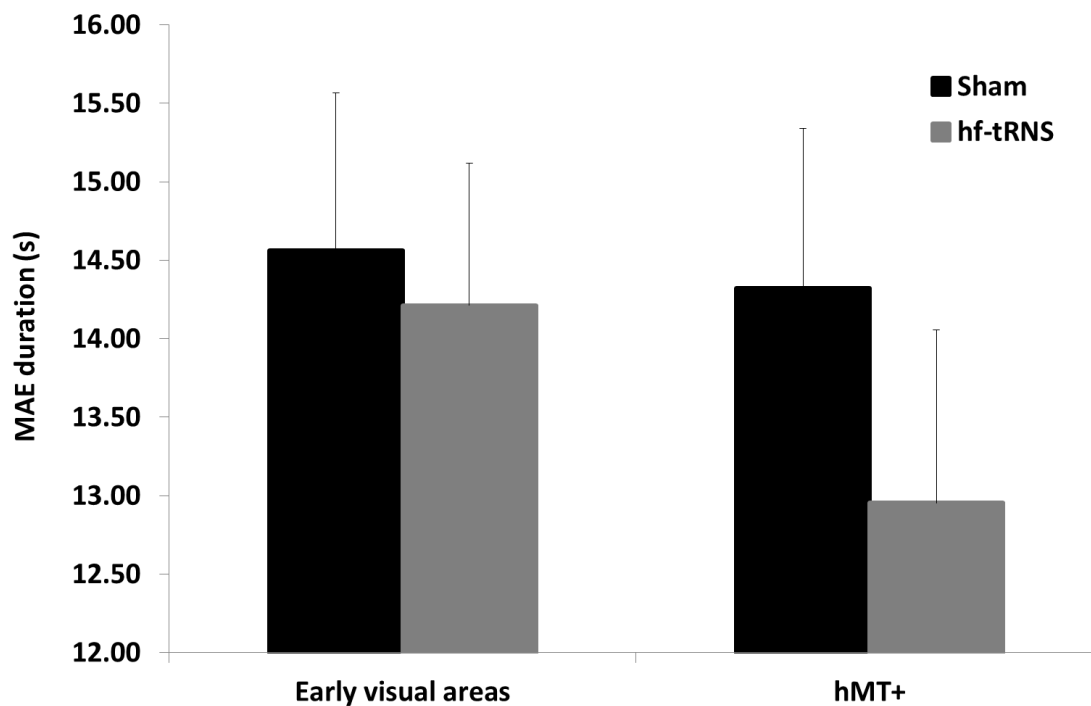


Figure 10: graph showing mean MAE duration when hf-tRNS or Sham was applied over V1 and MT+. Error bars represent the standard error of the mean (SE).

3.3.2 Experiment 2

The leftmost part of Figure 11 shows the mean MAE duration as a function of stimulation (Sham vs. hf-tRNS) when electrodes were positioned on frontal areas. The columns on the right show the mean MAE duration in the two

successive blocks with Sham stimulation when electrodes were positioned over area V5/MT bilaterally. No significant differences in mean MAE duration could be found when hf-tRNS was applied to the frontal lobe, with respect to Sham stimulation ($z = -0.078$, $p > .05$, $r = -0.016$), nor between two sequential sessions with Sham stimulation over the hMT+ complex ($z = -0.31$, $p > .05$, $r = -0.064$).

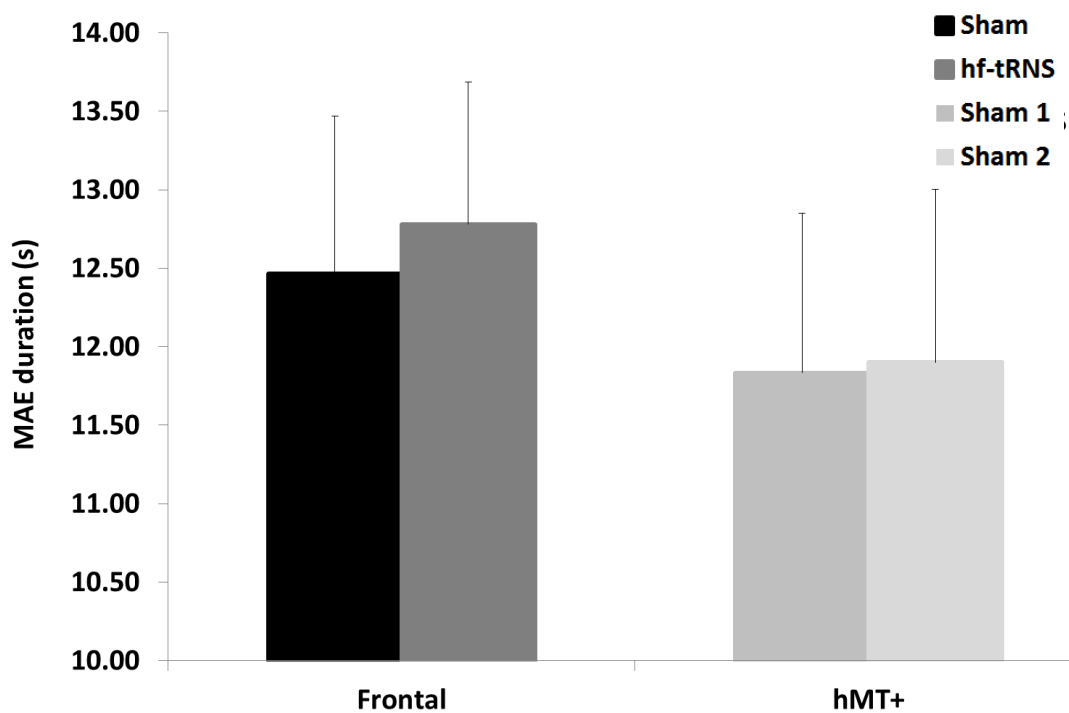


Figure 11: Graph showing mean MAE duration when hf-tRNS or Sham was applied over F7/F8 and double Sham condition over MT+. Error bars represent the standard error of the mean (SE).

3.3.3 Experiment 3

Figure 12 shows the mean MAE duration as a function of stimulation (Sham vs. lf-tRNS) and targeted areas (early visual areas vs. V5/MT - which as in the other experiments were counterbalanced). When lf-tRNS was applied over

the occipital pole (early visual areas), duration of the MAE did not significantly differ with respect to when Sham stimulation was applied ($z = -0.706$, $p > .05$, $r = -.14$). On the contrary, when stimulation was applied over V5/MT, the lf-tRNS condition yielded significantly longer durations (~1.5 s longer, on average) with respect to the Sham condition ($z = -2.353$, $p < .05$, $r = -.48$).

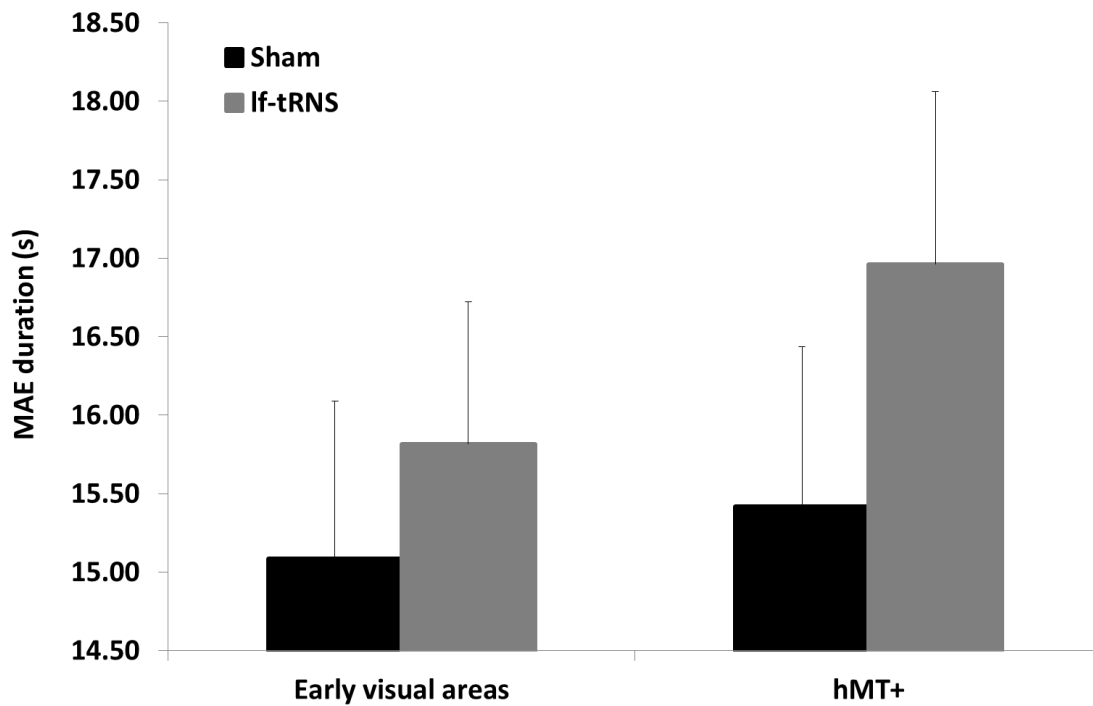


Figure 12: graph showing mean MAE duration for each block when lf-tRNS or Sham was applied over V1 and MT⁺. Error bars represent the standard error of the mean (SE).

3.4 Discussion

The present study investigated the neuro-modulatory effects of low and high frequency tRNS on the MAE illusion. Results point towards a specific role of area MT in the MAE with complex motion stimuli, since the modulation of the

MAE duration was only present when tRNS was applied over bilateral MT. No significant effects of either low or high frequency tRNS were observed when administered over early visual areas (V1) or over frontal areas. This finding corroborates with other studies exploring the MAE using non-invasive brain stimulation techniques. For example, Antal and colleagues (2004) found an effect of direct current stimulation only when it was administered over MT. No effects were reported when either anodal or cathodal stimulation were administered over the posterior occipital pole. Another study using alternating current stimulation at 10hz sought to investigate more specifically, which aspects of motion adaptation are affected by administering tACS during the adapting stimulus phase (Kar & Krekelberg, 2014). Like Antal and colleagues, they found that tACS, which also generates current flow of both polarities, reduced the MAE. Furthermore, this reduction was correlated with the improvement in motion sensitivity. Interestingly, they demonstrate that tACS had no reliable effect when administered prior to the adapting stimulus presentation nor when applied during recovery from motion adaptation. These findings postulate that perceptual effects of tACS resulted from an attenuation of adapted neurons, further suppressing them.

This is the first study to investigate the effects of random noise stimulation on illusory motion. It has been pointed out that tRNS acts on the visual system through repeated depolarisations across the neural membranes (Terney et al., 2008). These repeated depolarizations through the influx of sodium, may, for example, generate a cumulative cyclic response of sodium channels to continually repolarise and depolarise, and in this fashion may produce a

heightened effect of the tRNS, resulting in the classical increases in cortical excitability observed. Furthermore, studies have reported the state dependency effect of brain stimulation (Silvanto et al., 2008). These studies propose that the behavioural and perceptual effects of brain stimulation (for example TMS) depend on the state of adaptation of the neural population stimulated. Specifically, they point out that TMS perceptually facilitates the attributes encoded by the less active neural population. Keeping this in mind, the present study hypothesizes that both high and low frequency tRNS is acting by enhancing the neuronal firing responding to the weaker signal, the suppressed adapted neurons. Therefore, hf-tRNS evokes a strong depolarisation of the adapted neurons following adaptation, which further increases their firing, this in turn results in a reduced shift in neuronal firing and thus the balance between the two opposing neuronal population (adapting neurons versus MAE neurons) stabilizes faster (a similar interpretation was put forward by Kar & Krekelberg, 2014, using 10 Hz tACS). Similarly, in the present study, lf-tRNS may induce weak depolarisations which leads to an overall reduced firing rate of the already suppressed adapted neurons, resulting in an increased shift and a more prolonged MAE.

An important confound to the present work which limits us from constructing any direct causal relationship between tRNS and the underlying mechanism of the MAE is that tRNS was applied continuously during both adaptation induction and the subsequent static test stimulus. Hence, the effects on MAE duration brought about by the stimulation could have been the consequence of tRNS interference with any of these processes. Nonetheless, the

present work identifies tRNS as a powerful tool in modulating neural excitability and consequently, visual motion perception. More work needs to be done combining these techniques with brain imaging in order to paint a better picture of the mechanisms by which random noise stimulation acts on neural firing and subsequent movement perception.

CHAPTER 4 Investigating the effects of a single Gabor contrast detection perceptual learning paradigm on visual acuity and contrast sensitivity in mild myopia

4.1 Introduction

PL has been shown to be useful for improving visual functions such as VA and CS in individuals with amblyopia (Chung, Li, & Levi, 2006; Polat, Ma-Naim, Belkin & Sagi, 2004; Huang, Zhou & Lu, 2008) and also in those with refractive defects (Durrie & McMinn, 2007; Tan & Fong, 2008). It is also a promising technique for improving peripheral visual functions in patients with central visual loss (Maniglia, Pavan, Cuturi, Campana, Sato & Casco, 2011). Since the early eighties (e.g.: Fiorentini & Berardi, 1980), PL has been observed in many visual tasks and found to be specific for the trained stimulus characteristics and even for the trained eye (Ahissar & Hochstein, 1996; Karni & Sagi, 1991; Fiorentini & Berardi, 1980; Campana & Casco, 2003; Fahle & Poggio, 2002), pointing to neural plasticity at early cortical stages. This suggested that sensory plasticity extends much beyond the critical period, and into adulthood (Sagi, 2011). Neuroimaging and electrophysiological results give evidence that the striate cortical area V1 is often involved in PL and associated plastic changes (Casco, Campana, Grieco, & Fuggetta, 2004; Gilbert, Sigman, & Crist, 2001; Pourtois, Rauss, Vuilleumier, & Schwartz, 2008; Schwartz, Maquet, & Frith, 2002). The mechanisms underlying PL could be a fine tuning (or selective weighting) of independent early detectors or channels (gain control) (Saarinen &

Levi, 1995; Schwabe & Obermayer, 2005), a modification of interactions between detectors, either via horizontal (Polat & Sagi, 1993, 1994a, 1994b, 2006; Tanaka & Sagi, 1998) or feedback connections (Ahissar & Hochstein, 1993, 1996, 1997), or a reduction of external or internal noise (Huang, Lu & Zhou, 2009; Lu & Doshier 2004), that could occur either at the sensory level (Bejjanki, Beck, Lu, & Pouget, 2011), or at the decision stage (Yu et al., 2004).

