Università Campus Bio-Medico di Roma

Corso di dottorato di ricerca in
Scienze della plasticità d'organo e della rigenerazione tissutale per il recupero funzionale
XXIV ciclo anno 2009

Transcranial Electrical Stimulation offers new prospects to investigate neuroplasticity in visual perceptual learning

Cornelia Pirulli

Coordinatore
Prof. Raffaele Antonelli Incalzi

Tutor
Prof. Carlo Miniussi

29 Maggio 2012

"Ogni viaggio inizia con un primo passo…"

***Ad Anna**, compagna di questo meraviglioso viaggio nelle Neuroscienze*

CONTENTS

Figures and tables

Abbreviations

PL = perceptual learning

VPL = visual perceptual learning

TIPL = task-irrelevant perceptual learning

PET = positron emission tomography

EEG = electroencephalography

ERPs = event-related potentials

DTI = diffusion-tensor- imaging

tES = transcranial electrical stimulation

TMS = transcranial magnetic stimulation

tDCS = transcranial direct current stimulation

tRNS = transcranial random noise stimulation

tACS = transcranial alternating current stimulation

a-tDCS = anodal-tDCS

c-tDCS = cathodal-tDCS

hf-tRNS = high-frequency tRNS

lf-tRNS = low-frequency tRNS

MEP = motor evoked potential

PTs = phosphenes threshold

VEP = visual evoked potentials

MT = medial temporal

MAE = motion after-effect

PPC = parietal posterior cortex

NMDA = n-methyl d-aspartate

LTP = long-term potentation

LTD = long-term depression

M1 = primary motor cortex

V1= visual primary cortex

ODT = orientation discrimination task

WM = working memory

RTs = response times

Abstract

The term neuroplasticity refers to the ability of the nervous system to change its structure and function as part of the processes that underlie adaptations to environmental changes. These changes correlate with cognitive plasticity at behavioural level. The capacity of a system to acquire or improve skills and to adapt to new environments through a learning process has been defined 'cognitive plasticity' (Baltes and Willis 1982). Cognitive plasticity has been observed after brain lesions and in response to adaptation and perceptual learning (PL) (Fahle, 2002a, Thiele, 2004). Furthermore, recent development of transcranial electrical stimulation (tES) techniques to induce and evaluate cortical plasticity, constitutes a significant important step in understanding the relationship between cognitive plasticity and neuroplasticity.

The goal of this work is to investigate whether and how tES can modulate cognitive plasticity in healthy adult brain. Neural plasticity induced by tES protocols has the potential to offer important insights to understand the mechanisms that underlie phenomena of plasticity, and will help to focus and constrain neurocognitive theories of the behavior-brain relationship. We applied tES protocol during the execution of a PL task. PL is a form of implicit memory characterised by an improvement in sensory discrimination after repeated exposure to particular types of stimuli and is considered a manifestation of neural plasticity (Carmel and Carrasco, 2008, Gilbert et al., 2001, Li et al., 2004). In particular we focused on visual PL (VPL). Animal and human studies have demonstrated the specificity of the primary visual cortex (V1) for recognising basic stimulus characteristics, such as orientation and direction. This specificity implies the direct involvement of V1 cells in learning and discriminating stimulus characteristics (Carmel and Carrasco, 2008, Li et al., 2004). Focusing on PL and the visual system, neurophysiological evidences have demonstrated that V1 is highly plastic (Wandell and Smirnakis, 2009). In the first study, we aimed to test the interaction between PL and different tES techniques on V1 by applying tES while healthy participants execute an orientation discrimination task (ODT). In particular, we evaluated the effects of two tES, expected to induce facilitator effects at behavioural level, transcranial random noise stimulation (tRNS) and anodal transcranial direct current stimulation (a-tDCS). Although previous

studies have shown that tRNS and a-tDCS had similar effects on the motor system (Nitsche et al., 2000; Terney et al., 2008; Moliadze et al., 2011) our hypothesis was that these two different stimulation protocols would have different effects on visual system. Our results show a greater improvement in performance in the ODT when subjects were stimulated with tRNS than with a-tDCS. This result highlights the potential of the new tRNS technique and advances our knowledge on neuroplasticity induction approaches.

The next step was to understand if the tRNS was effective regardless of the timing of application in relation to the state of cerebral activation during the task. We asked ourselves what would happen applying the same stimulation protocols before the task execution. Consequently the aim of the second experiment was to understand if there was a critical timing for the application tES to obtain the induction of neuroplasticity in the V1 cortex. In this second experiment we applied tES (i.e., tRNS, a-tDCS and cathodal-tDCS) before (offline) or during (online) the execution of an ODT.

The results confirm that a critical and "ideal" timing of application exists, and it depends on the stimulation type. tRNS facilitation is present only if applied during the task execution, whereas it's better to apply anodal tDCS before the task in order to induce facilitation in VPL.

Overall, these results provide important indications for the designing of rehabilitation protocols, highlighting which of the two excitatory techniques is better to choose in relation to its timing of application. The opportunity to directly influence brain plasticity offered by these data opens new possibilities in cognitive neuroscience and neurorehabilitation.

CHAPTER 1

SCOPE OF THESIS AND WORK ORGANIZATION

This doctoral dissertation describes the research activity developed during the three years of PhD in "Scienze della plasticità d'organo e della rigenerazione tissutale per il recupero funzionale". In this period my research activity focused on the study of cognitive functions by the application of transcranial electrical stimulation (tES).

The scope of my thesis is to understand *which, when* and *how* stimulation protocols are the most effective to obtain the induction of neuroplasticity in the primary sensory cortex. The specific aim of my experiments is to study the behavioral effects of tES to better understand and to improve knowledge on these methods and some aspects in their utilization. Indeed, there are still many questions to answer on the tES application such as: it possible to modulate the behavioral performance of cognitive task by tES? How can tES induce cognitive plasticity facilitation? Which of the different types of tES is the best to induce and potentiate cognitive plasticity? What is the ideal time to apply tES, before or during the cognitive task execution?

tES is able to modulate the synaptic transmission efficacy, this modulation results in excitability and activity changes in specific cortical networks that are activated by the cognitive task's execution, and these changes correlate with cognitive plasticity at the behavioral level. To show the efficacy of tES to induce neuroplasticity and understand what are the best parameters of stimulation would allow us a correct application of the transcranial stimulation for the rehabilitation of patients with cognitive deficit (i.e. perception, learning, memory, language).

In the first part of **chapter 2,** I describe the main studies performed on VPL, specifically animals and humans studies have shown the involvement and the importance of the V1 in processing of visual information during the execution of VPL tasks. In the second part, I illustrate some of the tES studies that have contributed to understand how the induced current affects the behavior and how different tES parameters and different type of tES drive to different, even opposite, results. Among different types of electrical stimulation, tDCS is the most used in different tasks and in different cerebral areas (motor, visual, somatosensory etc.). However, many studies on functional effects of tDCS were performed on the primary motor cortex (M1) and in elementary task, such as reaction time task or implicit learning in the motor system. Therefore, I started from the work of Nitsche et al. (2003c), Terney et al.(2008) and Stagg et al. (2011a) that have applied tDCS and

tRNS to investigate the facilitator effects in the implicit motor learning. In the **chapter 3** my work has the aim to extend these results to a different area of brain (i.e., visual system), further aim of my research is to understand *which* stimulation protocols tES are the most effective to obtain the induction of neuroplasticity in V1. Once demonstrated the efficacy of these techniques, in the **chapter 4** the scope of work is to understand *what* is the ideal timing of their application in normal cognitive processes, it will be possible to extend these protocols also to patients with cognitive deficits (e.g., learning, memory, attention). In the last chapter (**chapter 5** – General discussion) the main findings of the entire work will be summarized and discussed.

CHAPTER 2

INTRODUCTION

2.1 Visual Perceptual Learning and Plasticity

A good starting point to define the concept of perceptual learning (PL) is given by Gibson (1963): "Any relatively permanent and consistent change in the perception of a stimulus array following practice or experience with this array will be considered perceptual learning". In other words, PL is considered a form of implicit memory characterised by an improvement in sensory discrimination after repeated exposure to particular types of stimuli (Carmel and Carrasco, 2008, Gilbert et al., 2001, Li et al., 2004). Perceptual learning differs from other forms of learning in that it can be highly specific to the stimulus features and involves structural and/or functional changes in primary sensory cortices. Moreover, in contrast to declarative forms of learning, PL seems to modify the neural population activation during the execution of the task, and not require intermediate consolidation storage. The neural modifications, that occur during learning of new skills, are the direct evidence of the presence of mechanisms of cortical plasticity in the brain. Indeed, behavioral expressions of learning can be associated with specific changes within a cortical network involved in the execution of a task. In addition, animal studies have shown that neuronal axonal structural plasticity and the anatomical and functional remodeling in the cerebral cortex can occur as consequence of learning (Watanabe, 2002). Regard to PL, plasticity can be defined as changing of the brain leading to more appropriate function, and serves to adjust the functional and anatomical organization of the central nervous system as a result of sensory experience (Fahle, 2002a). PL involves functional changes that affects cortical function throughout life. This finding overcome the old concept that the brain structure becomes immutable after childhood. Recently, PL has been considered a way to understanding the nature of experience-dependent changes in the cerebral cortex.

Perceptual learning in adult human observers has been documented in many perceptual tasks in visual, auditory, and somato-sensory domains (Fahle, 2002a). I concentrated on PL in the visual domain. It has been studied in different visual task, including detection or discrimination of visual gratings (Mayer, 1983), stimulus orientation discrimination (Matthews and Welch, 1997, Vogels and Orban, 1985), motion direction discrimination (Ball and Sekuler, 1982, 1987) texture

discrimination (Karni and Sagi, 1991, 1993), hyperacuity and vernier tasks (Fahle, 1993, McKee and Westheimer, 1978), and object recognition (Furmanski and Engel, 2000). The visual system has the ability to adapt to experience-dependent changes, these changes in the functional properties of neurons and in the network underlying these changes is known as plasticity (Gilbert et al., 2009). Moreover, plasticity can be seen at multiple stages in the visual pathway, starting from the primary visual cortex (Furmanski et al., 2004). Specifically, V1 seems to respond and to adapt to specific features of the stimuli. Indeed, studies of visual plasticity have psychophysically demonstrated that detection or discrimination thresholds can be reduced and showed a high degree of specificity respect to the trained visual stimuli (Seitz and Watanabe, 2005). Shoups et al. (1995) showed specificity of learning to the retinotopic location or to simple stimulus features such as orientation or direction. These characteristics are thought to involve cells in early visual areas that have small receptive fields. Nevertheless, these results do not exclude the possibility that higher-level visual or decisional areas are involved in PL (Ahissar and Hochstein, 2004).

As will be described in the next sections, low-level sensory plasticity involved in VPL has been confirmed by studies of electrophysiology in animal and functional imaging in human. Consequently, PL is considered a manifestation of neural plasticity (Carmel and Carrasco, 2008). Thus the study of the neural changes associated with VPL can increase our understanding on neuroplasticity induction in adult sensory cortices.

2.1.1 Animal studies

In the past, plasticity of early sensory cortices was thought to be limited to a period of the early postnatal life know as the critical period. The neural basis of the critical period were discovered by Hubel and Wiesel (1959), that found that the ocular dominance could be altered by restricting visual experience to one eye. They observed that the capacity of the visual cortex to undergo these changes were limited to the first months of life. However, in the following years further experiments have clarified that the critical period is limited to specific properties of the cortex (Merzenich, 1984, 1988). Much evidence of the presence of plasticity effects in the

sensory area come from animal studies (Kaas, 1991). Cellular correlates of long term use-dependent changes in attentive learning have been shown in the auditory and somatosensory cortices of awake animals applying natural sensory stimuli (Recanzone et al., 1992a). Other in vitro studies have shown change in cellular plasticity of visual cortical cells with electrical stimulation as well as in vivo studies by pairing of natural stimuli with artificial depolarization (Cahusac, 1995, Fregnac, 1988, 1992). Moreover, in animals, the pioneering studies in the sensory cortices investigated the effect of reorganization of the cortical topographic maps as a consequence of a cortical lesions (Eysel, 1980, Kaas, 1991). Gilbert and Wiesel (1992) demonstrated in adult monkey, through single-cell recording, that receptive fields in the V1 cortex can change positively after retinal lesions. Subsequent experiments in monkeys and cats have been developed applying PL paradigms to study plasticity effects in the V1 cortex. These studies showed that sensory experience response of specific neural receptive fields and the topographical maps of the cortex can be altered (Crist, 2001, Recanzone et al., 1992b). However, in the V1 cortex, there are contradictory results regarding the changes in the cortical area after extensive training in VPL tasks. Shoups et al. (2001) demonstrated, through single unit recording studies in monkeys, the presence of different response of cells in early visual cortex during an orientation discrimination task. The authors found a correlation between improved monkey orientation discrimination and V1 neuron orientation tuning curves while no plasticity was found in the same receptive field with stimuli presented at a different location. However, Ghose et al. (2002) found no orientation tuning changes in either V1 and V2 neurons whit the orientation discrimination task. In contrast, Raiguel et al. (2006) reported more significant orientation tuning changes in V4 neurons. Even so, these changes in V4 cells were still too small to account for behavioral response. Li et al. (2004) showed that V1 neurons responded very differently to ad identical visual stimulus under different visual discrimination tasks, in monkeys trained in a shape discrimination task. Interestingly, the corresponding neuronal changes were not seen when the trained task was performed by anesthetized monkeys (Li et al., 2004). New technologies offer promising tools for studying cortical plasticity. In the monkey, molecular tools are used to measure gene expression associated with cortical reorganization after

visual learning tasks (Chen et al., 2010). There is increasing evidence that experience-dependent plasticity of specific circuits in the visual cortex involves cell type-specific structural plasticity.

Overall all these results suggest endless plasticity in V1 and the presence of continuous changes after visual experience. Indeed, animal studies have demonstrated that changes in visual input are sufficient to induce functional plasticity within the visual system.

2.1.2 Human studies

PL in humans has been documented in a wide range of perceptual tasks in visual, auditory and somatosensory domain. In addition, PL has been investigated in recent years in different prospective such as psychophysics, neurophysiology (Pourtois et al., 2008), brain imaging (Schiltz et al., 1999) and lesion studies (Fahle and Daum, 2002b, Xu et al., 2010). Considering VPL in human, we should consider some aspects that can influence the performance, such as the role of attention, feedback and the type of task (serial vs. parallel). For example, it has been found that PL is only associated to the particular feature the participants pay attention (Ahissar et al., 2001). However, Watanabe and colleagues (2001) reported that PL can occur even in the absence of selective attention. In their study subjects identified a letter (target stimulus) in centre of a display while moving dots (irrelevant stimulus) were presented peripheral field. Successively, the authors found that subjects enhanced their ability of discrimination or detection only for the coherent motion direction to which they were previously exposed (Watanabe et al., 2001). This type of studies are called task-irrelevant PL (TIPL). TIPL for a stimulus depends on the relationship between that stimulus and important task events or upon stimulus reward contingencies (Seitz et al., 2009, Seitz and Watanabe, 2009). Feedback can also influence PL even if there are contradictory opinions. Some authors argued that feedback improves subjects' performance. Herzog a and Fahle (1997) showed that the subjects were facilitated by trial by trial feedback and also by feedback regarding the percentage of correct responses in Vernier acuity task. Shibata and coworkers (2009) showed that block-feedback facilitated PL if it was more positive than the

subject's actual performance. In contrast, Liu and colleagues (2010) demonstrated the same learning curves ODT with and without feedback. Finally, regarding the choice of the task, some studies indicated that VPL is task dependent. Indeed VPL of a specific feature did not transfer to another task involving the same or similar stimuli but using different procedure (Thiele, 2004). Psychophysical results distinguish an important difference between two types of task. In the first type (serial task), the target and its location are well defined and are known to the subjects. Most tasks used in studying PL are orientation discrimination of a line element, Vernier, bisection and contrast discrimination. In the other type (parallel task), the target is presented among many distracters, and subjects have to find a target placed in a contest composed of many distracters. An example of such a task is texture segmentation task or contour integration. Different studies have applied serial or distributed parallel tasks with the principal aim to understand the neuronal changes in the visual cortex. It is widely accepted that these neuronal modifications has been induced by training and learning of these different type of PL task (i.e., serial, parallel). Usually, the term "use-dependent plasticity" refers to plastic change that occurs whit the acquisition of perceptual skills (Fahle, 2002). Several studies have shown that the experience modifies low-level visual processes, attention free, in the V1 cortex (Fahle, 2002a). VPL of complex or simple features of the stimulus is associated with changes in the earliest visual area such as V1 (Karni and Sagi, 1991). The recent development of non invasive imaging technique has permitted to study the modifications in activity neuronal after VPL task. Several studies found that VPL is associated with increase in the BOLD signal in the region of V1 that corresponds to the location of the trained stimulus (Schwartz et al., 2002). However, Schiltz et al. (1999) found an activity decrease in positron emission tomography (PET) when they compared activation pattern before and after the training in orientation discrimination task. The V1 plasticity hypothesis is further sustained by the time course of the learning-dependent brain activity, measurable with electroencephalography (EEG) method. Both within and between session learning modulated the EEG component corresponding to V1 activity (Pourtois et al., 2008). Censor and Sagi (2009) investigated the relationship between visual performance and visual event-related potentials (ERPs) using a backward-masked texture segmentation task. Their results

showed a temporal correlation between ERP component (i.e., N1) and performance. It seems that practice reduces temporal interaction between successively stimuli, possibly by increasing the efficiency of target processing within early visual areas. The more recently introduced diffusion tensor imaging (DTI) method can be used to estimate anatomical changes associated with skill learning, corresponding to changes in white and gray matter density (Scholz et al., 2009). Yotzumoto et al. (2009) provided evidence supporting such structural changes in V1 associated with texture learning.

Overall, these studies support a localized increase in processing efficiency in the V1 as a result of practice. Such changes can be due both to inputs that directly arrives to V1 and to feedback input that V1 receives from other brain areas. Concluding, VPL may be a good tool to investigate plasticity effects in the V1 cortex. Furthermore the application of new non-invasive techniques of stimulation (see section 2.2 and 2.3) may offer important insights into the mechanisms that underlie human visual plasticity.

2.2 Transcranial electrical stimulation (tES)

The *tES* technique involves the application of weak electrical currents (~1-2 mA) directly to the head for several minutes (~5-30 minutes). The stimulation is delivered by a battery-driven current stimulator through a pair of electrodes (see Figure 1). These currents generate an electrical field that modulates neuronal activity according to the modality of the application. tES differs qualitatively from other brain stimulation techniques such as transcranial magnetic stimulation (TMS) because it doesn't induce neuronal action potentials. This is due to application of static field that in this range do not yield the rapid depolarization required to produce action potentials in neuronal membranes. Therefore tES might be considered a neuromodulatory interventions (Nitsche et al., 2008). However, it effectively modifies the evoked cortical response to afferent stimulation as well as the postsynaptic activity level of cortical neurons, presumably by inducing a shift in intrinsic neuronal excitability. In addition, tES do not produce the noise and discomfort produced by TMS and therefore changes in performance are not

attributable to unspecific effects. For the application of tES have to be considered several important parameters. Indeed, the efficacy of tES to induce a modifications of membrane polarity and consequently in behavioral performance depends on current density determined by induced electrical field strength and electrode size. Another important parameter of tES is stimulation duration. With constant current density, increasing stimulation duration determine the occurrence and the duration of after-effects both in human and animals (Bindman et al., 1964, Nitsche and Paulus, 2000, Nitsche et al., 2003a). Another important parameter to achieve the expected electrical stimulation effects is the orientation of the electric field, which is defined by the position of electrodes and polarity. For example, some studies have demonstrated that the role of reference electrode (Bikson et al., 2010) is very important because it was shown that only specific return electrode position resulted in tES-dependent cortical excitability alterations (Nitsche and Paulus, 2011). Since an extracephalic reference electrode could permit a more focal stimulation by avoiding an effect of this electrode on brain function, these effects have been investigated recently (Moliadze, 2011). This last parameter is especially important most of all for direct current electrical stimulation because it is a technique polarity-specific (see section 2.2.1). Indeed, there are different ways to distribute current which can be *direct* (transcranial direct current stimulation, tDCS), *random noise* (transcranial random noise stimulation, tRNS) or *alternating* (transcranial alternating current stimulation, tACS).

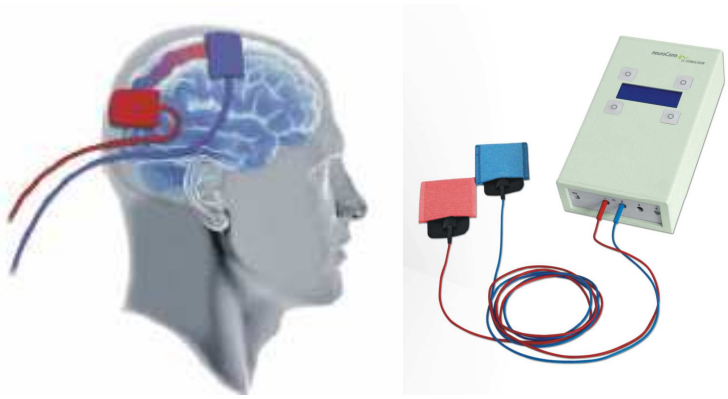

Figure 1. tES device composed by a battery-driven current stimulator with a pair of electrodes.

