# Low Intensity Transcranial Electrical Stimulation: Effects on Categorization and Methodological Aspects

Dissertation

zur Erlangung des mathematisch-naturwissenschaftlichen

Doktorgrades

**"Doctor rerum naturalium"**

der Georg-August-Universität Göttingen


vorgelegt von

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aus Orosháza, Ungarn

Göttingen, 2011

# Statement of originality

I hereby declare that this thesis is my own work and has been written independently with no other sources and aids than those quoted in the text, references and acknowledgments.

Göttingen, $14^{th}$ December, 2011

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# Chapter 1

# Introduction

Modulating, altering, disrupting or otherwise interfering with the activity of the cerebral cortex by non-invasive, external means not only offers the possibility of clinical intervention in neurological disorders and conditions, but provides us with a powerful research tool for understanding the workings of the intact human brain. In the past decades, a number of non-invasive brain stimulation (NIBS) techniques have been developed.

*Transcranial electrical stimulation* (TES, Merton & Morton, 1980), where short pulses (in the $\mu$s range) of currents are applied via small electrodes (1 cm in diameter) over the scalp above the targeted cortical area using a relatively high stimulation intensity, was shown to be able to elicit motor evoked potentials (MEPs) and phosphenes (Merton & Morton, 1980; Merton, Morton, Hill, & Marsden, 1982). Due to the relatively small size of the electrodes and the high intensity of the current

needed to produce a reliable response (e.g. a peak current intensity of $50A$ was used to elicit the perception of phosphenes), this method involves considerable discomfort.

*Transcranial magnetic stimulation* (TMS, Barker, Freeston, Jalinous, Merton, & Morton, 1985) uses rapidly changing magnetic fields to induce electrical currents in the underlying cortical tissue, and is also capable of eliciting MEPs. As no current is passing through the skin, this method involves less discomfort compared to TES.

Today, single pulse TMS is used in a number of different settings: in clinical diagnostics, for example, it is applied to assess the integrity of the functioning of the central motor pathway (Sandbrink, 2008), and in research it is used in the evaluation of methods designed to modulate cortical excitability (Nitsche & Paulus, 2000; Priori, Berardelli, Rona, Accornero, & Manfredi, 1998). Repetitive TMS (rTMS) has also been shown to be capable of influencing cortical processing (for a review see Fitzgerald, Fountain, & Daskalakis, 2006) and causing lasting changes in cortical excitability. For example, continuous stimulation with 5 Hz impulses of three 50 Hz bursts (continuous theta burst stimulation – TBS) at the 80% of the active motor threshold (AMT) suppresses the MEP size, whilst alternating 2 seconds of breaks with 2 seconds of stimulation (intermittent TBS – iTBS) enhances MEPs (Huang, Chen, Rothwell, & Wen, 2007).

*Low-intensity transcranial electrical stimulation* or, alternatively, *weak transcranial electrical stimulation* (tES) methods are a group of NIBS techniques where currents with intensities considerably lower than the motor threshold (typically 1 — 2 mA) are applied through the scalp (for a review, see Paulus, 2011). These techniques, though not capable of causing neuronal firing directly, have been shown to induce changes in cortical excitability outlasting the duration of the stimulation in a

spatially restricted and reversible manner. tES methods include transcranial direct current stimulation (tDCS), transcranial alternating current stimulation (tACS) and transcranial random noise stimulation (tRNS).

This thesis deals with studies conducted using low intensity transcranial electrical stimulation techniques.

## 1.1 Application of tES Intervention

The general guidelines for applying tDCS and communicating the results have been set in 2008 by collaborators from leading tES laboratories (for a review see Nitsche et al., 2008).

The mode of application is generally similar in the case of all tES interventions. The current is delivered by a battery-driven stimulator. The stimulation is usually applied using a pair of rubber electrodes encased in viscose sponge wrappers that are soaked in saline solution. Alternatively, the electrodes may be applied using conductive electrode paste. Rubber bands are used to fix the electrodes to the head. Typically, one electrode is defined as the stimulation or target electrode, which is positioned above the cortical region of interest. The other electrode is usually referred to as the reference (Nitsche et al., 2008), or return electrode (Bikson, Datta, Rahman, & Scaturro, 2010a). It is important to note that the return electrode can also be considered physiologically active; to reduce the confounding effect of the stimulation under this electrode, an extracephalic montage can be used, or the size of the electrode can be increased to reduce the current density (Nitsche et al., 2008).

## 1.2 Measuring the Effects of tES Intervention

A standard procedure in quantifying the effects of a newly developed tES technique is to measure the magnitude, direction, and time course of its effects on motor cortex excitability. This is achieved by using *TMS-elicited MEPs* (Nitsche & Paulus, 2000; Antal et al., 2008; Terney, Chaieb, Moliadze, Antal, & Paulus, 2008).

TMS stimulation over the visual cortex has the potential to elicit *phosphenes*. The effects of tES techniques may also be assessed by gauging the changes in TMS intensity thresholds that can elicit phosphenes, before and after the tES intervention (Antal, Kincses, Nitsche, & Paulus, 2003; Kanai, Paulus, & Walsh, 2010). Also, the effects tES interventions on cognitive functions are being assessed. Stimulation of the primary motor cortex during the application of the serial reaction time task (SRTT, Nissen & Bullemer, 1987), an implicit motor learning paradigm, has become a standard procedure (Antal et al., 2008; Nitsche, Schauenburg, et al., 2003; Terney et al., 2008). Another widely used task is the n-back task (Kirchner, 1958), a paradigm used to measure working memory storage and executive functions performance. Here, typically the dorsolateral prefrontal cortex (DLPFC) is targeted for stimulation (e.g. Fregni et al., 2005; Teo, Hoy, Daskalakis, & Fitzgerald, 2011).

The use of tES stimulation techniques in order to alter cognitive task performance, applied to the DLPFC is discussed later in this thesis in more detail.

## 1.3 Transcranial Direct Current Stimulation

Transcranial direct current stimulation is the most widely utilized of the tES techniques. tDCS polarizes neuronal membrane potentials, but the intensity delivered is

not sufficiently high to discharge action potentials directly; instead, it increases or decreases the spontaneous firing rate of the affected neurons. As pharmacological studies suggest, the effects of tDCS are most likely NMDA receptor and $Ca^{2+}$ channel dependent (Liebetanz, Nitsche, Tergau, & Paulus, 2002; Nitsche, Fricke, et al., 2003). The effects of tDCS are also current-direction dependent. As studies on the motor system have shown, anodal stimulation increases, while cathodal stimulation decreases cortical excitability (Nitsche & Paulus, 2000).

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tDCS is utilized in basic research in a variety of research areas such as learning and memory (Nitsche, Schauenburg, et al., 2003; Antal, Nitsche, Kincses, et al., 2004; Ambrus, Zimmer, et al., 2011), somatosensory perception (Rogalewski, Breitenstein, Nitsche, Paulus, & Knecht, 2004), emotions (Boggio, Zaghi, & Fregni, 2009; Penolazzi et al., 2010) and social neuroscience (Karim et al., 2010; Knoch et al., 2008), where it has been consistently demonstrated that DC stimulation applied to task-relevant areas is able to modulate task execution and behaviour.

Furthermore, the effectiveness of tDCS has been investigated in a wide spectrum of neurological disorders, such as stroke (Hummel et al., 2005a), depression (Fregni et al., 2006; Rigonatti et al., 2008), migraine (Antal, Kriener, Lang, Boros, & Paulus, 2011), aphasia (Monti et al., 2008; Schlaug, Marchina, & Wan, 2011), and substance addiction (Boggio et al., 2008; Fregni et al., 2008; Boggio, Liguori, et al., 2009), in addition to applications for both treatment and rehabilitation, with promising results.

## 1.4 Transcranial Alternating Current Stimulation

Transcranial alternating current stimulation, that is, applying a sinusoidal alternating current at a constant frequency, has been designed to interact with ongoing cortical oscillations. Early studies conducted using tACS applied frequencies in the physiological range to the intact human cortex. 10 Hz tACS over the M1 has been shown to facilitate motor learning (Antal et al., 2008), and the stimulation of the visual cortex has been reported to elicit phosphenes in a frequency-dependent manner (Kanai, Chaieb, Antal, Walsh, & Paulus, 2008; but see: Schutter & Hortensius, 2010, Schwiedrzik, 2009 and Paulus, 2010), and to influence phosphene thresholds measured by the application of TMS (Kanai et al., 2010). 140 Hz tACS over the M1 has been shown to induce aftereffects similar to those of anodal tDCS as measured using TMS-elicited MEPs (Moliadze, Antal, & Paulus, 2010a).

Recently the effects of tACS outside the physiological frequency spectrum, in the kHz range, have also been explored. It is argued that AC stimulation in the low kHz range might have a modulating effect on membrane excitation, thus shaping neuroplastic processes. Excitatory aftereffects have been reported using 1, 2 and 5 kHz tACS as measured by TMS-elicited MEPs (Chaieb, Antal, & Paulus, 2011).

A safety assessment of the application of tACS in the kHz range has been conducted in our laboratory, and it has been found to be safely applicable within the parameters specified within the original article and in accordance with the safety consensus on tES application guidelines (Chaieb et al., in preparation).

## 1.5 Transcranial Random Noise Stimulation

Transcranial random noise stimulation (tRNS) means the non-invasive application of a low-intensity alternating current where the intensity and the frequency of the current vary in a randomized manner. The experiments conducted using tRNS used varied intensities according to the Gaussian distribution, and applied frequencies in a "white noise" fashion between 1 and 640 Hz (Terney et al., 2008).

It has been suggested that, although their modes of action might differ, tRNS can have an effect that is comparable to that of anodal tDCS, that is enhancing the cortical excitability of the targeted cortical area; Terney and colleagues (2008) have shown that 10 minutes of tRNS applied over the M1 can cause excitatory aftereffects lasting up to 1.5 hours, and is capable of improving the performance in the acquisition and early consolidation phase of an implicit motor learning task (Terney et al., 2008). On the other hand, a study investigating the effects of tDCS and tRNS on the n-back test found that the significant improvement found in the anodal tDCS condition was not observable in the case of tRNS (Mulquiney, Hoy, Daskalakis, & Fitzgerald, 2011).

tRNS is considered by Edelmuth et al. to be an "early development NIBS device", that is, although promising initial results have been published using this technique, clinical trials are yet to be conducted (Edelmuth, Nitsche, Battistella, & Fregni, 2010).

## 1.6 Factors Influencing the Effects of tES Stimulation

In the case of tDCS, current polarity is a main determinant of the aftereffects, with anodal stimulation causing an increase, and cathodal stimulation causing a decrease

in cortical excitability. This is not an issue when considering tACS and tRNS, since they lack this polarity constraint.

As discussed earlier, in the case of tACS, and tRNS, frequency and frequency range are major factors regarding the efficacy of the intervention. As empirical (Moliadze, Antal, & Paulus, 2010b) and modeling (Bikson, Datta, Rahman, & Scaturro, 2010b) studies have shown, the position of the reference, or "return" electrode can also have an impact on the strength of the effects of the stimulation, as there is a negative correlation between the distance of the electrodes and the magnitude and duration of the aftereffects. Initial computer modeling studies have demonstrated that High-Definition tDCS (HD-tDCS), an emerging variant of the tDCS, might represent a significant improvement on the focality of the stimulation compared to the conventional design. (HD-tDCS) is modeled as having disk-shaped electrodes, 8 mm in diameter. The target electrode is surrounded by four return electrodes at a 3 cm distance (Datta et al., 2009). Recently, an empirical study using this approach with similar parameters (electrode diameter: 12 mm, target-return distance: 7 cm) has reported that anodal HD-tDCS of the motor cortex is able to modulate pain perception (Borckardt et al., 2011).

Current density, the quotient of the applied current intensity and the interfacing electrode surface area ($I = J/A$), determines the electrical field strength, is also an important parameter of tES, with larger current densities resulting in stronger effects (Nitsche et al., 2008). Reported in the literature are nominal values, as, for example, the shape of the electrode, or inhomogeneities of the electrode and also, possibly, the scalp surface (such as sweat glands, skin lesions) may cause the accumulation of currents that lead to local shifts in current density (Minhas, Datta, & Bikson, 2011a). The role of electrode geometry is discussed later in this thesis. Also, by

increasing the size of the return electrode (thereby reducing the current density) the stimulation under this electrode is suggested to be rendered functionally inert (Nitsche et al., 2007; Fregni et al., 2008).

Stimulation duration also influences the strength of the aftereffects - up to a point (when current density is kept constant), longer stimulation durations lead to more pronounced changes (Nitsche et al., 2008); although the relationship is not strictly linear. The application of anodal tDCS for 26 minutes resulted in inhibitory aftereffects (Monte-Silva et al., submitted, as cited by Paulus, 2011), and the inhibitory aftereffects lasted for a shorter duration with the application of 18 minutes of continuous cathodal tDCS compared to the proportional increase in duration observed comparing 5, 7, and 9 minutes of cathodal tDCS (Monte-Silva, Kuo, Liebetanz, Paulus, & Nitsche, 2010). Short duration stimulation over the M1 has also been shown to be capable of modulating cortical excitability, although the effects do not seem to outlast the stimulation itself (Nitsche et al., 2008).

Stimulation timing can also be a relevant issue, as it has been reported that anodal tDCS stimulation of the M1 during the execution of the SRTT task enhanced performance (Nitsche, Schauenburg, et al., 2003), while anodal tDCS before the execution of the task did not lead to altered task performance (Kuo et al., 2008).

The state of the cortex during stimulation might also be an important factor regarding the effects of tDCS (Silvanto, Muggleton, & Walsh, 2008), as the effect of stimulation differs significantly if it is applied to an active, rested or fatigued cortical area, as modulated by inactivity, cognitive, and motor activity (Antal, Terney, Poreisz, & Paulus, 2007). Also, in a study by Andrews and colleagues (Andrews, Hoy, Enticott, Daskalakis, & Fitzgerald, 2011) performance in the n-back task was

increased by the execution of a digit span task during anodal tDCS stimulation of the DLPFC, compared to stimulation without the digit span task, and sham tDCS with this secondary task.

Genetic and gender differences may also modulate the effects of tES interventions. In a retrospective analysis done by our laboratory, Antal and colleagues have found differential effects of stimulation using several NIBS techniques when they compared individuals with different alleles of the Val66Met single nucleotide polymorphism (rs6265) of the brain-derived neurotrophic factor (BDNF) gene (Antal et al., 2010). Results also coming from our lab indicate that gender can be a modulating factor in the visual system (Chaieb, Antal, & Paulus, 2008), and also in the motor system (Kuo, Paulus, & Nitsche, 2006).

# Chapter 2

# The Effects of Transcranial Electrical Stimulation of the Dorsolateral Prefrontal Cortex on Categorization

Changes in cortical excitability accompany learning processes (Pascual-Leone, Grafman, & Hallett, 1994), and tES techniques enable us to modulate cortical excitability, thus they offer the possibility to interact with learning performance (Nitsche, Schauenburg, et al., 2003).