Some perceptual tasks, however, have been reported to produce no or very little improvement with practice, except in individuals showing high initial thresholds (Fahle & Henke-Fahle, 1996) or after eliminating stimulus uncertainty (Swift & Smith, 1983). These findings suggest that some type of processing, probably occurring at low-sensory level, could be hard-wired and unchangeable, already exhibiting the best possible performance (Sagi, 2011). In the case of contrast detection or discrimination, Adini and colleagues (Adini, Sagi, & Tsodyks, 2002; Adini, Wilkonsky, Haspel, Tsodyks, & Sagi, 2004) found that contrast discrimination of a Gabor stimulus can improve with practice only if it is flanked by pairs of similar, high contrast Gabor stimuli. The mechanism underlying the improvement of contrast detection with flankers, known as lateral masking, has been attributed to an increase of the range of facilitation between collinear elements resulting from a cascade of local connections between detectors based on Hebbian synaptic mechanisms (Polat, 1999; Polat & Sagi, 1994b).

In the 1994 studies, Polat and his colleague, using a lateral masking paradigm, demonstrated that PL results in an increase in the cortical spatial range of lateral interactions by a factor of six (Polat & Sagi, 1994a; 1994b). In a

later study by Polat and colleagues (2004) trained adults with amblyopia using the same lateral masking technique found that learning generalized to higher level tasks such as letter recognition, and VA. This and other studies using lateral-masking paradigms point to plasticity of spatial interactions in adults following repetitive training on a target-flanker task.

Different PL paradigms however have been implemented in adults with amblyopia, which resulted in effective improvement of CS or VA. For example, training on a contrast detection task using single Gabor patches (in the absence of flankers), either with (Huang et al., 2009) or without (Zhou, Huang, Xu, Tao, Qiu, Li, & Lu, 2006) external noise. Zhou and his colleagues indicated that training improved VA and CSF in the amblyopic eyes, which were retained for up to 1-year post training. Other studies have trained amblyopic participants on Vernier tasks, showing that repetitive practice not only leads to significant improvement in measured Vernier acuity but also a substantial improvement in (standard) VA and CS measurements, which in some cases, reached up to normal vision. Despite the fact that these studies have shown that PL training on single targets (Gabor or Vernier stimuli) improved performance in the amblyopic eye, overall, the results obtained with the lateral masking paradigm seemed to be more efficient: the improvement in CS was of 9.5dB with the lateral masking paradigm, of 4.9dB with single Gabor training, and of 3.5dB with the Vernier task (Zhou et al., 2006). Indeed, the amount of VA improvement in Zhou and colleagues (2006) with no lateral masking was nearly half respect that obtained by Polat and colleagues (2004) where lateral masking has been used, although the number of

sessions administered in Zhou and colleagues' study was much less than that used by Polat and colleagues.

In myopia, the neuronal connectivity has developed normally in childhood and is capable of processing images efficiently; however, the visual input is limited by an optical de-focus. In individuals with myopia, visibility of high spatial frequencies (SFs) is perceived as low contrast even when their physical contrast is high, thus degrading VA (Tan & Fong, 2008). Despite the fact that perceptual or sensory training cannot modify the structure of the eye and the aforementioned ocular defects, positive results using PL have also been found using similar lateral masking techniques on refractive defects and thus individuals with myopia and presbyopia (Durrie & McMinn, 2007; Polat, 2009; Polat et al., 2012; Tan & Fong, 2008). The mechanism by which PL improves vision in refractive disorders is still a matter of debate. The current explanation is that, an increase of cortical processing efficiency can overcome the poor resolution of the image formed on the retina. The aim of this study is to investigate if an efficacious perceptual training, able to improve visual functions such as VA in mild myopia, really needs to be based on lateral interactions between detectors. It is still possible that cortical dysfunctions such as amblyopia, where connectivity between neurons is impaired, might obtain the most beneficial effects from a training based on lateral masking. However in cases of poor vision, not due to cortical dysfunctions, such as the case of mild myopia, does a perceptual training regime really need to be based on lateral interactions? A contrast detection training with single Gabor patches was used on individuals with mild myopia to assess if it can have equally positive effects on CS and VA,

similarly to those reported using a lateral interaction paradigm. A battery of tests, including VA measured with Landolt C, Vernier acuity, CS and a test of lateral interactions using the lateral masking procedure, were administered in order to assess changes in visual functions before and after the training.

4.2 Methods

4.2.1 Participants

Ten participants with mild myopia were recruited from the University of Padova (mean age of 24.22, ranging between 22 and 27), all of which fitting the following inclusion criteria: refractive error up to -2 diopter (D) in each eye (minimum was -0.75D), with astigmatism not exceeding -0.5D in either eye. The participants had a stable refractive index for the 6 months prior to training. Exclusion criteria included any other ocular condition or cause for reduced VA other than simple myopia and/or mild astigmatism; these include diabetes mellitus, pregnancy, presence of myopia-related ocular complications and any previous ocular surgery. To ensure the inclusion and exclusion criteria, prior to training every participant carried out a detailed assessment by an optometrist.

This study has been approved by the Local Ethics Committee. Informed consent was obtained from each participant prior to the enrolment in the study highlighting that at any point during the experiment, they were allowed to withdraw from the study. Two participants withdrew from the study following the complete training and post-training tests, thus they did not carry out the follow up two months post- training.

4.2.2 Experimental Procedure

Following the assessment carried out by the optometrist, each participant carried out a series of baseline visual functioning tests that served as the pre-test measurements. These tests measured VA, Vernier hyperacuity, CS and lateral interactions. Following the pre-test, participants carried out an 8-week behavioural training using a single Gabor contrast detection task, completing a total of 24 sessions each lasting approximately 45 minutes. The same battery of tests were re-administered at the end of the treatment (post-test) and 2 months follow up from the end of the treatment. Furthermore, In order to investigate any possible effects of the intervention on optical eye characteristics, each participant also carried out an eye examination before and after the training at a local optometrist, who also administered tests of pupil size (Polat et al., 2012).

4.2.3 Apparatus

Stimuli were displayed on a 22-inch Philips Brilliance 202P4 monitor with a refresh rate of 60 Hz. Both the stimuli used in the training and in the lateral interaction test were generated with the Matlab Psychtoolbox, whereas stimuli for measuring VA, CS and Vernier acuity were generated using the Freiburg Acuity and Contrast Test (FrACT 3.8) (Bach, 1996). All stimuli were presented in foveal vision. The screen resolution was 1280x1024 pixels, each pixel subtended 0.33 arcmin at a viewing distance of 3 meters, and 0.67 arcmin at a viewing distance of 1.5 meters. Viewing distance was equal to 3 meters for all tests except for the lateral interaction test and the training, which was administered from 1.5

meters. Display linearization was performed before the beginning of this study by means of a dedicated screen calibrator. Both the tests and training were carried out in a dark, silent room. Background screen luminance was 31.5 cd/m² for all stimuli (FrACT CS test, training and lateral interaction stimuli) measured using a Gossen Mavo-Monitor luminance meter.

4.2.4 *FrACT*

Stimuli used for measuring CS at the pre and post test measurements were sinusoidal gratings presented in a circular window with a narrow Gaussian taper. Size of the gratings was 3 deg, while grating orientations used were 0, 45, 90 or 135 deg. The task of the participant entailed discriminating the orientation of the grating (4AFC) at different spatial frequencies, ranging from 1 cpd to 15 cpd, in separate blocks. Landolt C optotypes were used to assess VA. The task of the participants was to indicate, in every trial, the orientation of the gap of the Landolt C out of eight possible orientations (8AFC). Vernier acuity was also assessed using two vertical lines, each 0.25 deg long, with no vertical separation between them, and with a variable horizontal offset. The task of the participants was to indicate, in every trial, the direction of the offset (left vs. right) of the upper line with respect to the lower line (2AFC). For all FrACT stimuli, the Best-Pest adaptive procedure was used to calculate the absolute threshold for each of these tests. Stimulus duration lasted until the participants' response. An auditory cue was presented upon stimulus presentation and a different auditory cue was implemented as feedback for error responses.

4.2.5 Gabor stimuli (training and lateral interactions in pre/post tests)

Stimuli used in the lateral interaction test and in the training comprised of Gabor patches consisting of a cosinusoidal carrier enveloped by a stationary Gaussian. Standard deviation of the luminance Gaussian envelope (σ) was equal to the sinusoidal wavelength (λ); that is, the size of the Gabor patch covaried with its spatial frequency. Additionally, the spatial phase of the cosinusoidal carrier equalled to zero (evenly symmetric Gabor patch). Stimulus duration lasted 200ms.

In the lateral interaction test two high-contrast Gabor patches (0.6 Michelson contrast), collinear to and with the same spatial frequency of a centrally presented low-contrast target Gabor, were located at various distances from the target (i.e., 2λ , 3λ , 4λ , and 8λ). Single spatial frequency, vertically oriented and collinear Gabor flankers were used. The spatial frequency used in the lateral masking task was the central spatial frequency amongst the three spatial frequencies used in the training (see “training” section). Results of this test allowed us to investigate whether individuals with mild myopia who train on single Gabor patches altered the strength of facilitatory (4λ) or inhibitory (2λ) collinear lateral interactions (Polat & Sagi, 1993).

4.2.6 Training Procedure

Following the baseline measurements, the participants undertook a series of training sessions using a single Gabor patch in a contrast detection task with a two interval forced choice (2IFC) procedure. In a typical training task, the

participant was presented with two consecutive displays where only one of the displays contained the target Gabor stimulus presented in the centre of the screen. Note that the same procedure was implemented in the lateral interaction test, with the exception that one interval contained both target and flankers, and one contained only the two flankers. Participants responded by pressing a key according to which interval he or she perceived the target. The threshold corresponding to 79% of correct discrimination was determined separately for each block by using a 1up/3down staircase procedure. In order to eliminate spatial or temporal uncertainty, and to avoid the possibility that practice improved performance by reducing uncertainty, both an auditory and a spatial cue were implemented. At the start of each block the participant was reminded to remain focused on the centre of the screen which was facilitated by providing a central fixation point (positional cue) preceding the presentation of each interval, as well as an auditory cue, indicating when the stimulus (if present) appeared. Performance feedback was also provided to the participants in the form of an auditory beep following an incorrect response.

During the training, the spatial frequency and orientation of the Gabor Patches were varied across sessions, starting with the lower spatial frequencies (e.g. 1 cpd, 3 cpd) and progressively presenting the higher ones (7 cpd, 9 cpd, 11 cpd, etc). Therefore, each participant trained on three different spatial frequencies which changed daily, and four different orientations which varied weekly (0 deg, 45 deg, 90 deg and 135 deg), thereby covering all stimulus orientations and three levels of spatial frequency; lower spatial frequencies serving as the easier training conditions at the start of the week and progressing

to the higher spatial frequencies for the final weekly session. The three trained spatial frequencies were chosen individually for each participant on the basis of individual performance on the pre- training CS (“Grating”) task.

Each training session comprised of 8 blocks, and each block contained 60 trials, amounting to a total training time of 40 minutes per session. The total duration of the training lasted 8 weeks. No more than one session per day was administered for three times per week. The battery of baseline tests were re-administered to each participant following 24 training sessions (8 weeks) in order to obtain post-training measurements of visual performance. Additionally, follow-up sessions were carried out two months following the end of the training in order to determine the long-term effectiveness of single Gabor techniques in improving visual functions.

Furthermore, in order to investigate any possible effects of the training on optical eye characteristics, each participant also carried out an eye examination prior to the training at a local optometrist, who also administered tests of pupil size (Polat et al., 2012). This optical examination was performed again at the end of the training. Finally, in case the training with single Gabor patches resulted in a VA improvement less than 0.5 LogMAR, participants were offered the chance to participate in a second training which employed the lateral masking paradigm (Polat, 2004; Tan & Fong, 2008; Durrie & McMin, 2007) this also allowed us to compare the results of training with single Gabors to lateral interaction training. The same battery of pre- and post-tests were administered after this second training, in order to assess the presence of any further improvement.

4.3 Results

Each Pre/Post-tests were analysed independently in order to identify whether any improvement has been made following the training. VA tests results (Landolt-C) were assessed by using a one-way ANOVA with "Time" (pre-, post-test and follow-up measurements) as main factor, followed by simple contrasts and Bonferroni-corrected post-hoc t-tests. The results revealed a significant main effect of time ($F_{2,14}=4.72$, $p<.05$, $\eta^2_p=0.4$). Simple contrast showed a significant VA improvement (see Figure 13) from pre- to post-test values ($F_{1,7}=7.95$, $p<.05$, $\eta^2_p=0.53$). However, no significant differences were found from pre- to follow-up test values ($F_{1,7}=3.64$, $p>.05$, $\eta^2_p=0.34$). The improvement of 1.6 LogMAR from pre- (0.427 LogMAR) to post-tests (0.267 LogMAR) decreased by only 10% at follow-up tests (0.283 LogMAR) despite yielding a statistically non-significant result. This may be due to a reduced sample size in the analysis since 2 participants dropped out following the post-tests and did not carry out the follow up assessment.