2.2.1 Transcranial Direct Current Stimulation (tDCS)

The tDCS is a non invasive technique that allows the modulation of cortical excitability in a polarity-specific manner. A direct current of low level intensity (see Figure 2) cross the cerebral cortex through two electrodes positioned on the scalp, one in correspondence of the cerebral area involved in task and the other positioned above an area not involved in task performance (cephalic or extracephalic site). This current is able to modulate the frequency rate of firing of the neural populations underneath (Nitsche and Paulus, 2000). In the anodal stimulation, the anode is collocated on the cerebral area of interest, whereas the cathode is collocated on a reference site. In the cathodal stimulation the electrodes positioning is reversed. The size of the electrodes is usually between 15 and 60 cm^2. The positive aspect of this relatively large electrode are its painfulness and the safety of the technique (Iyer et al., 2005; Poreisz et al.,2007), but the negative aspect is its low spatial resolution (Wagner et al., 2007). However, small electrodes might be less effective in terms of current density on the cerebral cortex compared to current strength induced through the stimulation electrodes (Miranda et al., 2009). Using these electrodes, current intensities vary between 1mA and 2 mA and are commonly applied for between 10 and 20 minutes.

Surely among different types of electrical stimulation, tDCS is the most used in many different tasks and in different cerebral areas (motor, visual, somatosensory, auditive etc.). The first studies on functional effects of tDCS were performed mainly on the primary motor cortex (M1) and in elementary task, such as reaction time task, implicit learning in the motor system (Nitsche et al., 2003c), visual and somatosensory perceptions (Antal et al., 2004 b, 2004c, Matsunaga et al., 2004). With regard to the efficacy and physiological effects of tDCS on M1, many studies have investigated excitability modifications monitored by TMS. These studies demonstrated that 5 minutes of anodal tDCS increased cortical excitability investigated in terms of amplitude in hand motor evoked potential (MEP) whereas cathodal tDCS leads to inhibition as evidenced by a decrease in MEP amplitude. The lasting effects depended on the duration of stimulation, indeed 13 minutes of anodal tDCS elicited 90 minutes of after effects and 9 minutes of cathodal tDCS caused a decrease in cortical excitability for almost an hour (Nitsche et al., 2003b; Nitsche and

Paulus, 2000). Quartarone and coworkers (2004) have shown that cathodal tDCS decreased MEP amplitudes with and without motor imagery, while anodal tDCS enhanced MEP amplitude only without motor imagery. However, behavioral effects of tDCS in healthy subjects do not directly mirror these physiological effects. Anodal tDCS applied to M1 during implicit learning task improves performance in test of motor speed (Nitsche et al., 2003c). Nevertheless, with the same task cathodal tDCS has no effect on learning or on simple reaction times (Nitsche et al., 2003c). In other study, Kuo and colleagues (2008) used the same task but applying tDCS before that the subjects executed the task, the rate of learning is reported to be unchanged both anodal stimulation and cathodal stimulation. Recently Reis et al. (2009) applied a motor skill task to investigate the effects of anodal and cathodal tDCS during the course of implicit motor learning. Reaction times and accuracy were measured within one training day and between training days. The experimental training group received 5 sessions of 20 min of anodal or cathodal tDCS over the left M1, whereas control group received sham stimulation. Their results showed a greater effects of anodal tDCS, on the total learning function for the whole training period, compared to cathodal or sham stimulation. These behavioral effects difference between the two studies depends on the timing with which it is applied relative to the learning task. So far, only one study has directly compared responses a procedural learning paradigm performed during and after tDCS (Stagg et al., 2011a). The authors found that anodal tDCS applied during the task increase the rate of motor sequence learning more than anodal stimulation applied before task performance. In contrast, there was no difference for cathodal stimulation. However, it is still not clear to what extent these findings are transferable to other areas of the cerebral cortex, and what happens with other types of stimulation (e.g., tRNS).

With regard to the impact of tDCS on visual, somatosensory and auditory functions, the number of studies is small and they present results that are contradictory. For the visual system, in section 1.4 it will be describe the main studies and more important results. For somatosensory perception, Bradnam et al. (2010) showed that cathodal tDCS of the M1 reduced cold perception threshold as well as pain perception induced by laser stimulation of the controlateral hand, whereas anodal reduced warm perception threshold. Regard to auditory system, cathodal tDCS over the superior

temporal and inferior frontal cortex decreased pitch matching ability (Loui et al., 2010). However, in the last years greater interest has been directed to more complex cognitive functions such as language, working memory and attention. The principal aim of these studies was to investigate the neuronal modulations induced by tDCS during the execution of cognitive task. In a recent study, Fertonani et al. (2010) showed that 10 minutes of anodal tDCS on the left dorsolateral prefrontal cortex improves naming performance, speeding up verbal reaction times after the end of the stimulation, whereas cathodal stimulation had no effect. tDCS applied on working memory (WM) task showed the involvement both of prefrontal cortices and parietal lobe in object WM task (Berryhill et al., 2010). Their results demonstrated a selective impairment of performance in recognition, but not recall, after cathodal tDCS whereas anodal stimulation was not effective. Finally, regarding attentional processes, Bolognini and coworkers (2010a) investigated the importance and specificity of right posterior parietal cortex for visual-auditory processing. The authors demonstrated that anodal tDCS applied on this area improves performance in a visuo-auditory attention task. In a successive study the same authors showed that training success in a visual search task was larger when the right, but not when the left posterior parietal cortex was stimulated (Bolognini et al., 2010b).

All these studies show that the tDCS may be applied to explore the role of different cortical areas with different tasks. Indeed, tDCS seems a promising tool for determination of the contribution of specific cortical areas to cognitive processes. Above all tDCS may be considered a valid tool to induce and modulate neuroplasticity in human. However, further studies are necessary to improve certain aspects of the technique such as optimizing stimulation protocols in terms of duration, timing to apply stimulation in relation to task performance, electrode positioning (especially position of reference electrode), and duration of after-effects. In addition, it is need clarify the physiological effects of the stimulation. This will improve our knowledge about the effects of tDCS, to have more consistent and to optimize protocols for clinical applications.

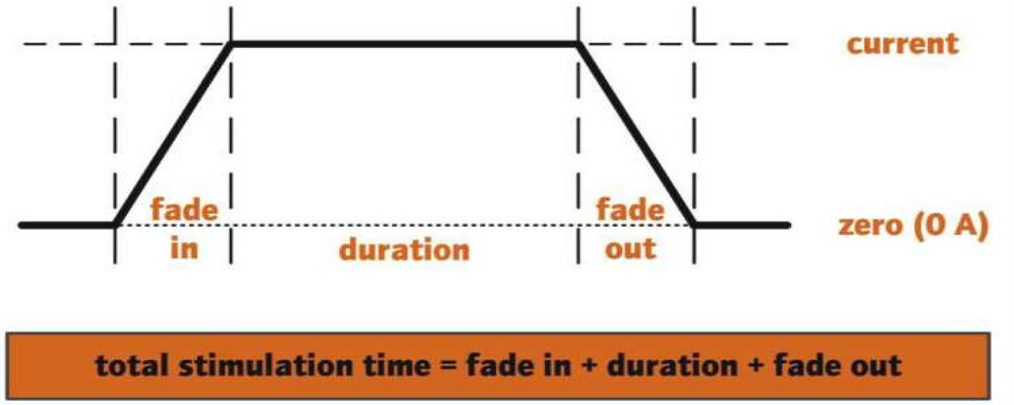

Figure 2. Current delivery during the direct current electrical stimulation. The total stimulation time include also a fade in period and fade out period.

2.2.2 Transcranial Random Noise Stimulation (tRNS)

More recently, interest has developed in a new electrical stimulation method: tRNS. It consists of the application of a repetitive, subthreshold, and alternating current at random frequencies over the cortex. Actually, tRNS can be applied at different frequency band ranges (low frequency from 0.1 to 100 Hz or high frequency from 101 to 640 Hz) of the entire spectrum from 0.1 to 640 Hz. The "random noise" signal contains all frequencies up to half of the sampling rate, i.e., a maximum of 640 Hz. This random level of current is generated for every sample (sampling rate 1280 sps) and is normally distributed. The probability density function follows a bell-shaped curve (see Figure 3). Transcranial application of weak random noise may appear to be a promising tool for neuroplasticity research, because a painless, selective, focal, non-invasive and reversible excitability modulation of the cortex without an adaptation effect. Indeed, tRNS is characterized by an oscillatory current and therefore does not have the polarity constraints of tDCS or the perceptible skin sensation when it is applied. tRNS could be expected to interact with ongoing cortical rhythms (Thut and Miniussi, 2009) and to induce a random frequency that should interfere with or potentiate the synchrony between neurons within an area or between areas within a network. However, these hypothesis have not yet been tested.

Actually there are only five studies that have applied tRNS in experimental protocol and with behavioural task. The first study that has applied tRNS is Terney et al. (2008). They demonstrated that ten minutes of tRNS at high frequency (101-640 Hz) to the motor cortex induces a positive modulation of cortical excitability (i.e., an increase in the amplitude of MEP) that persists after the end of stimulation. Furthermore, behavioural improvement in a motor learning task was obtained with the application of the entire frequency spectrum (0-640 Hz). The authors concluded that tRNS induced a facilitatory effect similar to that of anodal tDCS (Ambrus et al., 2010b, Terney et al., 2008), even if it was sustained by different mechanisms. In successive study, Chaieb and colleagues (2009) showed that short-duration application of tRNS (4 minutes at 1 mA) induced a reduction of the blood-oxygen-level dependency (BOLD) response in the motor cortex that can be seen on the fMRI during finger-tapping task. The authors showed that tRNS applied with different duration and in combination with sensory-motor task might result in different outcomes. In a recent study, Mulquiney et al. (2011) compared 10 minutes of 1mA anodal tDCS and tRNS during a WM task. Experimental sessions were separated by 1 week. Immediately prior and after each stimulation session the participants were measured on speed and accuracy of performance on a n-back task. Their results confirmed the efficacious of anodal stimulation in enhancing of some aspects of Dorsolateral Left Prefontal Cortex (DLPFC) functioning but do not support for the hypothesis that tRNS improves WM. Nevertheless, this study has some important methodological limitations that may have contributed to the lack of significant for tRNS (e.g., small sample size, lack of counterbalancing of the order sessions, low intensity of stimulation, reference electrode etc.).

However, until now, there are still few studies that have applied tRNS and that explore what are the effects and mechanisms of action of tRNS. Indeed, it is need further research to optimize parameters and efficacy, because considered the several advantages of this new technique (safety, lower perception cutaneous, good for placebo condition) tRNS could be the most effective technique in neurorehabilitation field. In addition, although its mechanism of action is yet unknown, tRNS may prove to be a useful powerful tool in understanding how neuroplasticity phenomena occur within cortical networks during the execution of cognitive task. Indeed, modulation

of synaptic transmission efficacy can result in excitability and activity changes in specific cortical networks that are activated by the task's execution, and these changes correlate with cognitive plasticity at the behavioral level.

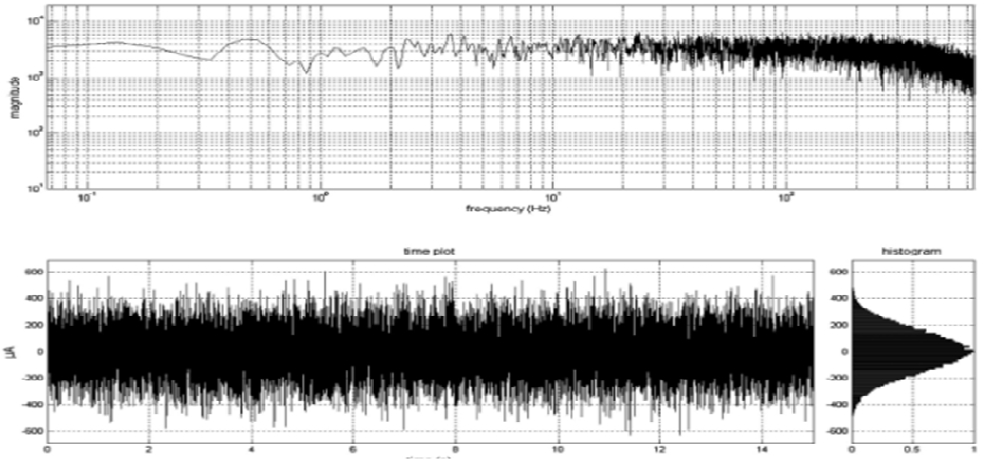

Figure 3. Random noise electrical stimulation. In the stimulation mode "noise", there is a random level of current generated for every sample (sampling rate 1280 sample/s). The random numbers are normally distributed. The amplitude of 1mA that 99% of all generated amplitude values were between +500uA and -500uA. (Terney et al., 2008)

2.3 Transcranial Electrical Stimulation and Visual Perception

The visual system, is considered among the most complex of all sensory systems and, it posses the ability to undergo induced and spontaneous neuroplastic changes (see section 2.1). Intensive researches revealed that distinct unimodal areas are involved in the information visual processing both in macaque and in human brain (Felleman and Van Essen, 1991, Tootell et al., 1996). Each area has distinct anatomical and functional properties and different visual areas are connected to each other in a hierarchical way (Yotsumoto et al., 2008). Visual information is first processed in earlier stage of visual system and after transmits its visual input to the ventral pathway and the dorsal pathway (Goodale and Kotagama, 2006). The complex visual information are progressively built through hierarchically connected cortical areas, resulting in the progressive increase of the complexity of the visual

neuronal representations. However, what has been known from animal and human studies is that V1 cortex is undoubtedly involved in the processing of visual information (see section 2.1.1 and 2.1.2). In the past years, important insights on the human visual processing are obtained with the application of non invasive technique such as electrical and magnetic stimulation of the brain. Indeed, transcranial stimulation (i.e., TMS and tES) produce a brief excitation or inhibition in the cerebral cortex and in that way interfere or potentiate normal ongoing cortical activity (for recent review see Miniussi et al., 2011). In recent years, many studies have demonstrated that tES seems to be a promising methods to induce visual cortical excitability and activity modulations (Antal and Paulus, 2008). Furthermore, there are prospects for the use of tES as tool to promote changes of visual cortex activity paralleled by learning/behavioural improvements. The first studies explored the effects of tDCS on the visual cortex excitability. In a study using large Gabor patch stimulus, Antal and co-workers (2001) applied anodal and cathodal tDCS for 7 minutes at 1mA intensity on the V1. They showed that only cathodal stimulation significantly decreased static and dynamic contrast sensitivities, whereas anodal stimulation did not influence contrast sensitivity (Antal et al., 2001). However, a recent study (Kraft et al., 2010) just demonstrated that anodal tDCS applied on the visual cortex can also cause a transient increase in contrast sensitivity for the central positions. In this study cathodal stimulation of the visual cortex did not affected contrast sensitivity. This might be due to different stimulation durations used in the two studies or to the specificities of the visual stimuli. Another possible demonstration of the efficacy of tDCS over visual areas is valuated through phosphenes threshold (PTs). TMS pulses applied to the occipital cortex can elicit spots of light or stars that appear with stimulation and disappear with its cessation, called phosphenes (Meyer et al., 1991). The mean TMS intensity required to elicit phosphenes is defined as the PT. Antal et al. (2003a, 2003b) demonstrated that tDCS over the visual cortex is able to modify the perception of phosphenes, evoked by single pulse or repetitive TMS of the V1. They showed that cathodal stimulation increased, whilst anodal stimulation decreased PT (Antal et al., 2003a, 2003b). Compared to PTs measurements, visual evoked potentials (VEP) are considered physiological measures more objective for evaluating visual excitability cortex.

Indeed, VEP reflect the feature of the entire visual pathway in response to visual activation. The amplitude and latency of the N70 and P100 VEPs were measured in two different studies that have obtained contradictory results. In the first study Antal et al., (2003b) showed that cathodal tDCS over the V1 decreased whilst anodal tDCS increased the amplitude of the N70 component of the VEP. However, significant effects can only be observed when low contrast visual stimuli were shown. High contrast stimuli probably activate the respective visual cortical pathways and areas optimally. Therefore subthreshold excitability modulation induced by tDCS could not produce a clear change in the size of the VEP amplitude in this case. In another study was observed the opposite effect than precedent study: anodal stimulation resulted in reduced P100 amplitude while cathodal stimulation increased it (Accornero et al., 2007). This discrepancy concerning the results of the two studies might be to the different visual paradigm and different electrode positions applied. In this second study had used pattern-reversal checkboard stimulation and a reference electrode or neck.

Recent human studies have showed the involvement of higher visual areas in the modulation of visual neural excitability using tDCS goes beyond V1 and could influence complex, visual adaptation related processes. Indeed, in a study Antal et al. (2004c) found that the percentage of correct tracking movements increase significantly during and immediately after cathodal tDCS over medial temporal (MT) areas (called V5), while anodal stimulation had no effect when already learned manual visuo-motor task was applied. The highly specificity of the effect of the stimulation might be explained by the complexity of perceptual information processing needed for this type of task. The complexity of the task could produce a "noisy" activation state in the neuronal population involved in the task and cathodal tDCS may decrease this "noisy" activation to increase the signal-to-noise ratio useful to improve performance (Antal et al., 2004a). In another experiment Antal et al. (2004c) supported the involvement of MT/V5 in motion adaption processes. In this study the authors demonstrated that both anodal and cathodal tDCS affected the strength of perceived motion after-effect (MAE), in the specific the stimulations reduced the perception of the MAE. The MAE plays an important role in visual neuroplasticity (Anstis et al., 1998) and is the perceptual manifestations of the

neuronal adaption processes. One possible explanation of this effect can be that tDCS affects the interaction between the neural representations of different motion directions in MT/V5. Modulation of the neural excitability with anodal and cathodal tDCS might result in an attenuated expression of the adaptation-induced imbalance in both cases and consequently in weakened motion after-effect. In addition, the stimulation of the right parietal posterior cortex (PPC) induced an enhancement of visual orienting and visual search. Unilateral stimulation of the PPC modulated the performance of healthy subjects in a visual dot detection task bidirectionally, anodal tDCS improved the detection of contralateral stimuli, while cathodal tDCS ameliorated the detection of ipsilateral stimuli (Schweid et al., 2008, Sparing et al., 2009). These findings are encouraging for future intervention in brain-damaged patients with sensory problems and with visuo-spatial disabilities. In recent years, few studies have applied a new method called transcranial alternating current stimulation (tACS). tACS consists of the application of sinusoidal/alternating current at specific frequencies over the cerebral cortex. Indeed, tACS can interact with ongoing rhythmic activities in the visual cortex in a frequency-specific fashion and induce visual experiences as phosphenes (Kanai et al., 2008). In a recent study, Kanai and colleagues (2008) applied an oscillatory current over V1 using different frequencies (5, 10, 20 and 40 Hz). The authors observed that tACS seems to induce phosphenes in a frequency dependent way, with a peak slightly lower in darkness than in brightness. In the specific, it was found that tACS at 20 Hz decreased TMS-induced PTs, in other words increased the excitability of the visual cortex, whilst other frequencies did not affected PTs. Recently, Zaehle et al. (2010) have reported the electrophysiological evidences of tACS. In this study, tACS was applied over the occipital cortex of healthy subjects to entrain the neuronal oscillatory activity in their individual alpha frequency range and compared results with those from a separate group of participants receiving sham stimulation. The tACS but not the sham stimulation elevated the endogenous alpha power in parieto-central electrodes of the EEG. These preliminary findings might lead to new implementations of rhythmic stimulation as tools in therapy and neurorehabilitation. Indeed, several recent studies indicate that interacting with cortical activity by TMS can positively influence cognitive performance in normal subject (Thut and Miniussi, 2009) and in patients

with pathological alterations of cortical excitability and neuroplasticity, deficit cognitive or dementia. The modification of cortical activity with rhythmic electrical stimulation might regulate maladaptive patterns of brain oscillations, and provide a possibility for inducing a new balance within the affected functional network. However these studies have applied only DC stimulation or tACS, till now no study has applied tRNS stimulation on the visual cortex and in visual cognitive task. In the next chapter will present the first experiment that used the tRNS stimulation to understand the plasticity phenomena in the visual cortex.