Due to its involvement in a large variety of cognitive processes, and because it is easily accessible to NIBS techniques, there is now a large, and still growing number of tES studies targeting the frontal cortical areas. Frontal tES intervention has

been shown to modulate working memory (Andrews et al., 2011; Fregni et al., 2005; Mölle, Marshall, Siebner, & Born, 2005), declarative memory (Javadi & Walsh, 2011; Marshall, Mölle, Hallschmid, & Born, 2004) and emotional processing (Peña Gómez, Vidal-Piñeiro, Clemente, Pascual-Leone, & Bartrés-Faz, 2011). Frontal areas have been targeted for intervention in a number of studies investigating the therapeutic potentials of tES intervention for neurological disorders. Encouraging results have been reported e.g. in the case of depression (Palm et al., 2009), tinnitus (Frank et al., 2011) and addiction disorders (Boggio, Liguori, et al., 2009; Boggio et al., 2008).

In 2004 the effects of PFC stimulation on a probabilistic classification task were measured in our laboratory (Kincses, Antal, Nitsche, Bártfai, & Paulus, 2004); it has been found that anodal stimulation during task execution can have a performance-enhancing effect in the weather prediction task (Knowlton, Squire, & Gluck, 1994).

In an experiment we have set out to investigate whether the changes of cortical excitability, as modulated by tDCS and tRNS stimulation over the DLPFC, can influence performance in a prototype learning task.

In the preceding decades prototype distortion tasks have been, and continue to be influential tools in the investigation of the cognitive processes underlying categorization performance in both humans and in animals. The stimuli presented during a prototype distortion task are generated by the modification of certain aspects of one or more prototype stimuli along a selected dimension or dimensions, with a given probability. The subject is exposed to these stimuli and, at some point during the experiment, has to make category judgments regarding these stimuli.

There are several variants of the prototype distortion task. Based on the number of categories, single category (*"A-not A"*, Posner & Keele, 1968; Reber, Stark, &

Squire, 1998a) and two categories (*"A or B"*, Seger et al., 2000; and *"A or B or none"*,Vogels, Sary, Dupont, & Orban, 2002) versions exist. Some variants require subjects to be informed about the existence of categories before the beginning of the training phase, while other versions prescribe mere exposure during category knowledge acquisition. In some versions, this learning is feedback-guided (Seger et al., 2000; Vogels et al., 2002). Also, there is variation in the visual stimuli used in the various versions of this paradigm: for example, dot patterns (Reber, Stark, & Squire, 1998b), color grids (Boettiger & D'Esposito, 2005; Seger et al., 2000), and cartoon animals (Zeithamova, Maddox, & Schnyer, 2008) have all been used in studies with human subjects.

## 2.1 Imaging and Categorization

Several imaging studies (mostly fMRI) using the prototype distortion task have been conducted to investigate the activation patterns associated with the execution of the task, mainly during the test phase. Testing the differences between the "A, not-A" and "A or B" variants of the task with cartoon figures as experimental stimuli, Zeithamova and colleagues (Zeithamova et al., 2008) found that the occipital and fusiform areas, inferior frontal regions, precentral gyrus and posterior hippocampus were shown to be active during both variants of the testing phase of the task, while frontal and parietal cortex and the parahippocampus was found to be active during the "A or B" variant, and posterior cortical region and the striatum was active during the "A, not-A" variant.

In the case of the "A, not A" variant, comparing classification of categorical and noncategorical stimuli during the testing phase, decreased activation in posterior

cortical regions and increased activity in both left and right lateral frontal cortex has been observed (Reber et al., 1998a). Comparing categorization and recognition using the "A, not-A" variant, Reber et al. (1998b) found decreased activation of the posterior occipital cortex associated with familiar stimuli, whereas familiar stimuli in the recognition phase increased activity in this area.

Studies comparing the activation patterns during categorization and a control task have also been conducted. An fMRI investigation by Reber, Wong, and Buxton (2002) tested categorization and recognition against a control involving a decision regarding the parity of the number of the dots constituting the stimulus pattern. This study revealed bilateral inferior prefrontal cortex and parietal cortex activation during categorization, and activation in the mediotemporal cortex, precuneus and visual areas during recognition. A PET study by Vogels et al. (2002) investigated the testing phase of a mixed, "A or B or none" variant of the prototype distortion task against a control condition where the position of the distorted pattern had to be judged. The orbitofrontal cortex and DLPFC has been shown to be active during categorization, while the activation of the intraparietal sulcus and the neostriatum has been observed to be active in both the categorization and the control condition.

At least two fMRI studies investigated the effects of information about the categories prior to the training phase. Using a version of the "A, not-A" prototype distortion task, Aizenstein et al. (2000) found increased activation of the V3 in subjects unaware of the existence of the categories, whereas increased activation in the frontal and mediotemporal regions as well as the V3 has been observed when the existence of the categories was revealed. Decreased activation in the parietal regions has been detected in both conditions. In a similar experiment, Reber, Gitelman, Parrish, and Mesulam (2003) reported decreased occipital activity for category members

in the case of uninformed participants, while increased activity in the hippocampus, right prefrontal cortex, precuneus and posterior cingulated cortex has been observed in the case of subjects who were informed about the category prior to the training phase.

Using fMRI, Seger et al. (2000) investigated the brain activity associated with categorization performance during the acquisition phase. The authors have used low distortion derivatives of two prototypes in a feedback-aided learning paradigm, and have found an increased activity in the right DLPFC and in the right inferior parietal areas in all subjects. The left DLPFC involvement corresponded selectively with high categorization performance only. According to the authors this phenomenon could indicate that the right hemispheric activation in this case is dissociable from category acquisition *per se*, and could be related to processes engaged in visuo-spatial analysis of the stimulus features.

The role of the DLPFC during the acquisition phase is difficult to assess using imaging techniques, especially when training requires mere exposure, and no feedback is involved. Modulation of the cortical excitability of this area using tES techniques offers an opportunity to gain knowledge about the role it plays in the acquisition of category knowledge.

## 2.2 The Impact of tES Intervention on Categorization in the Prototype Distortion Task

**In our experiment** (Ambrus, Zimmer, et al., 2011) we have chosen an "A, not-A" version of the prototype distortion task. In a parallel group design study, we have

stimulated the right DLPFC with anodal and cathodal tDCS and tRNS, and the left DLPFC with anodal tDCS stimulation (all simulation conditions: 1 mA / 35 $cm^2$, Cz-DLPFC montage). Sham stimulation was used as a control. The training phase of the task started approximately 8 minutes after the stimulation onset. During this training phase, subjects saw 20 low and 20 high distortion versions of a prototype pattern consisting of 9 dots. The prototype itself was not shown during training. Next, the subjects were told that the patterns previously seen belonged to a category, and that in the next phase of the task they will be presented with similar images, and they were required to decide whether the pattern presented does or does not belong to this previously established category. During the test phase, in a randomized order, 20 high and 20 low distortion patterns, and 40 unrelated patterns have been presented, and the prototype itself was also shown four times.

Our results have shown that, when compared to the sham group, both anodal tDCS and the tRNS group demonstrated decreased performance in identifying prototype and low-distortion patterns as category members, while the difference between the categorization performance of prototype items in the cathodal and the sham groups did not reach the level of statistical significance. Furthermore, the sham group exhibited the characterisic prototype effect, while it was missing in all active stimulation groups.

In this study we have demonstrated that the application of tES stimulation to the DLPFC before and during the "A, not-A" variant of the prototype distortion task can modify task performance. The findings, that the prototype effect disappeared in all verum stimulation conditions, and that anodal stimulation did not cause an enhancement in performance, were surprising, although not unprecedented, as there have already been studies that have shown results on task performance that diverged

from the conventional 'anodal enhancement, cathodal decrease' pattern (e.g. Antal, Nitsche, Kruse, et al., 2004)

An interpretation of this result has to take into account the limitations and confounding factors this experiment suffers from. As no direct evidence exists with regard to the time course of the tES aftereffects on the DLPFC, we had to refer to studies conducted on the motor system, where it has been shown that stimulation for only 2 minutes (the time required for the acquisition phase) is probably not enough to cause observable changes. We have thus timed the training phase to begin at the end of a 10 minute long stimulation session. This arrangement, however, causes uncertainty about the effective "time window", as it cannot be deduced whether the observed effects are due to the pre-task stimulation, the stimulation during the task, or depend on both. Furthermore, the "acquisition via mere exposure" study design did not allow for the direct assessment of acquisition performance, so it leaves open the question of the affected memory process/processes, and it adds further ambiguity to the time-course issue.

The theoretical implications of these confounding factors are dealt with in more detail in the Discussion section of the original article (Ambrus, Zimmer, et al., 2011, pp. 1978–1979). Finally, when interventions acting in opposite directions (in this case, anodal tDCS and tRNS: increase, cathodal tDCS: decrease in cortical excitability) cause the same behavioral outcome (the disappearance of the prototype effect), the balance of the system under study and the circumstances of the application of the intervention must be independently scrutinized.

It is conceivable that the level of excitability of the targeted region in healthy adults is optimal for this type of task, and any increase or decrease in the level

of excitability is going to be detrimental to performance. Furthermore, it can also be argued that the sham technique we have used has failed to control for that effect, although the fact that the performance in the categorization of prototypes was also severed in the tRNS condition, which has been shown to have a substantially higher threshold for cutaneous perception (50% response thresholds: tRNS: 1300 $\mu A$ vs. tDCS: 400 $\mu A$; Ambrus, Paulus, & Antal, 2010), makes this prospect unlikely. Nevertheless, we have decided to further investigate the efficacy of blinding and the cutaneous perception during stimulation, an investigation that forms the second part of this thesis.

# Chapter 3

# Issues of Procedural Discomfort and Blinding in tES research

In order to assess whether a new medical device has a significant clinical effect, randomized, controlled trials are necessary. One of the major issues regarding controlled studies is the availability of a "perfect placebo control condition" (Edelmuth et al., 2010).

As part of an ongoing project to assess the procedural sensations and the possible blinding potentials of different methods, we have also conducted a number of experiments (Ambrus et al., in press; Ambrus, Antal, & Paulus, 2011; Ambrus, Paulus, & Antal, 2010).

## 3.1 Cutaneous Sensations and Procedural Discomfort

Nitsche and colleagues in 2008 have collected the perceptual phenomena associated with tDCS in the literature published up to that date (Nitsche et al., 2008, pp. 208–216), and the first systematic review on the adverse effects of tDCS stimulation was published by Brunoni et al. (2011). By far the most widely reported phenomenon associated with both active and sham tES stimulation is the itching or tingling sensation under the electrode. The sensation of tingling is most commonly reported in studies that have utilized smaller electrodes (Brunoni et al., 2011); this is most probably due to the higher current density. Other, less frequently reported phenomena associated with the stimulation are burning sensations, headache, redness of skin, nausea and light flashes at the beginning and the end of the stimulation (Brunoni et al., 2011; Poreisz, Boros, Antal, & Paulus, 2007). The occurance and strength also seems to depend on electrode placement: an M1 – contralateral orbit montage may evoke more pronounced stimulation-induced cutaneous sensations than an Oz – Cz montage, probably due to the relatively higher sensitivity of the forehead compared to that of the piliferous vertex (Poreisz et al., 2007).

To assess the cutaneous perception characteristics of electrical stimulation methods at different current intensities, and to compare the blinding potentials of tDCS and tRNS, we have designed an experiment (Ambrus et al., 2010) in which we have asked 30 subjects, naïve to tES methods, experienced subjects, and investigators using tES in their research, to report the presence of cutaneous sensations when different intensities (200 – 2000 $\mu$A, with 100 $\mu$A increments, in a randomized order) of anodal, cathodal, tRNS stimulation, or no stimulation. The current was applied in an M1 – contralateral orbit montage in a 8 seconds ramp up, 15 seconds stimulation, 8 seconds ramp down manner. For the experimental setup, see Figure 1.

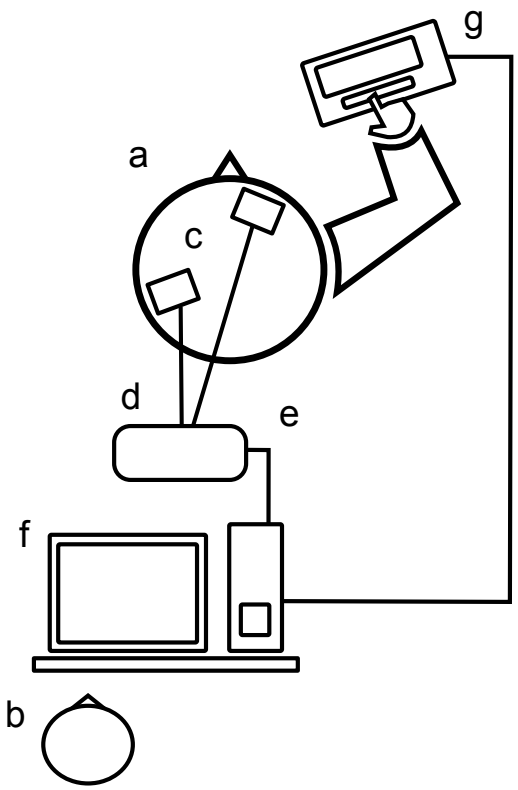


Figure 3.1: **Experimental setup used in the cutaneous perception characteristics experiments described in this chapter.** The subject (a) is sitting in a reclining chair. The investigator (b) is sitting behind the subject. Rubber electrodes in sponges (c) are positioned over the right M1 and the contralateral orbit. Stimulation is delivered by a battery driven constant current stimulator (d) which is triggered via parallel port connection (e) by a PC (f) located also behind the subject. In each trial the subject is instructed to start the stimulation and interrupt it in case they perceived the skin sensation by pressing the space bar on a standard PC keyboard (g).

Our results have shown that investigators had a lower false alarm rate in response to non-stimulation trials. Regarding stimulation intensities, more than 50% of the participants reported sensations at 400 $\mu$A in the case of both anodal and cathodal

tDCS, while this 50% threshold was at 1200 $\mu$A in the case of tRNS. Our data show that, when applied using the same current intensities, tRNS is not as noticeable as tDCS is, which suggests that when cutaneous perception is an issue, tRNS might be easier to blind (see Table 1/A).

## 3.2 Blinding

Blinding requires the successful application of a control intervention or interventions (usually a placebo, but can also be an additional active control) that are indistinguishable from the verum treatment. The procedure generally involves concealing the nature of the intervention applied (but see Kaptchuk et al., 2010)

Generally, medical devices represent a challenge to placebo intervention and blinding. It has been proposed that medical devices can elicit a higher placebo response (Kaptchuk, Goldman, Stone, & Stason, 2000; Kaptchuk et al., 2006), and when compared to pharmacological agents, they are more difficult to blind – not just the patients/participants, but the health care providers administering the intervention or those assessing the outcomes of treatment effects (Boutron, Tubach, Giraudeau, & Ravaud, 2004).

Considering NIBS device based interventions, tES methods are generally considered to be easier to blind than TMS-based methods (Priori, Hallett, & Rothwell, 2009). It is a non-invasive intervention, the type of stimulation cannot be judged by an outside observer, it is easily applicable, and it is widely described in the literature as mostly painless and free from side-effects.