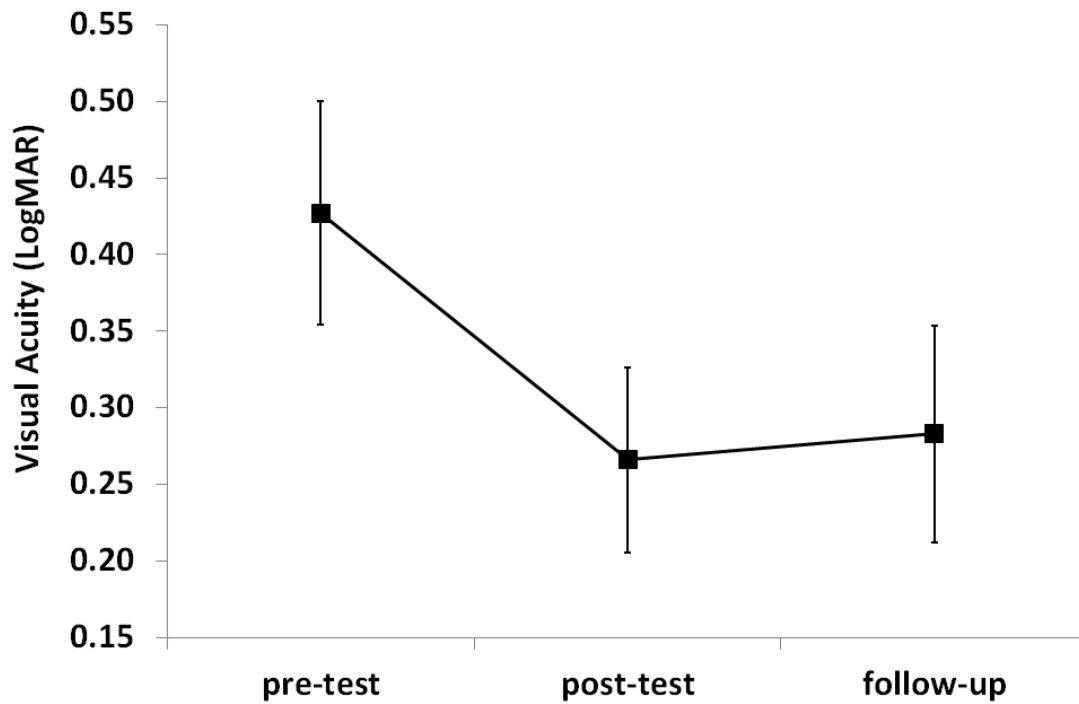


Figure 13: Mean VA measured with Landolt C before the training (pre-test), after the training (post-test) and at 2 months from the end of the training (follow-up). Error bars represent the standard error of the mean (SE).

For what concerns Vernier hyperacuity, despite average pre-training hyperacuity was reported as 92.78 arcsecs and post-training hyperacuity at 61.85 arcsecs, a paired-samples t-test did not reveal any significant difference ($t_9=1.85$, $p>.05$). CS test results (Figure 14) were assessed using a two-way ANOVA with “Time” (pre- post- measurements) and “Spatial Frequency” (1, 3, 5, 7, 9, 11, 15 cpd) as main factors. No significant main effect of time was yielded ($F_{1,8}=0.88$, $p>.05$, $\eta^2_p=0.1$). Despite a significant interaction time by spatial frequency ($F_{6,48}=2.83$, $p<.05$, $\eta^2_p=0.26$), and a trend of improvement following the training at

3 and 9 cpd, post-hoc t-tests revealed no statistically significant effect of time for any of the tested spatial frequencies (all $p > .05$).

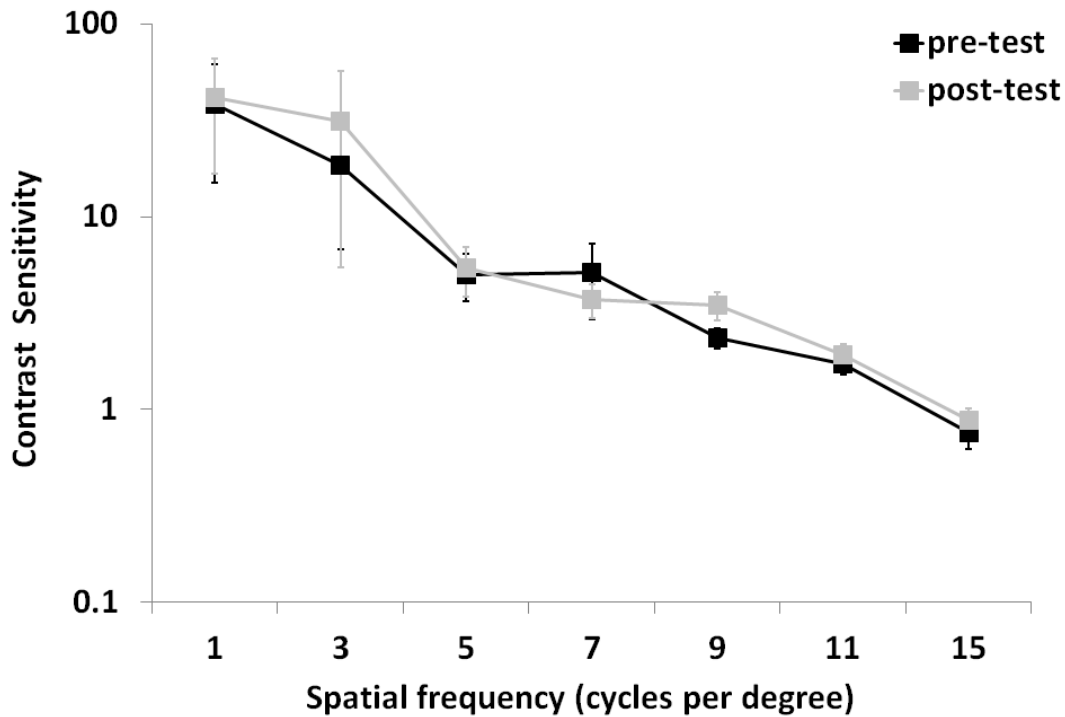


Figure 14. Mean CS function measured before (pre-test) and after the training (post-test). Error bars represent the SE.

Lateral interactions tests assessed using a two-way ANOVA with “Time” (pre-, post-test measurements) and “Target-to-Flankers Distance” (2, 3, 4 and 8 λ) as main factors, revealed no statistically significant results of time ($F_{1,9}=0.09$, $p > .05$, $\eta^2_p=0.01$), target-to-flankers distance ($F_{3,27}=2.56$, $p > .05$, $\eta^2_p=0.22$), or interaction ($F_{3,27}=2.5$, $p > .05$, $\eta^2_p=0.22$). In order to analyse the effects of single Gabor training on lateral interactions, the two spatial frequencies which were

used in the pre- and post-tests were grouped together and a facilitation index ($8l - 4l$) and an inhibitory index ($8l - 2l$) were calculated (Figure 15). A two-way ANOVA (pre- vs post-test, facilitatory vs inhibitory) revealed no significant differences between pre- and post-test results ($F_{1,9}=0.06$, $p>.05$, $\eta^2_p=0.007$), and no significant differences between facilitatory and inhibitory indices ($F_{1,9}=1.6$, $p>.05$, $\eta^2_p=0.15$), or interaction ($F_{1,9}=3.33$, $p>.05$, $\eta^2_p=0.27$).

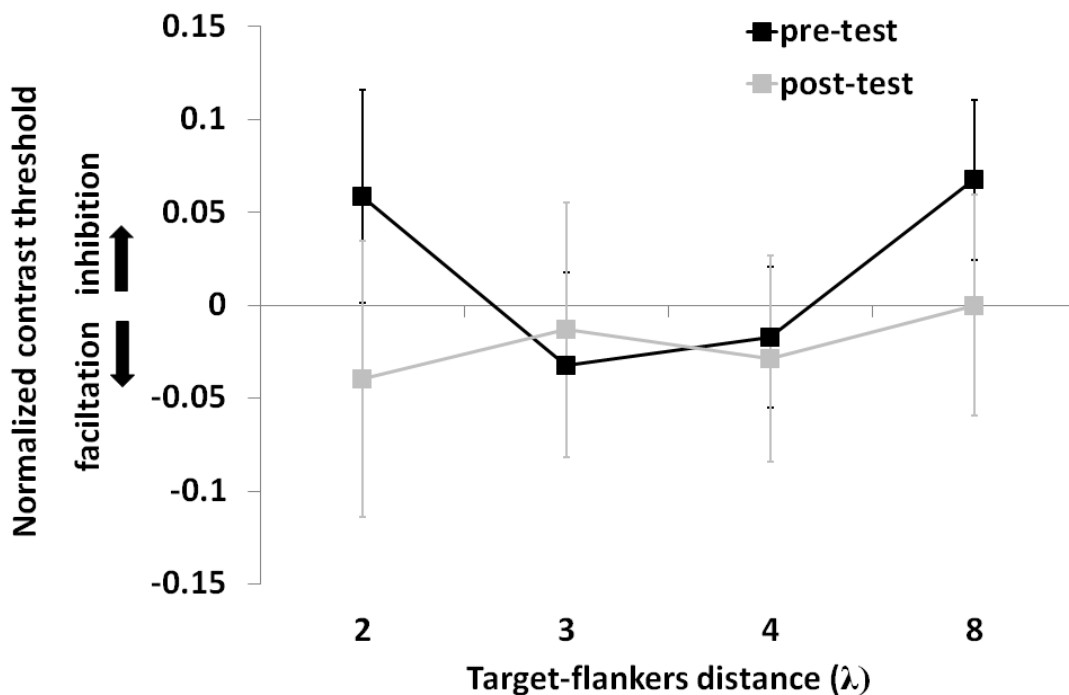


Figure 15. Mean normalized contrast thresholds (threshold differences respect to baseline thresholds without flankers) in the lateral masking paradigm as a function of target-flankers distance (λ), before (pre-test) and after the training (post-test). Positive values indicate inhibitory effects of the flankers, negative values facilitatory effects. Error bars represent the SE.

In order to assess whether training modified the optical characteristics of the eye, measurement of pupil size was taken in scotopic conditions for each

participant. Mean pupil size before and after training were respectively 4.33 mm and 4.35 mm for the right eye, and 4.32 mm and 4.37 mm for the left eye. A two-way ANOVA with time (pre vs post) and eye as factors showed no significant differences between pupil size before and after training ($F_{1,9}=0.17$, $p>.05$, $\eta^2_p=0.019$).

The three participants who obtained a VA improvement of less than 0.5 LogMAR agreed to participate in the 1 month re-training with lateral masking stimuli. Following the retraining, participants showed an additional improvement in their VA ranging from 1 to 2.8 LogMAR, and a consistent improvement in their CS at all tested spatial frequencies (improvement ranging from 10% to 100%). No further improvement in Vernier acuity was found in either participant.

4.4 Discussion

In the present study we investigated the effects of single Gabor training, in the absence of lateral masking, on the outcome of VA and CS in a group of individuals with mild myopia (maximum -2 Diopters). The results taken from this group of participants indicate that training using a single Gabor protocol resulted, on average, in a transfer of improvement on VA of 1.6 LogMAR following 24 training sessions, with 1 participant out of 10 that worsened his VA. This degree of improvement, although both statistically significant and clinically relevant, is not as strong in magnitude as that which is found in training protocols using lateral masking, which is reported as being an improvement of

2.2 LogMAR in various groups of visual difficulties, namely, myopia, presbyopia and amblyopia (Polat, 2004, 2012; Durrie & McMinn, 2007; Tan & Fong, 2008). The non-significant improvement in CS and Vernier acuity support the idea of only a limited effect of single Gabor training on visual cortical processing. In fact, the VA and CS improvement of 2 participants (who did not improve with single Gabor training) upon re-training with lateral masking paradigm, further suggests that optimal tuning of visual cortical processing able to overcome blurred images due to mild refractive defects, likely requires the strengthening of facilitatory and inhibitory lateral interactions between collinear detectors, brought about through lateral masking. The absence of significant differences between the various target-to-flanker distances and between facilitatory and inhibitory indices suggests that these (uncorrected) myopic participants might have altered lateral interactions between collinear detectors, that are not boosted with single Gabor training. In fact, looking at Figure 15, single Gabor training seems to flatten the (non-significant) trend of facilitation showed at 3λ and 4λ at pre-test. Indeed, the present results of the lateral interaction pre/post tests reveal that the single Gabor training has no effect in significantly modulating collinear lateral interactions between detectors.

Nevertheless and despite high inter-participant variability in VA improvement, the single Gabor training did improve VA up to 4.4 LogMAR. Although in principle, such improvement in VA could also be due to a more efficacious attentional focusing, the presence of an auditory cue both in the training task and in the VA task (besides the fact that in the VA task the stimulus was shown with no time limits) should have already produced an optimal focus

of attention, with not much room for further enhancement. Additionally, the specific improvement of VA performance (whereas CS did not improve) does not support the idea of high-level learning of rules for performing a visual discrimination task (Zhang, Cong, Levi, Klein & Yu, 2014).

The distinct organic differences between myopia and amblyopia, calls into question whether rehabilitation of visual functioning on an organic and functional level follows the same processes and thus requires the same conditions of learning. In amblyopia, studies reveal improved visual functioning following PL both in the presence and in the absence of flankers, although larger improvements are found with the lateral masking paradigm (Zhou et al., 2006, Polat et al., 2004 & Huang, et al., 2009; Zhang et al., 2014). The underlying mechanisms proposed are said to involve strengthening connections through Hebbian learning, resulting in recovery of function implicating various lateral, feedforward and feedback mechanisms (e.g. Rosa, Silva, Ferreira, Murta, & Castelo-Branco, 2013, Li & Levi, 2004; Polat et al., 2004). In myopia, thus far, no study has investigated whether training in the absence of flankers can transfer to improved VA and CS. The present study suggests that when there is no cortical deficit, such as in refractive defects, some sort of compensatory mechanism can take place at the cortical level through PL, even in the absence of lateral masking, which results in more effective processing of the received blurred input, although CS does not seem to be affected. Training to detect low-contrast, small Gabor stimuli could have increased the ability of the visual system to detect small signals in noise (blurred image), thus transferring this ability to VA, with no strong modifications of lateral interactions between detectors responding to

oriented, collinear stimuli. In fact, when lateral masking paradigms have been used (Durrie & McMinn, 2007; Tan & Fong, 2008), a larger improvement on VA and CS was found, indicating that, not just in amblyopia, but also in refractive defects, a modification of the strength of lateral interactions is necessary for an optimal recovery of blurred vision.