2.4 Mechanisms of functioning of tES at the neuronal level

The neuronal basis of electrical stimulation using DC were first described in the late 1950' s and 1960's (Bindman et al., 1962; Purpura and McMurtry, 1965; Terzuolo and Bullock, 1956). Single cell recording studies in rats and cats have demonstrated that DC is able to modulate the neuronal spontaneous firing rate through a shifting of the resting neuronal membrane potential, but does not evoke cortical potentials. Cathodal stimulation can reduce neuronal firing rates, whereas anodal stimulation is able to reverse this effects (Creutzfeldt et al., 1962). Studies in the human suggest that tDCS modulate cortical excitability during stimulation by non-synaptic changes of the cells, but there is increasing evidence that the aftereffects of tDCS are driven by synaptic modifications (Bindman et al., 1962, 1964). However, over the years, the vast majority of human studies have applied specific drugs for explore and manipulate the physiological effects induced by tES (Liebetanz et al., 2002). The motor cortex excitability changes in healthy human were tested with the use of sodium channel blooker flunarizine and drugs involving NMDA-receptor. These studies have shown that changes induced by anodal tDCS are dependent upon the N-methyl D-aspartate (NMDA) receptor (Ridding and Ziemann, 2010; Ziemann and Siebner, 2008). Empirical evidence supports this idea, changes induced by a single application of tDCS are reversible, last from a few minutes to more than an hour, and are dependent upon NMDA. Indeed, long-term tDCS effects are not observed after administration of an NMDA receptor antagonist (Liebetanz et al., 2002; Nitsche et al., 2003a). The similarities between changes induced by tDCS and those involved in

the induction of long term potentation (LTP) plasticity in the motor cortex strongly suggest that synaptic plasticity is occurring. Both LTP and its opposite, long term depression (LTD), have also been postulated to explain the persistent effects of tES on cortical activity (Fritsch et al., 2010; Nitsche et al., 2009; Thickbroom, 2007; Ziemann and Siebner, 2008). Neural plasticity induced by different type of tES at the level of brain networks might be determined by changes that alter the property of synaptic plasticity. Nevertheless, the neuronal depolarization or hyperpolarization induced by tES is likely to affect many of the synapse within the stimulated cortex, although these effects are not homogeneous across the cortex but depend on the orientation type, and depth of the stimulated neurons (Stagg and Nitsche, 2011b). Moreover additional aspects and effects should be considered in the relation to the differential stimulation characteristics. In addition, it is important to consider that the mechanism underlying cortical excitability changes induced by tES may differ during after stimulation. Indeed, the effect of tES stimulation depends by several aspects: on the current density, stimulus duration, intensity, timing and intervals of time between two stimulations. In a recent study, Fricke et al. (2011) explored how homeostatic plasticity depend on the time interval between the application of two plasticity-inducing protocols. The author measured the MEP amplitude after repeated tDCS stimulation of the motor cortex. In addition they investigated if the duration of stimulation is important in the induction of phenomena homeostatic. In the specific, they compared the aftereffect of a single 5 min session of anodal or cathodal tDCS with the effect of a 5min session preceded by an identical 5 min conditioning session 30, 3 or 0 min beforehand. The main result of their study was that the effects of repeated short period of motor cortex tDCS follow a time-dependent rule compatible with homeostatic mechanisms. Indeed, the authors showed that 5min of anodal tDCS increase excitability for about 5 min and they found opposite effect for cathodal stimulation. However, if two 5 min periods of tDCS are applied with 3 minutes of interval, then the second session has the opposite effect to 5min tDCS given alone. The reversal effect has been observed with the cathodal stimulation, where 3 minutes of break between two 5 min of stimulation induced a significant facilitation of MEP. Another recent study found that the efficacy of inhibitory cathodal tDCS was enhanced if a second period of stimulation was given during the aftereffects of the

first one. But it was reduced if the second stimulation was applied when the aftereffects of the first stimulation had vanished (Monte-Silva et al., 2010). This result is not surprising, considering the presence of numerous evidences in the literature on the phenomena of meta-plasticity with the application of techniques of brain stimulation (i.e., TMS, tDCS) (Gamboa et al., 2010; Gentner et al., 2008; Iyer et al., 2005; Siebner et al., 2004). In addition, this recent literature seems to confirm the involvement of mechanisms involving the voltage-gated Ca2+ channels (Wankerl et al. 2010) already demonstrated with experiments that have used specific drugs (Liebetanz et al., 2002; Nitsche et al., 2003a; Ridding and Ziemann, 2010). The studies described so far have mainly explored the motor cortex, indeed there are no currently available data about the underlying cellular-molecular mechanism of tES induced effect over the visual areas. Although it was hypothesized that tDCS acts similarly in the visual cortex and in the motor cortex because some of the functional effects are comparable, the results of the motor researches cannot be translated to the visual areas due to anatomical, neurochemical, and physiological difference (Antal et al., 2006). Indeed, the visual areas are rich of cholinergic and GABAergic innervations therefore it is possible that other neuromodulator take part in the effect of tDCS compared to the motor cortex that is influenced mainly NMDA receptors.

Regard to random noise stimulation the neuronal/molecular mechanism are not yet known. Terney et al. (2008), suggest that using tRNS sodium channels activity can be augmented. After a depolarization, repolarization of sodium channels would generally take some time, but if a repeated stimulation is applied these channels can be reopened in a shorter time (Schoen and Fromherz, 2008). Because tRNS is a repetitive, random, and subthreshold stimulation, it has been hypothesized that it would induce temporal summation of neural activity when the time-constant of a neuron is sufficiently long to permit the summation of two stimuli presented in close sequence (Fertonani et al., 2011). These mechanisms should not be present with tDCS because a direct and continuous stimulation may induce an adaptation of stimulated neurons that rebalance the modulation of ion channel conductance. In summary, a pure DC stimulus can open Na+ channel just once, whereas repeated pulse can induce multiple ionic influxes and obtain heightened effects. The interval at which the pulses are repeated must be short and related to time constant of the

nerve membrane (Terney et al., 2008). A further possible explanation for the effects induced by tRNS can be in the frame of the stochastic resonance phenomenon e.g.(Miniussi and Vallar, 2011) tRNS is by definition a stimulation that induce non-finalized random activity in the system i.e., noise. In general, noise decreases performance, but nonlinear systems, like the brain, can use noise to enhance performance through stochastic resonance (see Moss et al., 2004). Indeed, the noise can increase but also the signal will increase consequently and since the state of the system (running a task) the increase of the signal will be likely more consistent. Therefore the effect of tRNS may to explain facilitatory results (Terney et al., 2008) in terms of the relationship between noise and signal in the nervous system, so enhanced performance could be observed with an optimum level of noise (Antal et al., 2004b; Ruzzoli et al., 2010). However these are only speculations which must be verified by both modeling studies and animal studies. In addition further studies are necessary for understanding which are the mechanisms of action of this new methodic. Indeed, once it able to elucidate how tRNS and other varying methods of external electrical stimulation are effective in the mammalian cortex, it will be able to apply these properties into useful therapeutic tools.

CHAPTER 3

"Random noise stimulation improves neuroplasticity in visual perceptual learning"

3.1 Introduction

Neuroplasticity refers to the ability of the human brain to change its structure and function as part of the processes that underlie learning and memory to permit adaptation to environmental changes (Cooke and Bliss, 2006). A staple mechanism of neural plasticity is the strengthening or weakening of synaptic transmission between neurons. Changes in the efficacy of synaptic transmission result in excitability and activity modifications in a specific cortical area or within a more restricted cortical network. These changes correlate with cognitive plasticity at behavioural level. Cognitive plasticity has been observed after lesions and in response to adaptation and PL (Fahle 2002a, 2002b; Thiele, 2004) PL is a form of implicit memory characterised by an improvement in sensory discrimination after repeated exposure to particular types of stimuli and is a manifestation of neural plasticity (Carmel and Carrasco, 2008; Gilbert et al., 2001; Li et al., 2004).

Animal and human studies have demonstrated the specificity of the V1 for recognising basic stimulus characteristics, such as orientation and direction. This specificity implies the direct involvement of V1 cells in learning and discriminating stimulus characteristics (Li et al., 2004; Yotsumoto et al., 2008). Focusing on PL and the visual system, neurophysiological evidence has demonstrated that V1 is highly plastic (Wandell and Smirnakis, 2009). Thus, the study of the neural changes associated with PL can be used to increase our understanding of plasticity mechanisms in the adult sensory cortices.

In recent years, new techniques for the study of the plasticity mechanisms that underlie perceptual, motor and cognitive functions in the human brain have emerged. Today, several brain stimulation techniques are used to modulate cortical excitability in a non-invasive way. Among these techniques, the use of transcranial electrical stimulation (tES) is very promising for the investigation of plasticity. The effects of tDCS depend on the direction of the current. Usually, anodal stimulation increases excitability, whereas cathodal stimulation decreases neural activity (for a review see Nitsche et al., 2008). In particular, several studies have investigated the application of tDCS to the motor areas (Nitsche and Paulus, 2000; Nitsche et al., 2003c) and the induction of cortical plasticity (Siebner et al., 2004). These studies have shown that long-term action of tDCS involves the glutamatergic N-methyl D-aspartate (NMDA)

receptors (Liebetanz et al., 2002, Nitsche et al., 2003a). Therefore, long-term potentiation has been postulated as a likely mechanism underlying the effects of tDCS. However, these observations have been based mainly on the motor system, and few papers have focused on the visual system (Accornero et al., 2007; Antal and Paulus, 2008). Additionally, the effects on behaviour are often not univocal (Berryhill et al., 2010; Fertonani et al., 2010; Marshall et al., 2005).

tRNS consists of the application of an alternating current at random frequencies over the cortex. The noise signal contains all of the frequencies from 0.1 Hz to 640 Hz, a range that includes all cortical rhythms up to 600 Hz that have been individuated at cortical level (Buzsaki, 2006). One of the organising principles of rhythmic activity in the brain is the synchronisation of oscillations across neuronal elements. Based on this technical characteristic, tRNS could be expected to interact with ongoing cortical rhythms (Thut and Miniussi, 2009) and to induce a random frequency that should interfere with the synchrony between neurons within an area or between areas within a network. However, these results are not observed. In fact, Terney et al. (2008) demonstrated that ten minutes of tRNS at high frequency (101-640 Hz) to the motor cortex induces a positive modulation of cortical excitability (i.e., an increase in the amplitude of MEP that persists after the end of stimulation. Furthermore, behavioural improvement in a learning motor task was obtained with the application of the entire frequency spectrum (Terney et al., 2008)

If these results can be extended to other brain areas, then tRNS might be as effective as tDCS, as suggested previously by Terney et al. (2008). Furthermore, because of the characteristics of the signal used, tRNS might be even more effective in the modulation of neuronal activity. We hypothesised that tRNS might be more effective in inducing "facilitation" than tDCS for two reasons. First, tRNS may induce direct temporal summation of activity in the stimulated neural population. This mechanism occurs when the time constant of the activity of a neuron is sufficiently long to permit the summation of two stimuli presented in close sequence. In contrast to tDCS, tRNS utilises stimuli in a rapid temporal sequence, and therefore, it should be more effective than tDCS. The second and more important reason is related to the ability of neurons to adapt to a constant environment. In neurons, ion channels are subject to homeostatic phenomena, such as light adaptation (i.e., the ability of the

retina to adjust to various levels of darkness and light). Therefore, neurons that are embedded in a constant electrical field should adapt their membrane responses and return to an initial "resting" state. This return to a resting state should not occur when the electrical field that excites the neurons is constantly changing in an alternating random frequency mode, e.g., as used in tRNS. Given these considerations, we can also predict that high frequencies of the oscillating current should produce strong effects.

Clearly, this type of stimulation is not sufficient to induce an action potential; however, it is adequate to block the adaptation of the membrane potential and force the membrane to stay close to the action potential threshold. These effects should not occur with tDCS because only a repeated stimulation of neurons (i.e., a change of current direction that involves continuous sub-threshold polarisations) at high frequency can lead to a reinforcement of the active connections stimulating the neurons. We expect to observe greater performance improvement with high-frequency tRNS than with tDCS. Moreover, we predict that the improvement with tRNS will not induce adaptation, in contrast to the improvement with the tDCS, and therefore, it will last longer. However, tRNS is a new method and has never been compared with tDCS. Therefore, direct considerations cannot be drawn.

To test these hypotheses, we modulated behaviour-induced neuroplasticity, which normally happens in the adult brain during skill learning, with different types of tES (e.g., tDCS and tRNS). To establish the role of the frequency range, we applied low-frequency tRNS (lf-tRNS, 0.1-100 Hz) and high-frequency tRNS (hf-tRNS, 101-640 Hz). Furthermore, these conditions were compared to anodal-tDCS (a-tDCS), cathodal-tDCS (c-tDCS) and sham conditions. The stimulations were applied while healthy subjects were executing an orientation discrimination task (Matthews et al., 1999). The orientation discrimination task was particularly useful for our purposes because it is a widely studied visual PL task that involves V1 neurons (Ghose, 2004; Li et al., 2004).

Our data support the initial hypothesis of a higher efficacy of tRNS as measured by an improvement of the subjects' performance. Moreover, this effect was more pronounced with the application of hf-tRNS and highlights the potential of this new method for the induction of plasticity at the neuronal level.

3.2 Materials and Methods

3.2.1 Subjects

107 healthy subjects took part in the experiment. All of the participants were right-handed with normal or corrected-to-normal vision. We did not include subjects with a history of seizures, implanted metal objects, heart problems or any neurological disease. Moreover, as a standard procedure, subjects who did not reach an established learning performance in the task were excluded from the study, based on this criteria 8 participants, were excluded. The remaining 99 subjects were divided as follow: 6 participants (2 males, mean age ±standard deviation 35.0 ±7.2 years; range 29-48 years) were tested in a pilot behavioral experiment, 84 participants (42 males, mean age 21.7 ±2.5 years; range 19-30 years) took part in the main experiment, and 9 participants (3 males, mean age 31.7 ±3.9 years; range 24-38 years) were tested in a control hf-tRNS experiment. The 84 subjects participating in the main experiment were divided into six groups. All groups had 14 participants (7 males and 7 females), and the mean ages of the groups were: 22.4 ±2.8 years for hf-tRNS group, 21.7 ±2.9 years for lf-tRNS group, 21.8 ±2.3 years for a-tDCS group, 21.7 ±2.6 years for c-tDCS group, 21.6 ±3.0 years for sham group and 20.9 ±1.6 years for the Cz group, respectively.

The present study was approved by the Ethics Committee of the IRCCS San Giovanni di Dio Fatebenefratelli, Brescia, Italy. Safety procedures based on non-invasive brain stimulation approaches were adopted (Poreisz et al., 2007; Rossi et al., 2009), and informed consent was obtained from all participants prior to the beginning of the experiment.

3.2.2 Ovarian hormone influence on data variability

The influence of ovarian hormones on task performance has been demonstrated in many transcranial magnetic stimulation (TMS) studies (Inghilleri et al., 2004; Sale et al., 2007; Smith et al., 2002; Smith et al., 1999). Indeed, these studies have shown that the cortical excitability of male and female subjects is only similar during the

follicular phase of the menstrual cycle when progesterone levels are low and estrogen levels are high (Inghilleri et al., 2004). Furthermore, tDCS studies (Chaieb et al., 2008, Kuo et al., 2006) have highlighted gender differences in both visual and motor domains. These differences are likely due to hormonal effects, but no studies have controlled for this factor. The female participants in the present study were tested during the follicular menstrual phase (mean day from the first day of the menstrual period: 14.1 ±3.0, range 9-20 days) because this is the period when progesterone levels are low and estrogen levels are high. Under these conditions, the cortical excitability of male and female subjects was similar (Inghilleri et al., 2004).

3.2.3 Orientation discrimination task (ODT)

We chose the ODT because it is a widely studied VPL task (Shiu and Pashler, 1992; Vogels and Orban, 1985) that involves V1 neurons. Many studies have demonstrated that V1 cells are highly specific for basic stimulus characteristics, such as orientation and direction (Schiltz et al., 1999; Schoups et al., 2001; Ts'o et al., 1986; Yacoub et al., 2008).

In the ODT task, participants had to decide whether the presented stimulus was tilted clockwise or counterclockwise relative to the previously presented stimulus. All stimuli were black lines, and each line stimulus was 2° long and 5 min wide (in visual angle). In each trial, there were two lines, one called reference (with a fix orientation) and the other called target stimulus (different orientations). The orientation of the reference stimulus was 45° in the upper right and lower left hemifields and 135° in the upper left and lower right hemifields. The angular differences between the reference and the target stimulus were 1.1, 1.21, 1.33 and 1.46° (Matthews et al., 1999). The reference was presented first in half of the trials and second in the other half of the trials. All of experimental parameters just mentioned were balanced and randomized between blocks. The trial structure is described in Figure 4. The subjects were asked report the orientation of the second line (clockwise/counterclockwise) compared to the first. They were asked to respond as quickly and accurately as possible after the second stimulus was presented by pressing the left (counterclockwise) or right (clockwise) button of a response pad

with the left or right index finger, respectively. Auditory feedback (duration = 50 ms; frequency for the correct response = 700 Hz; frequency for the incorrect response = 350 Hz) informed the subjects about the correctness of their responses.

Stimuli were presented on a computer screen using Presentation software v. 12.0 (http://www.neurobs.com) in each of the four visual hemifields: upper left, upper right, lower left and lower right. In each trial, the two stimuli were presented in the same hemifield (Figure 4). To limit the area in which the stimuli were presented, a black piece of cardboard covered the screen except for a circle that was 10 cm in diameter located at the centre of the screen. A central fixation point was maintained for the duration of the trial.

In the main experiment, each block of the ODT consisted of 64 trials and lasted approximately 4 minutes. The ODT consisted of eight blocks plus a training block. The training block was similar to the trial blocks, but it had a different number of trials (only 8) and an increased rotation angle between the two stimuli (15° clockwise or counterclockwise). The last block (i.e., the eighth one) was a fictitious block that only consisted of 16 trials.

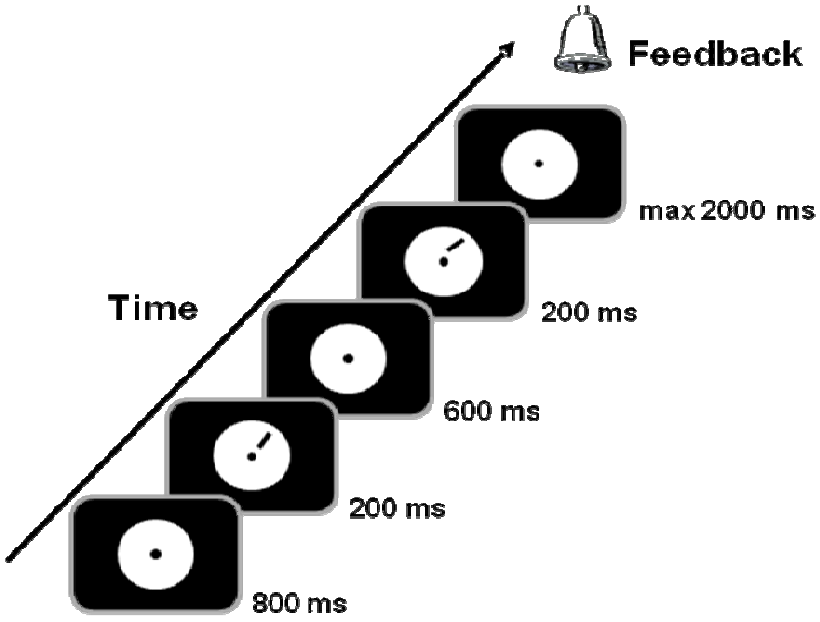

Figure 4. Trial structure. Example of a trial of the orientation discrimination task with the reference and target stimuli presented in the upper-right hemifield.

3.2.4 Pilot experiment

The pilot experiment aimed to determine the ideal parameters of the ODT. The subjects were asked to complete five blocks of 128 trials each, and the results are presented in Table 1. All of the subjects reported a sense of fatigue at the end of each of the five blocks, which was more pronounced for the last two blocks. For this reason, 8 shorter blocks (i.e., each block contained half as many trials as the original block) were presented in the main experiment.

	Block 1	**Block 2**	**Block 3**	**Block 4**	**Block 5**
Accuracy **d'** (±SD)	0.07 ± 0.35	0.31 ± 0.34	0.50 ± 0.19	0.57 ± 0.15	0.61 ± 0.16
RTs **ms** (±SD)	693 ± 134	608 ± 124	589 ± 120	556 ± 132	545 ± 125

Table 1. Pilot experiment: Results of the orientation discrimination task for the pilot experiment.

3.3 Main experiment

3.3.1 Stimulation techniques: tRNS and tDCS

The stimulations were delivered by a battery-driven stimulator (Eldith-Plus, NeuroConn GmbH, Ilmenau, Germany) through a pair of saline-soaked sponge electrodes. The tRNS consisted of an alternating current of 1.5 mA intensity with a 0 mA offset applied at random frequencies. The frequencies ranged from 0.1 to 100 Hz for lf-tRNS, or from 100 to 640 Hz for hf-tRNS. The intensity of stimulation did not induce a phosphene perception in either of the frequency bands (Kanai et al., 2008). The tDCS consisted of a direct 1.5 mA current.

In the main experiment, the stimulations were applied for approximately four minutes during each of the first five experimental blocks. The total duration of the stimulations was approximately 22 minutes. The active electrode had an area of 16 cm^2, whereas the reference had an area of 60 cm^2. The current density was maintained below the safety limits (varying between 25 and 60 $\mu A/cm^2$) (Poreisz et al., 2007). The electrodes were kept in place with elastic bands, and an electro-conductive gel was applied under the electrodes before the montage to reduce skin impedance. The active electrode was applied over the occipital cortex. When tDCS stimulation was applied, the polarity of the active electrode was anodal in the a-tDCS condition and cathodal in the c-tDCS condition. The individual target area was determined by examining the position in accordance with the 10-20 International EEG system. This procedure was performed starting at 10% of the nasion-inion distance above the inion. The mean position for V1 stimulation was determined to be 3.5 ±0.2 cm above the inion. The reference electrode was fixed extracephalically on the right arm. In the sham stimulations, the current was turned off 20 seconds after the stimulation began (Gandiga et al., 2006). Additionally, we employed a control condition to test the specificity of the hf-tRNS effects by administering active stimulation over the vertex, which is a brain area that is not involved in our task. All of the stimulation parameters were identical to hf-tRNS except for the active electrode, which was placed on the Cz location.

At the end of the experimental session, we asked all subjects to complete a questionnaire developed by our research laboratory about the sensations they experienced during the different stimulations (Fertonani et al., 2010).