**A**

| ◀Stim. | ◀Group | ◀Phase | Stimulation intensity (μA) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 200 | 300 | 400 | 500 | 600 | 700 | 800 | 900 | 1000 | 1100 | 1200 | 1300 | 1400 | 1500 | 1600 | 1700 | 1800 | 1900 | 2000 |
| tRNS | N | A | 0 | 30 | 20 | 30 | 30 | 30 | 30 | 30 | 40 | 60 | 60 | 50 | 60 | 40 | 70 | 60 | 60 | 80 | 60 |
| | | R | 0 | 10 | 0 | 10 | 0 | 20 | 0 | 0 | 20 | 0 | 0 | 20 | 30 | 10 | 20 | 20 | 20 | 40 | 30 |
| | E | A | 20 | 30 | 30 | 30 | 20 | 20 | 30 | 50 | 50 | 20 | 20 | 70 | 40 | 70 | 50 | 60 | 60 | 70 | 60 |
| | | R | 10 | 0 | 0 | 30 | 0 | 0 | 10 | 0 | 10 | 0 | 0 | 10 | 20 | 20 | 40 | 40 | 30 | 40 | 40 |
| | I | A | 0 | 10 | 10 | 20 | 10 | 20 | 20 | 20 | 40 | 40 | 40 | 50 | 50 | 70 | 80 | 80 | 70 | 90 | 100 |
| | | R | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 10 | 10 | 20 | 20 | 40 | 20 | 60 | 50 |
| | All | A | 6.7 | 23.3 | 20 | 26.7 | 20 | 23.3 | 26.7 | 33.3 | 43.3 | 40 | 40 | 56.7 | 50 | 60 | 66.7 | 66.7 | 63.3 | 80 | 73.3 |
| | | R | 3.3 | 3.3 | 0 | 13.3 | 0 | 6.7 | 6.7 | 0 | 10 | 0 | 0 | 13.3 | 20 | 16.7 | 26.7 | 33.3 | 23.3 | 46.7 | 40 |
| Anodal tDCS | N | A | 30 | 40 | 50 | 80 | 70 | 90 | 90 | 90 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | R | 10 | 10 | 30 | 30 | 60 | 40 | 60 | 50 | 60 | 60 | 60 | 70 | 70 | 90 | 80 | 100 | 90 | 100 | 90 |
| | E | A | 30 | 60 | 90 | 100 | 90 | 100 | 100 | 90 | 100 | 100 | 100 | 100 | 90 | 100 | 100 | 100 | 90 | 100 | 100 |
| | | R | 20 | 10 | 10 | 50 | 60 | 80 | 70 | 50 | 90 | 100 | 100 | 80 | 80 | 90 | 100 | 100 | 90 | 100 | 100 |
| | I | A | 10 | 20 | 30 | 50 | 80 | 100 | 100 | 90 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | R | 0 | 0 | 20 | 20 | 40 | 70 | 50 | 70 | 60 | 80 | 80 | 70 | 80 | 90 | 80 | 90 | 90 | 80 | 100 |
| | All | A | 23.3 | 40 | 56.7 | 76.7 | 80 | 96.7 | 96.7 | 90 | 100 | 100 | 100 | 100 | 96.7 | 100 | 100 | 100 | 96.7 | 100 | 100 |
| | | R | 10 | 6.7 | 20 | 33.3 | 53.3 | 63.3 | 60 | 56.7 | 70 | 80 | 80 | 73.3 | 76.7 | 90 | 86.7 | 96.7 | 90 | 93.3 | 96.7 |
| Cathodal tDCS | N | A | 30 | 50 | 50 | 70 | 70 | 70 | 80 | 90 | 80 | 90 | 90 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | R | 10 | 20 | 10 | 40 | 30 | 40 | 40 | 60 | 50 | 70 | 70 | 60 | 50 | 80 | 70 | 80 | 50 | 80 | 80 |
| | E | A | 30 | 40 | 70 | 70 | 80 | 80 | 90 | 80 | 90 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 100 | 100 | 100 |
| | | R | 10 | 10 | 10 | 30 | 20 | 70 | 60 | 60 | 70 | 80 | 80 | 80 | 80 | 70 | 80 | 90 | 80 | 80 | 80 |
| | I | A | 20 | 40 | 50 | 80 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | R | 0 | 0 | 20 | 30 | 50 | 60 | 60 | 80 | 80 | 70 | 70 | 90 | 60 | 90 | 90 | 100 | 100 | 100 | 90 |
| | All | A | 26.7 | 43.3 | 56.7 | 73.3 | 83.3 | 83.3 | 90 | 90 | 90 | 96.67 | 96.7 | 100 | 96.7 | 100 | 100 | 96.7 | 100 | 100 | 100 |
| | | R | 6.7 | 10 | 13.3 | 33.3 | 33.3 | 56.7 | 53.3 | 66.7 | 66.7 | 73.33 | 73.3 | 76.7 | 63.3 | 80 | 80 | 90 | 76.7 | 86.7 | 83.3 |

**B**

| ◀Stim. | ◀Group | ◀Phase | Stimulation intensity (μA) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 200 | 300 | 400 | 500 | 600 | 700 | 800 | 900 | 1000 | 1100 | 1200 | 1300 | 1400 | 1500 | 1600 | 1700 | 1800 | 1900 | 2000 |
| Rectangular | Rn | A | 16.6 | 8.33 | 16.6 | 33.3 | 16.6 | 8.33 | 25 | 8.33 | 41.6 | 41.7 | 41.6 | 41.6 | 41.6 | 50 | 58.3 | 50 | 50 | 66.6 | 66.6 |
| | | R | 0 | 0 | 0 | 8.33 | 0 | 0 | 16.6 | 0 | 0 | 8.33 | 8.33 | 16.6 | 8.33 | 16.6 | 8.33 | 8.33 | 16.6 | 33.3 | 41.6 |
| | An | A | 25 | 58.3 | 58.3 | 75 | 75 | 100 | 91.6 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | R | 16.6 | 8.33 | 33.3 | 41.6 | 50 | 75 | 75 | 83.3 | 75 | 66.7 | 75 | 75 | 91.6 | 91.6 | 91.6 | 100 | 91.6 | 91.6 | 100 |
| | | A | 18.1 | 54.5 | 54.5 | 90.9 | 81.8 | 81.8 | 90.9 | 81.8 | 90.9 | 100 | 100 | 90.9 | 90.9 | 100 | 100 | 90.9 | 100 | 100 | 90.9 |
| | | R | 0 | 9.1 | 27.2 | 45.4 | 36.3 | 54.5 | 72.7 | 81.8 | 90.9 | 83.3 | 81.8 | 90.9 | 63.6 | 100 | 81.8 | 90.9 | 100 | 90.9 | 81.8 |
| Round | Rn | A | 8.3 | 16.6 | 8.3 | 8.3 | 25 | 41.6 | 16.6 | 25 | 33.3 | 50 | 41.6 | 33.3 | 50 | 41.6 | 66.6 | 50 | 58.3 | 66.6 | 58.3 |
| | | R | 0 | 0 | 8.3 | 0 | 8.3 | 8.3 | 0 | 0 | 0 | 16.7 | 16.6 | 16.6 | 33.3 | 25 | 25 | 33.3 | 25 | 33.3 | 41.6 |
| | An | A | 41.6 | 66.6 | 66.6 | 66.6 | 83.3 | 83.3 | 91.6 | 91.6 | 100 | 91.7 | 91.6 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | R | 0 | 0 | 41.6 | 33.3 | 58.3 | 66.6 | 66.6 | 75 | 75 | 91.7 | 91.6 | 83.3 | 83.3 | 91.6 | 83.3 | 91.6 | 91.6 | 91.6 | 91.6 |
| | Cat | A | 41.6 | 50 | 50 | 58.3 | 66.6 | 75 | 83.3 | 75 | 83.3 | 100 | 100 | 83.3 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | R | 0 | 8.3 | 16.6 | 25 | 58.3 | 50 | 66.6 | 58.3 | 58.3 | 91.7 | 91.6 | 75 | 75 | 75 | 83.3 | 83.3 | 83.3 | 100 | 83.3 |

Figure 3.2: **Percentage of subject responding to different stimulation intensities** in (A) different stimulation (Ambrus, Paulus, & Antal, 2010), and (B) electrode geometry conditions (Ambrus, Antal, & Paulus, 2011). 50%+ responses are marked. R: during ramp-up; A: during all phases. N: naïve, E: experienced, I: investigator participants. An: anodal, Cat: Cathodal tDCS; Rn: tRNS. Reproduced with permission.

There are two basic approaches to blinding tES interventions: (1) attempting to abolish any sensations associated with the stimulation in the case of both verum and sham interventions, and, (2) applying a sham intervention that attempts to mimic the sensations associated with verum stimulation.

**Approaches based on reducing or abolishing the sensations associated with the stimulation**

When sponges soaked in NaCl solution are applied as electrode wrappers, decreasing ionic concentration within the solution may reduce the procedural discomfort (Dundas, Thickbroom, & Mastaglia, 2007; Minhas, Datta, & Bikson, 2011b); on the other hand, de-ionized solutions are suggested to contribute to the appearance of skin lesions via increasing the impedance at the site of the scalp-electrode interface (Palm et al., 2008). For this reason, decreasing sponge salinity is not considered to be a viable option at this point (McFadden, Borckardt, George, & Beam, 2011).

The application of topical anesthetics has been suggested to reduce and/or abolish the cutaneous sensations associated with tES (Nitsche et al., 2008). In 2011, McFadden and colleagues (McFadden et al., 2011) devised a study that compared the effects of topically applied Eutectic mixture of local anesthetics (EMLA) to a sham solution on procedural discomfort associated with tDCS (2 mA, 5 minutes). They have found that the application of ELMA had significantly reduced the cutaneous sensations when compared to the placebo cream condition.

Some alterations in the electrode montage have the potential of reducing the sensations normally associated with stimulation. Our second experiment compared the

cutaneous perception associated with tDCS and tRNS stimulation using circular and rectangular electrodes (Ambrus, Antal, & Paulus, 2011). Based on the assumption that when using a circle-shaped sponge electrode wrapper instead of a conventional rectangular wrapper, but with the same surface area, thereby eliminating the corners and shortening the perimeter of the stimulation electrodes, we reduce the accumulation of the current along the edges (see Figure 2), thereby reducing the associated skin sensations. We recruited 12 participants who were tested in a similar setting as in our first study. They received anodal, cathodal and tRNS stimulation with different intensities ranging from 200 – 2000 $\mu$A using rectangular and round electrode wrappers in a randomized, repeated measures design. Unfortunately, our results have shown no substantial difference between the two electrode configurations (see Table 1/B), meaning, that the round electrode geometry, at least when using sponge electrode pads, does not reduce the procedural sensations compared to the conventional rectangular design. In an effort to match our observations with computer simulations, researchers in the laboratory of Professor Bikson at the City College of New York confirmed this finding using finite element modeling-aided calculations (Minhas et al., 2011a).

**Methods based on the sham procedure mimicking the cutaneous sensations of the verum stimulation**

The *Fade-in – Short duration stimulation – Fade-out approach* (FiSsFo), where the current intensity is slowly ramped down after a few seconds of stimulation is based on mimicking the assumed initial presence and the consecutive disappearance of the cutaneous sensations associated with the verum stimulation. This method has been

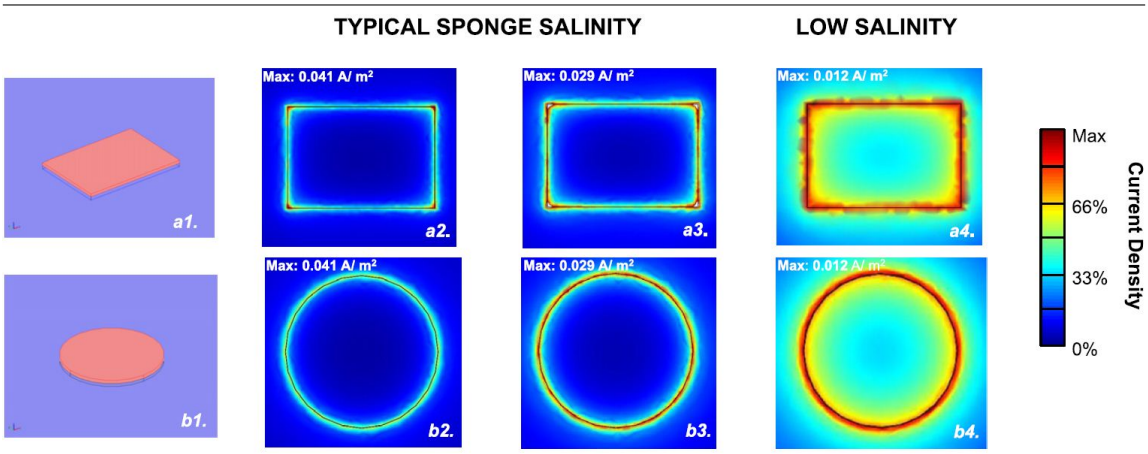


Figure 3.3: **Current density profiles of circular and rectangular electrode configurations.** Based on parameters by Ambrus et al. (2011), the models have been created by Minhas, Datta, & Bikson (2011). Used with permission.

shown to be an adequate method of blinding (Gandiga, Hummel, & Cohen, 2006). The FiSsFo approach and its underlying assumptions are further discussed in this thesis in more detail.

Active "sham stimulation" targeting cortical areas outside the region of interest represents another alternative. For example, in an experiment assessing the effects of DLPFC tDCS on working memory, Fregni and colleagues also applied tDCS to the M1 as an active control (Fregni et al., 2005). Similarly, Javadi and Walsh applied tDCS stimulation to the M1 as a control for DLPFC stimulation in an experiment investigating the influence of tDCS on declarative memory (Javadi & Walsh, 2011). Both studies reported differential performance according to electrode placement, supporting this method as a viable control alternative.

However, multiple issues have to be considered regarding this approach. First, tES methods have a relative low focality, and the behavior of the current on the

path between the two electrodes is still under investigation (Bikson et al., 2010b). Second, it is conceivable that task-relevant areas are stimulated via proxy connections (Boros, Poreisz, Münchau, Paulus, & Nitsche, 2008). For example, recent functional imaging experiments conducted by Polanía and colleagues suggest that functional coupling between the stimulated M1 and other cortical areas, as well as subcortical regions, such as the thalamus and the striatum, can be modulated (Polanía, Paulus, & Nitsche, 2011). Future behavioral, computer modeling and imaging studies might help us to refine this technique.

## 3.3 The Time-course of the Sensations Associated with Stimulation

The Fade-in – Short duration stimulation – Fade-out approach (FiSsFo) is the most widely used sham stimulation method in current studies.

The first studies using tDCS compared the differential aftereffects of anodal and cathodal stimulation, and had no direct sham control conditions, although one of the first seminal papers (Nitsche & Paulus, 2000) established that, tDCS, when applied to the motor cortex, with intensities below 0.6 mA or stimulation durations less than 3 minutes, might be considered inert.

Other initial studies reported using a non-stimulation condition as a control (e.g. Marshall et al., 2004; Nitsche, Schauenburg, et al., 2003), where the electrodes have been fixed to the head, but no current was administered during the session.

The first studies that explicitly state using initial short stimulation durations ($<$ 5 s) and low intensities ($<$ 500 mA) as sham were by Siebner and colleagues (Siebner et al., 2004) and Rogalewski and colleagues (Rogalewski et al., 2004), but no fade-in or fade-out has been reported either in the sham nor in the verum condition, though Nitsche and colleagues previously established the practice of ramping up and down the stimulation at the beginning and at the end of the stimulation to avoid the short light flashes associated with the current being turned on and off abruptly (Nitsche, Liebetanz, et al., 2003).

The first study using the FiSsFo approach in its currently established form was reported by Hummel and colleagues (Hummel et al., 2005b), who described using an intensity of 1 mA in both sham and verum sessions, with a stimulation duration of ca. 30 seconds in the former case, and a ramping of the current flow in both conditions. This approach has remained the standard practice ever since. A study by Gandiga and colleagues (Gandiga et al., 2006) has examined its effectiveness in a double-blind, sham controlled setting, and found that such designs are indeed feasible.