Our findings related to CS are not as suggestive as that which was found for VA improvement, as no statistically significant effects of single Gabor training were found on the improvement of CS. On the contrary, training protocols implementing lateral masking techniques have shown a transfer on both VA as well as CS. As mentioned earlier on, the combination of neural interactions at various spatial frequencies results in an individual's CSF (Polat, 1999). The CS results of the present study may signify that a lack of lateral masking may have hindered neuronal lateral interactions reported to be the building blocks of CSF. An alternative explanation of the disparity of results found with single Gabor versus lateral masking training could reside in the variations of training protocols and stimulus characteristics. This study has investigated single Gabor training using a fixed protocol which trained using 3 different spatial frequencies and 4 different orientations, whereas studies using lateral masking technique have used a more individualized algorithm where the choice of the stimulus parameters were tailored depending of the performance of each subject during the training. Although in the present study each participant trained on four orientations and three difficulty levels of spatial frequency according to individual performances in pre-training CS tests, stimulus selection was not tailor made for each participant using a specific algorithm. This may

account for the lower degree of improvement when compared to training using lateral interaction protocols (Durrie & McMinn, 2007; Tan & Fong, 2008). Another difference between the present study and lateral masking studies concerns the duration of the training. Whereas in this case the duration was fixed and equal to 24 sessions, in other studies, the training duration was also tailored on the performance of participants and was, on average, slightly longer. For example, in the study by Tan and Fong an improvement of 2.1 logMAR was found over a training period between 20 and 30 sessions over 3 months. Another study using lateral masking in myopia also found an improvement of 2.2 logMAR following 30 training sessions (Durrie & McMinn, 2007). An improvement of 2 logMAR was also achieved in a study on presbyopia using lateral masking over 37.4 (± 10.7) training sessions (Polat et al., 2012).

However, despite the fact that these alternative explanations cannot be ignored, the single data obtained on re-training with the lateral masking technique on participants that did not improve with the single Gabor training, suggests stronger reliability of lateral masking as a training for improving visual functions. Indeed, the participants who achieved small improvements on either VA or CS with 24 sessions of training with single Gabors, seemed to have improved both in both measures with an additional 12 sessions of training using a lateral masking paradigm. Due to the small sample size, this retraining data is only clinically indicative and adequate statistical analysis is not possible.

Acknowledgements

A version of this chapter has been published in the Journal: *Attention Perception & Psychophysics* and has been accordingly cited in the reference section: Camilleri, R., Pavan, A., Ghin, F., & Campana, G. (2014a). Improving myopia via perceptual learning: is training with lateral masking the only (or the most) efficacious technique? *Attention, Perception, & Psychophysics*, 76(8), 2485-2494.

CHAPTER 5 The application of online transcranial random noise stimulation and perceptual learning in the improvement of visual functions in mild myopia

5.1. Introduction

In adults, visual sensory maps are plastic, able to undergo network reorganization in response to injury and experience. From a structural standpoint, brain plasticity entails the potential of neurons to change their synaptic connections (Ashford and Jarvik, 1985). While the mechanisms involved are still an ongoing query, it is clear that visual cortex plasticity at the synaptic and cellular level is achievable in adults (Frégnac, Shulz, Thorpe & Bienenstock, 1988; Godde, Leonhardt, Cords & Dinse, 2002; Karni & Sagi, 1991; Sale, De Pasquale, Bonaccorsi, Pietra, Olivieri, Berardi & Maffei, 2011; Walsh, Ashbridge & Cowey, 1998). This notion of visual network plasticity is paramount not only in helping us achieve a better understanding of the human visual system and of visual plasticity mechanisms, but also in identifying non invasive treatment tools and protocols to provide visual rehabilitation. In the case of refractive defects such as myopia, being able to manipulate neuroplasticity might help us achieve visual recovery through compensatory strategies.

As was discussed in great detail in Chapter 2, to date, the most common non-invasive, behavioural method implemented to boost visual network plasticity and achieve recovery of function in a variety of visual disorders is known as PL, which is the improvement on a visual task following repeated

practice on the same or on a related task. Such improvements following practice are seen as a manifestation of neural plasticity, and since these functional improvements are long lasting, LTP is likely the mechanism underlying such visual gains (Nitsche et al., 2009; Levi & Li, 2009; Polat, 2009; Sagi, 2011). In general, most studies point to a localized increase in processing efficiency in V1 following practice on a visual perceptual task. This change can be attributed to a specific and localised plasticity in V1 or alternatively to a more complex network involving changes in the inputs V1 receives from other higher order brain regions following practice (top-down modulation). So far, PL has been shown to be effective in improving, among other dysfunctions, visual abilities in amblyopia (Campana et al., 2014; Hussain et al., 2012; Levi and Li, 2009; Li et al., 2005; Polat et al., 2004; Polat, 2009; Zhou et al., 2006), mild refractive defects (myopia: Tan and Fong, 2008; Camilleri et al., 2014a; presbyopia: Polat et al., 2012), central or peripheral vision loss and cortical blindness (Chung, 2011; Das et al., 2014; Huxlin et al., 2009; Kasten et al., 1998, Sabel et al., 2005).

Despite its proven effectiveness, PL techniques require lengthy protocols in order to yield effective outcomes (usually a minimum of two months training of up to three to four times weekly) (e.g. Camilleri et al., 2014a; Polat et al., 2004; Tan and Fong, 2008). Random noise stimulation could optimize the effects of a behavioural training with measurable changes in the brain by modulating neuronal excitability that are involved in LTP (Fritsch et al., 2010; Stagg et al., 2009) which may ultimately lead to neuroplasticity. tRNS is an innovative method of boosting neural plasticity and accelerating the neuro-plastic effects of PL through the application of a weak alternating current at random frequencies

(0.1–640 Hz). Unlike tDCS, tRNS has only recently been explored within the visual domain (Camilleri et al, 2014b; Campana et al., 2014; Fertonani et al., 2011; Pirulli et al., 2013). The question still remains as to what is accountable for this accelerated improvement and which treatment protocols are most suitable.

The aforementioned studies were unable to determine whether it is specifically the combined use of the techniques that brought about this fast improvement or whether tRNS alone is able to achieve the same outcome on the visual system in the absence of any behavioural training or whether there may be any potential placebo effect of the electrical stimulation. This may also address the issue on the mechanisms by which random noise stimulation, as opposed to direct current stimulation, influences neural plasticity. By using a between-groups approach where participants are trained, using a contrast detection training (Camilleri et al., 2014a,b; Zhou et al., 2006), with concurrent tRNS, or Sham stimulation, or else receive tRNS with no behavioural training, the aim of this study is to isolate the contribution of PL, tRNS and the combination of both. Given the previous results (discussed in chapter 4) demonstrating some effect of single Gabor contrast detection training in mild myopia, the present study chose to implement the same PL protocol in order to evaluate its effectiveness when combined with tRNS. Furthermore, this work focuses on the potential application of TES, specifically tRNS, together with behavioural trainings, as a new approach to further ameliorate visual outcomes of existing training paradigms.

5.2. Methods

5.2.1 Participants

Thirty participants with mild myopia were recruited from the University of Padova (mean age of 25.31, ranging between 19 and 29). The participants were randomly assigned to one of three different treatment groups each consisting of 10 participants. The first group carried out a 2-week (8 sessions) behavioural training using a contrast detection task combined with online high frequency tRNS (hf-tRNS) for 25 minutes of stimulation. The second group took part in the same training protocol but combined with Sham stimulation. The third group of participants carried out 25 minutes of hf-tRNS in the absence of any behavioural training. This was done in order to compare the effects of combining behavioural training with tRNS with the effects of behavioural training alone (without tRNS) and tRNS alone on UCVA and UCCS.

All participants fit the following inclusion criteria: refractive error up to 2 diopters (D) in either eye (minimum was -0.75D), with astigmatism not exceeding -0.5D in either eye. All tests and the behavioural training were administered binocularly and without the use of optical corrections. All participants had a stable refractive index for the 6 months preceding the training. Exclusion criteria included the presence of any other condition for reduced VA other than simple myopia and/or mild astigmatism, including pregnancy, diabetes mellitus, presence of myopia-related ocular complications and any previous ocular surgery. To ensure the inclusion and exclusion criteria were met, prior to commencement of the training, the participants carried out a detailed assessment by an optometrist. Additionally, each participant in the two tRNS

groups filled in a questionnaire to check that all were eligible to undergo non-invasive brain stimulation (e.g.: no history of seizures, no internal metal objects or previous traumatic brain injury). The Local Ethics Committee approved this study.

5.2.2 Experimental Procedure

Prior to (pre-tests) and after the training (with and without tRNS) (post-tests), uncorrected VA and uncorrected CS were measured for each participant by using Landolt C and Grating tests of the Freiburg Visual Acuity Test (FrACT, Bach, 1996, 2007). The Best-Pest adaptive procedure was used to calculate the absolute threshold for each of these tests. Stimulus duration lasted until the participants' response. An auditory cue was presented upon stimulus presentation and a different auditory cue was implemented as feedback for error responses.

The Landolt C test was used to assess uncorrected VA. The task of the participants was to indicate, in every trial, the orientation of the gap of the Landolt C out of eight possible orientations. Contrast stimuli for the uncorrected CS assessment consisted of sinusoidal gratings presented in a circular window with a narrow Gaussian taper. Size of the gratings was 3 deg. Grating orientations used were 0, 45, 90 or 135 deg. The task of the participant was to discriminate the orientation of the grating at different spatial frequencies, ranging from 1 to 15 cpd, in separate blocks.

The behavioural (training) paradigm consisted of a two-interval forced choice (2IFC) task where the participants had to detect the presence of a single

Gabor Patch, which changed in contrast according to the performance of the participant. The threshold corresponding to 79.4% of correct detection was determined by using a 1up/3down staircase procedure (Levitt, 1971). Stimuli used in the training were comprised of Gabor patches consisting of a cosinusoidal carrier enveloped by a stationary Gaussian. Standard deviation of the luminance Gaussian envelope (σ) was equal to the sinusoidal wavelength (λ); therefore, the size of the Gabor patch covaried with its spatial frequency. Additionally, the spatial phase of the cosinusoidal carrier equalled to zero (evenly symmetric Gabor patch). Stimulus duration lasted 200ms. In order to reduce spatial and temporal uncertainty both an auditory and a spatial cue were implemented. On each trial a central fixation point preceded the presentation of each interval, and an auditory cue indicated when the stimulus (if present) appeared. Performance feedback was also provided to the participants in the form of an auditory beep following an incorrect response.

Participants in groups 1 and 2 underwent 8 training sessions over 2 weeks (4 consecutive sessions each week) and trained on 4 different orientations of the stimulus with a single spatial frequency (ranging from 6 to 15 cpd), which were chosen according to the individual's cutoff performance in the pretest uncorrected CS measurement, defined as the spatial frequency at which the estimated contrast threshold from pre-training uncorrected CS measurements was closest to 0.50 (Michelson contrast) (Zhou et al., 2006). Since interleaving different stimulus conditions (roving) has been shown to hinder PL (Herzog, Aberg, Frémaux, Gerstner, et al., 2012; Kuai, Zhang, Klein, Levi, et al., 2005), in order to increase the efficacy of PL, participants were trained on the same

orientation for 2 consecutive days. Each session consisted of 8 blocks each containing 60 trials, which lasted for approximately 45 minutes.

Participants in group 1 were administered hf-tRNS (1.5mA) during the first 25 minutes of each session, which covered the first 5 blocks (Fertonani et al., 2011). Participants in group 2 underwent Sham stimulation for the same length of time. In the Sham condition, the stimulation was a placebo for all eight blocks and was delivered for 20s at the beginning of each block. Participants in group 3 were administered hf-tRNS (1.5mA) for 25 minutes, without any concurrent task. This matches the stimulation parameters of group 1.

5.2.4 Apparatus and tRNS

Both the behavioural training and pre/post tests were displayed on a 22-inch Philips Brilliance 202P4 luminance-calibrated (gamma-corrected with $\gamma = 1$) monitor with a refresh rate of 60 Hz and a resolution of 1280 x 1024 pixels. The stimuli used in the training were created with the Matlab Psychtoolbox (Brainard, 1997; Pelli, 1997), whereas stimuli for measuring VA in the pre and post-tests were generated using the Freiburg Acuity and Contrast Test (FrACT 3.8, Bach, 1996, 2007). Spatial dithering (Bach, 1997) and colour bit stealing (Tyler, 1997) for increasing the depth of contrast resolution (12 bits) were enabled on the FrACT, thus allowing precise CS measurement. The screen resolution was 1280x1024 pixels, each pixel subtended 0.33 arcmin at a viewing distance of 3 meters, and 0.67 arcmin at a viewing distance of 1.5 meters. Viewing distance was equal to 3 meters for pre- and post-tests, whereas the training was administered from 1.5 meters. All stimuli were presented centrally and both the

tests and training were carried out in a dark, silent room. Background screen luminance (corresponding to mean luminance of Gabor stimuli) was 31.5 cd/m².

The high frequency tRNS was delivered to groups 1 and 3 using a battery-driven stimulator (BrainSTIM, EMS) through a pair of saline-soaked sponge electrodes. The tRNS consisted of an alternating current of 1.5 mA intensity with a 0mA offset applied at random frequencies. The frequencies ranged from 100 to 640Hz (high frequency range). This stimulation protocol has been demonstrated efficacious in boosting PL in previous studies (Camilleri et al., 2014b; Fertonani et al., 2011; Pirulli et al., 2013). The active electrode had an area of 16 cm² and was placed over the occipital cortex measured at ~3 cm above the inion. The reference electrode had an area of 60 cm² and was placed on the forehead. The current density was always maintained below the safety limits (below 1 A/m²) (Poreisz et al., 2007). The electrodes were kept in place with bandages.