3.3.2 Procedure

The participants were seated in front of a computer screen in a quiet, semi-dark room. A 57 cm distance from the screen was maintained through the use of a chin rest. The experiment was a between-subjects design with six stimulation conditions: hf-tRNS, lf-tRNS, a-tDCS, c-tDCS, sham stimulation and Cz.

The subjects began the ODT 10 seconds after the onset of the stimulation. The procedure is depicted in Figure 5.

In the real stimulation conditions (hf-tRNS, lf-tRNS, a-tDCS, c-tDCS and Cz), the stimulation was only delivered during the first five blocks of the task. In the last three blocks, sham stimulation was applied for 20 seconds at the beginning of each block. In the sham condition, the stimulation was a placebo for all eight blocks and was delivered for 20 seconds at the beginning of each block. The duration of the entire experimental session was approximately 45 minutes.

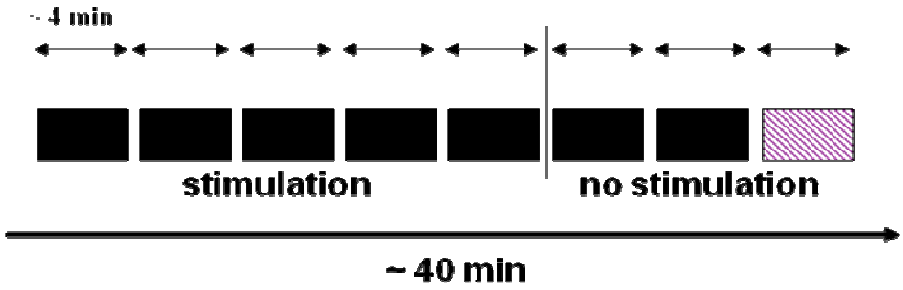

Figure 5. Procedure of the experiment. The experiment was designed to be between-subjects with six stimulation conditions: hf-tRNS, lf-tRNS, a-tDCS, c-tDCS, CZ and sham. Subjects were stimulated only in the first five blocks (label "stimulation"). In the sixth and seventh block the stimulation was a placebo one (label "no stimulation"). The black blocks represent the real trials, whereas the white block was the fictitious block.

3.3.3 Data analysis

The average orientation sensitivity was calculated in terms of a d' value for each subject and each block, separately for each stimulation condition. Because the ODT is a two-alternative forced-choice task, a value of d' = 1 corresponded to a 75% accuracy rate.

As an index of learning rate, we analyzed the relationship between d' values and the log transformation of block numbers using linear regression analysis. This analysis allowed us to associate a slope value with each subject.

We also analyzed the mean reaction times (RTs) of the correct response trials for each subject and each block.

For all data (d' values, slopes and RTs), the Kolmogorov-Smirnov test confirmed the normality of the distribution, and the data were analyzed using a repeated-measures analysis of variance (ANOVA). The data sphericity was tested using the Mauchly test where appropriate. When the test results were statistically significant, the data were corrected using the Huynh–Feldt correction. A p-value < 0.05 was considered significant for all statistical analyses. For multiple comparisons, we used Fisher's Least Significant Difference (LSD) method to test our specific "a priori" hypotheses (i.e., to compare the different stimulation conditions). For all other comparisons, the p-values were corrected using a Bonferroni correction.

Data from the sensations induced by tES were analyzed using a one-way ANOVA for each sensation to compare the different stimulation types. The p-values were corrected using Bonferroni corrections. In addition, we calculated an intensity of perceived sensations index for each subject (i.e., the sum of the values reported in the questionnaire for each sensation) and correlated it with the mean d' values in the five blocks of real stimulation.

3.4 Results

3.4.1 Orientation sensitivity – d'

We performed a repeated-measures ANOVA with *block* (from 1 to 7) as a within-subjects factor and *stimulation condition* (hf-tRNS, lf-tRNS, a-tDCS, c-tDCS, sham, and Cz) and *gender* (male, female) as between-subjects factors. We observed a significant main effect for *block* [$F(6, 432) = 29.34$; $p < 0.001$], *stimulation condition* [$F(5, 72) = 3.49$; $p < 0.01$] and *gender* [$F(1, 72) = 6.82$; $p < 0.05$]. Regarding the main effect of *block*, multiple post-hoc comparisons revealed a statistically significant difference between block 1 and blocks 3, 4, 5, 6 and 7; between block 2 and blocks 4, 5, 6 and 7; between block 3 and blocks 6 and 7; and between blocks 4 and 7.

Regarding the main effect of *stimulation condition*, multiple post-hoc comparisons revealed that hf-tRNS (mean d' ±SEM = 0.770 ±0.130) was significantly different from sham (0.375 ±0.116), a-tDCS (0.509 ±0.134), c-tDCS (0.377 ±0.125), and Cz

(0.376 ±0.119) conditions (Figure 6). The difference between hf-tRNS and lf-tRNS (0.617 ±0.124), however, was not significant (p = 0.220), and the difference between lf-tRNS and sham stimulation was marginally significant (p = 0.053). The *gender* factor was statistically significant. Males were more accurate than females in all conditions (males: 0.597 ±0.125; females: 0.411 ±0.131). The absence of any interaction between the *gender* and *stimulation condition* factors, however, discouraged us from examining the gender factor in more detail.

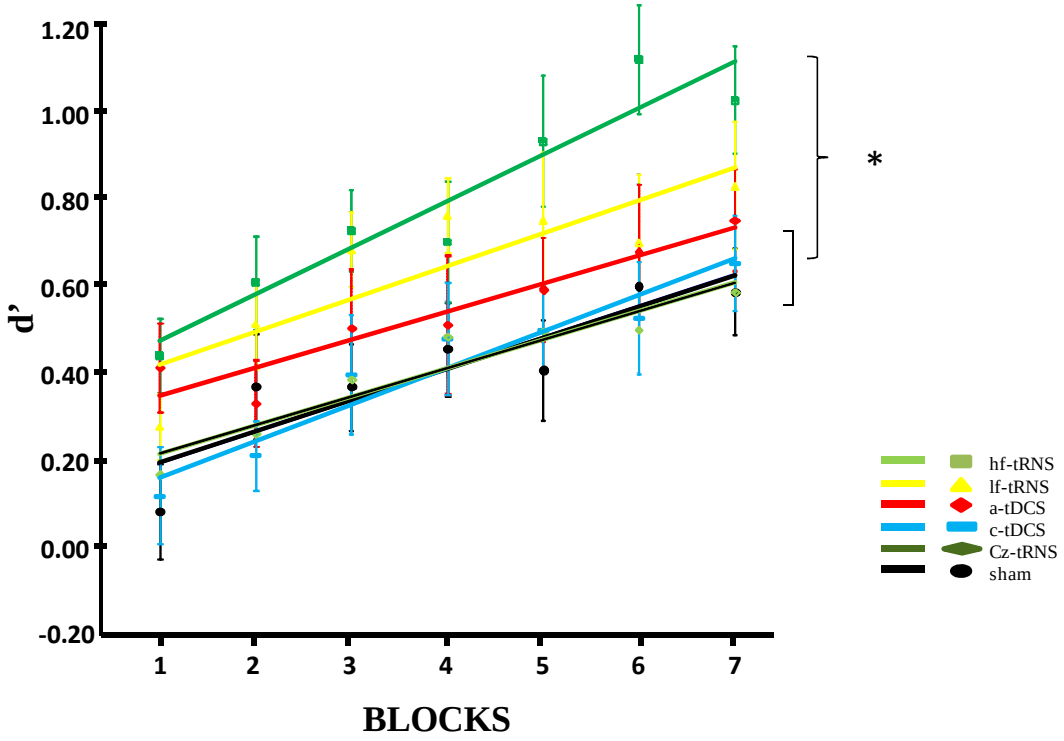

Figure 6. Main experiment results. Data are represented as the mean SEM. The lines represent the fit of each condition: the green line corresponds to hf-tRNS, the yellow line corresponds to lf-tRNS, the red line represents a-tDCS, the blue line represents c-tDCS, dark green line represents Cz stimulation and, the black line corresponds to sham. The asterisk near the curly bracket indicates the statistically significant differences between hf-tRNS and the conditions in the square bracket ($p < 0.05$).

Repeated measures ANOVA of each stimulation condition in the five blocks with real stimulation highlighted the main effect of *block* ($p < 0.01$) for all stimulation conditions except a-tDCS. Post-hoc comparisons in the hf-tRNS condition revealed that block 1 was different from blocks 3, 4 ($p = 0.065$) and 5, and block 2 differed from block 5. In the lf-tRNS condition, block 1 was different from blocks 3, 4 and 5. In the c-tDCS condition, block 1 was different from blocks 4 and 5, and block 2 was different from block 5. In the sham and Cz conditions, block 1 was different from blocks 4 and 5. No statistically significant differences were observed in the a-tDCS condition, which suggested the absence of a learning effect during a-tDCS.

To evaluate the stimulation effects at different time points of the protocol (blocks 1 and 5: beginning and end of stimulation, respectively; blocks 6 and 7: after the stimulation ended), we compared the different stimulations by separately considering each block. In block 1, there was a significant main effect of the *stimulation condition* [$F(5, 78) = 2.58$; $p < 0.05$]. Post-hoc comparisons revealed differences between the hf-tRNS and the sham, c-tDCS, and Cz conditions ($p < 0.05$) and between the a-tDCS and the sham and c-tDCS conditions ($p < 0.05$). In block 5, there was a significant main effect of the *stimulation condition* [$F(5, 78) = 2.42$; $p < 0.05$]. Post-hoc comparisons revealed a difference between the hf-tRNS and a-tDCS, sham, c-tDCS, and Cz conditions ($p < 0.05$). In block 6, after the stimulation ended, there was a significant main effect of the *stimulation condition* [$F(5, 78) = 2.89$; $p < 0.05$]. Post-hoc comparisons revealed differences between the hf-tRNS and all other conditions ($p < 0.05$). In block 7, only a marginally significant effect of the *stimulation condition* [$F(5, 78) = 1.99$; $p = .09$] was present. Post-hoc comparisons revealed a difference between the hf-tRNS and the sham, c-tDCS, and Cz conditions ($p < 0.05$).

The present data support the initial hypothesis that tRNS was more efficacious than a-tDCS (measured by improvement in the subjects' performance). Among the excitatory stimulations, this result confirms that hf-tRNS had the most prominent effect

3.4.2 Learning rate – slopes

To specifically evaluate learning rate differences for hf-tRNS and a-tDCS, the two main types of stimulation that increase cortical excitability, we compared learning performance during hf-tRNS and a-tDCS. In this analysis, the *stimulation condition* results were significant [$F(1, 26) = 3.17$; $p = 0.05$], which suggested the presence of a different learning rate between the two groups (i.e., a lower rate with a-tDCS). Moreover, to evaluate the overall effects while considering the other conditions, we performed a one-way ANOVA with *stimulation condition* as a between-subjects factor. The result was not statistically significant [$F(5, 72) = 1.03$; $p = 0.32$].

3.4.3 Response times (RTs)

We performed a repeated measures ANOVA with *block* as a within-subjects factor and *stimulation condition* and *gender* as between-subjects factors. These analyses showed a significant main effect for *block* [Epsilon = 0.74, $p < 0.001$; Huynh–Feldt $F(4.44, 319.36) = 25.94$; $p < 0.001$] and *gender* [$F(1, 72) = 7.31$; $p < 0.01$]. The factor *stimulation condition* was not statistically significant [$F(5, 72) = 1.03$; $p = 0.40$]. Significant interactions between *block* and *stimulation condition* [$F(30, 432) = 2.12$; $p < 0.001$] and between *block*, *stimulation condition* and *gender* [$F(30, 432) = 1.79$; $p < 0.001$] were present, but post-hoc comparisons (Bonferroni corrections) did not confirm their relevance.

Regarding the main effect for *block*, multiple post-hoc comparisons revealed a statistically significant difference between block 1 and the other blocks; between block 2 and the other blocks but block 3; and between block 3 and blocks 6 and 7. As expected, the subjects were slower at the beginning of the task than at the end. The main effect for *gender* showed that males were faster than females in task execution (mean RT ±SEM: male = 682 ±27 ms, female = 773 ±28 ms).

3.4.4 Sensations induced by different types of tES

Only one recently published paper (Ambrus et al., 2010a) has considered the skin perception thresholds for both tDCS and tRNS. Nevertheless, Ambrus and colleagues (2010a) only compared the detection rates of the tDCS and tRNS and found that tRNS-induced sensations were less frequently perceived than sensations induced by tDCS. Based on these data, they proposed using tRNS application as a possible alternative to tDCS. In addition to analyzing the differences between the stimulation types, we also analyzed all of the possible induced sensations in detail. Each participant completed a questionnaire at the end of the experiment (Fertonani et al., 2010) and reported having tolerated the stimulation without discomfort. The results of the questionnaire are reported in Table 2. Participants were unable to distinguish the real stimulation from the placebo stimulation. A one-way ANOVA for each sensation was performed to compare the different stimulations. No difference between the tRNS and sham conditions was found. Interestingly, the analysis highlighted a statistically significant difference ($p < 0.01$) between both a-tDCS and c-tDCS and the other conditions with respect to itching. Furthermore, the a-tDCS was different ($p < 0.01$) from the other conditions with respect to irritation and burning. In general, the tDCS-induced sensations were perceived more strongly (Ambrus et al., 2010a; Poreisz et al., 2007) than the tRNS- or sham-induced sensations. With tDCS, pain, heat, iron taste and fatigue were comparable to the sham condition, but irritation, burning, and itching were perceived with real, but not sham, stimulation. In contrast, hf-tRNS and lf-tRNS were indistinguishable from sham conditions for all of the sensations examined. This characteristic makes tRNS an optimal tool for experimental designs in which sham stimulations should not differ from real stimulations.

Furthermore, we calculated, for all the experimental conditions, the correlation between the intensity of perceived sensations index (calculated for each subject as the sum of the values reported in the questionnaire for each sensation) and the mean d' values in the five blocks of real stimulation. The analysis was not statistically significant [$r(82) = 0.04$, $p > 0.05$]. We also tested the correlation for the a-tDCS group because the subjects reported slightly higher perceptions of discomfort. Similarly, this analysis was not statistically significant [$r(12) = -0.09$, $p > 0.05$]. This

result confirms the absence of a correlation between perceived discomfort and task performance.

tES		Irritation	Pain	Burning	Heat	Itch	Iron taste	Fatigue	Effect on performance
hf-tRNS	**Intensity**	**0.1**	**0.0**	**0.1**	**0.3**	**0.3**	**0.0**	**0.5**	**0.4**
	Subjects (%)	14	0	7	21	29	0	43	36
lf-tRNS	**Intensity**	**0.0**	**0.1**	**0.0**	**0.0**	**0.4**	**0.2**	**0.1**	**0.1**
	Subjects (%)	0	7	0	0	36	14	7	14
a-tDCS	**Intensity**	**1.1**	**0.2**	**0.7**	**0.4**	**1.4**	**0.1**	**0.4**	**0.5**
	Subjects (%)	79	14	50	29	86	7	29	36
c-tDCS	**Intensity**	**0.6**	**0.1**	**0.3**	**0.1**	**1.4**	**0.1**	**0.2**	**0.1**
	Subjects (%)	50	7	21	7	93	7	14	14
Sham	**Intensity**	**0.2**	**0.0**	**0.1**	**0.1**	**0.2**	**0.0**	**0.4**	**0.5**
	Subjects (%)	21	0	7	14	14	0	29	29
Cz	**Intensity**	**0.3**	**0.0**	**0.1**	**0.1**	**0.4**	**0.1**	**0.1**	**0.3**
	Subjects (%)	29	0	7	14	36	7	7	21

Table 2. Transcranial electrical stimulation-induced sensations: Mean intensity of the sensations reported by subjects after tES, and the percentage of subjects who reported each sensation. Sensation intensity was evaluated on a 5-point scale: 0 = none, 1 = mild, 2 = moderate, 3 = considerable, and 4 = strong. The column "Effect on performance" indicates the participants' subjective feelings relative to the tES-induced sensation's effect on performance.

3.5 Control hf-tRNS experiment

In the main experiment we found that hf-tRNS applied over V1 improves the performance in the ODT. This effect is immediately evident (i.e., from the first block, see Figure 7) and persist till the end of the stimulation, differently from a-

tDCS. To confirm that this initial effect was due to the stimulation, and not to baseline differences across groups, we tested an additional group of subjects in an experimental design that included an initial baseline block without stimulation.

3.5.1 Procedure

In this experiment the participants executed six blocks of the ODT. The first block was without stimulation while in the following five hf-tRNS was applied over V1. In this way all the parameters of the task and of the stimulation were maintained as in the main experiment, except for the moment in which the stimulation was applied (first vs. second block).

3.5.2 Results

The performance of the control-hf-tRNS group is depicted in Figure 7. A repeated measures ANOVA on the six blocks of the task highlighted the main effect of *block* ($p < 0.01$). Post-hoc comparisons revealed that block 1 was different from blocks 2, 3, 4, 5 and 6, and blocks 2, 3 and 4 differed from blocks 5 and 6.

Furthermore we compared the results of this group with the sham and hf-tRNS groups of the main experiment. We performed a repeated-measures ANOVA with *block* (from 1 to 6) as a within-subjects factor and *stimulation condition* (control-hf-tRNS, hf-tRNS and sham) as between-subjects factor. We observed a significant main effect for *block* [$F(5, 170) = 24.70$; $p < 0.001$] and *stimulation condition* [$F(2, 34) = 5.59$; $p < 0.01$]. Regarding the main effect of *block*, post-hoc comparisons revealed a statistically significant difference between block 1 and blocks 2, 3, 4, 5 and 6; between blocks 2, 3 and 4 and blocks 5 and 6; between block 5 and block 6.

Regarding the main effect of *stimulation condition*, as was expected post-hoc comparisons revealed that control-hf-tRNS (mean d' ±SEM = 0.614 ±0.142) was significantly different from sham (0.375 ±0.116). The difference between hf-tRNS (0.770 ±0.130) and sham was also statistically significant.

Student's t-test revealed that in the first block the performance of control-hf-tRNS group was not different from sham ($t = 0.771$, $df = 21$, $p = 0.45$) whereas it was

different from hf-tRNS ($t = -2.091$, $df = 21$, $p < 0.05$). In the last block vice versa the control-hf-tRNS performance was different from sham ($t = 2.556$, $df = 21$, $p < 0.05$), but not from hf-tRNS ($t = -0.645$, $df = 21$, $p = 0.53$).

To specifically evaluate learning rate differences for control-hf-tRNS vs. hf-tRNS, a-tDCS and sham, we performed a one-way ANOVA on the slope values with *stimulation condition* as a between-subjects factor. The result was statistically significant [$F(3, 47) = 2.99$; $p < 0.05$]. Post-hoc comparisons revealed a statistically significant difference between control-hf-tRNS and both sham and a-tDCS groups. The difference between hf-tRNS and a-tDCS obtained in the main experiment was therefore confirmed.

These new results strengthen our main experiment data. As showed in Figure 7, the performance in the control-hf-tRNS group is very similar to the hf-tRNS one, except for the first block in which the control-hf-tRNS performance is similar the sham one.

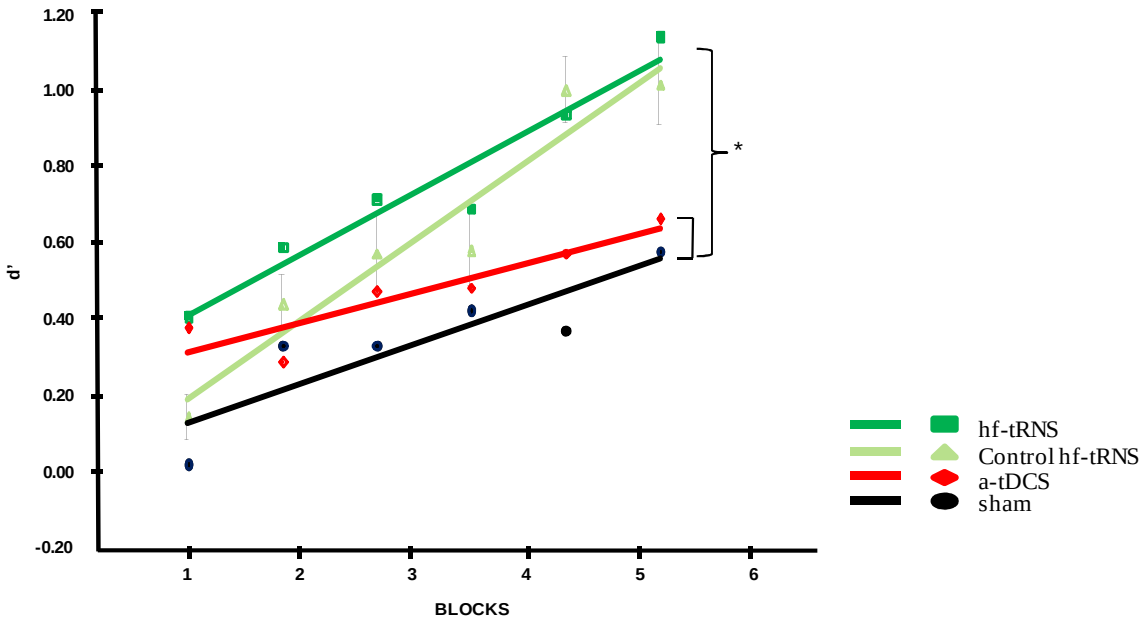

Figure 7. Control-hf-tRNS experiment results compared with main experiment hf-tRNS and sham groups. The lines represent the fit of each condition: the dark green line corresponds to hf-tRNS, the light green line corresponds to control-hf-tRNS, the red line corresponds to a-tDCS, and the black line

corresponds to sham. The asterisk near the curly bracket indicates the statistically significant differences between both control-hf-tRNS and sham ($p < 0.05$) and hf-tRNS and sham ($p<0.05$).