The rationale for adopting the FiSsFo method was to mimic the time course of the appearance and disappearance of the cutaneous sensations associated with the verum stimulation. It is widely assumed in the literature that the phenomena associated with tDCS are mostly restricted only to the initial phase of the intervention:

> "At the beginning of stimulation, most subjects will perceive a slight itching sensation, which then fades in most cases." (Nitsche et al., 2008)

> "In tDCS there are minimal or no scalp sensations with stimulation (and subjects tend to get habituated to it after a few seconds of stimulation)." (Fregni & Pascual-Leone, 2007)

> "Although, previous studies have reported that perceived sensations on the skin usually fade out within the first 30 s of stimulation [...]" (Dundas et al., 2007)

> [tDCS - quality of sensations] "Only in the initial few seconds of application, then fades" (Gandiga et al., 2006)

> "This protocol has been tested in about 500 subjects in our laboratory so far without any side-effects, apart from a slight tingling sensation under the electrode during the first seconds of stimulation [...]" (Nitsche, Liebetanz, et al., 2003)

However, systematic, quantitative analyses investigating this claim have not so far been published. In contrast, there have been sporadic reports describing persistent sensations outlasting the initial phase of the verum stimulation (Dundas et al., 2007) and the presence of similar phenomena after the cessation of sham stimulation (Gandiga et al., 2006).

In the light of these reports it seemed conceivable that the FiSsFo approach is not an effective method of blinding because it mimics the cessation of cutaneous sensations presumably associated with verum stimulation, but because in both verum and FiSsFo cases, these sensations are (at least, subjectively) present for approximately equal time intervals.

In a third study (Ambrus et al., in press), we have set out to investigate the time-course of the cutaneous sensations associated with verum and sham (FiSsFo)

tDCS in a double-blind experiment, recruiting naïve, experienced and investigator participants. In this study we have applied either 10 minutes of verum (anodal and cathodal) stimulation or 30 seconds of sham stimulation (both with 10 seconds of fade in and out) in a repeated measures design. We have asked the subjects to report the strength of the perceived stimulation and the site of the sensation at specific intervals (1.5 minutes apart) during the session. We have found that, generally, the cutaneous sensations did not disappear completely either in the verum or in the sham condition. We also asked the participants to tell us if they thought they had received sham or verum stimulation. Here, we have found that naïve and experienced subjects had a strong bias towards reporting "real stimulation" in verum and also sham sessions, whereas investigators in most cases could correctly distinguish between verum and sham stimulation.

Our study demonstrated that when taking naïve and experienced subjects into account, the FiSsFo sham stimulation mimicked the perceived strength of the phenomena during the whole course of the stimulation. We have also found that although the cutaneous perception associated with both the verum and the sham stimulation procedure did show a reduction with time, but contrary to the claims previously reported, it did not fade away completely.

The distinction between the two possibilities – sham mimicking the disappearance of cutaneous perception, or sham eliciting approximately the same level and quality of perception – may not be trivial.

Cutaneous perception of the stimulation, depending on the perceived discomfort, may contribute to increased levels of arousal and stress. The increase in arousal may lead to diversion of attention and a reduction in the levels of concentration and

task performance; or conversely, it can lead to a greater efficiency, depending on the type of the task. The fact that tDCS has already been reported to be able to modulate attention (Stone & Tesche, 2009) and stress (Antal, Chaieb, & Saiote, in preparation), may further complicate the issue.

As described by Yerkes and Dodson (1908), the relationship between arousal and performance is not a linear one. For every task there exists an optimal level of arousal, above and beyond which performance is going to decrease. Performance increases with arousal up to that point, and any further increase in the level of arousal decreases efficiency. This optimal level of arousal changes with the type of task in question; it is lower in tasks that burden attention and cognitive resources, while it is higher in tasks that are less demanding.

Possible correlates of the interaction between arousal/stress levels and task performance in the prefrontal cortex (PFC) are extensively discussed in the review article by Diamond and colleagues (Diamond, Campbell, Park, Halonen, & Zoladz, 2007). They propose that if the task performance relies on the PFC, such as working memory, executive processing, divided attention and decision making, a higher level of arousal is more likely to have a detrimental effect on performance, while task less reliant on PFC-mediated cognitive processes benefit from increased arousal (see Ariely, Gneezy, Loewenstein, & Mazar, 2009). They found evidence that intermediate levels of dopamine and norepinephrine in the PFC are required for the efficient execution of complex tasks.

The question remains: does tES stimulation, via the associated cutaneous perception and discomfort, cause an increase in the levels of arousal? If the answer is

yes, does this increase manifest itself in task performance? Is there a measurable difference between sham and verum stimulation in this regard?

The closest to assessing arousal levels during stimulation that can be found in the literature are pre- and post-stimulation reports of attention and fatigue as gauged before, during and after the stimulation. Gandiga and colleagues found that ratings of attention have not shown to be significantly altered by stimulation (Gandiga et al., 2006), while Poreisz and colleagues have found a significant difference between during and after stimulation regarding self-reported occurrence of fatigue (as assessed by a post-stimulation questionnaire, Poreisz et al., 2007).

An argument can be made that the assessment of changes in the level of attention based on post facto questionnaires of self-reported sleepiness, fatigue, or concentration is not sensitive enough to show any differential effect of perceived discomfort during sham and verum stimulation. More sensitive behavioral measurements, such as vigilance tests with varied levels of cognitive demand, combined with assessments of cutaneous perception during stimulation, could be conducted to clarify this issue.

Also, it could be worthwhile to conduct such time-course experiments on cutaneous perception with the stimulation electrodes fixed to the head, but without any stimulation (no-stimulation condition) to assess what proportion of the phenomena that can be attributed to circumstantial properties of the setup, such as the wet sponge surface touching the skin, or the discomfort arising from the pressure from the rubber bands. In this thesis I argue, in agreement with Brunoni et al. (2011), that the reporting of adverse effects and side-effects of stimulation administered needs to be improved, and more studies on the issue of blinding need to be conducted.

# Chapter 4

# Summary

The cognitive part of the thesis at hand presents evidence that applying tES stimulation to the DLPFC can influence categorization performance in the "A, not A" version of the prototype distortion test; the results show a disappearance of the prototype effect when this area is stimulated. This study also establishes that in the case of the "A, not A" task, the effects of tRNS stimulation are similar to those of anodal tDCS, further supporting tRNS as a promising method in research and for clinical applications. Future studies are needed to shed light on the effects of various stimulation parameters, such as the effects of stimulation timing. Functional imaging techniques should investigate the activity correlates of the influence of stimulation in categorization tasks. This, and further studies may not only help us investigate the role of targeted brain regions involved in categorization, but also to fine-tune the parameters of tES interventions for research and clinical purposes.

The methodological part of the thesis delineates studies dealing with the cutaneous sensations associated with tES methods.

tRNS and anodal tDCS have been described to have similar effects. We have quantified the cutaneous perception characteristics of both tDCS and tRNS for short duration stimulations at different intensities, and have found that the application of tRNS involves substantially less procedural discomfort, therefore when cutaneous perception is an issue (e.g. blinding) tRNS may be better suited for certain purposes. The physiological effectiveness of tRNS, however, needs further confirmation.

We have also tested the cutaneous perception characteristics of tDCS and tRNS using round and rectangular electrodes to test whether the application of round electrodes reduces the procedural sensations associated with the stimulation. We have found that the round electrodes did not have an advantage in that regard.

The time-course of the procedural discomfort associated with tDCS and the FiSsFo sham method has been investigated in our third methodological study. We have found that investigators could more easily distinguish between verum and sham stimulation than naïve and experienced subjects; which can have far-reaching consequences when investigators are recruited e.g. as test subjects in pilot studies. Furthermore, we have found that the cutaneous perception does not disappear in the first phase of the stimulation as previously reported, but has never been quantitatively assessed. Nevertheless, when taking only naïve and experienced participants into account, no differences in the levels of perceived stimulation strength could be observed, thus the FiSsFo method may be considered a reliable approach to blinding in tDCS research, at least when using 1 mA stimulation intensity. The changes procedural discomfort can cause in the levels of arousal and stress, and its effect on task performance, has to be the subject of future investigations.

In conclusion, tES methods are promising techniques in both research and within a clinical setting. Future investigations will address both stimulation parameters for

optimizing efficacy, and technical issues for the reduction of procedural discomfort and for more applicable placebo stimulation within controlled studies.

# Original Articles In the Thesis

## Original Articles in the Cognitive Section of the Thesis

**Ambrus, G. G.**, Zimmer, M., Kincses, Z. T., Harza, I., Kovács, G., Paulus, W., Antal, A. (2011). The enhancement of cortical excitability over the DLPFC before and during training impairs categorization in the prototype distortion task. *Neuropsychologia, 49(7), 1974-1980.*

The study design was devised by Prof. Paulus, Prof. Antal and Dr. Gyula Kovács and Tamás Kincses. The experimental program was created by Dr. Tamás Kincses. The measurements have been conducted by Géza Gergely Ambrus, Irén Harza and Márta Zimmer. The article was written by Géza Gergely Ambrus, Prof. Antal and Prof. Paulus with contributions from all authors.

Neuropsychologia 49 (2011) 1974–1980

Contents lists available at ScienceDirect

Neuropsychologia

journal homepage: www.elsevier.com/locate/neuropsychologia

# The enhancement of cortical excitability over the DLPFC before and during training impairs categorization in the prototype distortion task

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ARTICLE INFO

*Article history:*
Received 22 October 2010
Received in revised form 16 March 2011
Accepted 18 March 2011
Available online 31 March 2011

*Keywords:*
Transcranial direct current stimulation
Transcranial random noise stimulation
Prototype distortion task
Visual categorization
Dorsolateral prefrontal cortex

ABSTRACT

The present study investigated the effects of transcranial weak electrical stimulation techniques applied to the right and left dorsolateral prefrontal cortex (DLPFC) on categorization learning measured using a variant of the prototype distortion task.

During the training phase of this task subjects saw low- and high distortions of a prototype dot-pattern. 60 participants received 10 min of either anodal or cathodal transcranial direct current (tDCS), transcranial random noise (tRNS) or sham stimulation before and during the training. We have assessed the effects of the intervention during a test phase, where the subjects had to decide whether the consecutive high- and low-distortion versions of the prototype or random patterns that were presented belonged to the category established in the training phase.

Our results show that the categorization of prototypes is significantly impaired by the application of anodal tDCS and tRNS to the DLPFC. The prototype-effect, observable in the case of the sham stimulation group, was severed in all active stimulation conditions.



## 1. Introduction

Categorization plays an important role in guiding behavior and thus the survival of animals. Efficient categorization requires the economical allocation of cognitive resources and the adequate mapping of categories along relevant features of the perceived environment. Consensus leans toward a multi-system approach of human category learning, with a system for rule-based acquisition and another one involving implicit learning (Ashby & Waldron, 1999; Poldrack & Foerde, 2008), although attempts are being made to construct single-system models to account for the experimental results (Zaki, 2004).

One of the most widely applied methods used to study categorization in the human is the prototype distortion paradigm (Posner & Keele, 1968). The procedure usually consists of a training and a testing phase (see Fig. 1). In the case of the "A, not-A" version the subject is exposed to various distorted stimuli derived from a single prototype during the training phase. In the testing phase the participant is shown stimuli similar in appearance, and has to decide whether a presented stimuli belongs to the category established in the training phase. In the case of the "A vs. non-A" variant stimuli are generated using two distinct prototypes, and participants have to place the presented patterns in one of these two categories. Subjects can be unaware of the existence of the category/categories during the training phase, or they can be guided through feedback-aided learning. Several versions of this paradigm exist; the stimuli of the task in most cases consist of dot patterns, color grids or cartoon animals. It has been consequently shown that healthy participants are able to assign prototypes and derivatives of the prototype pattern to the category in the testing phase of the task. Despite the fact that the prototype patterns are not presented during the training phase, the rate of correct categorization decisions is consequently higher for these stimuli than of the distorted versions of the prototypes. This phenomenon is known as the prototype effect, and, in addition to humans it has been reported in experiments involving birds (Jitsumori, 1996) and non-human primates (Smith, Redford, & Haas, 2008) as well (for reviews, see Jitsumori, 2006; Jitsumori & Delius, 2001), although the issue is still being debated (Vauclair, 2002).

Data from amnestic patients with impaired declarative memory suggest a retained implicit ability to acquire category-level knowledge via exposure to multiple instances of the given category in the prototype distortion task (Knowlton & Squire, 1993; Squire & Knowlton, 1995). Alzheimer's patients with affected prefrontal

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doi:10.1016/j.neuropsychologia.2011.03.026

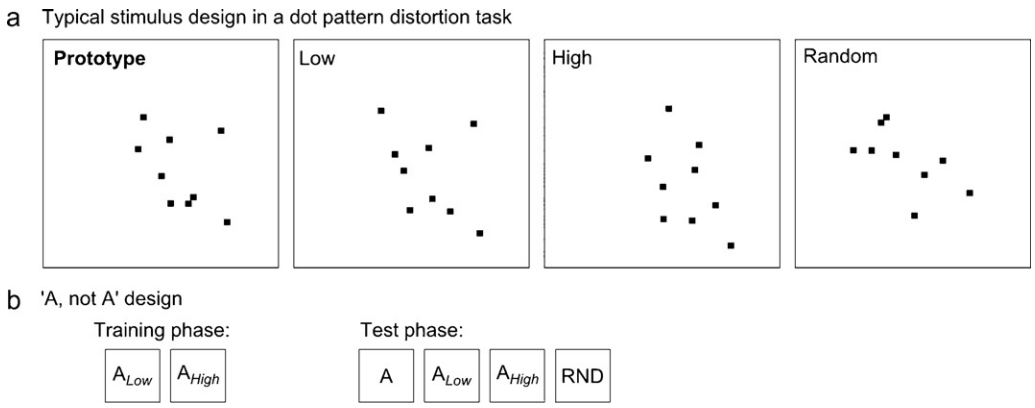


**Fig. 1.** Experimental procedure of the prototype distortion task. (a) A typical stimuli design of the dot pattern variant: a pattern designated as prototype, low and high distortion patterns derived from the prototype and a random pattern. (b) Basic example for the experimental workflow in the "A, not A" (see e.g. Reber, Stark, & Squire, 1998a, 1998b) variant.

regions show more impairment in rule-based categorization than in similarity-based classification (Smith & Grossman, 2008).

A number of imaging studies have been conducted using the prototype distortion task in order to identify the activation patterns in the brain during the execution of the task. Almost all of these studies so far concentrated on performance in the testing phase rather than acquisition during training. Table 1 summarizes the parameters and findings of these investigations. Although there was a considerable variation among the experimental paradigms used in these studies, frontal and occipital areas of the cortex were among the sites most frequently identified. Learning in the training phase is possibly more difficult to operationalize. One study by Seger et al. (2000) investigated category learning in the acquisition phase; here right frontal and parietal activation patterns during the learning phase of a categorization learning task were observable in subjects both with high and with low task performances. The authors argue that this right-hemispheric activation is dissociable from category acquisition *per se*, and is related to processes engaged in visuo-spatial analysis of the stimulus features. However, interpreting results acquired using imaging techniques should take into account that a correlation between the observed activity and the investigated effect does not necessarily imply functional causation.