5.3. Results

All data were subject to the Kolmogorov-Smirnov test of normality. Pre- and post-tests of uncorrected VA measurements, for each of the three groups, were normally distributed ($p > .05$), therefore ANOVAs and t-tests were used for this data. For what concerns Log-transformed UCCS, pre- and post-tests with various spatial frequencies in the three groups, were not normally distributed ($p < .05$), therefore the non-parametric Wilcoxon signed-rank test was used, separately for each group and for each spatial frequency, to assess differences between pre- and post-tests.

A mixed design ANOVA with 'group' as the between subject factor was used to compare pre- and post-test measurements of uncorrected VA. A significant interaction of pre/post by group indicated that the groups differed in their pre versus post outcome ($F_{2,27}=14.481$, $p<.0001$, $\eta^2_p=0.518$). Post hoc analysis was carried out using t-tests which revealed a significant difference for pre and post test in group 1 (PL with tRNS) ($t_9=4.474$, $p<.01$). Results indicate an improvement of 0.171 LogMAR from a baseline measurement of 0.337 LogMAR to a post-test reading of 0.166 LogMAR (Figure 16). No significant differences were observed for either group 2 (PL with Sham stimulation, $t_9=-1,221$, $p>.05$) or group 3 (tRNS alone, $t_9=-0,295$, $p>.05$).

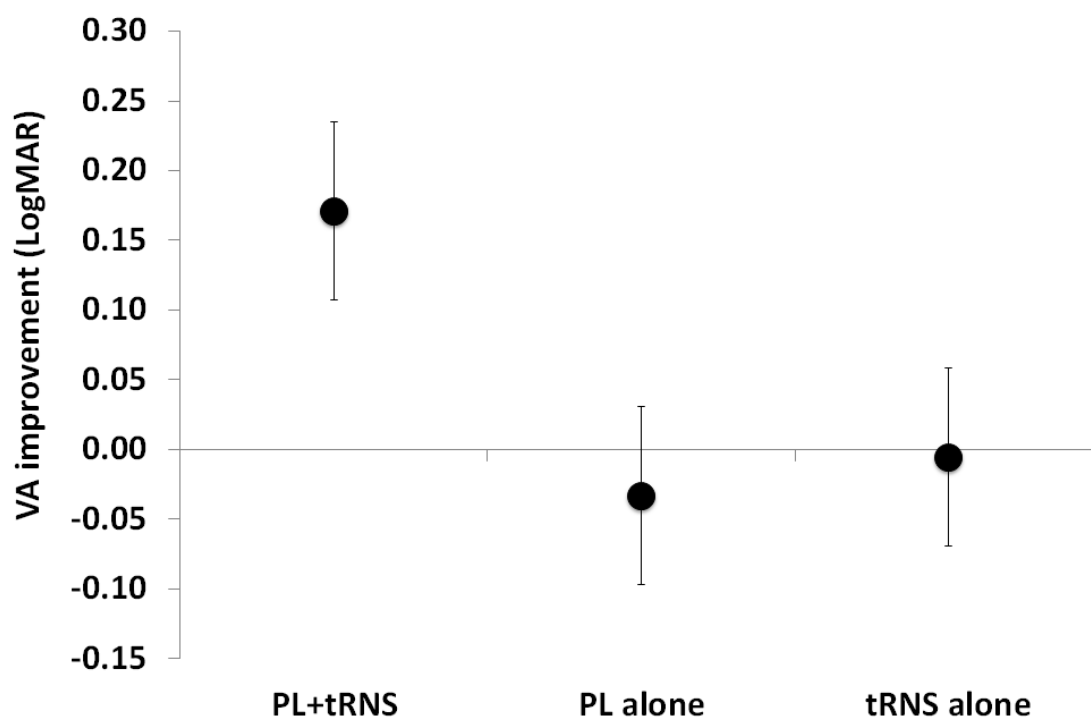


Figure 16: The mean uncorrected VA improvement (LogMAR difference) between pre- and post-test is shown for each of the three groups of participants. Error bars represent ± 1 SEM.

With regards to UCCS, pre- and post-test measurements were compared with a Wilcoxon signed-rank test on the Log-transformed CS data. Participants undergoing both random noise stimulation and behavioural training (group 1) improved significantly at the following spatial frequencies: 3 cpd ($Z=-1.988$, $p<.05$), 5cpd ($Z=-2.293$, $p<.05$), 7 cpd ($Z=-2.09$, $p<.05$), 9 cpd ($Z=-2.191$, $p<.05$), and 11 cpd ($Z=-2.599$, $p<.01$). No significant improvements were seen at the lowest (1 cpd) and highest (15 cpd) tested spatial frequencies (Figure 17). In group 2 (PL plus Sham stimulation), although at 15cpd the pre-post difference was approaching significance ($Z=-1.886$, $p=.064$), no significant differences were observed at any tested special frequency. Interestingly in group 3, which included only tRNS, a significant difference was found at 9cpd ($Z=-1.988$, $p=<.05$), while 15cpd was close to being significant ($Z=-1.886$, $p=.064$).

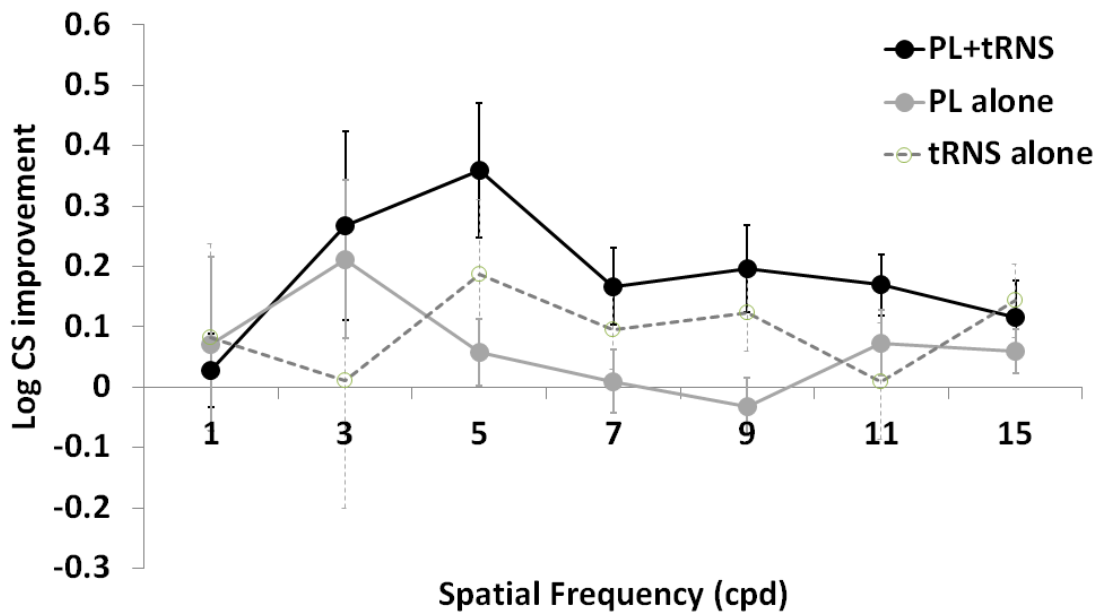


Figure 17: The mean UCCS improvement (Log-transformed difference) between pre- and post-test is shown for each tested spatial frequency, separately for each of the three groups of participants. Error bars represent ± 1 SEM.

5.4. Discussion

The present study focuses on the application of tRNS together with a visual training, specifically, a single Gabor contrast detection paradigm to further enhance the outcome of existing PL regimes so as to improve visual defects. In line with a previous study carried out on participants with myopia, this work identifies tRNS as a valuable tool for improving visual defects in mild myopia (Camilleri et al., 2014b). Moreover, it seeks to investigate the specific role of tRNS in visual PL, i.e. whether it is only effective when combined with a behavioural task or whether similar results can also be attained with a Sham group or

perhaps in the absence of a behavioural task (stimulation alone). The obtained improvements in UCVA and UCCS in the combined treatment group suggest a specific mechanism underlying the effects by which tRNS acts: it seems to require external sensory (visual) input, thereby acting on the neurons activated by the task at hand. This idea is further strengthened by the lack of significant improvement when tRNS was applied in isolation of a behavioural task. Interestingly however, in the absence of the behavioural training, tRNS resulted in improved CS at 2 high spatial frequencies (9 and 15cpd). Some improvement in CS with the sole use of brain stimulation, so far, has been observed in individuals with amblyopia after the administration of either anodal tDCS (Spiegel, Byblow, Hess & Thompson, 2013) or high-frequency repetitive TMS (Clavagnier, Thompson & Hess, 2013; Thompson, Mansouri, Koski & Hess, 2008).

Although here, improvement on CS is much smaller than when combined with PL, suggesting a weaker effect of tRNS in the absence of a behavioural task. These results may be explained by the underlying phenomenon of stochastic resonance (Terney et al., 2008). In the present study, the random noise stimulation at frequencies between 100 and 650 Hz may be interacting with the neurons already activated by the low contrast Gabor stimuli and by doing so, enhance their firing response (increasing signal to noise ratio), whilst preventing the network from becoming desensitized and progress to a homeostatic state as can occur with tDCS. On the other hand, when tRNS is applied in the absence of any input, the stimulation is unspecific and may simply add noise to the system. In fact, there is emerging consensus that the effects of brain stimulation

techniques are highly dependent on the state of the stimulated neuronal population (Silvanto, Muggleton & Walsh, 2008). For example, in a study investigating the effects of tRNS on motor evoked potentials (MEPs), the authors conclude that external induction of neuronal plasticity (such as in the case of brain stimulation) is highly dependent on the state of the participant during stimulation (Terney et al., 2008).

Since the seminal paper of Bliss & Lomo (1973), it is well established that high frequency stimulation is able to produce LTP through strengthening of synaptic connections. More recently, it has been suggested that also noisy electrical fluctuations are able to boost synaptic signals (Moss et al., 2004). Interestingly, oscillations within a frequency range of 80–200 Hz included in the high frequency band, have been associated with plasticity processes (Grenier et al., 2001) and learning (Ponomarenko et al., 2008). Another recent study by Fertoni and colleagues (2011) explains how the repetitive action of tRNS may induce direct temporal summation of neural activity and may desynchronise (pathological or inefficient) rhythms by increasing the signal to noise ratio. A very recent study proposes that, unlike tDCS, tRNS-induced plasticity is independent of NMDA receptors and involves the modulation of voltage-gated sodium channels (Chaieb, Antal & Paulus, 2015). Due to the recurring potentiation of sodium channels, its aftereffects through LTP may outlast those observed after tDCS stimulation. The aftereffects of tRNS on cortical excitability have recently been evaluated in the motor cortex by measuring the participants MEPs following 4, 5 and 6 minutes of stimulation (Chaieb et al., 2011). The researchers observed that increased cortical excitability following 5 minutes of

tRNS lasted only for 10 minutes. Whereas 6 minutes of tRNS induced an even stronger excitability increase of up to 30 minutes post stimulation.

The application of TES as a potential tool in neuro-rehabilitation is a relatively young concept. Yet many studies are seeking to understand the mechanisms by which different TES techniques can complement an existing cognitive training (e.g. Yun, Chun & Kim, 2015, Dhaliwal, Meek & Modirrousta, 2015; Krause & Kadosh, 2013). TES is non invasive and if used correctly should not lead to any aversive effects, it is relatively cheap and can be implemented in various contexts as an adjunct to existing techniques, which although are effective in isolation, might not be offering the most optimal treatment to patients. tRNS, a younger sister of tDCS, has not featured in as many studies, yet due to the mechanisms by which it acts and its lack of discomfort, is starting to capture the attention of many clinical researchers. The present study identifies the potential this device has in assisting existing visual rehabilitation methods, such as PL, and encourages further insight into the exact mechanisms by which it is acting. In line with the present work, a recent study by Campana and colleagues, demonstrated how hf - tRN stimulation applied to V1 in combination with a lateral masking paradigm results in a significant improvement in VA and CS in the amblyopic eye of participants (Campana et al., 2014). Furthermore, the improvements following combined tRNS and PL in mild myopia, using the same protocol as in the present study, have been shown to be maintained for up to 3 months post training (Camilleri et al., 2014b).

Following these positive results, a larger clinical study is paramount in order to investigate more reliably, the effectiveness of these techniques in other

clinical populations. In addition, it is necessary that follow-up measures are taken post-training to establish long-term effects while allowing for flexible re-application of the training. It is still unclear what relevance these improvements will have in a real-life setting outside the laboratory. Additional use of questionnaires and self-reports assessing day-to-day improved vision is essential.

CHAPTER 6 Probing neural plasticity in the amblyopic cortex through the combined use of transcranial random noise stimulation and perceptual learning

6.1 Introduction

Amblyopia, also referred to as “lazy eye”, is a developmental disorder explained by impairments in spatial vision in the absence of any organic ocular defects (Ciuffreda, Levi & Selenow, 1991; McKee, Levi & Movshon, 2003; Robaei, Rose, Ojaimi, Kifley, et al., 2006). Impairments comprise of a reduction in VA, CSF and Vernier acuity, abnormal spatial interactions (Levi, Hariharan & Klein, 2002; Polat, Sagi & Norcia, 1997) or deficiencies in stereopsis (Wallace, Lazar, Melia, Birch, Holmes, Hopkins, et al., 2011). It is believed to be due to an atypical pattern of functional connectivity within the primary visual cortex, in particular of neurons selective for orientation and spatial frequency (Polat, 1999), thus causing abnormal processing of visual information coming from one or both eyes (but typically only one eye is involved). Until recently, amblyopia was thought to be untreatable after the “critical period” spanning up to the first decade of life (Epelbaum, Milleret, Buisseret, & Dufier, 1993; Greenwald & Parks, 1999; Loudon, Polling & Simonsz, 2002), due to diminished neural plasticity within the visual cortex that would limit any anatomical, physiological or functional changes (Berardi, Pizzorusso, Ratto, & Maffei, 2003).