3.6 Discussion

We observed an improvement in performance in the ODT when subjects were stimulated with hf-tRNS. With a-tDCS, we observed an initial facilitation, which was followed by a reduction of the learning phenomena. With c-tDCS and Cz stimulation, the performance was identical to the performance with sham stimulation. A not well defined role is that of lf-tRNS. With this stimulation we observe an improvement, but the performance was not statistically different nor from sham nor from hf-tRNS.

Terney et al. (2008) reported a modulation of cortical excitability after 10 minutes of hf-tRNS to the primary motor cortex. The increase in MEP amplitude, which persisted after the end of the stimulation, was greater than the increase that is usually obtained with a-tDCS. Based on these results, Terney et al. (2008) proposed that tRNS induced a facilitatory effect similar to that of a-tDCS (Ambrus et al., 2010b; Terney et al., 2008), even if it was sustained by different mechanisms. They also showed behavioral improvement in a motor learning task, but only when the full frequency spectrum (from 0.1 to 640 Hz) was used, which does not allow for determination of whether a more restricted frequency range is responsible for the observed effect. For this reason, in our study, we applied the low and high frequencies separately.

Changes induced by tES, are considered to be dependent upon the NMDA receptor (Ridding and Ziemann, 2010). Short- and long-term tDCS effects are not observed after administration of an NMDA receptor antagonist or blocking Na+ channels (Liebetanz et al., 2002; Nitsche et al., 2003a, 2003b). On this basis both LTP and its opposite, LTD, have been postulated to explain the persistent effects of brain stimulation on cortical activity (Fritsch et al., 2010, Nitsche et al., 2009, Thickbroom, 2007, Ziemann and Siebner, 2008). Nevertheless additional aspects should be considered in the relation to the differential stimulation characteristics and effects. Plasticity induced by different type of tES at the level of brain networks might be determined by changes that alter the property of synaptic plasticity. In the present

study, we demonstrated that tRNS and a-tDCS have different behavioral effects, at least in the visual domain, which led us to conclude that the two types of stimulation are not fully interchangeable. In fact, the facilitation effect induced during the stimulation by tRNS was not present with a-tDCS. Terney et al. (2008), suggest that using tRNS sodium channels activity can be augmented. After a depolarization, repolarization of sodium channels would generally take some time, but if a repeated stimulation is applied these channels can be reopened in a shorter time (Schoen and Fromherz, 2008). Because tRNS is a repetitive, random, and subthreshold stimulation, we hypothesized that it would induce temporal summation of neural activity when the time-constant of a neuron is sufficiently long to permit the summation of two stimuli presented in close sequence (i.e., the high frequency range used in our experiment). These mechanisms should not be present with a-tDCS because such a polarizing stimulation may induce an initial facilitation, which could be followed by adaptations to rebalance the modulation of ion channel conductance. It might be likely that in the a-tDCS's initial phase, the cortical excitability shifted because of membrane polarization, and this shift induced a strengthening of the neural circuitry that improved performance. The initial increase in excitability, however, was followed by an adaptation of the neural system through mechanisms likely based on rebalancing the modulation of voltage-dependent ion channel conductance. The tDCS effects possibly relied mainly on the self-regulatory actions of voltage-dependent channels to induce inactivation during sustained depolarization (Levitan and Kaczmarek, 2002). Interestingly, some ion-channels undergo a progressive decrease of activation in response to constant activation by a voltage change. In some cases, these voltage-dependent channels become inactive (i.e., closed) after an earlier activation, even if depolarization is maintained (Kurachi and Ishii, 2004). The inactivation of voltage-dependent channels is fundamental to determine whether there is a progressive decrease in the neuronal response to sustained exposure to a stimulus. This change in channel activity is termed the "rate of inactivation", and it is primarily related to faster sodium channels. Potassium channels also undergo inactivation, but the rate is much slower than sodium channels (Levitan and Kaczmarek, 2002). Importantly, potassium channels also participate in determining the actual rate at which a neuron fires, which makes them important

contributors to the final outcome. Fast inactivation of calcium conductance during prolonged depolarization has also been reported in voltage-clamp studies of calcium currents (Eckert et al., 1977; Kostyuk and Krishtal, 1977).

This inactivation due to adaptation of ion channels may justify the absence of learning differences between the first and the last block of stimulation in the a-tDCS condition. Similar to our results Antal et al. (2004a) found that a-tDCS over V5 only improved performance in the first block of a visuomotor learning task. Consistent with Antal and colleagues (2004a), the present results showed that a-tDCS immediately enhanced cortical excitability and ameliorated stimuli perceptions, but this effect disappeared in later blocks. We concluded that the dynamics of the induced effects differ between a-tDCS and tRNS because we found a difference in learning rates between the two types of stimulation.

Based on the homeostasis theory, we would expect c-tDCS to induce an initial performance deterioration followed by realignment to the normal trend of the sham group in subsequent blocks. In the present study, however, it was impossible to observe an impairment in performance because of a "floor effect" in the first block (i.e., it is impossible to have a performance lower than chance level). Therefore, the cathodal stimulation was completely ineffective from a behavioral perspective. The absence of a cathodal effect has also been reported in previous studies (Antal et al., 2004b, 2004c; Fertonani et al., 2010; Kraft et al., 2010) although several papers have reported either inhibitory (Antal et al., 2003a, 2003b; Antal et al., 2001) or facilitatory effects (Accornero et al., 2007, Antal et al., 2004b). This discrepancy is probably due to several factors, such as the different experimental tasks (e.g., pattern-reversal checkerboard vs. sinusoidal luminance gratings) and the differences in stimulation parameters (e.g., intensity, duration, electrode size and the location and direction of the current flow) (Nitsche et al., 2008). The placement of the reference electrode, which influences the direction and shunting of the current flow, seems particularly important. These aspects highlight the role of methodological differences in shaping the effects of tES (Antal and Paulus, 2008).

Importantly, the results obtained within the motor system are not always equivalent to the results obtained in the visual system or other areas. The cytoarchitectonic and myeloarchitectonic differences, including differences in neuron diameters, may

explain the differential inductions observed in different regions. The principal feature of the motor cortex is the large pyramidal cells of the 5th layer of the Giant pyramidal cells of Betz (Brodal, 1981), which are not present in the visual areas. Neuronal threshold is inversely related to axon diameter; axons with a large diameter have a lower impedance, and large axons have a large membrane surface. This difference in structure means that changes in polarization may, in theory, be more "easily" induced over the motor cortex compared with other cortices because of the presence of large fibers. Nevertheless, there are some limitations related to the dendritic structure of pyramidal neurons (Spruston, 2008; Stuart and Spruston, 1998). In addition, the striate visual cortex is different from the precentral motor cortex; the former is granular and the latter is agranular. Indeed, the visual cortex has greater morphological variability (Dougherty et al., 2003), stellate cells are present instead of pyramidal neurons, and the striate cortex is characterized by its cortical thinness (Brodal, 1981). Taken together, these anatomical differences can explain the non overlapping data for the same stimulation parameters over different cortices (Terney et al., 2008). Thus, for anatomical geometrical reasons and differences in current characteristics, hf-tRNS may induce a more robust effect than tDCS over the visual cortex.

Our most interesting finding was the broad performance enhancement that was obtained with tRNS. There are different mechanisms that can explain the induced behavioral effects and their dissimilarity to the effects induced by tDCS. In contrast to a-tDCS, we hypothesized that tRNS does not permit homeostasis of the system. Because of its particular wave shape, tRNS might induce temporal summation of small depolarizing currents, which could interact with the activity of the engaged neurons (Cash and Yuste, 1998) and enhance performance. Therefore, tRNS of neurons provides the driving force for a synaptic potentiation-like phenomenon. The effect was more pronounced with hf-tRNS, which was likely due to the frequency range applied (100-640 Hz). Because the time constant of the cell body and dendrites is between 1 and 10 ms (Kandel et al., 2000), stimulation between 100 and 1,000 Hz may be optimal for affecting neuronal communication.

In the low-frequency range, the random stimulation effect was weaker and not statistically different from sham or from hf-tRNS which may be due to the presence

of relatively high frequencies of stimulation (from 80 to 100 Hz) at the upper end of this low-frequency range. These higher frequencies could interact analogously to hf-tRNS. This point should be clarified in future studies with the application of more specific frequency ranges (e.g., 40-60 Hz vs. 80-100 Hz). In this respect, studies have shown that different classes of neurons are activated at different frequencies (Freeman et al., 2010). Because different cortical areas contain different neuronal types, a specific band of frequencies might lead to the response of a subpopulation of neurons.

A further possible explanation for the effects induced by tRNS can be in the frame of the stochastic resonance phenomenon (e.g., Miniussi et al., 2010). tRNS is by definition a stimulation that induce non-finalized random activity in the system i.e., noise. In general, noise decreases performance, but 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 of the signal, thereby rendering the noise in the signal. So the noise can increase but also the signal will increase consequently and since the state of the system (running a task) the increase of the signal will be likely more consistent (the to be activated neurons by the task are neurons closer to threshold). Therefore the effect of tRNS on neuronal activity may not be just random addition of noise. In this framework, it is possible to explain facilitatory results in terms of the relationship between noise and signal in the nervous system, so enhanced performance could be observed with an optimum level of noise (Antal et al., 2004b; Ruzzoli et al., 2010). Even if the term random noise stimulation can evocate such explanation the present data do not let us to do draw a consideration in this framework since we cannot characterize the temporal coding between neural populations (i.e., neural synchronization or phase locking) by the present protocol.

In conclusion the present data confirm the efficacy of hf-tRNS as a technique to improve performance in a VPL task and show its superiority over tDCS in inducing facilitatory effects. We also demonstrated the high specificity of this stimulation over the V1 cortex, since hf-tRNS applied over the vertex, a brain area that is not involved

in the ODT task, was totally ineffective, and subject performance was similar to performance after sham stimulation.

We suggest that the mechanism of action of tRNS is based on the repeated subthreshold stimulations that prevent homeostasis of the system. This effect might potentiate the activity of the neural populations involved in cognitive tasks that facilitate brain plasticity by strengthening synaptic transmission between neurons. Modulation of synaptic transmission efficacy can result in excitability and activity changes in specific cortical networks that are activated by the task's execution, and these changes correlate with cognitive plasticity at the behavioral level.

CHAPTER 4

"Which is the best timing for neuroplasticity induction in a visual perceptual learning task?"

4.1 Introduction

As reported so far there are different types of tES, differentiated by specific modalities of current erogation (e.g., direct vs. alternating). However all have the ability to induce modifications in the neuronal excitability in a non-invasive and painless way. In a precedent research (chapter 3) we have compared different types of tES: anodal and cathodal tDCS and high and low frequency tRNS. In this study we have shown the superiority of tRNS on tDCS to improve the behavioral performance in a VPL task. However, the effects of tES depend on factors both internal and external to the technique. Internal factors are for example current density, intensity, duration of the stimulation, timing of application and intervals of time between two stimulations. Instead when we speak about external factors we indicate the cortical area of interest, the choose of the task and the kind of experimental sample (e.g., healthy subjects vs. patients). For example several studies have investigated the importance of the current intensity to obtain a significant modulation of the MEP amplitude (Nitsche and Paulus, 2000), or have highlighted different effects depending on the stimulated area (Antal et al., 2011; Nitsche and Paulus, 2011). To understand the way in which all these parameters modulate the effects of tES is fundamental for its appropriate application in the therapeutic field.

Recently it has been demonstrated that different effects can been obtained depending on the moment in which the stimulation is applied (Stagg et al., 2011a).

Nitsche et al. (2003c) have demonstrated that anodal tDCS applied during the execution of an implicit learning task lead to an improvement in the rate of learning of that task. On the other hand, if the same task is performed after ten minutes from the end of the stimulation, there is not effect (Kuo et al., 2008). These data are reinforced by Stagg (2011a). Indeed this is the only work that has directly compared two timing of stimulation, analyzing the responses given on a explicit motor learning task performed after or during tDCS. The authors found that anodal tDCS increase the rate of motor learning only when applied during the execution of the task. With the cathodal stimulation they don't found any effect. However, it is still not clear to what extent these findings are transferable to other areas of the cerebral cortex. Indeed, to date no study has investigated the timing effects of tES in the visual cortex. In this study, we first compared different types of tES (i.e., tDCS vs. tRNS)

applying them at different timings (before vs. during the task execution). We hypothesized that in the visual system the effects of tES are highly dependent on the state of cerebral activation. Therefore the same type of stimulation, applied in different moments, may have different effects. The principal objective of this work is to investigate if there is an "ideal" timing for the application of tES to obtain the induction of neuroplasticity in the V1 cortex. Therefore we applied hf-tRNS, a-tDCS and c-tDCS before (offline) or during (online) the execution of an orientation discrimination task.

Our results confirm that exists a critical timing of application, depending on the type of stimulation. High frequency tRNS is efficacious only if applied during the task execution, whereas it's better to apply anodal tDCS before the task. These results provides important indications for the designing of rehabilitation protocols, highlighting which among the two excitatory techniques is better to choose in relation to its timing of application.

4.2 Materials and Methods

3.2.1 Subjects

108 healthy subjects took part in the experiment. All of the participants were right-handed with normal or corrected-to-normal vision. We did not include subjects with a history of seizures, implanted metal objects, heart problems or any neurological disease. Moreover, as a standard procedure, subjects who did not reach an established learning performance in the task were excluded from the study, based on this criterion 10 participants, were excluded. The remaining 98 subjects (49 males, mean age 21.8 ±2.6 years; range 19-30 years) took part in the experiment. They were divided into seven groups. All groups had 14 participants (7 males and 7 females), and the mean ages of the groups were: 22.4 ±2.8 years for online-hf-tRNS group, 21.8 ±2.3 years for online-a-tDCS group, 21.7 ±2.6 years for online-c-tDCS group, 21.6 ±2.7 years for offline-hf-tRNS group, 21.7 ±3.4 years for offline-a-tDCS group, 21.6 ±1.9 years for offline-c-tDCS group and 21.6 ±3.0 years for sham group, respectively.

The present study was approved by the Ethics Committee of the IRCCS Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy. Safety procedures based on non-invasive brain stimulation approaches were adopted (Poreisz et al., 2007; Rossi et al., 2009), and informed consent was obtained from all participants prior to the beginning of the experiment.

4.2.2 Ovarian hormone influence on data variability

Even for this second experiment we have considered the influence of ovarian hormones on task performance. Indeed, the importance of this influence has been previously demonstrated by many TMS studies (for a detailed description see chapter 3.2.2). The female participants in the present study were tested during the follicular menstrual phase (mean day from the first day of the menstrual period: 12.8 ±2.9, range 11-20 days) because this is the period when progesterone levels are low and estrogen levels are high. Under these conditions, the cortical excitability of male and female subjects was similar (Inghilleri et al., 2004).

4.2.3 Orientation discrimination task (ODT)

We chose the ODT as reported before (see chapter 3.2.3), because it is a widely studied visual-perceptual learning task (Shiu and Pashler; 1992, Vogels and Orban, 1985) that involves V1 neurons.

In the ODT, participants had to decide whether the presented stimulus was tilted clockwise or counterclockwise relative to the previously presented stimulus. All stimuli were black lines, and each line stimulus was 2° long and 5 min wide (in visual angle). The orientation of the reference stimulus was 45° in the upper right and lower left hemifields and 135° in the upper left and lower right hemifields. The angular differences between the reference and the target stimulus were 1.1, 1.21, 1.33 and 1.46° (Matthews et al., 1999). The reference was presented first in half of the trials and second in the other half of the trials. All of experimental parameters just mentioned were balanced and randomized between blocks. The trial structure is described in Figure 8. The subjects were asked to respond as quickly and accurately as possible after the second stimulus was presented by pressing the left

(counterclockwise) or right (clockwise) button of a response pad with the left or right index finger, respectively. Auditory feedback (duration = 50 ms; frequency for the correct response = 700 Hz; frequency for the incorrect response = 350 Hz) informed the subjects about the correctness of their responses.

Stimuli were presented on a computer screen using Presentation software v. 12.0 (http://www.neurobs.com) in each of the four visual hemifields: upper left, upper right, lower left and lower right. In each trial, the two stimuli were presented in the same hemifield (Figure 8). To limit the area in which the stimuli were presented, a black piece of cardboard covered the screen except for a circle that was 10 cm in diameter located at the centre of the screen. A central fixation point was maintained for the duration of the trial.

In the experiment, each block of the ODT consisted of 64 trials and lasted approximately 4 minutes. The ODT consisted of five blocks plus a training block. The training block was similar to the trial blocks, but it had a different number of trials (only 8) and an increased rotation angle between the two stimuli (15° clockwise or counterclockwise).

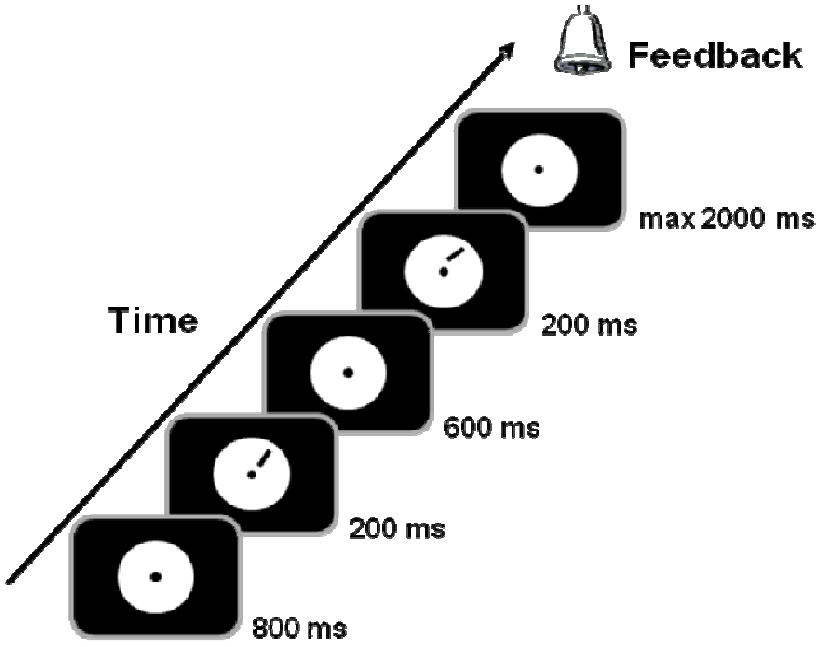

Figure 8. Trial structure. Example of a trial of the orientation discrimination task with the reference and target stimuli presented in the upper-right hemifield

4.2.4 Stimulation techniques: tRNS and tDCS

The stimulations were delivered by a battery-driven stimulator (Eldith-Plus, NeuroConn GmbH, Ilmenau, Germany) through a pair of saline-soaked sponge electrodes. The tRNS consisted of an alternating current of 1.5 mA intensity with a 0 mA offset applied at random frequencies. The frequencies ranged from 101 to 640 Hz (i.e., hf-tRNS). The intensity of stimulation did not induce a phosphene perception (Kanai et al., 2008). The tDCS consisted of a direct 1.5 mA current.

In the online conditions, the stimulations were applied for approximately four minutes during each of the first five experimental blocks. The total duration of the stimulations was approximately 22 minutes. In the offline conditions, the stimulations were applied before the execution of the task, maintaining the same time-intervals used in the online condition (i.e., ~4 minutes of stimulations – 2 minutes of pause – ~4 minutes of stimulation and so on). See Figure 9 for details of the procedure.

The active electrode had an area of 16 cm^2, whereas the reference had an area of 60 cm^2. The current density was maintained below the safety limits (varying between 25 and 60 $\mu A/cm^2$) (Poreisz et al., 2007). The electrodes were kept in place with elastic bands, and an electroconductive gel was applied under the electrodes before the montage to reduce skin impedance. The active electrode was applied over the occipital cortex. When tDCS stimulation was applied, the polarity of the active electrode was anodal in the a-tDCS condition and cathodal in the c-tDCS condition. The individual target area was determined by examining the position in accordance with the 10-20 International EEG system. This procedure was performed starting at 10% of the nasion-inion distance above the inion. The mean position for V1 stimulation was determined to be 3.5 ±0.2 cm above the inion. The reference electrode was fixed extracephalically on the right arm. In the sham stimulations, the current was turned off 20 seconds after the stimulation began (Gandiga et al., 2006).

At the end of the experimental session, we asked all subjects to complete a questionnaire developed by our research laboratory about the sensations they experienced during the different stimulations (Fertonani et al., 2010).

4.2.5 Procedure

The participants were seated in front of a computer screen in a quiet, semi-dark room. A 57 cm distance from the screen was maintained through the use of a chin rest. The experiment was a between-subjects design with seven stimulation conditions: online-hf-tRNS, online-a-tDCS, online-c-tDCS, offline-hf-tRNS, offline-a-tDCS, offline-c-tDCS and sham stimulation.

In the online conditions, the subjects began the ODT 10 seconds after the onset of the stimulation, whereas in the offline condition the stimulation was applied before the execution of the task. In the offline condition during the stimulation the subjects listened to a tale. The procedure is depicted in Figure 9.