Changes in cortical excitability have been shown to accompany learning processes (Pascual-Leone, Grafman, & Hallett, 1994). Weak transcranial electrical stimulation techniques, such as transcranial direct current (tDCS) or random noise (tRNS) stimulation enable us to modulate cortical excitability, thus they offer the possibility to interact with learning performance (Nitsche et al., 2003). Furthermore, they give us the opportunity to investigate the role of different cortical regions in cognitive processes by selectively targeting specific cortical areas in different phases of a given task. The majority of studies involving DC stimulation focused so far mainly on effects on motor and visual areas. The application of cathodal tDCS over the primary motor cortex (M1) diminishes the amplitude of transcranial magnetic stimulation (TMS) elicited motor-evoked potentials (MEPs), whereas anodal stimulation increases it. Similarly, tDCS can also modulate the excitability of the visual cortex (for a review, see Antal, Nitsche, & Paulus, 2006). The after-effects of DC stimulation can be prolonged by increasing the current intensity or the duration of tDCS (Nitsche & Paulus, 2000; Nitsche & Paulus, 2001). tRNS is a new non-invasive transcranial stimulation method involving the application of a random electrical oscillation spectrum. Terney, Chaieb, Moliadze, Antal, and Paulus (2008) reported that tRNS with a frequency range of 0.1–640 Hz increases M1 excitability, lasting for 60 min post-stimulation – an effect similar to that of anodal tDCS. With regard to cognition, a growing number of studies on the effects of weak transcranial electrical stimulation on cognitive functions have been published recently. tDCS has been demonstrated to have a beneficial effect on simple reaction times and implicit motor learning in healthy human subjects when the M1 was stimulated (Nitsche et al., 2003), and it also improves the learning of a visuo-motor coordination task when the M1 or the visual area V5 is stimulated (Antal, Kincses, Nitsche, Bartfai, & Paulus, 2004). tDCS is reported to improve performance in classification learning using stimulation over the prefrontal cortex (Kincses, Antal, Nitsche, Bártfai, & Paulus, 2004). tRNS has also been shown to increase performance significantly in the acquisition and early consolidation phase of an implicit motor learning task (Terney et al., 2008).

As the imaging studies have shown, the prototype distortion task offers a number of possible cortical areas as easily accessible targets for investigation using electrical stimulation techniques, including the visual cortex and frontal regions. Systematic investigation of these areas with electrical stimulation techniques may shed light on the role these areas play in the encoding, storage and retrieval stages of memory processes.

The right DLPFC was chosen for investigation from the aforementioned areas, as the study by Seger et al. (2000) reported that this region of the brain is active during the whole duration of the acquisition phase of a category learning task, regardless of the level of task performance. We have also investigated the effects of anodal stimulation on the left DLPFC, as this region was selectively implicated in participants with high categorization performance in the same study. In the recent years it has been demonstrated that tDCS stimulation of the DLPFC is indeed feasible and can lead to alterations of performance in cognitive tasks (Fregni et al., 2005; Kincses et al., 2004; Knoch et al., 2008; Priori et al., 2008).

In a previous study we have shown that the cutaneous perception associated with low intensity electrical stimulation is less prominent in the case of tRNS when compared to tDCS, suggesting that tRNS could be more efficiently blinded (Ambrus, Paulus, & Antal, 2010). Initial studies hinted that the effects of anodal tDCS and tRNS can be similar (Terney et al., 2008). To establish tRNS as a potential alternative to anodal tDCS, further studies comparing the efficacy of the two are warranted. Thus, our aim was also to compare the effects of tDCS to the effects of tRNS on the performance in this version of the prototype distortion task.

Based on previous investigations we have predicted DLPFC stimulation to differentially modulate categorization performance. In line with the findings of stimulation studies targeting the DLPFC our expectation was that cathodal stimulation would decrease performance, while anodal tDCS and tRNS were expected to increase categorization effectiveness.

**Table 1**
Imaging studies using various versions of the prototype distortion task.

| Study | Experimental paradigm | Imaging | Imaging phase | Findings |
|---|---|---|---|---|
| Reber et al. (1998a) | Prototype distortion task (dot patterns), *A–not A* | fMRI | *Testing phase*; Categorical vs. non-categorical stimuli | *Categorical patterns*: less activation in posterior occipital areas; increased activity in left and right anterior frontal regions, right inferior lateral frontal cortex |
| Reber et al. (1998b) | Prototype distortion task (dot patterns), *A–not A*<br>Control: pattern recognition | fMRI | *Testing phase*; familiar vs. non-familiar stimuli in membership judgement/recognition | *Categorization*: familiar stimuli associated with decreased activity in the posterior occipital cortex<br>*Recognition*: increased activity in occipital regions for familiar stimuli |
| Aizenstein et al. (2000) | Prototype distortion task (dot patterns), *A–not A* | fMRI | *Testing phase*; informed vs. uninformed | *Both conditions*: decreased activation of parietal regions<br>*Implicit condition*: decreased activation of V3<br>*Explicit condition*: increased activation of V3, MTL, frontal regions |
| Seger et al. (2000) | Prototype distortion task (visual concept learning – color grids) *A or B: learning with feedback* | fMRI | *Acquisition phase*; learning vs. baseline; correlation of activation patterns with performance | *Throughout the task execution*: right DLPFC and right inferior parietal areas<br>*High performance only*: left DLPFC |
| Vogels, Sary, Dupont, and Orban (2002) | Prototype distortion task (dot patterns), mixed: *A or B or none*<br>Control: judgement of position of distorted patterns | PET | *Testing phase* | *Both test and control*: activation in intraparietal sulcus and neostriatum<br>*Categorization*: activation in orbitofrontal cortex, DLPFC |
| Reber et al. (2002) | Prototype distortion task (dot patterns), *A–not A*/*pattern recognition*<br>Subjects were informed about the category only after training<br>Control: counting the dots. | fMRI | *Testing phase*; comparison between category membership judgement/recognition vs. dot counting | *Categorization*: bilateral inferior PFC and parietal cortex<br>*Recognition*: MTL, precuneus, visual areas |
| Reber, Gitelman, Parrish, and Mesulam (2003) | Prototype distortion task (dot patterns), *A–not A*<br>Subjects: informed/uninformed about the existence of a category before the training phase | fMRI | *Testing phase*; comparison between category members/non-members in the intentional/incidental groups | *Uninformed*: decreased occipital activity for category members<br>*Informed*: increased activity in hippocampus, rPFC, lITC, precuneus, posterior cingulate for category members |
| Zeithamova, Maddox, and Schnyer (2008) | Prototype distortion task (cartoon animals)<br>*A or B*: learning with feedback during training<br>*A–not A*: informed about the category before training | fMRI | *Testing phase*; *A or B* vs. *A–non A* | *Both tasks*: occipital and fusiform areas, inferior frontal cortex, precentral gyrus, bilat. posterior hippocampus<br>*A or B*: frontal and parietal cortex, parahippocampus<br>*A–not A*: posterior cortical regions, striatum |

## 2. Materials and methods

### 2.1. Subjects and experimental design

60 healthy subjects, students and employees of the University of Göttingen (23 male, age range: 19–40) participated in the study. The visual acuities were normal or corrected to normal. None of the participants had any previous history of neurological or psychological disorders, drug or alcohol abuse, and had no metal implants. They were not taking any chronic or acute medication. All subjects gave informed consent before participating. The experiment was conducted in accordance with the guidelines of the Declaration of Helsinki, and with the approval of the ethics committee of the University of Göttingen.

Participants have been randomly assigned to the 5 different stimulation condition groups (right anodal (male: 4), left anodal (male: 5) or right cathodal tDCS (male: 5), right tRNS (male: 5) and sham (male: 4), all groups: $n = 12$), none of the subjects participated in more than one session. The difference in the mean age of the five groups was not statistically significant [$F(4, 55) = 0.182$, $p = 0.946$]. All of the participants were naïve to the implicit categorization task and have been blinded to the stimulation condition.

A between-group design was used to rule out the effects of practice that could have resulted from a multiple measurement design. Any repeated measure design would require stimuli derived from multiple prototypes, each for every session. As of now, we do not have standardized set of prototype stimuli shown to be equivalent on a large sample, thus a lack of control over more, or less "learnable" stimuli would have confounded our findings. Furthermore, in accordance with the previous studies using this method (Knowlton & Squire, 1993; Reber, Wong, & Buxton, 2002) the existence of the category and the task to categorize is only to be revealed to the participant in the second part of the session (the "test phase"). In a repeated measurement design the actual purpose of the task would have been known to the subject from the second session on.

### 2.2. Stimuli

For the purpose of this investigation we have used an "A, not-A" version of the prototype distortion task (Ashby & Maddox, 2005; Posner & Keele, 1968). Stimuli consisted of patterns made up of nine black dots (rectangles, each 0.148 cm × 0.149 cm in area) placed within a 12 cm × 12 cm area of the otherwise white computer screen. Four types of stimuli were used. A dot pattern was created and was designated as the prototype. "Low" and "high" distortions of this central prototype were generated with varying degrees of displacement of the nine dots. During stimulus preparation, virtual boundaries with different sizes (in a radius of 0.592 cm for the low distortion displacement and in a radius of 2.368 cm for the high distortion displacement) were constructed around the dots of the prototype pattern. Distortions were generated by moving each dot relative to its position in the prototype pattern inside these boundaries. Random items with a pattern independent of the prototype have also been presented during the experiment. The software

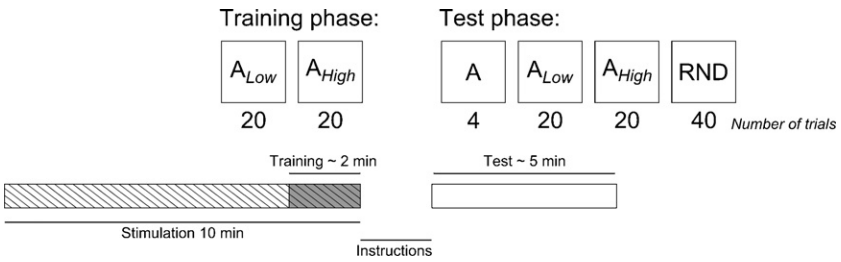


**Fig. 2.** Experimental workflow. Stimulation has been applied for 10 min, the training phase (observation of 20 low and 20 high distortion patterns, presented in a random order) took place in the last 2 min. Thereafter the participants received their instructions for the remainder of the session. The categorization phase followed, in which the participants had to make category membership judgements for 4 prototypes, 20 low distortion, 20 high distortion and 40 random patterns, again, presented in a random order.

was written in MATLAB 6.5 (Mathworks Inc.). A Dell Flat Panel Monitor (refresh rate 75 Hz) was used for stimuli presentation. Eye–display distance was 75 cm.

### 2.3. Procedure

To assess categorization performance the "A, not-A" variant of the prototype distortion task was used. Stimulation was applied prior to- and during the acquisition phase of the task (see Fig. 2).

Stimulation was delivered by a battery-driven constant current stimulator (neuroConn GmbH, Ilmenau, Germany). The current was transferred by a pair of standard electrodes (3 cm × 3.5 cm) placed in rectangular sponges (5 cm × 7 cm) soaked in isotonic sodium chloride solution.

Stimulation electrodes were placed on the scalp in a Cz – DLPFC montage. A similar montage has been proven to be effective in a previous study (Kincses et al., 2004). Talairach coordinates of the DLPFC ($x = 51$, $y = 38$, $z = 22$) were converted to the 10/20 system (http://wwwneuro03.uni-muenster.de/ger/t2tconv/). We have calculated the electrode position for an average tragus-to-tragus distance (38 cm) and used this position for all of the subjects in the study (9 cm to the right/left and 7 cm anterior relative to the Cz). In the case of DC stimulation the electrode to which polarity refers to was the one over the DLPFC. The electrodes were fixed to the head with elastic rubber bands.

Stimulation has been applied for 10 min with an intensity of 1.0 mA. Sham stimulation was administered similarly as active stimulation with the difference that the stimulator was slowly ramped down after approximately 30 s of stimulation, in order to reproduce the slight prickling sensation under the electrodes as produced by active stimulation. This method (Gandiga, Hummel, & Cohen, 2006) is considered to be an effective way to administer sham stimulation and is a standard procedure in tDCS studies. In the case of sham stimulation a *Cz and right DLPFC* montage was used, and direction of the current has been randomly assigned for each individual subject in the sham group. The tRNS stimulation had been administered using a 0.1–640 Hz noise signal frequency (see Terney et al., 2008).

The training phase of the task begun 8 min after the start of the stimulation. Stimulation was administered for 2 more minutes, the approximate time needed for the completion of the training task. During the training phase, 20 "low" and 20 "high" distortions of the prototype pattern were presented in a randomized order, each for 93 ms. After the disappearance of each stimulus a blank screen with a white background had been presented for at least 1 s, and the subjects had to press a button to make the next pattern appear. The participants were instructed to look at the center of the screen during the whole course of the training phase.

Prior to the end of the training phase participants were not informed about the existence of a category, or any subsequent task. Only after the completion of the training task, were the participants told that the previously seen patterns all belonged in the same category ("*in the same sense that if a series of dogs had been presented, they would comprise the members of the category 'dog'*" (Squire & Knowlton, 1995)).

In the testing phase, 4 prototypes, 20 new "low", 20 new "high" distortion patterns and 40 random patterns were presented in a pseudorandom order. Participants have been instructed to respond 'yes' (press the left mouse button) if the presented item belonged in the category seen before in the training phase and 'no' (right mouse button) if it did not. In the category judgement task the subjects' response was not strictly time-limited. Categorization performance was defined as the percentage of successful classification judgements – marking the prototypes/distortion patterns as category members and identifying random patterns as non-members.

### 2.4. Analysis

The $d'$ sensitivity index has been calculated for each subject using the overall hit and false alarm rates. These $d'$ values have been compared across the stimulation conditions using a one-way ANOVA. The percentage of correct responses were entered into a 5 (stimulation: sham, right anodal tDCS, right cathodal tDCS, right tRNS, left anodal tDCS) × 4 (stimuli: prototype, high distortion, low distortion, random patterns) Brown–Forsythe $F$-test. Games–Howell test (for unequal cross-group variances) was used for *post hoc* comparisons. Paired $t$-test between prototype and distorted pattern categorization performance measures has been used to assess the prototype-effect in each stimulation group. All comparisons have been conducted with a significance level of 5%.

## 3. Results

All of the subjects tolerated tDCS and tRNS stimulation and reported no side-effects during or after the experimental session.

Categorization performance of the stimulation groups is shown in Fig. 3.

### 3.1. Sensitivity index

The mean of the $d'$ sensitivity index was calculated for each group (sham mean = 0.188, $SD = 1.256$; right anodal tDCS mean = −0.026, $SD = 1.159$; left anodal tDCS mean = 0.308, $SD = 0.575$; right cathodal tDCS mean = 0.546, $SD = 0.813$; right tRNS mean = 0.277, $SD = 1.220$). Comparing the $d'$ indices across stimulation groups the one-way ANOVA analysis did not reveal any significant differences [$F(4, 55) = 1.1028$, $p = 0.364$].