Numerous studies, however, have reported large and stimulus-specific performance improvements (PL) in normal adults following training in a variety of visual tasks (Fiorentini & Berardi, 1981; Karni & Sagi, 1991; Poggio, Fahle & Edelman, 1992; Schoups, Vogels & Orban, 1995; see Sagi, 2011 for a review), pointing to neuronal plasticity at early levels of the adult visual system (Pourtois, Rauss, Vuilleumier & Schwartz, 2008; Schoups, Vogels, Qian & Orban, 2001). In fact, over the past 15 years, marked improvements of various visual functions in adults with amblyopia, following extensive sessions of PL, have been reported (see Levi & Li, 2009 and Polat, 2009; Astle, Webb, & McGraw, 2011 for recent reviews). As pointed out earlier, the task that obtained the largest improvement ratio on both VA and CS measurements was a contrast detection task using a lateral masking procedure (Polat et al., 2004). Focusing on the abnormal spatial interactions in amblyopia, Polat and colleagues (2004) used a training procedure that allowed a strengthening of facilitatory lateral interactions and a weakening of inhibitory lateral interactions between detectors tuned to specific orientations and spatial frequencies, thus obtaining a large and consistent improvement in VA (78% gain, equal to 0.25 LogMAR improvement) and CSF (improvement ranging from 2.05 to 4.23 times) in adults with amblyopia. A well-known drawback however of this, and similar training paradigms, however, is the large number of sessions required to achieve the reported improvements (from 30 to 80 sessions) which could either discourage patients from starting the training or may lead to a high number of dropouts. In light of this, recent studies have pointed out how non-invasive transcranial brain stimulation techniques are able to boost PL in normal observers. In particular, it has been shown that online

transcranial electrical stimulation using random frequencies in the high-frequency range (hf-tRNS), is the most efficacious type of electrical stimulation for enhancing and accelerating within-session contrast detection (Fertonani et al., 2011; Pirulli et al., 2013).

In the present study, the effects of a short PL (8 sessions) combined with hf-tRNS or Sham stimulation, was investigated on the resulting VA and CS improvement in two groups of patients with anisometric amblyopia.

6.2 Methods

6.2.1 Participants

Seventeen participants with anisometric amblyopia were recruited at the San Paolo Ophthalmic Center of San Antonio Hospital (Padova, Italy) during routine ophthalmological assessment (mean age of 35.4, ranging between 26 and 52). The participants were divided into two groups, both of which were enrolled in a 2-week (8 sessions) behavioural training programme using a contrast detection task under lateral masking conditions (Polat et al., 2004; Polat, 2008). Group 1 (PL plus tRNS) underwent online hf-tRNS during the first 20 minutes of the training while the second group underwent Sham stimulation (PL plus Sham). It is necessary to point out that due to blindness in the non-amblyopic eye of one participant in the Sham group, data from the untrained eye is missing. Furthermore, one participant in group 1 did not register CS values for the lowest and highest spatial frequencies and one participant in group 2 (for the highest spatial frequency) due to a fault in the programme. Participants were unaware of the type of stimulation being administered and were informed that two different

types of stimulation parameters were being investigated. At the end of the training, participants in the Sham group (group 2) were given the chance to participate in another session using hf-tRNS.

All pre/post tests were administered monocularly on either eye and with the best optical correction. Perceptual training was also administered monocularly on the amblyopic eye with the best optical correction. Exclusion criteria included any other ocular condition or cause for reduced VA other than amblyopia, myopia, presbyopia, hypermetropia and/or astigmatism; these include diabetes mellitus, pregnancy, presence of myopia-related ocular complications and any previous ocular surgery. Exclusion criteria also included incompatibility with transcranial electrical stimulation, as assessed with a questionnaire (e.g. history of seizures, skin problems, migraine, etc.). The local Ethics Committee approved the study.

2.2 Experimental Procedure

Before (pre-tests) and after the training (with tRNS) (post-tests), VA and CSF were assessed for each participant by using respectively Landolt C of the Freiburg Visual Acuity Test (FrACT, Bach, 1996), and the CRS Psycho 2.36 test (Cambridge Research Systems Ltd, Rochester, UK) from a viewing distance of 1.5 meters.

VA was measured with an orientation discrimination task (8 possible orientations of the gap of the Landolt C). The Best-Pest adaptive procedure was used to calculate the threshold corresponding to 62.5% of correct discrimination. Stimulus duration lasted until the participants' response. An auditory cue was

presented upon stimulus presentation and a different auditory cue was used as feedback for incorrect responses.

CS was measured with the method of adjustment by asking the participant to adjust the contrast of a vertical sinusoidal grating covering the whole screen (21.3 x 16 deg), with four ascending (from lower to higher grating contrast) and four descending (from higher to lower grating contrast) series. The initial contrast on the first descending series was set according to pilot experiments, ranging from -15 dB (17.78 % contrast) at intermediate spatial frequencies, to 0 dB (100% contrast) at high spatial frequencies. On successive series the starting contrast for each tested spatial frequency was set as the contrast threshold obtained in the previous series, plus (in descending series) or minus (in ascending series) a factor between 6 dB and 10 dB (randomly selected). Increments/decrements were equal to 1 dB. The resulting contrast threshold was the arithmetic mean of the last selected contrast for each of the eight series, independently for each spatial frequency. Each tested spatial frequency (ranging from 0.8 to 14.5 cpd) was presented sequentially starting from the lower spatial frequency and progressively moving on to the higher spatial frequencies; five different spatial frequencies were tested. For each participant, CS at each tested spatial frequency was calculated by averaging across series.

The behavioural training (PL) consisted of a two-interval forced choice (2IFC) task where the participants had to detect the presence of a central Gabor, which changed in contrast according to the performance of the participant, flanked by two high-contrast (0.6 Michelson contrast) collinear Gabors (Figure 1). Gabors were made of a cosinusoidal carrier enveloped by a stationary Gaussian.

Standard deviation of the luminance Gaussian envelope (σ) was equal to the sinusoidal wavelength (λ); that is, the size of the Gabor patches covaried with their spatial frequency. Additionally, the spatial phase of the cosinusoidal carrier equalled to zero (evenly symmetric Gabor patch). Centre-to-centre distance between target and flankers was varied across blocks (1.5, 3, 4 and 8λ). On each session two blocks were administered with the same centre-to-centre distance. The order of presentation always started with the largest distance and ended with the smallest distance. Stimulus duration lasted 200ms. Contrast threshold, corresponding to 79% of correct responses, was determined by using a 1up/3down staircase procedure on the last 8 reversals (Levitt, 1971). In order to reduce spatial and temporal uncertainty both an auditory and a spatial cue were implemented. On each trial a central fixation point preceded the presentation of each interval. Performance feedback was also provided to the participants in the form of an auditory beep following an incorrect response.

Participants underwent 8 training sessions during 2 weeks (4 consecutive sessions per week), and trained on 4 different orientations of the stimulus (that changed every 2 days) with a single spatial frequency, chosen according to the individual's cut-off performance in the pretest CS measurement, defined as the spatial frequency at which the estimated contrast threshold from pre-training CS measurements was 0.50 (Michelson contrast) (Zhou et al., 2006). Trained spatial frequencies ranged from 3 to 12 cpd. Each session consisted of 8 blocks each containing 60 trials, which lasted for approximately 45 minutes. The total training time for each participant, across the two weeks was approximately 6 hours. Follow-up sessions were carried out six months following the end of the

training in order to determine the long-term effectiveness of tRNS combined with PL on VA in amblyopia.

2.3 Apparatus

Training and VA tests were displayed on a 22-inch Philips Brilliance 202P4 monitor with a refresh rate of 60 Hz and a resolution of 1280 × 1024 pixels. The monitor was luminance-calibrated (gamma-corrected with $\gamma = 1$). The stimuli used in the training were generated with the Matlab Psychtoolbox (Brainard, 1997; Pelli, 1997), whereas stimuli for measuring VA were generated using the Freiburg Acuity and Contrast Test (FrACT 3.8, Bach, 1996). All stimuli were presented centrally. Viewing distance was equal to 3 meters for VA tests, whereas the training was administered from 1.5 meters (Polat et al., 2004). Background screen luminance (corresponding to mean luminance of Gabor stimuli) was 31.5 cd/m².

CS tests were displayed on a 17-inch CRT monitor (Brilliance 107P; Philips) with a refresh rate of 70 Hz and a resolution of 1024 × 768 pixels. The monitor was luminance-calibrates with $\gamma = 1$. The stimuli were generated with the CRS Psycho 2.36 test (CRS Psycho 2.36; Cambridge Research Systems Ltd, Rochester, UK) on a computer equipped with a 12-bit resolution graphics card (Cambridge Research Systems Ltd VSG2/3). Viewing distance was equal to 1.5 meters. Background screen luminance (corresponding to mean luminance of the gratings) was 48.5 cd/m². All tests and the training were carried out in a dark and silent room.

2.4 Stimulation Parameters

High frequency transcranial random noise stimulation was delivered using a battery-driven stimulator (BrainSTIM, EMS) through a pair of saline-soaked sponge electrodes. The tRNS consisted of an alternating current of 1.5 mA intensity with a 0mA offset applied at random frequencies. The frequencies ranged from 100 to 640Hz.

The stimulations were applied for approximately 4 minutes (equalling the duration of a training block) during each of the first five training blocks (Fertonani et al., 2011); thus, the total duration of the stimulation was ~20 minutes. This stimulation protocol has been demonstrated efficacious in boosting PL in previous studies (Fertonani et al., 2011; Pirulli et al., 2013). The active electrode had an area of 16 cm² and was placed over the occipital cortex measured at ~3 cm above the inion. The reference electrode had an area of 60 cm² and was placed on the forehead. The current density was maintained well below the safety limits (always below 1 A/m²) (Poreisz et al., 2007). The electrodes were kept in place with bandages. Participants in group 2 underwent Sham stimulation, which was delivered by linearly increasing current intensity for 30s up to 1.5 mA, and decreasing it during the successive 30s up to 0 mA, just before the beginning of the block.

6.3 Results

All data were subject to the Kolmogorov-Smirnov test of normality. Pre- and post-tests were normally distributed ($p > .05$), therefore ANOVAs and t-tests

were used. VA and CS data were analysed, for each group of participants (PL+tRNS vs PL+Sham), with a repeated measures ANOVA with Time (pre-post-test, and follow-up), and Spatial Frequency (for CS only: 0.2, 0.8, 2.9, 5.8, 9.7, 14.5, and 21.8 cpd) as within-subjects factors, and Eye (amblyopic/trained vs. non-amblyopic/untrained) as a between-subjects factor. When data violated the assumption of sphericity, as assessed with the Mauchly's test, we applied the Greenhouse-Geisser correction of the degrees of freedom. As expected, VA in the amblyopic eye was significantly different from that of the non-amblyopic eye both in the PL plus tRNS ($F_{1,8}=21.55$, $p < .01$, $\eta^2_p = 0.57$) and in the PL plus Sham group ($F_{1,13}=8.8$, $p < .01$, $\eta^2_p = 0.40$).

Following eight sessions of a contrast detection training with lateral masking coupled with tRNS, VA significantly improved in both the trained and untrained eye ($F_{2,32}=31.2$, $p < .01$, $\eta^2_p = 0.66$). The interaction between Training Time and Eye was also significant ($F_{2,32}=2.75$, $p < .05$, $\eta^2_p = 0.19$), suggesting that trained and untrained eyes did not result in the same amount of improvement. In fact, despite Bonferroni-corrected t-tests showed that improvement in VA was significant at post-test and maintained at follow-up for both trained (pre- vs. post-test: $t_8=7.73$, $p < .01$; pre- vs- follow-up: $t_8=5.16$, $p < .01$) and untrained eye (pre- vs. post-test: $t_8=3.12$, $p < .05$; pre- vs- follow-up: $t_8=2.82$, $p < .05$), subsequent t-tests conducted on the differences between pre- and post-tests comparing trained and untrained eye, showed that the trained eye had a larger improvement ($t_{16}=2.33$, $p < .05$), and such larger improvement was maintained at follow-up ($t_{16}=2.13$, $p < .05$). Overall the mean improvement at post-test of the trained amblyopic eye was close to 2 LogMAR lines (0.18 LogMAR, that is from

0.44 LogMAR to 0.26 LogMAR) and equal to 0.1 LogMAR, that is from 0 LogMAR to -.1 LogMAR in the untrained eye (Figure 18).



Figure 18: Graph showing mean VA improvement at post-test and follow up in the trained and untrained eye for group 1 (PL plus tRNS). Error bars represent ± 1 SEM.

No significant difference in VA between pre- and post-test ($F_{1,13}=0.65$, $p > .05$, $\eta^2_p=0.05$), nor any interaction with trained vs. untrained eye ($F_{1,13}=0.39$, $p > .05$, $\eta^2_p=0.03$) was found when using PL in conjunction with Sham stimulation (figure 19).



Figure 19: Graph showing mean VA improvement at post-test in the trained and untrained eye for group 2 (PL plus Sham). Error bars represent ± 1 SEM.