The duration of the entire experimental session was approximately 45 minutes on the online condition, whereas was about 90 minutes in the offline condition.

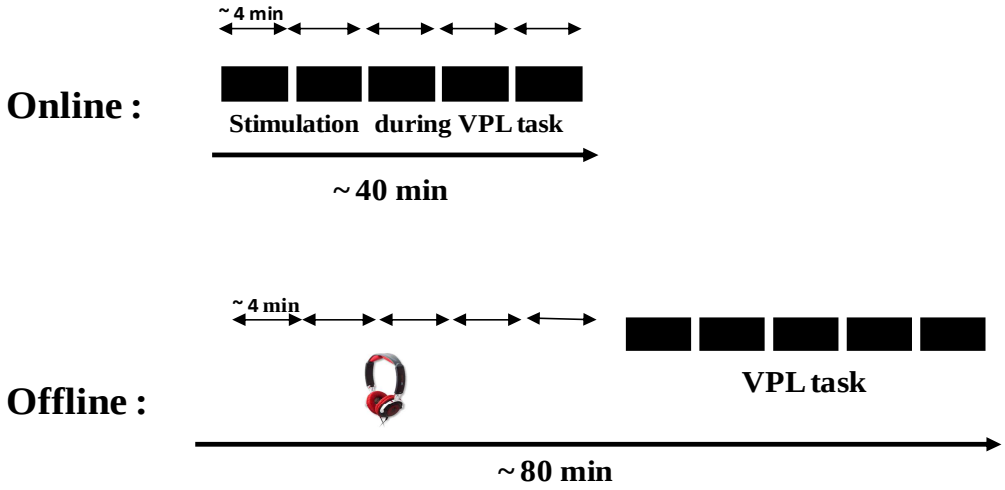

Figure 9. Procedure of the experiment. The experiment was designed to be between-subjects with six stimulation conditions: hf-tRNS, lf-tRNS, a-tDCS, c-tDCS, CZ and sham. Subjects were stimulated before the execution of the task whereas they listened to a tale. The stimulations were applied maintaining the same time-intervals used in the online condition. The black blocks represent each block of the VPL task.

4.2.6 Data analysis

The average orientation sensitivity was calculated in terms of a d' value for each subject and each block, separately for each stimulation condition. Because the ODT is a two-alternative forced-choice task, a value of d' = 1 corresponded to a 75% accuracy rate.

For all data (d' values and slopes), the Kolmogorov-Smirnov test confirmed the normality of the distribution, and the data were analyzed using a repeated-measures analysis of variance (ANOVA). The data sphericity was tested using the Mauchly test where appropriate. When the test results were statistically significant, the data were corrected using the Huynh–Feldt correction. A p-value < 0.05 was considered significant for all statistical analyses. For multiple comparisons, we used Fisher's Least Significant Difference (LSD) method to test our specific "a priori" hypotheses (i.e., to compare the different stimulation conditions). For all other comparisons, the p-values were corrected using a Bonferroni correction.

Data from the sensations induced by tES were analyzed using a one-way ANOVA for each sensation to compare the different stimulation types. The p-values were corrected using Bonferroni corrections.

4.3 Results

4.3.1Orientation sensitivity – d'

We performed a repeated-measures ANOVA with *block* (from 1 to 5) as a within-subjects factor and *stimulation condition* (online-hf-tRNS, online-a-tDCS, online-c-tDCS, offline-hf-tRNS, offline-a-tDCS, offline-c-tDCS and sham) and *gender* (male, female) as between-subjects factors. We observed a significant main effect for *block* [$F(4, 336) = 27.35$; $p < 0.001$], *stimulation condition* [$F(6, 84) = 3.00$; $p = 0.01$] and *gender* [$F(1, 84) = 6.54$; $p = 0.01$]. Regarding the main effect of *block*, multiple post-hoc comparisons revealed a statistically significant difference between block 1 and blocks 3, 4, 5 and between block 2 and blocks 4, 5.

Regarding the main effect of *stimulation condition*, multiple post-hoc comparisons revealed that online-hf-tRNS (mean d' ±SEM = 0.625 ±0.121) was significantly different from offline-hf-tRNS (0.389 ±0.102), online-c-tDCS (0.304 ±0.121) and sham (0.299 ±0.116) and marginally from online a-tDCS (p=0.07) (0.437 ±0.127). Moreover, offline-c-tDCS (0.616 ±0.122) was significantly different from online-c-tDCS and sham and marginally from offline-hf-tDCS (p=0.06). Finally offline-a-tDCS (0.574 ±0.112) results different from online-c-tDCS and sham. See Figure xx.

The *gender* factor was statistically significant. Males were more accurate than females in all conditions (males: 0.587 ±0.115; females: 0.401 ±0.131). The absence of any interaction between the *gender* and *stimulation condition* factors, however, discouraged us from examining the gender factor in more detail.

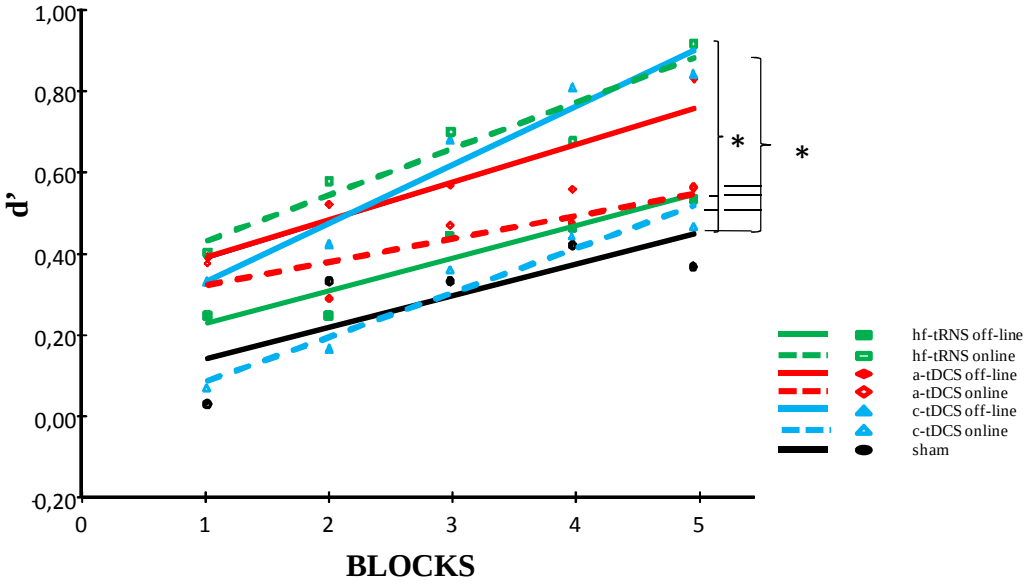

Figure 10. Experiment results. The lines represent the fit of each condition: the continue green line corresponds to hf-tRNS off-line, the broken green line corresponds to hf-tRNS on-line, the continue red line represents a-tDCS off-line, the broken red line represents a-tDCS on-line, the continue blue line represents c-tDCS off-line, the broken blue line represents c-tDCS on-line, the black line corresponds to sham. The asterisk near the curly bracket indicates the statistically significant differences between hf-tRNS on-line and the conditions in the square bracket ($p < 0.05$), and between c-tDCS off-line and the conditions in the square bracket ($p < 0.05$).

Repeated measures ANOVA performed separately for each stimulation condition highlighted the main effect of *block* ($p < 0.01$) for all stimulation conditions except online-a-tDCS and offline-hf-tRNS. Post-hoc comparisons in the online-hf-tRNS condition revealed that block 1 was different from blocks 3, 4 ($p = 0.065$) and 5, and block 2 differed from block 5. In the online-c-tDCS condition, block 1 was different from blocks 4 and 5, and block 2 was different from block 5. In the offline-a-tDCS condition blocks 1, 2, 3 and 4 were different from block 5. In the offline-c-tDCS condition, block 1 was different from blocks 3, 4 and 5, and block 2 was different from blocks 4 and 5. In the sham condition, block 1 was different from blocks 4 and 5. No statistically significant differences were observed in the online-a-tDCS condition and in the offline-hf-tRNS condition, which suggested the absence of an enhancement in performance with these stimulations.

The present data support the initial hypothesis about the importance of stimulation timing. Indeed, online-hf-tRNS was more efficacious than offline-hf-tRNS. In contrast, a-tDCS was more efficacious in the offline condition. Finally, the performance in online-c-tDCS condition was identical to the sham one but in the offline-c-tDCS we observed a surprising enhancement in the task execution. Indeed the levels of performance reached with offline-c-tDCS and online-hf-tRNS are similar.

4.3.2 Sensations induced by different types of tES

Each participant completed a questionnaire at the end of the experiment (Fertonani et al., 2010) and reported having tolerated the stimulation without discomfort. The results of the questionnaire are reported in Table 3 (these are the results of offline stimulations, the online conditions are reported in table 2 of the chapter 3). Also in this second experiment as for the first (see chapter 3.4.2) the participants were unable to distinguish the real stimulation from the placebo stimulation. A one-way ANOVA for each sensation was performed to compare the different stimulations. No difference between the offline-tRNS and sham conditions was found. Interestingly, the analysis highlighted a statistically significant difference ($p < 0.01$) between both offline-a-tDCS and offline-c-tDCS and the other conditions with respect to itching.

Furthermore, the offline-a-tDCS was different ($p < 0.01$) from the other conditions with respect to irritation and burning. In general, the tDCS-induced sensations were perceived more strongly (Ambrus et al., 2010a; Poreisz et al., 2007) than the offline tRNS- or sham-induced sensations. With tDCS, pain, heat, iron taste and fatigue were comparable to the sham condition, but irritation, burning, and itching were perceived with real, but not sham, stimulation. In contrast, hf-tRNS was indistinguishable from sham conditions for all of the sensations examined. This characteristic makes tRNS an optimal tool for experimental designs in which sham stimulations should not differ from real stimulations.

tES		Irritation	Pain	Burning	Heat	Itch	Iron taste	Fatigue
hf-tRNS	**Intensity**	**1.0**	**0.5**	**0.5**	**0.8**	**0.7**	**0.1**	**1.7**
	Subjects (%)	14	14	14	36	21	7	21
a-tDCS	**Intensity**	**1.8**	**1.0**	**1.5**	**1.3**	**1.9**	**1.5**	**1.0**
	Subjects (%)	86	14	79	29	100	14	14
c-tDCS	**Intensity**	**1.1**	**2.0**	**1.3**	**1.0**	**1.6**	**1.0**	**1.5**
	Subjects (%)	50	14	43	14	71	7	14
Sham	**Intensity**	**0.2**	**0.0**	**0.1**	**0.1**	**0.2**	**0.0**	**0.4**
	Subjects (%)	21	0	7	14	14	0	29

Table 3. Transcranial electrical stimulation-induced sensations by offline stimulation: Mean intensity of the sensations reported by subjects after tES, and the percentage of subjects who reported each sensation. Sensation intensity was evaluated on a 5-point scale: 0 = none, 1 = mild, 2 = moderate, 3 = considerable, and 4 = strong.

4.4 Discussion

In this study we have shown that the effect of a specific tES is different in accordance with the timing in which it is applied. Indeed, we observed a significant improvement of the performance when a-tDCS is applied before the task (i.e., offline), but no when it is applied during the task execution (i.e., online). On the contrary, with offline hf-tRNS the behavioral performance is not different from sham condition whereas with online hf-tRNS we have a great improvement. Surprisingly a similar strong improvement is present with offline c-tDCS whereas online c-tDCS is totally super imposable to sham group.

Therefore, at least in the visual system, the effects of tES are highly dependent on the stimulation timing. These results confirm our hypothesis on the importance of the state of cerebral activation when a stimulation is applied. Moreover, our data show that the direction of the effects of stimulation is extremely dependent from the stimulated area (i.e., visual vs. motor system).

Indeed the relation between timing and motor learning has been yet highlighted by previous motor studies (Kuo et al., 2008, Nitsche et al., 2003c, Stagg et al., 2011a), obtaining effects different from those revealed in this work. In a first study, Nitsche and colleagues (2003c) have demonstrated a facilitation effect of anodal stimulation applied during an implicit motor learning task. A similar facilitation trend was seen for cathodal stimulation. Subsequently Kuo et al., (2008) have investigated tDCS effects on the same task applying the stimulations before the task execution. They found an effect of a-tDCS only when it was applied in concomitance with the assumption of a partial NMDA-receptor agonist. However when tDCS was applied without drugs they failed to show any effect of both anodal and cathodal stimulation. Recently Stagg et al. (2011a) has investigated the timing-dependent interactions between tDCS and learning in an explicit motor learning paradigm. They demonstrated that ten minutes 1 mA anodal tDCS increase the rate of learning if applied during the execution of the task but not if given before the task. Nevertheless the effect of c-tDCS was not timing-dependent, slowing the rate of learning in both conditions. These few studies suggest to apply a-tDCS during a motor task execution for maximum effectiveness. However, as we have shown, these data are not

generalizable to the visual cortex. The disagreement between motor and visual studies may be explained by the differences between the two neural systems. Indeed, the results obtained within the motor area are not always equivalent to the results obtained in the visual system or other areas because there are cyto- and myeloarchitectonic differences, including differences in neuron diameters, that may explain a differential current diffusion (see section 3.6 for major details).

The main difference with motor studies regards the facilitator effect of offline c-tDCS. This datum overcomes the simplistic view of the cathodal stimulation as inhibitory one and is in line with a metaplasticity hypothesis (Bienenstock et al., 1982). Based on this theory, the effect of the task depends on the previous state of neural activation. In this case, a prolonged c-tDCS hyperpolarize all the neurons of the stimulated area, but when they are called to response to execute the task, we observe a rebound effect in the engaged neurons which result in a performance improvement. Indeed the metaplasticity mechanisms consent to maintain the neurons in an optimal state of activity, making them more reactive after a prolonged inhibitory stimulation (Lang et al., 2004). This preconditioning effect is not observable if the stimulation is applied during the task execution. In this case we speculate the involvement of homeostatic mechanisms. As we have explained in a previous work (Fertonani et al., 2011) in online c-tDCS we expected an initial decrement of performance followed by a realignment to the sham trend. However the "floor effect" present in the first block don't permit to observe this initial impairment.

In our study the metaplasticity hypothesis explain well the enhancement effects of a prolonged inhibitory stimulation delivered before the task. But when we applied a facilitator stimulation seem to be involved other plasticity mechanisms. Our data shows that a-tDCS is better if applied offline than online. We observed with online a-tDCS an initial facilitation, which was followed by a reduction of the learning phenomena (see section 3.3.3 for detailed analysis), that may be due to the activation of homeostatic mechanisms. The initial improvement of performance could be caused by the strengthening of the neural circuitry provoked by the neuronal membrane depolarization. Both the prolonged delivery of a-tDCS and the sustained exposure to a stimulus (that induce LTP-like phenomena) cause a sustained

depolarization, with the intracellular increase of Ca2+, Na+ and K+. The excessive intracellular presence of these ions, induce a saturation in the neural system and the activation of self-regulatory mechanisms of voltage-dependent ion channels (Levitan and Kaczmarek, 2002). Therefore the rebalancing in the voltage-dependent ion channel conductance (in particular Na2+ and K+) doesn't permit a further improvement in the behavioral performance. These saturation mechanisms should not occur when the stimulation is given alone. Indeed the facilitation effect of offline a-tDCS is probably given by an optimal level of intracellular Ca+ and Na+, which remains also after the stimulation, during the execution of the task. Although the delivery of offline-a-tDCS induce a facilitation effect, this one doesn't reach that obtained with the online-hf-tRNS. This effect could be explained by the hypothesis of temporal summation. Indeed because of its particular wave shape the tRNS (repetitive, random, and subthreshold stimulation) would induce temporal summation of neural activity if the time-constant of a neuron is sufficiently long to permit the summation of two stimuli presented in close sequence. Terney et al. (Terney et al., 2008), suggest that using tRNS Na channels activity can be augmented. After a depolarization, repolarization of Na channels would generally take some time, but if a repeated stimulation is applied these channels can be reopened in a shorter time (Schoen and Fromherz, 2008). However, this mechanism induce a facilitation effect (i.e., a performance improvement) only if it can reinforce a neural population activated by the execution of a task (Cash and Yuste, 1998). This seems to be confirmed by the absence of effect with offline-hf-tRNS.

In this study we have not only compared different kind of stimulation, but also investigated the ideal timing for the application of each different tES. Indeed, our data highlight for the first time that the effect of a particular type of stimulation depends critically from the activation state of the cortex. This result becomes even more important for the design of rehabilitative protocols.

In the interpretation of our results need to be considered also the presence of intervals (i.e., pauses) during the stimulation. In the online condition the stimulation was applied only during the execution of each block of the task, and not during the 2 min interval between the blocks. We have maintained the same pattern of stimulation in the offline condition. This methodological choose, in the light of recent works

(Fricke et al., 2011, Monte-Silva et al., 2010), may have influenced our results. Indeed these studies have demonstrated that the repetition of tDCS has a different impact on mechanisms of plasticity, depending on the break between the stimulations. In particular Fricke and colleagues (2011) have shown that two sessions of c-tDCS (5-min duration) separated by 3 minutes of break have an opposite effect on MEP amplitude (i.e., enhancement) in respect to continue cathodal stimulation. These interesting data suggest the importance of the intervals, nevertheless it would be important to study these effects with more breaks and also in motor learning.

In conclusion, we overcome the simplistic view of the anodal/cathodal stimulation as the excitatory/inhibitory one (Jacobson et al., 2011; Miniussi et al., 2010). Indeed we have highlighted, in the visual domain, the impressive enhancement effect of a prolonged offline cathodal stimulation. Moreover this work highlights the specificity of the tRNS potentiality, linked to the state of activation of the engaged neuronal population.

These results increase our knowledge about tES-induced plasticity mechanisms to make the most of the potentiality of each type of electrical stimulation, and consequently are the first step towards a more effective, theoretically and empirically motivated use of tES methods for the study of cognitive plasticity.

CHAPTER 5

GENERAL CONCLUSIONS

tES is widely employed in order to test the brain-behaviour relationship in many different cognitive domains (Nietsche et al., 2011). tES is able to generate an electrical field in the stimulated area that modulates neuronal activity according to the modality of the application (Nietsche et al., 2000). The efficacy of tES to induce a modifications of membrane polarity and consequently in behavioral performance depends by different important parameters (intensity, duration, electrodes, area stimulated, type of task etc). Notwithstanding in recent years have seen much progress on the effectiveness and application of tES many question are still open. In this doctoral dissertation we have improved our knowledge by answering at some important questions still open on tES application. Indeed, understand whether and how cognitive plasticity can be induced and modified by means of tES in the healthy adult brain is the matter of debate of the present dissertation

An objective of our experiments has been to utilize a basic learning model (i.e., VPL) to determine which of the available tES methods most effectively induces plasticity, the ideal timing of application and whether the induced effect reflects a general potentiating effect or a specific change in learning ability.

In the first experiment (chapter 3) we have demonstrated that is possible potentiate cognitive plasticity by means tES, in the specific we have shown that different type of tES may to induce different effects on the behavioral performance. Indeed, plasticity induced by different type of tES at the level of brain networks might be determined by changes that alter in different way the property of synaptic plasticity. We demonstrated that tRNS and a-tDCS have different behavioral effects (see chapter 3), at least in the visual domain, this result which led us to conclude that the two types of stimulation are not fully interchangeable as affirmed in previous studies (Terney et al., 2008). We argue that this important finding is given by different ways in which current is delivered (direct vs. alternate). Indeed, because tRNS is a repetitive and random stimulation, we speculate that it would induce temporal summation of neural activity when the time-constant of a neuron is sufficiently long to permit the summation of two stimuli presented in close sequence. tRNS might induce temporal summation of small depolarizing currents, which could interact with the activity of the engaged neurons (Cash and Yuste, 1998) and enhance performance. However these mechanisms should not be present with a-tDCS because

such a polarizing stimulation may induce an initial facilitation, which could be followed by adaptations to rebalance the modulation of neuron conductance.

A further important question involves the timing relationship between tES and task execution. In literature several studies have shown that the stimulation both before and during the execution of task may facilitate the behavioral performance (Nitsche et al., 2011). However, the effects of facilitation tES depend by different factors that are current density, intensity, duration of the stimulation, timing of application and intervals of time between two stimulations. In addition, other external factors are important as the cortical area of interest, the choose of the task and the kind of experimental sample (e.g., healthy subjects vs. patients). Recently it has been demonstrated that different effects can been obtained depending on the moment in which the stimulation is applied (Stagg et al., 2011a). But, to date no published studied have compared different tES techniques to evaluate the different induced effects in the same experimental task and in different time. In the second experiment of this dissertation (see chapter 4) we first compare different type of tES before and after cognitive task highlighting that same type of stimulation, applied in different moments, have different effects. If in the first research we have shown the superiority of tRNS on tDCS during of the execution of the task, in the second study we have shown that the same technique of stimulation may have no effect when applied before execution of the cognitive task. We suggest the importance of the state of cerebral activation when a stimulation is applied. In our study the metaplasticity hypothesis explain well the enhancement effects of a prolonged inhibitory stimulation delivered before the task. But when we applied a facilitator stimulation seem to be involved other plasticity mechanisms. In this last case, our data show that a-tDCS is better if applied offline than online. Although the delivery of offline-a-tDCS induce a facilitation effect, this one doesn't reach that obtained with the online-hf-tRNS. However, our data contradict the results obtained in the motor system (Nietsche et al., 2003, Kuo et al., 2008), that demonstrated the efficacy of tDCS applied during a motor learning task. Indeed, our data show that the direction of the effects of stimulation is extremely dependent from the stimulated area (i.e., visual vs. motor system).