### 3.2. Comparison of correct response rates

The highest percentage of correct responses in the categorization of prototype patterns was in the sham stimulation group: 91.66% (SEM = 3.55). The prototype correct response rate in the right anodal tDCS group was 43.75% (SEM = 12.35), while the left anodal tDCS group reached 58.33% (SEM = 9.89). This value in the right cathodal tDCS stimulation group was at 75%

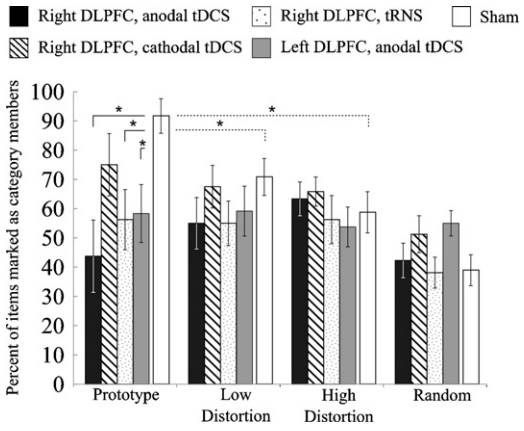


**Fig. 3.** Classification performance of prototype, low distortion, high distortion and random stimuli in the five stimulation groups: left- and right anodal, right cathodal tDCS, tRNS and sham stimulation. Significant differences have been observed between the left- and right anodal tDCS and the sham group as well as between the tRNS and the sham group in the correct categorization rates of prototype stimuli. The prototype-effect was only observable in the sham stimulation group. Error bars denote SEM.

(SEM = 10.66) and 56.25% (SEM = 10.26) in the case of the right tRNS group.

Regarding the percentages of correct responses in the case of low distortion patterns the sham group scored 70.8% (SEM = 6.31). The right anodal tDCS group performance was 55.0% (SEM = 8.79), the left anodal tDCS group performed at 59.16% (SEM = 8.52), while the right cathodal group reached 67.5% (SEM = 7.29) and the right tRNS group achieved 55.0% (SEM = 7.87).

In the case of correct response rates in the highly distorted pattern categorization, the sham group's performance was 58.8% (SEM = 5.28), the right anodal tDCS group reached 63.3% (SEM = 5.47), the left anodal tDCS group performed at 53.75% (SEM = 6.80), the right cathodal tDCS group scored 65.8% (SEM = 4.99) while the right tRNS group performed at 56.3% (SEM = 8.23).

Regarding the random patterns 39.0% (SEM = 7.04) of the sham group categorized these as category members; this rate was 42.3% (SEM = 5.90) in the right anodal tDCS, 55% (SEM = 4.33) in the left anodal tDCS, 51.3% (SEM = 6.31) in the right cathodal tDCS, and 38.1% (SEM = 5.28) in the case of right tRNS group.

The Brown–Forsythe *F*-test revealed cross-group differences in the categorization of prototype patterns [$F(4, 45) = 3.587$, $p = 0.013$]. The Games–Howell *post hoc* analysis indicates that the categorization performance for prototype patterns was significantly higher in the sham group than that in the right anodal tDCS ($p = 0.018$), left anodal tDCS (0.046) and right tRNS ($p = 0.039$) groups. The correct response rate for prototype categorization did not differ significantly between the sham and the right cathodal stimulation groups ($p = 0.589$).

No between-group differences have been found between stimulation groups for low distortion [$F(3, 44) = 0.731$, $p = 0.539$], high distortion [$F(3, 44) = 0.682$, $p = 0.568$] or random [$F(3, 44) = 0.983$, $p = 0.410$] patterns.

Results show that both left and right anodal DC and tRNS stimulation significantly impaired performance in categorizing prototype patterns compared to sham stimulation. Performance in the right cathodal DC group also tended to be lower compared to the sham group, but the difference was not statistically significant.

Pairwise comparisons between categorization performance for prototypes and distorted stimuli have shown that the prototype effect (significantly higher performance for prototype items) was only observable in the sham stimulation group (prototype compared to low distortion: $t(22) = 4.110$, $p = 0.002$; prototype compared to high distortion: $t(22) = 4.647$, $p = 0.001$). No significant differences have been found in categorization performance between the prototype and distorted stimuli in the active stimulation groups: right anodal tDCS (prototype compared to low distortion: $t(22) = -1.393$, $p = 0.191$; prototype compared to high distortion: $t(22) = -1.361$, $p = 0.201$), left anodal tDCS (prototype compared to low distortion: $t(22) = -0.159$, $p = 0.875$; prototype compared to high distortion: $t(22) = -0.288$, $p = 0.778$), right cathodal tDCS (prototype compared to low distortion: $t(22) = 1.067$, $p = 0.309$; prototype compared to high distortion: $t(22) = 0.827$, $p = 0.427$) and right tRNS (prototype compared to low distortion: $t(22) = 0.175$, $p = 0.864$; prototype compared to high distortion: $t(22) = 0.000$, $p = 1.000$).

The interaction effect of stimulation site and distortion type in the case of anodal tDCS was analysed separately. A factorial ANOVA has shown no significant interaction effect [$F(3, 88) = 1.199$, $p = 0.314$].

## 4. Discussion

Our investigation indicates that anodal tDCS, applied both to the left and the right DLPFC, and tRNS over the right DLPFC during the training phase had a significant impact on categorization performance in the prototype distortion task. We have observed a significant decrease in performance accuracy in identifying prototype and low-distortion patterns as category members both in the anodal tDCS and in the tRNS groups when compared to the sham group. The difference between the categorization performance of prototype items in the cathodal and the sham groups was not significant. Furthermore, the prototype-effect apparent in the sham stimulation group was missing in all active stimulation groups.

### 4.1. Methodological considerations

The paradigm that we used in this study is frequently used to investigate implicit learning in healthy and in clinical populations. Using the "A, not-A" version of the prototype distortion task, Knowlton and Squire (Knowlton & Squire, 1993; Squire & Knowlton, 1995) found intact categorization in amnestic patients, but an impaired performance in recognition. Based on their results the authors argue for separate and parallel memory systems for consciously remembering individual items and implicitly acquiring category-level information derived from multiple examples. Also by using the "A, not-A" prototype distortion task, Reber and Squire (1999) found no significant difference between categorization and recognition performance in Parkinson's disease patients compared to control subjects. The authors note that the frontal dysfunction described in Parkinson's disease patients did not seem to influence the cognitive capabilities investigated.

For the purposes of the current investigation, strength and duration of the active stimulation were based upon previous investigations. These parameters have been shown to be effective and reliable concerning blinding (Antal et al., 2004; Kincses et al., 2004; Nitsche & Paulus, 2000; Rosenkranz, Nitsche, Tergau, & Paulus, 2000; Terney et al., 2008).

The feasibility of altering task performance and behavior using tDCS stimulation of prefrontal areas has been demonstrated by multiple studies. Kincses et al. (2004) tested if the electrical stimulation of the left prefrontal cortex could modify probabilistic classification learning (PCL) and have shown that implicit PCL could be modified by weak anodal tDCS. Fregni et al. (2005) investigated the effects of anodal stimulation of the DLPFC in a sequential-letter memory task. Their results indicate that anodal stimulation of the left DLPFC increases the accuracy of the task performance. Examining the possibility of modifying working memory by bifrontal tDCS, Marshall, Mölle, Siebner, and Born (2005) found slowing in reaction time during both anodal and cathodal stimulation indicating that stimulation detained neuronal processing related to response selection and preparation in the n-back task. Zaehle, Sandmann, Thorne, Jancke, and Herrmann (2011) also investigated the effects of tDCS on the n-back working memory task, and have found increased performance after anodal stimulation of the DLPFC.

As the DLPFC has been shown to play vital roles in a number of processes involving memory, such as the executive functions of working memory, attention, inhibition of irrelevant information (Smith & Jonides, 1999), set shifting, decision making, from the data available, our findings can be the result of interference in any of these processes.

### 4.2. Possible explanations and confounding factors

In the light of the results of the aforementioned reports our finding in this present study is surprising, since the level of task performance in the anodal group was significantly lower than that of the sham stimulation group, and most studies show increased performance when anodal tDCS is applied to task-relevant cortical areas during task execution.

This finding might be explained with the timing of stimulation. It was already observed that stimulation started before the training phase of a given task can also be a critical factor with regard to the results. For example, in a study involving motor cortex tDCS and an implicit motor learning paradigm, Nitsche et al. (2003) found that anodal stimulation *during* the execution enhances the performance, while anodal tDCS *before* the execution of the task did not lead to altered task performance (Kuo et al., 2008). This discrepancy could be explained in terms of homeostatic plasticity. Alterations in excitability, as postulated by the Bienenstock–Cooper–Munro model (BCM, Bienenstock, Cooper, and Munro, 1982), are to be influenced by the prior history of activity; the probability that a neuroplastic event induces facilitation is reduced if it is preceded by a high-level of activity. As, in our case, the performance of the task is assumed to be dependent on the activation state of the cortex, homeostatic down-regulation of activity due to the pre-task stimulation could explain the impaired performance in the anodal tDCS group. However, following the same logic cathodal stimulation should therefore have increased performance and, that was not the case in our study.

A study conducted in our laboratory (Antal et al., 2004) gave a possible explanation to a similarly puzzling finding in a visuomotor coordination task. In this study, cathodal tDCS stimulation of the V5 increased task performance, while anodal tDCS had no effect. The authors suggest that the global decrease in cortical excitability caused by cathodal tDCS lowered the activational state of presumed neuronal patterns associated with properties suboptimal in relation to the task, below the threshold of execution, leaving the optimal pattern above threshold still. Applying the same logic to our present findings, it is possible, that, by increasing the overall cortical excitability using anodal tDCS (and tRNS), we have also elevated the activational state of suboptimal neuronal patterns, thus enhanced the chance of the implementation of incorrect responses.

A multi-session, counter-balanced, cross-over study by Dockery, Hueckel-Weng, Birbaumer, and Plewnia (2009) has found that both anodal *and* cathodal stimulation of the left DLPFC *improved* executive functions as measured by the Tower of London test. The authors attribute the performance-enhancing effect of the cathodal stimulation to the overall reduction of noise in the targeted region, thus aiding executive performance in the early training sessions, while the anodal stimulation is suggested to have an improving effect in later sessions when subjects have sufficiently mastered the task. The investigators linked these effects to the amount of dopamine available, previously described by Kuo et al. (2008) to have an influence on stimulation effects. A commentary by Smith and Clithero (2009) on this study, however, raised the possibility that an order-effect, and/or distal effects of stimulation on remote brain areas might have influenced these results.

An inherent confounding attribute of non-invasive electrical stimulation itself is that both electrodes, when placed on the scalp, must be considered active. Also, the location of the "non-target" electrode can have an effect on the cortical electric fields (as demonstrated by Bikson, Datta, Rahman, & Scaturro, 2010 in modeling studies). Furthermore, the effects of the stimulation might not be restricted to the intended target area, as data from neuroimaging studies can be interpreted to implicate an influence also on other, remote brain areas (Kwon et al., 2008; Smith & Clithero, 2009).

Furthermore, we must also take into account that the effects of the electrical stimulation outlast the duration of the actual stimulation itself. The effects of 10 min of tDCS at 1 mA intensity over the M1 outlast the stimulation duration by more than 1 h (Nitsche et al., 2008). The duration of the after-effects following the stimulation of visual cortical areas is relatively shorter, lasting about 10–20 min (Antal et al., 2004). Further investigation is needed to determine the duration of the after-effects of the application of various weak electrical stimulation techniques to the DLPFC. Nevertheless, it is reasonable to assume that the stimulation in our present study not only affected the acquisition phase but also influenced subsequent retention and recall. Experiments with shorter stimulation durations and selective intervention during the different phases of the task are in order to further investigate the role of the DLPFC in these memory processes during this task.

### 4.3. Conclusion

Our results show that categorization performance in the prototype distortion task can be modified by weak anodal tDCS and tRNS of the DLPFC prior to- and during the acquisition phase. The investigation found that these stimulation techniques with the described parameters have specifically influenced the categorization of prototype patterns. It was also our aim to assess the similarity between the effects of anodal tDCS and tRNS. Since tRNS is less prone to be perceived cutaneously, it can be considered as an alternative with a better potential regarding blinding if it has similar effects to that of anodal tDCS. Our findings indicate that the effects of tRNS in this task are comparable to those of anodal tDCS, a finding that further embraces tRNS as a research method.

## Funding

This study was initiated and funded by an unrestricted grant awarded by the Rose Foundation and by the German Ministry for Education and Science via the Bernstein Centre for Computational Neuroscience (BCCN), Göttingen (Grant No. 01GQ0432).

## Conflicts of interest

The authors declare no conflicts of interest.

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# Original Articles in the Methodological Section of the Thesis

**Ambrus, G. G.**, Paulus, W., & Antal, A. (2010). Cutaneous perception thresholds of electrical stimulation methods: Comparison of tDCS and tRNS. *Clinical Neurophysiology, 121(11), 1908-1914.*

The study design was devised by Prof. Paulus, Prof. Antal and Géza Gergely Ambrus. The experimental program was created and the measurements have been conducted by Géza Gergely Ambrus. The article was written by Géza Gergely Ambrus, Prof. Antal and Prof. Paulus.

**Ambrus, G. G.**, Antal, A., & Paulus, W. (2011). Comparing cutaneous perception induced by electrical stimulation using rectangular and round shaped electrodes. *Clinical Neurophysiology, 122(4), 803-807.*

The study design was devised by Prof. Antal, Prof. Paulus and Géza Gergely Ambrus. The experimental program was created and the measurements have been conducted by Géza Gergely Ambrus. The article was written by Géza Gergely Ambrus, Prof. Antal and Prof. Paulus.

**Ambrus, G.G.**, Al-Moyed, H., Chaieb, L., Sarp, L. Antal, A., & Paulus, W. (in press). The fade-in - short stimulation - fade out approach to sham tDCS - reliable at 1mA for naive and experienced subjects, but not investigators. *Brain Stimulation.*

The study design was devised by Prof. Antal, Prof. Paulus and Géza Gergely Ambrus. The experimental program was created by Géza Gergely Ambrus. The measurements have been conducted by Hanan Al-Moyed, under the supervision of Géza Gergely Ambrus and Dr. Leila Chaieb. The processing and analysis of the data was conducted by Géza Gergely Ambrus, Lena Sarp, Hanan Al-Moyed and Leila Chaieb. The article was written by Géza Gergely Ambrus, with contributions from all authors.

Clinical Neurophysiology 121 (2010) 1908–1914

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

# Cutaneous perception thresholds of electrical stimulation methods: Comparison of tDCS and tRNS

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ARTICLE INFO

*Article history:*
Accepted 21 April 2010
Available online 14 May 2010

*Keywords:*
tDCS
tRNS
Sham stimulation
Sensory perception
Motor cortex

ABSTRACT

*Objective:* Controlled blinded studies using transcranial electrical stimulation (tES) paradigms need a validated sham stimulation paradigm since an itching or tingling sensation on the skin surface under the electrode can be associated with current flow.
*Methods:* Here we investigated the skin perception thresholds of transcranial direct current stimulation (tDCS) and transcranial random noise stimulation (tRNS) for current intensities ranging from 200 to 2000 μA and additional non-stimulation trials using a motor cortex–contralateral orbit montage in three different healthy subject groups: subjects naïve to tES methods, subjects with previous experience with these techniques and investigators, who use these methods in their research.
*Results:* Taking the whole sample into consideration the 50% perception threshold for both tDCS conditions was at 400 μA while this threshold was at 1200 μA in the case of tRNS. Anodal and cathodal tDCS are indistinguishable regarding sites of perception. Experienced investigators show a significantly higher anodal stimulation detection rate when compared to the naïve group, furthermore investigators performed significantly better than naïve subjects in non-stimulation discrimination.
*Conclusions:* tRNS has the advantage of higher cutaneous perception thresholds and lower response rates in when compared with tDCS. Further investigation in blinding methods (such as placebo itching) is warranted in order to improve sham control.
*Significance:* As tRNS has been shown to have similar aftereffects as anodal tDCS, this finding points to the application of tRNS as a possible alternative with a better blinding control.