In the PL plus tRNS group CS significantly improved after training ($F_{1,14}=17.8$, $p < .01$, $\eta^2_p=0.56$), regardless the eye (interaction Time by Eye: $F_{1,14}=.13$, $p > .05$, $\eta^2_p=0.01$) (figures 20 and 21). As expected, there was also a large CS variation across the different spatial frequencies tested ($F_{1.57,22.08}=46.2$, $p < .01$, $\eta^2_p=0.76$), a significant difference in CS between the two eyes ($F_{1,14}=9.76$, $p < .01$, $\eta^2_p=0.41$), and a significant interaction Time by Spatial Frequency ($F_{3.16,44.26}=8.45$, $p < .01$, $\eta^2_p=0.37$), suggesting that the CS improvement could have occurred only, or mainly, at certain spatial frequencies. In order to test this hypothesis, a further analysis was performed combining both eyes and separately for each spatial frequency. Repeated-measures ANOVAs with Training Time (pre- vs. post-Test) as a within-subject factor, and Eye (trained vs. untrained) as a between-subjects factor showed a significant difference between pre- and post-test at most the

tested spatial frequencies (0.8 cpd: $F_{1,16}=10.2$, $p < .01$, $\eta^2_p=0.39$; 2.9 cpd: $F_{1,16}=14.48$, $p < .05$, $\eta^2_p=0.47$; 5.8 cpd: $F_{1,16}=16.5$, $p < .01$, $\eta^2_p=0.5$; 9.7 cpd: $F_{1,16}=9.14$, $p < .01$, $\eta^2_p=0.36$; 14.5 cpd: $F_{1,16}=6.9$, $p < .05$, $\eta^2_p=0.30$; 21.8 cpd: $F_{1,14}=5.3$, $p < .05$, $\eta^2_p=0.27$), except for the lowest tested spatial frequency (0.2 cpd: $F_{1,14}=3.8$, $p > .05$, $\eta^2_p=0.21$), and regardless of the eye (interaction Time by Eye was not significant in any of the tested spatial frequencies). In terms of percentage improvement with respect to pre-test, CS in the trained eye had more than a two-fold improvement (averaged across participants and spatial frequencies), ranging from 163% to 440% at the highest tested spatial frequency, whereas CS in the untrained eye had a mean CS improvement of 160% (averaged across participants and spatial frequencies), ranging from 123% to 200%.

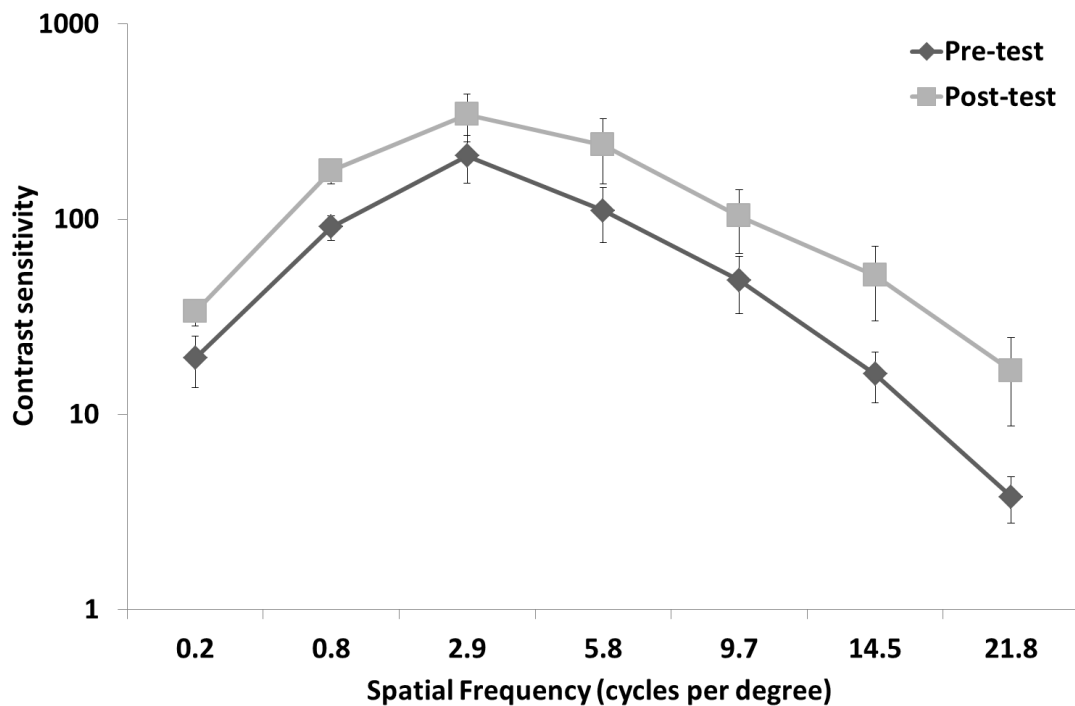


Figure 20: Graph showing mean CS improvement in the trained amblyopic eye at each tested spatial frequency for group 1 (PL plus tRNS). Error bars represent ± 1 SEM.

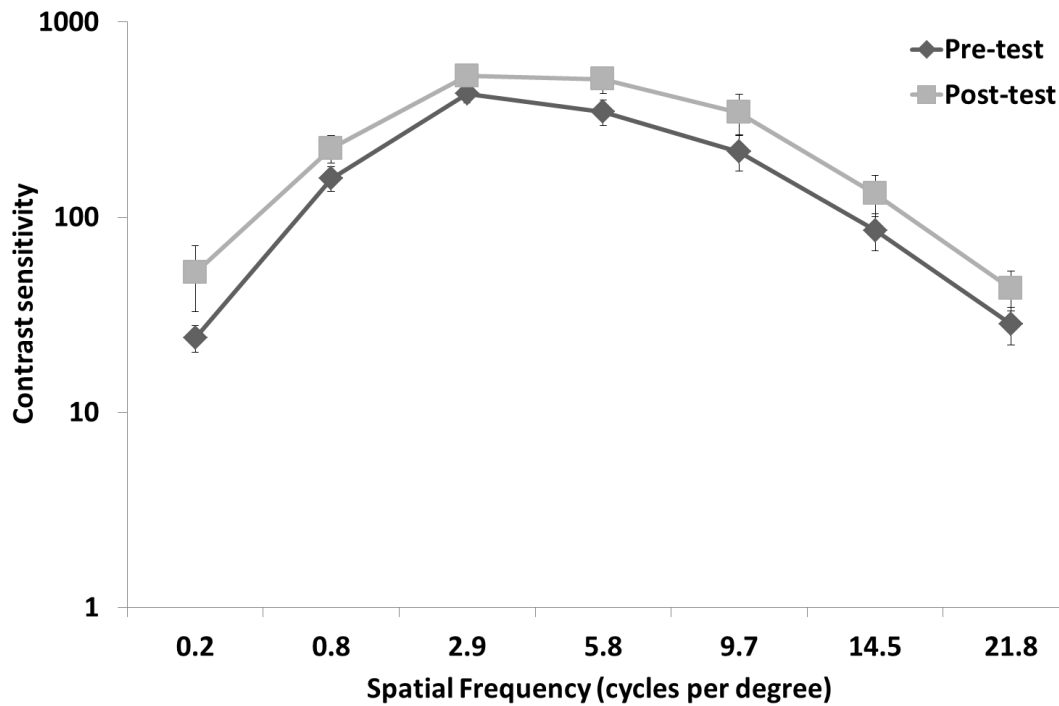


Figure 21: Graph showing mean CS improvement in the untrained eye at each tested spatial frequency for group 1 (PL plus tRNS). Error bars represent ± 1 SEM.

Interestingly, the PL plus Sham group also improved significantly after training in CS ($F_{1,13}=5.79$, $p < .05$, $\eta^2_p=0.32$), regardless of the eye tested (interaction Time by Eye: $F_{1,12}=0.58$, $p > .05$, $\eta^2_p=0.046$) (figures 22 and 23). As in the other group, there was a large CS variation across the different spatial frequencies tested ($F_{2.09,27.18}=68.8$, $p < .01$, $\eta^2_p=0.84$), and a significant difference in CS between the two eyes ($F_{1,13}=7.43$, $p < .05$, $\eta^2_p=0.36$). No significant interaction Time by Spatial Frequency was found ($F_{2.04,24.53}=2.9$, $p > .05$, $\eta^2_p=0.19$), suggesting that the improvement in CS for the Sham group has occurred to a similar extent at all tested spatial frequencies.

In the PL plus Sham group CS in the trained eye had a 160% improvement (averaged across participants and spatial frequencies), ranging from 125% to 235% at the highest tested spatial frequency, whereas CS in the untrained eye

had a mean CS improvement of 140% (averaged across participants and spatial frequencies), ranging from 121% to 200%.

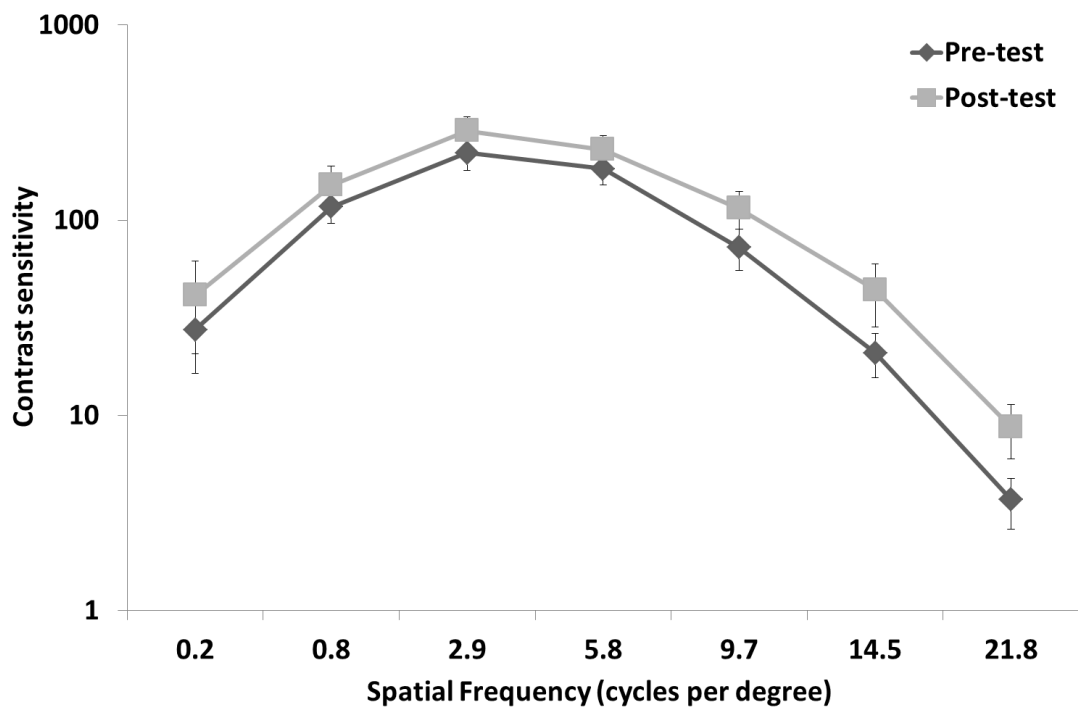


Figure 22: Graph showing mean CS improvement in the trained amblyopic eye at each tested spatial frequency for group 2 (PL plus Sham). Error bars represent ± 1 SEM.

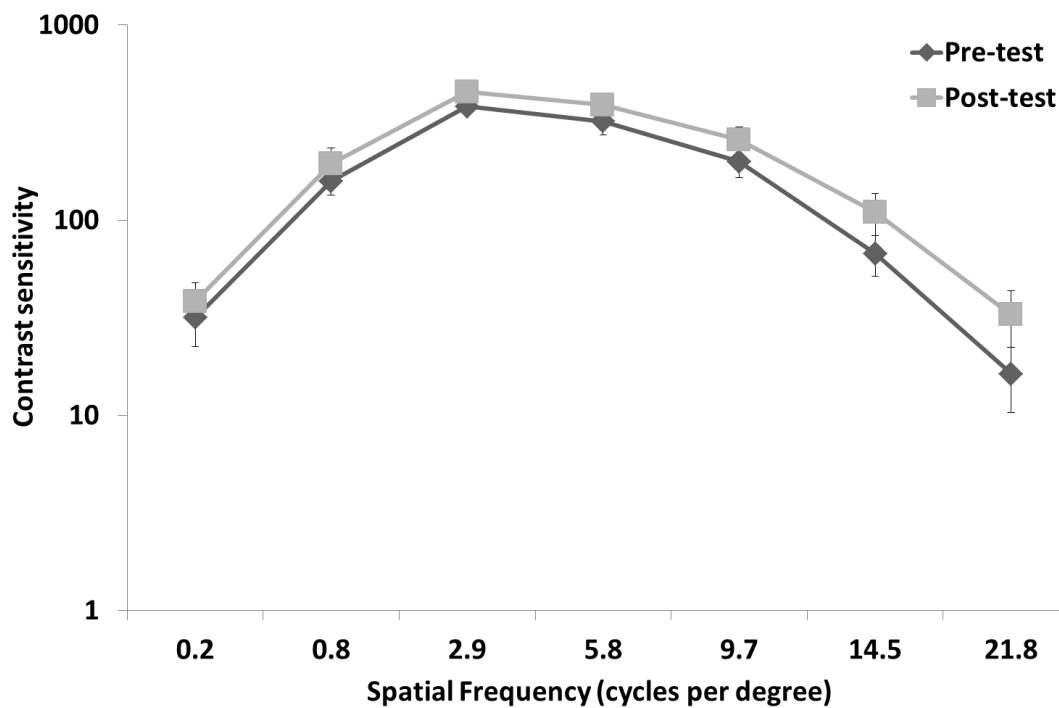


Figure 23: Graph showing mean CS improvement in the untrained eye at each tested spatial frequency for group 2 (PL plus Sham). Error bars represent ± 1 SEM.

6.4 Discussion

The present work investigated the effects of hf-tRNS compared to Sham stimulation combined with a short lateral masking monocular PL training on VA and CS improvement in patients with anisometric amblyopia. Eight sessions of monocular PL both with random noise stimulation and with Sham stimulation resulted in some visual improvement. With regards to VA, the group that underwent real online stimulation achieved a much larger improvement in the trained eye compared to the Sham group (0.18 logMar compared to 0.05 logMar), which was maintained until at least 6 months as observed in the follow-up assessment. This finding gives evidence for the enhanced effect of PL and its

transfer to untrained visual functions such as VA brought about by hf-tRNS of the visual cortex, which has also been reported in participants with myopia (Camilleri et al., 2014b). Surprisingly, an improvement in CS across a broad range of spatial frequencies was observed in both tRNS and Sham groups, despite the fact that only 8 sessions of PL were administered. However, the data point towards a larger improvement in the tRNS group, as can be seen in the graphs and from the percentage improvements in the amblyopic trained eye (up to 440% in the tRNS group vs. up to 235% in the Sham group), suggesting a more robust effect of PL when tRNS is concurrently applied.