In conclusion, our work shed light to some critical points debated in the tES literature. First of all, we overcome the simplistic view of the anodal/cathodal stimulation as the excitatory/inhibitory one (Jacobson et al., 2011). Without a doubt, the facilitation or inhibition doesn't depend only from the type of stimulation, but become decisive both the stimulated area and the timing of the application, and this may explain many contradictory data reported by the literature. Indeed we have highlighted, in the visual domain, the impressive enhancement effect of a prolonged offline cathodal stimulation. Moreover this work highlights the specificity of the tRNS potentiality, linked to the state of activation of the engaged neuronal population.

These results increase our knowledge about tES-induced plasticity mechanisms to make the most of the potentiality of each type of electrical stimulation. The development of tES techniques to study the cognition constitutes a significant breakthrough in our understanding of the changes in brain state that may account for behavioral modifiability. Understanding the basis of adult cognitive plasticity is extremely important and will have an immediate impact on basic neuroscience and clinical practice. The development of new protocols aimed at improving cognitive performance in patients with brain damage as well as slowing the cognitive decline.

Appendix :

Survey of sensations related to transcranial direct current stimulation (Published in Fertonani et al., 2010)

Subject code: _____ **Date:**____/____/____

Experiment: _____

Have you experienced any sensation during the direct current stimulation? Please answer to the following questions regarding the different sensations, indicating the degree of intensity of your perception according to the following scale:

- ***None*** **= I have not felt the described sensation**
- ***Mild*** **= I have mildly felt the described sensation**
- ***Moderate*** **= I have felt the described sensation**
- ***Considerable*** **= I have felt the described sensation to a considerable degree**
- ***Strong*** **= I have strongly felt the described sensation**

In the first stimulation block

Itchiness:	□ None	□ Mild	□ Moderate	□ Considerable	□ Strong
Pain:	□ None	□ Mild	□ Moderate	□ Considerable	□ Strong
Burning:	□ None	□ Mild	□ Moderate	□ Considerable	□ Strong
Warmth/Heat:	□ None	□ Mild	□ Moderate	□ Considerable	□ Strong
Pinching:	□ None	□ Mild	□ Moderate	□ Considerable	□ Strong
Iron taste:	□ None	□ Mild	□ Moderate	□ Considerable	□ Strong
Fatigue:	□ None	□ Mild	□ Moderate	□ Considerable	□ Strong
Other_____:	□ None	□ Mild	□ Moderate	□ Considerable	□ Strong

When did the sensations begin?

□ At the beginning of the block □ About the middle of the block □ Towards the end of the block

How long did they last?

□ They stopped soon □ They stopped in the middle of the block □ They stopped at the end of the block

How much did these sensations affect your performance?

□ Not at all □ A little □ Considerably □ Much □ Very much

In the second stimulation block

…

If you want to provide more details, please briefly describe the experimented sensations in relation to:

- Itchiness:
- Pain:
- Burning:
- Warmth/Heat:
- Pinching:
- Iron taste:
- Fatigue:
- Other:

References

Accornero N., Li Voti P., La Riccia M. and Gregori B. (2007). Visual evoked potentials modulation during direct current cortical polarization. Experimental Brain Research, 178(2), 261-266.

Ahissar M. and Hochstein S. (2004). The reverse hierarchy theory of visual perceptual learning. Trends Cogn Sci, 8(10), 457-464.

Ahissar M., Laiwand R. and Hochstein S. (2001). Attentional demands following perceptual skill training. Psychol Sci, 12(1), 56-62.

Ambrus G.G., Antal A. and Paulus W. (2010a). Comparing cutaneous perception induced by electrical stimulation using rectangular and round shaped electrodes. Clin Neurophysiol, 122(4), 803-807.

Ambrus G.G., Paulus W. and Antal A. (2010b). Cutaneous perception thresholds of electrical stimulation methods: comparison of tDCS and tRNS. Clin Neurophysiol, 121(11), 1908-1914.

Antal A., Kincses T.Z., Nitsche M.A. and Paulus W. (2003a). Manipulation of phosphene thresholds by transcranial direct current stimulation in man. Experimental Brain Research, 150(3), 375-378.

Antal A., Kincses T.Z., Nitsche M.A. and Paulus W. (2003b). Modulation of moving phosphene thresholds by transcranial direct current stimulation of V1 in human. Neuropsychologia, 41(13), 1802-1807.

Antal A., Nitsche M.A., Kincses T.Z., Kruse W., Hoffmann K.P. and Paulus W. (2004a). Facilitation of visuo-motor learning by transcranial direct current stimulation of the

motor and extrastriate visual areas in humans. European Journal of Neuroscience, 19(10), 2888-2892.

Antal A., Nitsche M.A., Kruse W., Kincses T.Z., Hoffmann K.P. and Paulus W. (2004b). Direct current stimulation over V5 enhances visuomotor coordination by improving motion perception in humans. Journal of Cognitive Neuroscience, 16(4), 521-527.

Antal A., Nitsche M.A. and Paulus W. (2001). External modulation of visual perception in humans. Neuroreport, 12(16), 3553-3555.

Antal A., Nitsche M.A. and Paulus W. (2006). Transcranial direct current stimulation and the visual cortex. Brain Res Bull, 68(6), 459-463.

Antal A. and Paulus W. (2008). Transcranial direct current stimulation and visual perception. Perception, 37(3), 367-374.

Antal A., Paulus W. and Nitsche M.A. (2011). Electrical stimulation and visual network plasticity. Restor Neurol Neurosci, 29(6), 365-374.

Antal A., Varga E.T., Nitsche M.A., Chadaide Z., Paulus W., Kovacs G. and Vidnyanszky Z. (2004c). Direct current stimulation over MT+/V5 modulates motion aftereffect in humans. Neuroreport, 15(16), 2491-2494.

Ball K. and Sekuler R. (1982). A specific and enduring improvement in visual motion discrimination. Science, 218(4573), 697-698.

Ball K. and Sekuler R. (1987). Direction-specific improvement in motion discrimination. Vision Res, 27(6), 953-965.

Baltes P.B. and Willis S.L. (1982). Plasticity and enhancement of intellectual functioning in old age: Penn State's Adult Development and Enrichment Project ADEPT. Craik and Trehub (Eds.), New York: Plenum Press.

Berryhill M.E., Wencil E.B., Branch Coslett H. and Olson I.R. (2010). A selective working memory impairment after transcranial direct current stimulation to the right parietal lobe. Neuroscience Letters, 479(3), 312-316.

Bienenstock E.L., Cooper L.N. and Munro P.W. (1982). Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. J Neurosci, 2(1), 32-48.

Bikson M., Datta A., Rahman A. and Scaturro J. (2010). Electrode montages for tDCS and weak transcranial electrical stimulation: role of "return" electrode's position and size. Clin Neurophysiol, 121(12), 1976-1978.

Bindman L.J., Lippold O.C. and Redfearn J.W. (1962). Long-lasting changes in the level of the electrical activity of the cerebral cortex produced bypolarizing currents. Nature, 196, 584-585.

Bindman L.J., Lippold O.C. and Redfearn J.W. (1964). The Action of Brief Polarizing Currents on the Cerebral Cortex of the Rat (1) During Current Flow and (2) in the Production of Long-Lasting after-Effects. J Physiol, 172, 369-382.

Bolognini N., Fregni F., Casati C., Olgiati E. and Vallar G. (2010a). Brain polarization of parietal cortex augments training-induced improvement of visual exploratory and attentional skills. Brain Res, 1349, 76-89.

Bolognini N., Olgiati E., Rossetti A. and Maravita A. (2010b). Enhancing multisensory spatial orienting by brain polarization of the parietal cortex. Eur J Neurosci, 31(10), 1800-1806.

Bradnam L.V., Stinear C.M., Lewis G.N. and Byblow W.D. (2010). Task-dependent modulation of inputs to proximal upper limb following transcranial direct current stimulation of primary motor cortex. J Neurophysiol, 103(5), 2382-2389.

Brodal A. (1981). Neurological Anatomy in Relation to Clinical Medicine (Third Edition ed.). New York: Oxford University Press.

Buzsaki (2006). Rythms of the brain. New York: Oxford University Press.

Cahusac P.M. (1995). Synaptic Plasticity induced in single neurones of the primary somatosensory cortex in vivo. Experimental Brain Research, 107, 241-253.

Carmel D. and Carrasco M. (2008). Perceptual learning and dynamic changes in primary visual cortex. Neuron, 57(6), 799-801.

Cash S. and Yuste R. (1998). Input summation by cultured pyramidal neurons is linear and position-independent. J Neurosci, 18(1), 10-15.

Censor N. and Sagi D. (2009). Global resistance to local perceptual adaptation in texture discrimination. Vision Res, 49(21), 2550-2556.

Chaieb L., Antal A. and Paulus W. (2008). Gender-specific modulation of short-term neuroplasticity in the visual cortex induced by transcranial direct current stimulation. Visual Neuroscience, 25(1), 77-81.

Chaieb L., Kovacs G., Cziraki C., Greenlee M., Paulus W. and Antal A. (2009). Short-duration transcranial random noise stimulation induces blood oxygenation level dependent response attenuation in the human motor cortex. Exp Brain Res, 198(4), 439-444.

Chen J., Yamahachi H. and Gilbert C.D. (2010). Experience-dependent gene expression in adult visual cortex. Cereb Cortex, 20(3), 650-660.

Cooke S.F. and Bliss T.V. (2006). Plasticity in the human central nervous system. Brain, 129(Pt 7), 1659-1673.

Creutzfeldt O.D., Fromm G.H. and Kapp H. (1962). Influence of transcortical d-c currents on cortical neuronal activity. Exp Neurol, 5, 436-452.

Crist R.E., Li, W. and Gilbert, C. D. (2001). Learning to see: Experience and attention in primary visual cortex. Nature Neuroscience, 4, 519-525.

Dougherty R.F., Koch V.M., Brewer A.A., Fischer B., Modersitzki J. and Wandell B.A. (2003). Visual field representations and locations of visual areas V1/2/3 in human visual cortex. J Vis, 3(10), 586-598.

Eckert R., Tillotson D. and Ridgway E.B. (1977). Voltage-dependent facilitation of Ca2+ entry in voltage-clamped, aequorin-injected molluscan neurons. Proc Natl Acad Sci U S A, 74(4), 1748-1752.

Eysel U.T., Gonzales-Aguilar, F. and Mayer, U (1980). A functional sign of reorganization in the visual system of adult cats: Lateral geniculate neurons with displaced receptive fileds after lesion of the nasal retina. Brain Research, 181, 285-300.

Fahle M. (1993). Visual learning in the hyperacuity range in adults. Ger J Ophthalmol, 2(2), 83-86.

Fahle M.a.P., T (2002a). Perceptual Learning. Fahle and Poggio (Eds), Cambridge, Massachusetts: MIT press.

Fahle M. and Daum I. (2002b). Perceptual learning in amnesia. Neuropsychologia, 40(8), 1167-1172.

Felleman D.J. and Van Essen D.C. (1991). Distributed hierarchical processing in the primate cerebral cortex. Cereb Cortex, 1(1), 1-47.

Fertonani A., Pirulli C. and Miniussi C. (2011). Random noise stimulation improves neuroplasticity in perceptual learning. J Neurosci, 31(43), 15416-15423.

Fertonani A., Rosini S., Cotelli M., Rossini P.M. and Miniussi C. (2010). Naming facilitation induced by transcranial direct current stimulation. Behavioral Brain Research, 208(2), 311-318.

Freeman D.K., Eddington D.K., Rizzo J.F., 3rd and Fried S.I. (2010). Selective activation of neuronal targets with sinusoidal electric stimulation. Journal of Neurophysiology, 104(5), 2778-2791.

Fregnac Y., Schulz. D., Thorpe, S. and Bienenstock. E. (1988). A cellular analogue of visual cortical plasticity. Nature, 333, 367-370.

Fregnac Y., Schulz. D., Thorpe, S. and Bienenstock. E. (1992). Cellular anologs of visual cortical epigenesis 1. Plasticity of orientation selectivity. Journal of Neuroscience, 12, 1280-1300.

Fricke K., Seeber A.A., Thirugnanasambandam N., Paulus W., Nitsche M.A. and Rothwell J.C. (2011). Time course of the induction of homeostatic plasticity generated by repeated transcranial direct current stimulation of the human motor cortex. J Neurophysiol, 105(3), 1141-1149.

Fritsch B., Reis J., Martinowich K., Schambra H.M., Ji Y., Cohen L.G. and Lu B. (2010). Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. Neuron, 66(2), 198-204.

Furmanski C.S. and Engel S.A. (2000). Perceptual learning in object recognition: object specificity and size invariance. Vision Res, 40(5), 473-484.

Furmanski C.S., Schluppeck D. and Engel S.A. (2004). Learning strengthens the response of primary visual cortex to simple patterns. Curr Biol, 14(7), 573-578.

Gamboa O.L., Antal A., Moliadze V. and Paulus W. (2010). Simply longer is not better: reversal of theta burst after-effect with prolonged stimulation. Exp Brain Res, 204(2), 181-187.

Gandiga P.C., Hummel F.C. and Cohen L.G. (2006). Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. Clinical Neurophysiology, 117(4), 845-850.

Gentner R., Wankerl K., Reinsberger C., Zeller D. and Classen J. (2008). Depression of human corticospinal excitability induced by magnetic theta-burst stimulation: evidence of rapid polarity-reversing metaplasticity. Cereb Cortex, 18(9), 2046-2053.

Ghose G.M. (2004). Learning in mammalian sensory cortex. Current Opinion in Neurobiology, 14(4), 513-518.

Ghose G.M., Yang T. and Maunsell J.H. (2002). Physiological correlates of perceptual learning in monkey V1 and V2. J Neurophysiol, 87(4), 1867-1888.

Gilbert C.D., Sigman M. and Crist R.E. (2001). The neural basis of perceptual learning. Neuron, 31(5), 681-697.

Gilbert C.D., Li W. and Piech V. (2009). Perceptual learning and adult cortical plasticity. J Physiol, 587(Pt 12), 2743-2751.

Gilbert C.D.a.W., T. N. (1992). Receptive field dynamics in adult primary visual cortex. Nature, 356, 150-152.

Goodale E. and Kotagama S.W. (2006). Context-dependent vocal mimicry in a passerine bird. Proc Biol Sci, 273(1588), 875-880.

Herzog M.H. and Fahle M. (1997). The role of feedback in learning a vernier discrimination task. Vision Res, 37(15), 2133-2141.

Hubel D.H.a.W., T. N. (1959). Receptive fields of single neurone in the cat's striate cortex. Journal of Physiology, 148, 574-591.

Inghilleri M., Conte A., Curra A., Frasca V., Lorenzano C. and Berardelli A. (2004). Ovarian hormones and cortical excitability. An rTMS study in humans. Clinical Neurophysiology, 115(5), 1063-1068.

Iyer M.B., Mattu U., Grafman J., Lomarev M., Sato S. and Wassermann E.M. (2005). Safety and cognitive effect of frontal DC brain polarization in healthy individuals. Neurology, 64(5), 872-875.

Jacobson L., Koslowsky M. and Lavidor M. (2011). tDCS polarity effects in motor and cognitive domains: a meta-analytical review. Exp Brain Res, 216(1), 1-10.

Kaas J.H. (1991). Plasticity of sensory and motor maps in adult mammals. Annu Rev Neurosci, 14, 137-167.

Kanai R., Chaieb L., Antal A., Walsh V. and Paulus W. (2008). Frequency-dependent electrical stimulation of the visual cortex. Current Biology, 18(23), 1839-1843.

Kandel E.R., Schwartz J.H. and Jessell M.T. (2000). Principle of neural sciences (4 ed.): McGraw-Hill.

Karni A. and Sagi D. (1991). Where practice makes perfect in texture discrimination: evidence for primary visual cortex plasticity. Proc Natl Acad Sci U S A, 88(11), 4966-4970.

Karni A. and Sagi D. (1993). The time course of learning a visual skill. Nature, 365(6443), 250-252.

Kostyuk P.G. and Krishtal O.A. (1977). Effects of calcium and calcium-chelating agents on the inward and outward current in the membrane of mollusc neurones. J Physiol, 270(3), 569-580.

Kraft A., Roehmel J., Olma M.C., Schmidt S., Irlbacher K. and Brandt S.A. (2010). Transcranial direct current stimulation affects visual perception measured by threshold perimetry. Experimental Brain Research, 207(3-4), 283-290.

Kuo M.F., Paulus W. and Nitsche M.A. (2006). Sex differences in cortical neuroplasticity in humans. Neuroreport, 17(16), 1703-1707.

Kuo M.F., Unger M., Liebetanz D., Lang N., Tergau F., Paulus W. and Nitsche M.A. (2008). Limited impact of homeostatic plasticity on motor learning in humans. Neuropsychologia, 46(8), 2122-2128.

Kurachi Y. and Ishii M. (2004). Cell signal control of the G protein-gated potassium channel and its subcellular localization. J Physiol, 554(Pt 2), 285-294.

Lang N., Siebner H.R., Ernst D., Nitsche M.A., Paulus W., Lemon R.N. and Rothwell J.C. (2004). Preconditioning with transcranial direct current stimulation sensitizes the motor cortex to rapid-rate transcranial magnetic stimulation and controls the direction of after-effects. Biol Psychiatry, 56(9), 634-639.

Levitan I.B. and Kaczmarek L.K. (2002). The Neuron. Cell and molecular biology (Third edition ed.). New York: Oxford University Press.

Li W., Piech V. and Gilbert C.D. (2004). Perceptual learning and top-down influences in primary visual cortex. Nature Neuroscience, 7(6), 651-657.

Liebetanz D., Nitsche M.A., Tergau F. and Paulus W. (2002). Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. Brain, 125(Pt 10), 2238-2247.

Liu J., Lu Z.L. and Dosher B.A. (2010). Augmented Hebbian reweighting: interactions between feedback and training accuracy in perceptual learning. J Vis, 10(10), 29.

Loui P., Hohmann A. and Schlaug G. (2010). Inducing Disorders in Pitch Perception and Production: a Reverse-Engineering Approach. Proc Meet Acoust, 9(1), 50002.

Marshall L., Molle M., Siebner H.R. and Born J. (2005). Bifrontal transcranial direct current stimulation slows reaction time in a working memory task. BMC Neuroscience, 6, 23.

Matsunaga K., Nitsche M.A., Tsuji S. and Rothwell J.C. (2004). Effect of transcranial DC sensorimotor cortex stimulation on somatosensory evoked potentials in humans. Clin Neurophysiol, 115(2), 456-460.

Matthews N., Liu Z., Geesaman B.J. and Qian N. (1999). Perceptual learning on orientation and direction discrimination. Vision Research, 39(22), 3692-3701.

Matthews N. and Welch L. (1997). Velocity-dependent improvements in single-dot direction discrimination. Perception and Psychophysics, 59(1), 60-72.

Mayer M.J. (1983). Practice improves adults' sensitivity to diagonals. Vision Res, 23(5), 547-550.

McKee S.P. and Westheimer G. (1978). Improvement in vernier acuity with practice. Percept Psychophys, 24(3), 258-262.

Merzenich M.M., Nelson, R. J., Stryker, M. P., Cynader, M. S., Schoppmann, A. and Zook J.M. (1984). Somatosensory cortical map changes following digit amputation in adult monkey. J. Comp- Neurol., 224, 591-605.

Merzenich M.M., Recanzone G., Jenkins, W. M., Allard T. T. and Nudo, R. J. (1988). Cortical representational plasticity. Neurobiology of Neocortex, 41-67.

Meyer B.U., Diehl R., Steinmetz H., Britton T.C. and Benecke R. (1991). Magnetic stimuli applied over motor and visual cortex: influence of coil position and field polarity on

motor responses, phosphenes, and eye movements. Electroencephalogr Clin Neurophysiol Suppl, 43, 121-134.

Miniussi C., Ruzzoli M. and Walsh V. (2010). The mechanism of transcranial magnetic stimulation in cognition. Cortex, 46(1), 128-130.

Miniussi C. and Vallar G. (2011). Brain stimulation and behavioural cognitive rehabilitation: a new tool for neurorehabilitation? Neuropsychol Rehabil, 21(5), 553-559.

Miranda P.C., Faria P. and Hallett M. (2009). What does the ratio of injected current to electrode area tell us about current density in the brain during tDCS? Clin Neurophysiol, 120(6), 1183-1187.

Moliadze V., Antal A. and Paulus W. (2011). Electrode-distance dependent after-effects of transcranial direct and random noise stimulation with extracephalic reference electrodes. Clin Neurophysiol, 121(12), 2165-2171.

Monte-Silva K., Kuo M.F., Liebetanz D., Paulus W. and Nitsche M.A. (2010). Shaping the optimal repetition interval for cathodal transcranial direct current stimulation (tDCS). J Neurophysiol, 103(4), 1735-1740.

Moss F., Ward L.M. and Sannita W.G. (2004). Stochastic resonance and sensory information processing: a tutorial and review of application. Clin Neurophysiol, 115(2), 267-281.