## 1. Introduction

Transcranial electrical stimulation (tES) methods – transcranial direct current stimulation (tDCS), and transcranial random noise stimulation (tRNS) – are non-invasive methods used in neuroplasticity research (Ziemann et al., 2008; Huang et al., 2009), in a variety of research areas such as learning and memory (Nitsche et al., 2003a; Antal et al., 2004; Fregni et al., 2005), somatosensory perception (Rogalewski et al., 2004), emotions (Boggio et al., 2009a) and social neuroscience (Knoch et al., 2008; Karim et al., 2009). The potential of their clinical applications in the acute treatment and rehabilitation of various neuropsychological conditions and disorders (Miniussi et al., 2009) – including e.g. depression (Liebetanz et al., 2006), aphasia (Monti et al., 2008), addictions (Boggio et al., 2008, 2009b) – are currently being evaluated (Langguth et al., 2008; George et al., 2009).

Among the important aspects of evaluating the efficiency of these methods are the possibilities of experimental control of sham – placebo or nocebo – stimulation. Hitherto the most frequently used transcranial electrical stimulation technique is tDCS. tDCS induces focal and remote changes in cortical excitability primarily depending on the electrode polarity: anodal tDCS causes depolarization thus increases; cathodal tDCS induces hyperpolarization, thus decreases cortical excitability (for a recent review see Nitsche et al., 2008). tDCS as a method is relatively free from adverse effects when used according to safety guidelines (Poreisz et al., 2007; Nitsche et al., 2003b). However, a number of sensations associated with the application of tDCS are reported in the literature. The most prominent phenomenon is the itching or tingling of the skin surface under the electrode (Poreisz et al., 2007); other effects include burning sensation, redness of skin and light flashes at the beginning/end of stimulation (Poreisz et al., 2007; for an overview see Nitsche et al., 2008). However, in a safety study it was reported that using $5 \times 5$ cm$^2$ size electrodes in a motor cortex–contralateral orbit montage with 1 mA intensity, neither the participants nor experienced investigators conducting the measurements were able to distinguish between the verum and the

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1388-2457/$36.00 
doi:10.1016/j.clinph.2010.04.020

sham stimulation using a double-blind paradigm (Gandiga et al., 2006).

The number of studies using tRNS is limited (Terney et al., 2008). In contrast to tDCS, tRNS has no constraint of current flow direction sensitivity. Terney et al. (2008) reported that tRNS with a frequency range of 0.1–640 Hz improved performance significantly in the acquisition and early consolidation phase of an implicit motor learning task and increased motor cortex excitability, lasting for 60 min poststimulation. Using these methods no adverse effects were reported and, generally, less sensory sensations were documented during stimulation. Therefore it can be argued that, if these two methods have similar aftereffects, the application of tRNS might be better suited for placebo-controlled studies.

The present study aims to assess the cutaneous perception thresholds of tDCS and tRNS regarding stimulation intensity in healthy subjects. Furthermore, we have also investigated how subjects with different levels of experience with regard to electrical stimulation can discriminate between sham and active stimulations. Therefore three subject groups – naïve, experienced and investigator – have been created for the purpose of this investigation. Sites of the perceived stimulation have also been assessed.

## 2. Materials and methods

### 2.1. Participants

Thirty healthy volunteers participated in the study (15 male; mean age = 25.9 years; SD: ±3.6). Participants had no previous history of neurological or psychological disorders, drug or alcohol abuse, and had no metal implants. They were not taking regular medication relevant to the study. All subjects gave informed consent before participating. The experiment was conducted in accordance with the guidelines of the Declaration of Helsinki, and with the approval of the ethics committee of the University of Göttingen.

Special attention has been given to the participants' previous experience with these methods. Three groupings have been created according to prior knowledge and exposure to the stimulation methods. Inclusion criteria for the different experimental groups were the following:

Group 1. *Naïve subjects* ($n = 10$; 6 male; mean age = 24.3 - years; SD: ±3.1). Newly recruited participants with no previous experience with tES methods.

Group 2. *Experienced subjects* ($n = 10$; 4 male; mean age = 26.2 years; SD: ±3.9). Participants who took part in at least one study involving tES prior to this current experiment.

Group 3. *Investigators* ($n = 10$; 5 male; mean age = 27.4 years; SD: ±3.4). Investigators at the Department of Clinical Neurophysiology, University of Göttingen, who took part and have conducted experiments involving tES methods.

Stimulation was delivered by a battery-driven constant current stimulator (neuroConn GmbH, Ilmenau, Germany). The current was transferred by a pair of standard electrodes ($3 \times 3.5$ cm) placed in sponges ($5 \times 7$ cm) soaked in isotonic sodium chloride solution. One electrode was placed over the left supraorbital area; the other electrode was placed contralaterally, over the C3, the approximate location of the M1. The electrodes were fixed to the head with elastic rubber bands. The stimulator was triggered by a personal computer via parallel port connection. The tRNS stimulation has been administered using a 0.1–640 Hz noise signal frequency (see Terney et al., 2008).

### 2.2. Experimental design

Participants were seated in a reclining chair and were given a keyboard. In order to reduce the observer-expectancy effect caused by communication between the investigator and the subject the computer was placed outside the visual field of the participant.

Every subject participated in three sessions, each time with a different stimulation condition. The order of conditions was counterbalanced for each subject, with at least a 24 h break between experimental sessions. Participants were instructed to start the trial by pressing the space bar on the keyboard. They were told that in some trials they might not feel any effect or they are not going to receive any stimulation at all. They were instructed to press the space bar again if they perceived any skin sensation during the trial. Approximately 30 s elapsed between consecutive trials.

One experimental session consisted of 26 trials: 19 trials of verum stimulation with an intensity of 200–2000 μA, with 100 μA increments, and seven non-stimulation trials. These trials were randomized in each session.

Each trial with verum stimulation consisted of a lead-in random interval (0–5 s – no stimulation), a fade-in phase (8 s – increasing the intensity to the specified value); the stimulation with the specified intensity (15 s), and a fade-out phase (8 s – decreasing the intensity to zero). Non-stimulation trials lasted from 31 to 36 s, mimicking the random lead-in interval, fade intervals and the stimulation interval of the trials with verum stimulation. Each trial was interrupted when the subject pressed the space bar again.

In the case of trials with verum stimulation, reaction time was measured from the start of the fade-in phase until the subjects' button press marking the perception of the stimulation. If no such feedback was given, the trial was registered as a "no response" and no reaction time was assigned to it.

The reaction time measurement in the non-stimulation trials started immediately after the participants' starting button press. The registration of the reaction time ended as in the case of verum stimulation trials: with a second button press marking the start of the perceived skin sensation or with the elapse of the time specified for the trial.

After each trial the participant was asked to describe the location of the perceived sensation.

### 2.3. Data acquisition and analysis

A perception threshold has been defined as the lowest intensity in a condition where more than 50% of the subjects reported cutaneous perception. This threshold has also been identified for responses during the ramp-up phases of verum trials.

Responses to non-stimulation trials have been analyzed separately. The number of responses to these non-stimulation trials ("false positives") have been divided by the number of non-stimulation trials (=7). These values have been averaged for each of the groups and also for each of the active stimulation conditions they were associated with. Verum detection rates have been calculated for every subject in every condition as the number of positive responses to verum trials divided by the number of verum trials (=19). Similarly, sham false positive rates have been also calculated for every participant in each condition as the number of positive responses to sham trials divided by the number of non-stimulation trials. These values have been compared across conditions using paired sample *t*-tests and across subject groups using two sample *t*-tests. Paired samples *t*-tests have been used for the comparison of rates of responses regarding the sites of perceived stimulation between stimulation conditions. All of the comparisons have been conducted with a significance level of 5% ($p > 0.05$).

## 3. Results

All of the subjects tolerated tDCS and tRNS stimulation and reported no side-effects during or after the experimental sessions.

None of the participants have pressed the button during the initial non-stimulation lead-in intervals of the verum stimulation trials.

### 3.1. Responses in verum stimulation trials

The number of subjects responding to verum trials has been summed up for each stimulation intensity, each stimulation condition and subject group. Generally, lower number of participants responded to tRNS trials (252 responses/570 trials) than to both anodal (495 responses/570 trials) and cathodal (488 responses/570 trials) stimulation trials. This has manifested in significantly higher detection rates in the case of verum stimulation trials in the tDCS conditions when compared to tRNS (tRNS compared to anodal tDCS: $t = -7.89$, $p = 0.000$; tRNS compared to cathodal tDCS: $t = -8.30$, $p = 0.000$). No difference was found in verum detection rates between the anodal and cathodal conditions ($t = 0.47$, $p = 0.636$).

The subject response rates to verum stimulation trials, broken down into individual subject groups, including type and intensity of stimulation, are shown in Table 1.

#### 3.1.1. tRNS

In the case of tRNS stimulation trials the first stimulation intensity where more than 50% of all the subjects reported skin perception was 1200 μA. More than 50% of the naïve subjects gave positive responses at 1100 μA, while experienced subjects reached this limit at 900 μA and investigators at 1200 μA. Only the investigator group reached the 100% response rate, at 1800 μA.

There was no intensity where the positive response rate of all subjects reached 50% during the ramp-up phase. The 50% threshold during the ramp-up period in the case of tRNS has only been reached by the investigator group, at 1800 μA, where 60% of the subjects reported cutaneous perception.

Two sample *t*-tests did not show any differences between subject groups in verum stimulation detection rates within the tRNS condition (naïve compared to experienced: $t = -0.03$, $p = 0.973$; naïve compared to investigators: $t = 0.08$, $p = 0.934$; experienced compared to investigators: $t = -0.04$, $p = 0.968$). Two naïve subjects did not respond to any tRNS intensities at all.

#### 3.1.2. Anodal tDCS

Considering participants from all three subject groups more than 50% gave positive responses at 400 μA in the case of anodal tDCS trials during the whole course of the trial. Reported positive responses from all subjects reached 100% first at 1000 μA. The naïve group reached 50% response rate at 400 μA, and 100% at 1000 μA. In the case of experienced subjects the 50% limit has been reached at 300 μA, and the 100% limit at 500 μA. The rate of positive responses reached 50% at 500 μA, while it reached 100% at 700 μA in the case of investigators.

The 50% perception threshold for all subjects in the case of anodal stimulation has been reached at 600 μA, where 53% of the subjects gave positive responses during the ramp-up phases of the trials. More than 50% of the naïve subjects reported cutaneous perception at 600 μA (60%), this limit has been reached at 500 μA by the experienced subjects, while in the case of the investigators this intensity was 700 μA (70%) during the ramp-up phase of the anodal condition.

A two sample *t*-test showed that the experienced subject group had a higher verum stimulation detection rate in the anodal tDCS condition than the investigator group had ($t = 2.92$, $p = 0.009$). Naïve–experienced and naïve–investigator comparisons did not show any differences in this regard (naïve compared to experienced: $t = -1.16$, $p = 0.260$; naïve compared to investigators: $t = 0.75$, $p = 0.457$).

#### 3.1.3. Cathodal tDCS

In the cathodal tDCS trials considering all participants the 50% perception limit has been reached at 400 μA and 100% was first reached at 1200 μA. 50% of naïve subject reported perceiving the effects of the stimulation at 300 μA, while at 1200 μA 100% of the naïve subjects gave positive response. Positive response rate of the experienced subjects in the case of cathodal trials reached 50% at 400 μA, and 100% at 1100 μA. 50% of the investigators gave positive response at 400 μA, and 100% gave a positive response at 600 μA.

700 μA was the intensity where more than 50% of all the subjects reported cutaneous perception during the ramp-up phase of the cathodal stimulation (56.7%). Naïve subjects reached the 50% perception threshold at 900 μA (60%), experienced subjects at 700 μA (70%) and investigators at 600 μA (50%) during the ramp-up period.

Two sample *t*-tests have failed to show any differences between subject groups in verum stimulation detection rates within the cathodal tDCS condition (naïve compared to experienced: $t = -0.31$, $p = 0.758$; naïve compared to investigators: $t = -1.02$, $p = 0.317$; experienced compared to investigators: $t = -0.554$, $p = 0.585$).

### 3.2. Reaction times in verum stimulation trials

Reaction time parameters of the three stimulation conditions have been characterized by cumulative reaction time curves.

For every stimulation condition, the cumulative percentage of positive responses for every second of each intensity has been calculated. These values have been averaged in each condition. A curve plotted from these values illustrates the lower response rates in the tRNS condition compared to the tDCS conditions in general as well as relatively lower response rate in case of tRNS in the initial few seconds of the stimulation (Fig. 1A).

To visualize the reaction time characteristics of the stimulation conditions in contrast to the non-stimulation trials, a second cumulative curve of the percentage of positive responses relative to the actual number of positive responses for each second of the trial for all stimulation and non-stimulation trials has also been plotted (Fig. 1B).

### 3.3. False positives in non-stimulation trials

Paired sample *t*-tests did not yield any differences across stimulation conditions between false positive rates in non-stimulation trials (tRNS compared to anodal tDCS: $t = 0.40$, $p = 0.687$; tRNS compared to cathodal tDCS: $t = 1.16$, $p = 0.251$; anodal tDCS compared to cathodal tDCS: $t = 1.31$, $p = 0.198$).

When averaging false positive rates in all conditions for each individual, a group difference emerged between the naïve and the investigator groups ($t = 2.50$, $p = 0.022$), compared with two sample *t*-tests, with the investigator group having the lower false positive rate. No significant difference has been found comparing false positive rates between naïve and experienced ($t = -0.29$, $p = 0.772$), and the experienced and investigator groups ($t = 1.67$, $p = 0.111$).