The underlying mechanisms of how tRNS is able to boost visual plasticity are still a matter of speculation. Nonetheless, a few studies propose an enhancement of neural activity that are specific to the task at hand when adding noise to the system (Terney et al., 2008). This mechanism, known as stochastic resonance, implies that the random noise frequencies being received by the cortex, increases the signal to noise ratio thereby boosting the activity specifically associated to the task being undertaken. The excitatory effects of tRNS have been postulated to result from the potentiation of voltage-gated sodium channels (Terney et al., 2008). The temporal summation of weak depolarizing currents at the neuronal level may enhance the communication between specific neurons firing at the same rate (in response to a stimulus) thereby contributing to LTP-like changes, reflected in the reported long term effects. In fact, it has been shown that random noise stimulation works better online during task execution (Pirulli et al., 2013) compared to its use in isolation (offline). In the present case, when tRNS was applied to the contrast detection training using Gabor stimuli, an

increased signal to noise ratio may have resulted in more efficient processing of the stimulus parameters. The after effects of tRNS, as observed in the long term improvements measured at a 6 month follow up, have been recently attributed to sodium channel modulations, unlike tDCS which have been demonstrated to be dependent upon NMDA receptor modulations. This is indeed a striking finding since sodium channels are one of the most abundant voltage-gated ion channels present on the cell membrane (Yu and Catterall, 2003).

An interesting finding of the present study is the transfer of improvement of VA and CS to the untrained, healthy eye. This finding is in line with other studies investigating the effects of PL with and without brain stimulation on the amblyopic visual cortex (Polat et al., 2004) and reflects strong intraocular connectivity. Understanding the neuro-anatomical underpinnings of the amblyopic cortex is crucial in order to speculate on the underlying mechanisms of visual improvement following electrical stimulation. Amblyopia is a disorder characterized by poor intraocular communication and is associated with impairments in both monocular and binocular vision (McKee et al., 2003). Interestingly, recent findings suggest that the binocular cells and their connections in the amblyopic visual cortex may be actively suppressed rather than absent (Hess et al., 2011; Mansouri, Thompson & Hess, 2008). Thus, improvement of visual functioning in the amblyopic cortex following combined electrical stimulation and PL may be due either to an increase in excitability of connections leading to the suppressed eye, likely through an increased response of glutamatergic connections or conversely, the combined treatment may induce plasticity in the networks responding to the amblyopic eye by reducing the

GABAergic inhibition from the more dominant, non-amblyopic eye, resulting in reduced intraocular suppression. In fact, a common theme emerging in neuroplasticity research is the critical role of the balance between neural excitation and inhibition in gating plasticity (Jiao, Zhang, Zhang, Wang, et al., 2011; Micheva & Beaulieu, 1995, 1996; Zheng & Knudsen, 1999; Zhou, et al., 2011).

Current research on treatment practices of visual defects in amblyopia report improved visual functioning through the administration of PL (for a review see Levi et al., 2009); dichoptic training (Hess et al., 2012; To, Thompson, Blum, Maehara, et al., 2011 & Li et al., 2013); and video gaming (Achtman, Green & Bavelier, 2008; Li et al., 2011). The underlying mechanisms proposed are said to involve the strengthening of weak connections through Hebbian learning, resulting in recovery of function implicating various lateral, feedforward and feedback mechanisms (e.g. Rosa et al., 2013, Li & Levi, 2004; Polat et al., 2004). Despite the positive results obtained with behavioural training interventions, most of these are lengthy and time consuming procedures that require monitoring the treatment progress over long periods in order to ensure participants reach their asymptotic level (for a review see Tsirlin, Colpa & Goltz, 2015). In light of this, developments in the field have moved towards a combined approach in the rehabilitation of visual defects using NIBS, in order to boost neural visual plasticity and enhance the effects of existing behavioural regimes (e.g. Thompson et al., 2008; Spiegel et al., 2013). For example Spiegel and colleagues (2013) investigated the effects of dichoptic treatment alone and treatment combined with visual cortex tDCS on measures of binocular and

monocular visual function. They found that the combined treatment resulted in greater improvements in stereo-acuity than the dichoptic treatment alone. Their results corroborate with the present findings in that NIBS over the visual cortex can enhance the efficacy of the combined behavioural training outcomes (Spiegel et al., 2013).

In conclusion, these findings support the notion that the mature amblyopic visual cortex possesses a considerable amount of plasticity and that visual function can improve even beyond the critical period of visual development. The results demonstrate that a short perceptual training combined with online hf-tRNS is more effective than PL with Sham stimulation in inducing brain plasticity in the amblyopic visual cortex. Furthermore, the combined treatment can considerably improve visual functions in the amblyopic eye, whilst also resulting in some transfer of improvement onto the non-amblyopic eye. Further studies, comparing monocular training to binocular training are needed to confirm existing uncertainties related to intraocular suppression, which is a central problem of amblyopia. Furthermore, a larger sample of participants needs to be tested in order to strengthen and replicate these findings, and to estimate the best ratio between extent of improvements of visual functions and duration of the perceptual training combined with hf-tRNS. Finally, combining these interventions with neuro-imaging techniques will provide deeper insight into the underlying processes of neural plasticity and the resulting behavioural outcomes.

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CHAPTER 7 General conclusions and future directions

The general aim of this doctoral thesis was to shed light on the neuro-modulatory and behavioural effects of tRNS on the visual system as well as to investigate its therapeutic effects when combined with visual PL in comparison with the prevailing PL training regimes. The present work set out to design a practical and effective intervention technique combining brain stimulation, specifically, random noise stimulation, with existing PL protocols to improve visual abilities of people affected by visual deficits such as myopia and amblyopia. In order to understand and accomplish this, four experiments were conducted which explored the effects of tRNS and/or PL on the adult visual cortex.

The first experiment set out to investigate the underlying mechanisms by which low and high frequency random noise stimulation differently modulated neural excitability in the visual cortex, specifically by probing the robust phenomenon of visual MAE in area MT⁺/V5 with low or high frequency tRNS. The results of this experiment demonstrated that hf-tRNS decreased the duration of the MAE whereas lf-tRNS increased it. The outcomes of this experiment led to the speculation of the underlying mechanisms of action of low and high frequency tRNS. It was concluded that random noise stimulation acts on the weaker, less active sub-population of neurons, which in the case of the MAE, are the adapted, suppressed neural motion-direction detectors. The excitability action of hf-tRNS likely acted on these neurons thereby reducing their suppression and increasing their activity, stabilizing the imbalance between the

two motion direction neurons, resulting in a shorter MAE duration. Low frequency random noise on the other hand resulted in a longer MAE duration, possibly due to the reduced excitability of the already suppressed neurons, by lf-tRNS, which further decreased their activity and thus, increased the imbalance of activity between the two sub-population of neurons. In light of these results, it was established that hf-tRNS has an excitability effect on the more suppressed visual cortical neurons leading to an overall increased activation of the underlying neural processes.

The subsequent experiment, explained in chapter 4, explored the efficacy of a contrast detection training (single Gabor PL regime) on the improvement of VA and CS in a group of participants with mild myopia wearing no optical corrections. The results reveal a positive effect of the training on VA and less so on CS. The data further indicates that individuals with mild myopia might have altered lateral interactions. Moreover, the effect of training on lateral interactions revealed that single Gabor training does not modulate collinear lateral interactions between detectors. One important limitation of this study is the relatively small sample size, thus future studies should recruit a larger cohort in order to obtain more robust conclusions. Furthermore, this research highlights the importance of introducing brain imaging and brain electrophysiological techniques in order to better understand the underlying neural mechanisms by which PL takes place under different conditions. Group studies undergoing different training protocols is also required in order to allow for direct comparisons of different PL paradigms.

The present study raises two main queries: 1. Is there a gold standard training protocol for PL to be most efficient and effective to daily visual functioning? 2. If so, by which neuronal mechanism is this transfer made possible, raising the importance of introducing electrophysiological techniques in combination with a PL paradigm for visual defects. Understanding these two questions is key in order to put into practice the current literature on psychophysics and PL as a rehabilitation tool for improving visual functions in a variety of visual defects.

Despite the effectiveness and pervasive use of PL techniques in the treatment of visual defects, this behavioural technique usually involves lengthy protocols, making it impractical for patients. Following the observed excitability effects of hf-tRNS in the first experiment, as well as the positive outcomes of hf-tRNS on an orientation discrimination task reported in a recent study by Fertonani and colleagues (Fertonani et al., 2011), the third experiment of this thesis set out to combine hf-tRNS with a single Gabor PL training regime as a means of improving VA and CS in mild myopia. This experiment is described in Chapter 5. The results proved to be very promising and demonstrated that with just 8 sessions of combined hf-tRNS and PL with a contrast detection task, an improvement in VA and CS was found which was equal to that reported following a 2 month training regime of just PL using the same task, as seen in the data reported in chapter 4. Additionally, 8 sessions of hf-tRNS alone and PL alone did not result in any significant improvement in VA or CS. Furthermore a two-month follow up revealed that the improvements seen in the combined group are long lasting. The results of the present work demonstrate that a short perceptual training combined with online hf-tRNS is more effective than hf-tRNS

or PL alone in inducing brain plasticity mechanisms in the adult myopic visual cortex, likely due to compensatory processes. A final experiment sought to explore this combined technique of hf-tRNS and PL in a group of people with a cortical visual defect, namely, amblyopia. The results once again revealed a positive effect of hf-tRNS and a monocular lateral masking training on VA and CS. In particular, no transfer of PL to VA was observed in the absence of tRNS. Furthermore, the results of this experiment showed that the effects of monocular training transferred to the untrained, healthy eye for both VA and CS. Importantly, these results support further investigation into the application of tRNS to the existing treatments of amblyopia. Despite the positive results, a larger sample size is deemed necessary so as to better isolate the effects of tRNS on PL.

The underlying mechanisms of tRNS in bringing about an improvement in VA and CS, specifically when combined with a visual PL protocol are so far in the speculation stages. The effects of tRNS are said to be attributed to mechanisms of stochastic resonance, which, as explained earlier, is the process of boosting a weak signal by adding white noise to the system that contains a wide spectrum of frequencies. The frequencies in the white noise that correspond or resonate with the original signal's frequencies will tune with each other, thereby amplifying the signal and increasing the signal-to-noise ratio. This is likely why tRNS works best when administered online, together with a behavioural visual task (PL), by interacting with the concurrent activity of cortical neurons, tuned to specific orientations and spatial frequencies, thereby not only inducing specific synaptic potentiation but also enhancing performance on the task. Similarly, it

has been pointed out that neurons with a history of suppression preferentially respond to excitatory stimulation (Silvanto et al., 2008), thus another reason to support the idea that the excitatory effects of tRNS act on weak, (suppressed) neurons, where, in the case of amblyopia, the activity of V1 cortical neurons responding to the low contrast Gabor stimulus are suppressed and less responsive when compared to healthy controls.

Notwithstanding the optimistic results on visual cortical plasticity achieved through the combination of random noise stimulation and visual PL in both cortical and refractive visual defects, the underlying neurophysiological processes are still largely unexplored. In light of this, part of this research project has dedicated itself in answering this issue. A pilot study has been conducted using event related potentials (ERPs) in twelve healthy participants in order to investigate the spatial and temporal characteristics of neural mechanisms of the combined action of hf-tRNS and PL lateral masking training. A contrast discrimination task akin to that used in a study by Khoe and colleagues was implemented in order to record the ERPs (Khoe et al., 2004). Since stimulating both banks of the calcarine sulcus when presenting the stimulus centrally, may generate dipoles of opposite orientation that can cancel out one another, the stimuli of both the ERP-recording task and the lateral interactions training were presented in the perifovea (for more information on the EEG task see Khoe et al., 2004). Using 32-Channel electrodes, ERPs were recorded before (Pre test) and after (post test) 8 sessions of combined hf-tRNS and PL using a lateral masking paradigm akin to the one used in the amblyopia study, only in the present study, the stimuli were presented in the perifovea (Khoe et al., 2004). Preliminary

results demonstrate a significant modulation of amplitude (increase) in the later components (P250 - P500 range) observed at frontal and fronto-parietal sites following 8 sessions of combined training. Strangely, the results of this pilot study did not yield any significant differences in the earlier components (visual evoked potentials) before and after the combined training. This may be due, partly, to a ceiling effect, since this cohort of participants already had normal or corrected to normal vision and thus the early visual components may have been harder to modulate. Additionally, no significant differences in latency effects were observed. With regards to the EEG-task behavioural data, only 7 out of 12 participants improved following the training. These preliminary results, specifically the modulation of amplitude at later components over frontal-parietal sites, give some indication of a top down input on visual PL. In light of this, it is worthwhile investigating, using a larger sample size and a control group using Sham stimulation, the underlying electrophysiological mechanisms of a combined lateral interactions PL paradigm and random noise stimulation training on healthy participants as well as patients with cortical visual defects such as amblyopia and hemianopia (Huxlin, et al., 2009) to name a few. Exploring the fundamental neurophysiological defects in these patient groups could offer insight not only into the underlying deficits but also into the mechanisms sub-serving the functional improvements observed following a short, combined behavioural and tRNS training paradigm.

In sum, the experiments presented in this thesis, for the first time, authenticate the use and applicability of tRNS, with and without visual PL on visual cortical plasticity in healthy adult participants, those with myopia and

amblyopia. Furthermore, the underlying mechanisms of tRNS, specifically in combination with a visual task (for example the MAE and PL), on visual cortex excitability have been put forward. The main limitation of the present work is the relatively small sample sizes in each of the treatment groups (myopia and amblyopia). It would be worthwhile replicating and expanding this research on a larger scale to confirm and validate the use of this combined technique in bringing about visual neural plasticity and aid in the recovery of vision loss.

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