Mulquiney P.G., Hoy K.E., Daskalakis Z.J. and Fitzgerald P.B. (2011). Improving working memory: exploring the effect of transcranial random noise stimulation and transcranial direct current stimulation on the dorsolateral prefrontal cortex. Clin Neurophysiol, 122(12), 2384-2389.

Nitsche M.A., Cohen L.G., Wassermann E.M., Priori A., Lang N., Antal A., Paulus W., Hummel F., Boggio P.S., Fregni F. and Pascual-Leone A. (2008). Transcranial direct current stimulation: State of the art 2008. Brain Stimulation, 1(3), 206-223.

Nitsche M.A., Fricke K., Henschke U., Schlitterlau A., Liebetanz D., Lang N., Henning S., Tergau F. and Paulus W. (2003a). Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. The Journal of Physiology, 553(Pt 1), 293-301.

Nitsche M.A., Kuo M.F., Grosch J., Bergner C., Monte-Silva K. and Paulus W. (2009). D1-receptor impact on neuroplasticity in humans. J Neurosci, 29(8), 2648-2653.

Nitsche M.A., Nitsche M.S., Klein C.C., Tergau F., Rothwell J.C. and Paulus W. (2003b). Level of action of cathodal DC polarisation induced inhibition of the human motor cortex. Clinical Neurophysiology, 114(4), 600-604.

Nitsche M.A. and Paulus W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. The Journal of Physiology, 527 Pt 3, 633-639.

Nitsche M.A. and Paulus W. (2011). Transcranial direct current stimulation - update 2011. Restor Neurol Neurosci, 29(6), 463-492.

Nitsche M.A., Schauenburg A., Lang N., Liebetanz D., Exner C., Paulus W. and Tergau F. (2003c). Facilitation of implicit motor learning by weak transcranial direct current stimulation of the primary motor cortex in the human. J Cogn Neurosci, 15(4), 619-626.

Poreisz C., Boros K., Antal A. and Paulus W. (2007). Safety aspects of transcranial direct current stimulation concerning healthy subjects and patients. Brain Res Bull, 72(4-6), 208-214.

Pourtois G., Rauss K.S., Vuilleumier P. and Schwartz S. (2008). Effects of perceptual learning on primary visual cortex activity in humans. Vision Res, 48(1), 55-62.

Purpura D.P. and McMurtry J.G. (1965). Intracellular Activities and Evoked Potential Changes During Polarization of Motor Cortex. J Neurophysiol, 28, 166-185.

Quartarone A., Morgante F., Bagnato S., Rizzo V., Sant'Angelo A., Aiello E., Reggio E., Battaglia F., Messina C. and Girlanda P. (2004). Long lasting effects of transcranial direct current stimulation on motor imagery. Neuroreport, 15(8), 1287-1291.

Raiguel S., Vogels R., Mysore S.G. and Orban G.A. (2006). Learning to see the difference specifically alters the most informative V4 neurons. J Neurosci, 26(24), 6589-6602.

Recanzone G.H., Merzenich M.M. and Dinse H.R. (1992a). Expansion of the cortical representation of a specific skin field in primary somatosensory cortex by intracortical microstimulation. Cereb Cortex, 2(3), 181-196.

Recanzone G.H., Merzenich M.M., Jenkins W.M., Grajski K.A. and Dinse H.R. (1992b). Topographic reorganization of the hand representation in cortical area 3b owl monkeys trained in a frequency-discrimination task. J Neurophysiol, 67(5), 1031-1056.

Reis J., Schambra H.M., Cohen L.G., Buch E.R., Fritsch B., Zarahn E., Celnik P.A. and Krakauer J.W. (2009). Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation. Proc Natl Acad Sci U S A, 106(5), 1590-1595.

Ridding M.C. and Ziemann U. (2010). Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects. J Physiol, 588(Pt 13), 2291-2304.

Rossi S., Hallett M., Rossini P.M., Pascual-Leone A. and Safety of TMS Consensus Group (2009). Safety, ethical considerations, and application guidelines for the use of

transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol, 120(12), 2008-2039.

Ruzzoli M., Marzi C.A. and Miniussi C. (2010). The neural mechanisms of the effects of transcranial magnetic stimulation on perception. J Neurophysiol, 103(6), 2982-2989.

Sale M.V., Ridding M.C. and Nordstrom M.A. (2007). Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation. Experimental Brain Research, 181(4), 615-626.

Schiltz C., Bodart J.M., Dubois S., Dejardin S., Michel C., Roucoux A., Crommelinck M. and Orban G.A. (1999). Neuronal mechanisms of perceptual learning: changes in human brain activity with training in orientation discrimination. Neuroimage, 9(1), 46-62.

Schoen I. and Fromherz P. (2008). Extracellular stimulation of mammalian neurons through repetitive activation of Na+ channels by weak capacitive currents on a silicon chip. J Neurophysiol, 100(1), 346-357.

Scholz J., Klein M.C., Behrens T.E. and Johansen-Berg H. (2009). Training induces changes in white-matter architecture. Nat Neurosci, 12(11), 1370-1371.

Schoups A., Vogels R., Qian N. and Orban G. (2001). Practising orientation identification improves orientation coding in V1 neurons. Nature, 412(6846), 549-553.

Schoups A.A., Vogels R. and Orban G.A. (1995). Human perceptual learning in identifying the oblique orientation: retinotopy, orientation specificity and monocularity. J Physiol, 483 (Pt 3), 797-810.

Schwartz S., Maquet P. and Frith C. (2002). Neural correlates of perceptual learning: a functional MRI study of visual texture discrimination. Proc Natl Acad Sci U S A, 99(26), 17137-17142.

Schweid L., Rushmore R.J. and Valero-Cabre A. (2008). Cathodal transcranial direct current stimulation on posterior parietal cortex disrupts visuo-spatial processing in the contralateral visual field. Exp Brain Res, 186(3), 409-417.

Seitz A. and Watanabe T. (2005). A unified model for perceptual learning. Trends Cogn Sci, 9(7), 329-334.

Seitz A.R., Kim D. and Watanabe T. (2009). Rewards evoke learning of unconsciously processed visual stimuli in adult humans. Neuron, 61(5), 700-707.

Seitz A.R. and Watanabe T. (2009). The phenomenon of task-irrelevant perceptual learning. Vision Res, 49(21), 2604-2610.

Shibata K., Yamagishi N., Ishii S. and Kawato M. (2009). Boosting perceptual learning by fake feedback. Vision Res, 49(21), 2574-2585.

Shiu L.P. and Pashler H. (1992). Improvement in line orientation discrimination is retinally local but dependent on cognitive set. Percept Psychophys, 52(5), 582-588.

Siebner H.R., Lang N., Rizzo V., Nitsche M.A., Paulus W., Lemon R.N. and Rothwell J.C. (2004). Preconditioning of low-frequency repetitive transcranial magnetic stimulation with transcranial direct current stimulation: evidence for homeostatic plasticity in the human motor cortex. The Journal of Neuroscience, 24(13), 3379-3385.

Smith M.J., Adams L.F., Schmidt P.J., Rubinow D.R. and Wassermann E.M. (2002). Effects of ovarian hormones on human cortical excitability. Annals of Neurology, 51(5), 599-603.

Smith M.J., Keel J.C., Greenberg B.D., Adams L.F., Schmidt P.J., Rubinow D.A. and Wassermann E.M. (1999). Menstrual cycle effects on cortical excitability. Neurology, 53(9), 2069-2072.

Sparing R., Thimm M., Hesse M.D., Kust J., Karbe H. and Fink G.R. (2009). Bidirectional alterations of interhemispheric parietal balance by non-invasive cortical stimulation. Brain, 132(Pt 11), 3011-3020.

Spruston N. (2008). Pyramidal neurons: dendritic structure and synaptic integration. Nat Rev Neurosci, 9(3), 206-221.

Stagg C.J., Jayaram G., Pastor D., Kincses Z.T., Matthews P.M. and Johansen-Berg H. (2011a). Polarity and timing-dependent effects of transcranial direct current stimulation in explicit motor learning. Neuropsychologia, 49(5), 800-804.

Stagg C.J. and Nitsche M.A. (2011b). Physiological basis of transcranial direct current stimulation. Neuroscientist, 17(1), 37-53.

Stuart G. and Spruston N. (1998). Determinants of voltage attenuation in neocortical pyramidal neuron dendrites. J Neurosci, 18(10), 3501-3510.

Terney D., Chaieb L., Moliadze V., Antal A. and Paulus W. (2008). Increasing human brain excitability by transcranial high-frequency random noise stimulation. J Neurosci, 28(52), 14147-14155.

Terzuolo C.A. and Bullock T.H. (1956). Measurement of Imposed Voltage Gradient Adequate to Modulate Neuronal Firing. Proc Natl Acad Sci U S A, 42(9), 687-694.

Thickbroom G.W. (2007). Transcranial magnetic stimulation and synaptic plasticity: experimental framework and human models. Exp Brain Res, 180(4), 583-593.

Thiele A. (2004). Perceptual learning: is V1 up to the task? Current Biology, 14(16), R671-673.

Thut G. and Miniussi C. (2009). New insights into rhythmic brain activity from TMS-EEG studies. Trends in Cognitive Sciences, 13(4), 182-189.

Tootell R.B., Dale A.M., Sereno M.I. and Malach R. (1996). New images from human visual cortex. Trends Neurosci, 19(11), 481-489.

Ts'o D.Y., Gilbert C.D. and Wiesel T.N. (1986). Relationships between horizontal interactions and functional architecture in cat striate cortex as revealed by cross-correlation analysis. J Neurosci, 6(4), 1160-1170.

Vogels R. and Orban G.A. (1985). The effect of practice on the oblique effect in line orientation judgments. Vision Res, 25(11), 1679-1687.

Wagner T., Fregni F., Fecteau S., Grodzinsky A., Zahn M. and Pascual-Leone A. (2007). Transcranial direct current stimulation: a computer-based human model study. Neuroimage, 35(3), 1113-1124.

Wandell B.A. and Smirnakis S.M. (2009). Plasticity and stability of visual field maps in adult primary visual cortex. Nature Reviews Neuroscience, 10(12), 873-884.

Wankerl K., Weise D., Gentner R., Rumpf J.J. and Classen J. (2010). L-type voltage-gated Ca2+ channels: a single molecular switch for long-term potentiation/long-term depression-like plasticity and activity-dependent metaplasticity in humans. J Neurosci, 30(18), 6197-6204.

Watanabe T. (2002). Greater plasticity in lower-level than higher-level visual motion processing in a passive perceptual learning task. Nature Neuroscience(5), 1003-1009.

Watanabe T., Nanez J.E. and Sasaki Y. (2001). Perceptual learning without perception. Nature, 413(6858), 844-848.

Xu P., Lu Z.L., Wang X., Dosher B., Zhou J., Zhang D. and Zhou Y. (2010). Category and perceptual learning in subjects with treated Wilson's disease. PLoS One, 5(3), e9635.

Yacoub E., Harel N. and Ugurbil K. (2008). High-field fMRI unveils orientation columns in humans. Proc Natl Acad Sci U S A, 105(30), 10607-10612.

Yotsumoto Y., Chang L.H., Watanabe T. and Sasaki Y. (2009). Interference and feature specificity in visual perceptual learning. Vision Res, 49(21), 2611-2623.

Yotsumoto Y., Watanabe T. and Sasaki Y. (2008). Different dynamics of performance and brain activation in the time course of perceptual learning. Neuron, 57(6), 827-833.

Zaehle T., Rach S. and Herrmann C.S (2010). Transcranial alternating current stimulation enhances individual alpha activity in human EEG. PLoS One, 5(11), e13766.

Ziemann U. and Siebner H.R. (2008). Modifying motor learning through gating and homeostatic metaplasticity. Brain Stimul, 1(1), 60-66.

Published papers

M. Ruzzoli, **C. Pirulli**, D. Brignani, C. Maioli, C. Miniussi, (2012). Sensory memory during physiological aging indexed by mismatch negativity (MMN). Neurobiology of Aging, 33, 3: 21-30.

A. Fertonani, **C. Pirulli,** C. Miniussi (2011). Random noise stimulation improves neuroplasticity in perceptual learning. Journal of Neuroscience, 31, 43:15416-23.

Manuela Ruzzoli, Simone Gori, Andrea Pavan, **Cornelia Pirulli**, Carlo A. Marzi and Carlo Miniussi, (2011) The neural basis of the Enigma illusion: a transcranial magnetic stimulation study. Neuropsychologia . 8

Pellicciari M.C., Veniero D., Marzano C., Moroni F., **Pirulli C.,** Curcio G., Ferrara M., Miniussi C., Rossini P.M., De Gennaro L. "Heritability of intracortical inhibition and facilitation". The Journal of Neuroscience 2009, July 15, 29(28):8897– 8900

De Gennaro L., Fratello F., Marzano C., Moroni F., Curcio G., Tempesta D., Pellicciari M.C., **Pirulli C.,** Ferrara M., Rossini P.M (2008)."Cortical plasticity induced by transcranial magnetic stimulation during wakefulness affects electroencephalogram activity during sleep". PloSOne June 3 (6): e2483.

Published abstracts

C. Pirulli, A. Fertonani, C. Miniussi (2011). Neuroplasticity induction in a perceptual learning task. Which is the best timing to apply tES? Archives Italiennes de Biologie, Vol: 149, 3. 19th Congress of the Italian Society of Psychophysiology (SIPF), Brescia, November 14-16

A. Fertonani, C. **Pirulli, C**. Miniussi (2011). Random noise stimulation improves neuroplasticity in perceptual learning. Archives Italiennes de Biologie, Vol: 149, 3. 19th Congress of the Italian Society of Psychophysiology (SIPF), Brescia, November 14-16.

C. Pirulli, A. Ragazzoni, D. Veniero, S. Rossi, C. Miniussi, M. Feurra, M. Cincotta, F. Giovannelli, R. Chiaramonti, M. Lino (2011). Cortico-cortical connectivity, Vegetative State and Minimally Conscious State: an investigation by Transcranial Magnetic Stimulation-Evoked Potentials. Clinical Neurophysiology 122, 1, S53.14th European Congress of Clinical Neurophysiology and 4th International Conferences on Transcranial Magnetic and Direct Current Stimulation. Rome, June 21-24

C. Pirulli, A. Fertonani, P.M. Rossini, C. Miniussi (2010). Plasticity effects in visual perceptual learning: a transcranial electrical stimulation (tES) study. 18th Congress of the Italian Society of Psychophysiology (SIPF), Palermo, November 24-26.

D. Brignani, **C. Pirulli,** M. Ruzzoli, C. Miniussi (2010). Sensory memory in physiological and pathological aging indexed by the mismatch negativity (MMN). 18th Congress of the Italian Society of Psychophysiology (SIPF), Palermo, November 24-26.

C. Pirulli, A. Fertonani, P.M. Rossini, C. Miniussi (2010). Platicity effects in visual perception learning: a transcranical electrical stimulation (tES) study. Workshop Nuove Prospettive della Stimolazione Elettrica Transcranica: tra sperimentazione e clinica. Brescia, November 12.Neuropsychological Trends, 8, 23.

S. Rossi, A. Ragazzoni, **C. Pirulli**, D. Veniero, C. Miniussi, M. Feurra, M. Cincotta, F. Giovannelli, R Chiaramonti, M. Lino (2010). Cortico-cortical connectivity,

Vegetative State and Minimally Conscious State: an investigation by TMS-Evoked Potentials (TEPs). International Congress of Clinical Neurophysiology, Kobe, October 28 - November 1, Clinical Neurophysiology, 121, 1.

M.C. Pellicciari, C. Marzano, **C. Pirulli,** P. Sammarco, C. Miniussi, L. De Gennaro (2010). Topografia corticale dell'EEG durante il sonno di pazienti Alzheimer. 9th Congress of the AFaR, Brescia, September 27-29.

M.C. Pellicciari, C. Marzano, **C. Pirulli,** S. Cordone, C. Miniussi, L. De Gennaro (2010). Variazioni topografiche dell'EEG di sonno in pazienti depressi sottoposti a trattamento rTMS. 9th Congress of the AFaR, Brescia, September 27-29.

A. Fertonani, **C. Pirulli,** P.M. Rossini and C. Miniussi (2010). Plasticity effects in visual perceptual learning: a transcranial random noise stimulation study. 9th Congress of the AFaR, Brescia, September 27-29.

C. Pirulli, A. Ragazzoni, D. Veniero, S. Rossi, M. Feurra, M. Cincotta, F. Giovannelli, R. Chiaramonti, M. Lino, C. Miniussi (2010). Cortico-cortical connectivity, Vegetative State and Minimally Conscious State: an investigation by TMS-Evoked Potentials (TEPs). 9th Congress of the AFaR, Brescia, September 27-29.

C. Pirulli, A. Fertonani, P.M. Rossini and C. Miniussi (2010) .Plasticity effects in visual perceptual learning: a transcranial random noise stimulation study. International Workshop on Synaptic Plasticity from Bench to Bed Side, Taormina, April 28 – May 1.

Attended workshops/courses:

XIX congress SIPF - Società Italiana di Psicofisiologia - Brescia 14 – 16 novembre 2011.

XIV congress of the Società Europea di Neurofisiologia – Roma 21 – 24 giugno 2011.

IV International Conference on Transcranial Magnetic and Direct Current Stimulation – 25 giugno 2011.

Workshop of "Biostatistica applicata alla ricerca clinica e sperimentale", Brescia 18 - 21 Aprile, 16 – 19 Maggio, 6 - 8 Giugno 2011.

XVIII congress SIPF - Società Italiana di Psicofisiologia - Palermo 24-27 novembre 2010.

Workshop "Nuove prospettive della Stimolazione elettrica Transcranica: tra sperimentazione e clinica"; - Brescia 12 novembre 2010.

Workshop "Corso teorico pratico di Neuronavigazione e di utilizzo del sistema Softaxic Optic 2,0"; - Brescia 11 novembre 2010.

X Congresso Nazionale AFaR; - Brescia 27-29 settembre 2010.

Doctoral School – Piattaforma didattica comune, programma di formazione relativo al dottorato di ricerca svoltosi presso l'Università Campus Bio-Medico di Roma - 15-18 giugno 2010.

Workshop di "Synaptic Plasticity" Taormina 28 aprile - 1 maggio 2010.

The third annual workshop – "Attention and Awareness" – Rovereto - 29-31 ottobre 2009

Formative Course: "Efficacia clinica delle terapie di gruppo". Brescia - 19 settembre 2009.

Conference: "Meccanismi di sincronizzazione neurale della corteccia dell'uomo rivelate da avanzate tecniche EEG" Brescia - 18 settembre 2009.

Doctoral School – Piattaforma didattica comune, programma di formazione relativo al dottorato di ricerca svoltosi presso l'Università Campus Bio-Medico – Roma, 22-25 giugno 2009.

Conference: "Alterazioni microstrutturali della sostanza bianca nel decadimento cognitivo lieve e nella malattia di Alzheimer"; – Brescia - 15 giugno 2009

TMS Summer School-Magstim, "Attention, Perception and Motor Cognition"; - London 29-30 maggio 2009.

Conference: "Terapia delle demenze frontotemporali"; – Brescia 9 marzo 2009

Ringraziamenti

Il mio percorso nelle neuroscienze inizia con il prof. Carlo Miniussi. A lui devo un Grazie particolare perché per me è stato una guida apparentemente assente ma costantemente presente, un punto di riferimento. Un "capo" che sia lavorativamente che umanamente, sono sicura, molti vorrebbero avere sulla propria strada.

Al laboratorio di Neuroscienze Cognitive di Brescia, ogni confronto anche quello più vivace ha caratterizzato questi tre anni e ha permesso la mia crescita lavorativa e personale. Non c'è una sola volta in cui, pensando ad un "domani" senza di loro, il mio cuore si rattristi.

A Maria Concetta, che dovrebbe rientrare nel ringraziamento precedente, ma lei prima che collega è amica, e sarò ripetitiva visto che nella mia tesi di laurea le ho scritto la stessa frase, ma è "un'amica di cui non posso più fare a meno".

A Manuela e Sandra, che insieme raggiungono il giusto equilibrio di durezza e dolcezza, che da angolature diverse mi hanno sempre spronato a fare del mio meglio in questo percorso.

Alla mia famiglia, che ha sempre cercato di accorciare l'inevitabile distanza che ci divide, facendomi sentire sempre la loro presenza

A Gianvito, compagno di vita da una vita, a lui devo la pazienza nel sapermi stare vicino in questo percorso, credendo sempre nelle mie capacità e amandomi sempre nello stesso modo, grazie.

A Giuliana, l'altra parte di me, lei che da sempre abita nel mio cuore.

Ai miei amici del Sud: Dominga, Carmilia, Rino, Gianni, Gaetano, Andrea, Alessandra, Francesco, Marinella. Ai miei amici del Nord: Maria, Laura, William,

Alberto, Marcella, Alessandro, Stefano e Sara. Vivendoli ho capito che l'amicizia ha lo stesso valore da Nord a Sud, e ha la stessa capacità di riempire la mia vita di magnifici ricordi.

Alla mia nipotina Claudia, che ha reso e rende trepidante ogni mio ritorno a casa. Ai suoi in bocca al lupo per telefono prima detti in modo confuso e poi diventati sempre più chiari, mi hanno emozionato.

E ci sarebbero tanti altri nomi da fare, pagine da riempire, allora:

"Semplicemente Grazie a chi mi ha accompagnato più o meno consapevolmente per questo meraviglioso viaggio nelle Neuroscienze, per questo pezzo di strada della mia vita".