### 3.4. Site of perception

After each trial with a positive response subjects have been asked about the location of the perceived stimulation. Four types of answers have been given: under the electrode over the motor

**Table 1**
Percentage of subject responding to stimulation intensities. This table shows the percentage of positive responses given by the subjects in different subject groups in the different verum stimulation trials. The percentages of positive responses during the initial ramping phase are also presented here.

| Stimulation type | Group | Phase | Intensity (μA) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 200 | 300 | 400 | 500 | 600 | 700 | 800 | 900 | 1000 | 1100 | 1200 | 1300 | 1400 | 1500 | 1600 | 1700 | 1800 | 1900 | 2000 |
| tRNS | Naïve | All | 0 | 30 | 20 | 30 | 30 | 30 | 30 | 30 | 40 | 60 | 50 | 60 | 40 | 70 | 60 | 60 | 80 | 60 | 50 |
| | | Ramp up | 0 | 10 | 0 | 10 | 0 | 20 | 0 | 0 | 20 | 0 | 20 | 30 | 10 | 20 | 20 | 20 | 40 | 30 | 30 |
| | Experienced | All | 20 | 30 | 30 | 30 | 20 | 20 | 30 | 50 | 50 | 20 | 70 | 40 | 70 | 50 | 60 | 60 | 70 | 60 | 60 |
| | | Ramp up | 10 | 0 | 0 | 30 | 0 | 0 | 10 | 0 | 10 | 0 | 10 | 20 | 20 | 40 | 40 | 30 | 40 | 40 | 30 |
| | Investigator | All | 0 | 10 | 10 | 20 | 10 | 20 | 20 | 20 | 40 | 40 | 50 | 50 | 70 | 80 | 80 | 70 | 90 | 100 | 70 |
| | | Ramp up | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 10 | 10 | 20 | 20 | 40 | 20 | 60 | 50 | 40 |
| | All subjects | All | **6.67** | **23.33** | **20** | **26.67** | **20** | **23.33** | **26.67** | **33.33** | **43.33** | **40** | **56.67** | **50** | **60** | **66.67** | **66.67** | **63.33** | **80** | **73.33** | **60** |
| | | Ramp up | 3.33 | 3.33 | 0 | 13.33 | 0 | 6.67 | 6.67 | 0 | 10 | 0 | 13.33 | 20 | 16.67 | 26.67 | 33.33 | 23.33 | 46.67 | 40 | 33.33 |
| tDCS anodal | Naïve | All | 30 | 40 | 50 | 80 | 70 | 90 | 90 | 90 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | Ramp up | 10 | 10 | 30 | 30 | 60 | 40 | 60 | 50 | 60 | 60 | 70 | 70 | 90 | 80 | 100 | 90 | 100 | 90 | 100 |
| | Experienced | All | 30 | 60 | 90 | 100 | 90 | 100 | 100 | 90 | 100 | 100 | 100 | 90 | 100 | 100 | 100 | 90 | 100 | 100 | 100 |
| | | Ramp up | 20 | 10 | 10 | 50 | 60 | 80 | 70 | 50 | 90 | 100 | 80 | 80 | 90 | 100 | 100 | 90 | 100 | 100 | 100 |
| | Investigator | All | 10 | 20 | 30 | 50 | 80 | 100 | 100 | 90 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 |
| | | Ramp up | 0 | 0 | 20 | 20 | 40 | 70 | 50 | 70 | 60 | 80 | 70 | 80 | 90 | 80 | 90 | 90 | 80 | 100 | 80 |
| | All subjects | All | **23.33** | **40** | **56.67** | **76.67** | **80** | **96.67** | **96.67** | **90** | **100** | **100** | **100** | **96.67** | **100** | **100** | **100** | **96.67** | **100** | **100** | **96.67** |
| | | Ramp up | 10 | 6.67 | 20 | 33.33 | 53.33 | 63.33 | 60 | 56.67 | 70 | 80 | 73.33 | 76.67 | 90 | 86.67 | 96.67 | 90 | 93.33 | 96.67 | 93.33 |
| tDCS cathodal | Naïve | All | 30 | 50 | 50 | 70 | 70 | 70 | 80 | 90 | 80 | 90 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | Ramp up | 10 | 20 | 10 | 40 | 30 | 40 | 40 | 60 | 50 | 70 | 60 | 50 | 80 | 70 | 80 | 50 | 80 | 80 | 90 |
| | Experienced | All | 30 | 40 | 70 | 70 | 80 | 80 | 90 | 80 | 90 | 100 | 100 | 100 | 100 | 100 | 90 | 100 | 100 | 100 | 100 |
| | | Ramp up | 10 | 10 | 10 | 30 | 20 | 70 | 60 | 60 | 70 | 80 | 80 | 80 | 70 | 80 | 90 | 80 | 80 | 80 | 90 |
| | Investigator | All | 20 | 40 | 50 | 80 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | Ramp up | 0 | 0 | 20 | 30 | 50 | 60 | 60 | 80 | 80 | 70 | 90 | 60 | 90 | 90 | 100 | 100 | 100 | 90 | 100 |
| | All subjects | All | **26.67** | **43.33** | **56.67** | **73.33** | **83.33** | **83.33** | **90** | **90** | **90** | **96.67** | **100** | **96.67** | **100** | **100** | **96.67** | **100** | **100** | **100** | **100** |
| | | Ramp up | 6.67 | 10 | 13.33 | 33.33 | 33.33 | 56.67 | 53.33 | 66.67 | 66.67 | 73.33 | 76.67 | 63.33 | 80 | 80 | 90 | 76.67 | 86.67 | 83.33 | 93.33 |

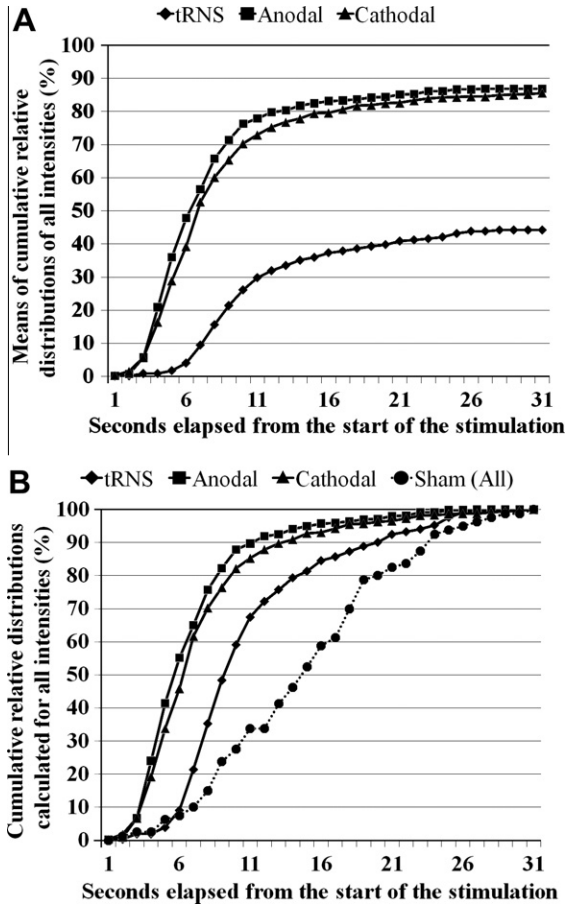


**Fig. 1.** Cumulative relative distribution of reaction times of positive answers in verum trials in different stimulation conditions based on the possible number of positive responses (A) and the actual number of positive responses (B).

cortex, under the electrode over the contralateral orbit, under both electrodes and on the whole scalp surface.

In the case of verum stimulation trials generally the great majority, 76.66% of the perceived sensation came from under the orbital electrode, 17.12% from under the electrode over the motor area, 6.09% from the skin surface under both electrodes and 0.13% from the whole scalp surface.

Considering non-stimulation trials responses from all subjects and all experimental sessions the motor cortex was identified in 55.13% of the trials with positive responses as the site of perception, followed by the orbit (38.46%), both electrodes (2.56%) and the whole scalp surface (3.85%).

When comparing the ratio of the responses identifying the site of perception as the orbit or as the skin surface over the motor cortex in all subjects across stimulation conditions there was no difference between the anodal and the cathodal condition when compared with a paired samples *t*-test (orbit: anodal tDCS compared to cathodal tDCS: $t = 0.44$, $p = 0.659$; M1: anodal tDCS compared to cathodal tDCS: $t = -1.98$, $p = 0.062$). Significant differences have been found, however, between tRNS and both tDCS conditions (orbit: tRNS compared to anodal tDCS: $t = -2.74$, $p = 0.013$; tRNS compared to cathodal tDCS: $t = -2.73$, $p = 0.013$; M1: tRNS compared to anodal tDCS: $t = 4.58$, $p = 0.000$, tRNS compared to cathodal tDCS: $t = 4.18$, $p = 0.000$).

## 4. Discussion

tES techniques are increasingly used for therapeutic purposes. Best studied so far is tDCS. Its therapeutic window is small, with an electrode size of 25 $cm^2$ intensities lower than 0.4 mA are probably not sufficient (Nitsche and Paulus, 2000), whereas 3 mA starts to be painful (Furubayashi et al., 2008). Several approaches are pursued to minimize skin perception, while other methods such as tACS or tRNS designed for different purposes provide as a "side effect" a smaller skin perception at the same intensity. Our study has found significantly less positive responses and higher cutaneous perception thresholds for tRNS compared to both anodal and cathodal tDCS. The 50% detection threshold for verum stimulation in case of all participants has been found at 400 μA for both anodal and cathodal tDCS and 1200 μA for tRNS. While anodal stimulation at 1 mA (current density: ~0.029 mA/$cm^2$) has been detected by all the subjects and cathodal stimulation by 90%, tRNS stimulation at 1 mA did not reach the 50% detection threshold (43.33% of all subjects detected it). During the ramp-up phase at 1 mA tRNS was detected only by 10% of the subjects, while anodal and cathodal stimulation has been detected by 70% and 66.67%, respectively.

Regarding the site of the perceived sensations the forehead has been identified in most of the verum stimulation trials, while the M1 has been most frequently reported in false positive trials in non-stimulation measurements. Anodal and cathodal tDCS has shown to be indistinguishable regarding skin sensation sites. In contrast, both anodal and cathodal stimulation differed from tRNS in that regard. The likely explanation for this observation is that the current flow in the case of tDCS is unidirectional while tRNS is polarity-independent.

Dundas et al. (2007) found that during stimulation the cutaneous sensations associated with tDCS were most prominent under the electrode placed on the forehead, in their case, the anode. The authors attribute this finding to the greater sensibility of the forehead rather than to current direction. Our results are in agreement with this reasoning; no difference was observed between anodal and cathodal tDCS concerning the ratio of responses identifying the location of the sensation as the orbit or the skin surface over the motor cortex.

No overall differences have been observed between verum detection rates between subject groups. Only in the anodal condition was the experienced group better than the investigator group, otherwise no significant differences have been detected. The individuals in the naïve group had higher average false positive rates than the experienced group had. Comparing false positive rates in case of non-stimulation trials between subject groups no specific differences have been detected in the in-condition comparisons.

The need for efficient sham trials in clinical evaluations is obvious due to the placebo effect. Based on a review by Kaptchuk et al. (2000) and Wassermann and Lisanby (2001) argues that device-based treatments (like repetitive transcranial magnetic stimulation [rTMS]) might have a more pronounced placebo effect compared to that of placebo pills, partially due to the elaborate procedure of application and the presence of high technology. For example, prominent placebo responses have been reported to be associated with various stimulation techniques in pain studies (Cruccu et al., 2007). A tDCS chronic pain study by Antal et al. (2010) reports nocebo-like responses, as moderate fatigue has been observed by 44.4% of the participants who received verum (anodal) stimulation, and by 64.7% by participants who received sham stimulation; also, tiredness has been reported by 33.3% of the patients with verum and by 70.6% of the patients with sham stimulation.

Various techniques are in use to reduce phenomena associated with tDCS. For example, ramping up the current flow at the beginning of the stimulation and ramping it down at the end is reported to reduce skin sensations as well as other side-effects. When an investigation requires longer stimulation sessions applying active stimulation only for a short period of time at the beginning of the designated sham trial may also prove to be an efficient way of designing sham-controlled experiments, as active stimulation

in such a short interval does not seem cause significant changes in brain functions (Nitsche et al., 2008). Gandiga et al. (2006) conducted a study on healthy subjects and stroke patients applying 20 min of tDCS using 1 mA intensity (with an additional fade-in phase lasting for about 10 s) and sham stimulation (stimulation started, but turned off after 30 s) to investigate the possibilities of double-blind sham-controlled experimental designs with the subjects' self-reported measures of adverse effects. During the 170 sessions of this study none of the participants or (blinded) investigators were able to distinguish between the verum and the sham stimulation. In their conclusion the authors argue for the feasibility of such designs.

This method is considered to be an effective way to administer sham stimulation at least when applying the current at 1 mA intensity (Ziemann et al., 2008) and is standard procedure in tDCS studies. On the other hand, a study by Dundas et al. (2007) reported that participants still perceived the current flow at 1 mA even 1 min after the stimulation onset. This study, however did not have a control for sham, and did not report the use of ramping either at the beginning or at the end of the stimulation.

Devices specially designed to deliver sham stimulations present another alternative. These stimulators have the advantage that they are capable to store and deliver multiple active stimulation setups beside sham. Preprogrammable stimulators are essential in double-blind studies, as the investigator working with the participants should not be aware of the type of stimulation being applied. This kind of device is indispensable in clinical evaluations likely to be conducted in the near future, when patients are given a stimulator to use it at their homes for a longer period of time.

An obvious practice is to place the stimulator outside the participants visual field. Alternatively, modified stimulators may also be used to conceal the type of stimulation. Fregni et al. (2006) describe a device which has a switch in the back with which the current flow can be stopped while the display still shows normal functioning.

The application of topical local anesthetics in order to decrease the intensity of the perceptual sensation under the electrodes (Nitsche et al., 2008) might also be a considered as an option, especially in cases where ramping is not possible or the paradigm requires stimulation parameters entailing more intense sensations. To our present knowledge no publication reported using this method.

In any case, post-trial questioning of the subjects can be helpful to evaluate the effectiveness of the measures taken in order to reduce the subjects' capability to differentiate between types of stimulations.

In summary, consequent whole-scale group differences between naïve, experienced and investigator participants have not been observed regarding verum detection rates. Differences in that regard have only been found in anodal verum stimulation detection rates, where the investigator group had a higher verum stimulation detection rate than the naïve group had. Naïve subjects performed worse in non-stimulation discrimination than investigators.

Our investigation has found a significant difference in skin perception characteristics between tRNS and tDCS stimulations manifested in lower response rates and higher thresholds. Generally, it can be concluded that tRNS is not as noticeable as tDCS regarding skin perception. While the cutaneous sensation for verum stimulation was localized mainly under the orbital electrode, the surface under the electrode above motor cortex was identified in the majority of the trials in the non-stimulation condition. Patterns in sites identified as the location of the perceived stimulation did not differ between anodal and cathodal tDCS, but both were different from tRNS. As tRNS has been shown to have similar aftereffects as anodal tDCS, this finding braces tRNS as a possible alternative with a better blinding control. Further research needs to be conducted, however, in order to ascertain whether the same neurophysiological or behavioral aftereffects can be achieved using anodal tDCS or tRNS, at the same current intensity.

## Funding

This study was initiated and funded by an unrestricted grant awarded by the Rose Foundation and by the Bernstein Center for Computational Neuroscience Göttingen.

## Conflicts of interest

The authors declare no conflicts of interest.

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Clinical Neurophysiology 122 (2011) 803–807

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

# Comparing cutaneous perception induced by electrical stimulation using rectangular and round shaped electrodes

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See Editorial, pages 637–638

ARTICLE INFO

*Article history:*
Available online 25 October 2010

*Keywords:*
tDCS
tRNS
Sham stimulation
Cutaneous perception
Motor cortex
Electrode geometry

HIGHLIGHTS

- We have tested the cutaneous perception characteristics of tDCS and tRNS in the intensity range of 200–2000 μA using a circle-shaped and a rectangle-shaped sponge electrode configuration.
- No substantial differences between the two configurations have been observed.
- Round sponge electrodes do not have better blinding properties compared to the rectangular electrode configuration.

ABSTRACT

*Objective:* We have investigated the cutaneous perception differences for anodal and cathodal transcranial direct current stimulation (tDCS) and transcranial random noise stimulation (tRNS) between two electrode configurations: a standard, rectangle-shaped, and a circle-shaped, round geometry with the same surface area, and thus, same nominal current distribution. We have aimed to find whether a smaller perimeter length and the absence of corners in the case of the round configuration would lead to altered skin perception characteristics when compared to the rectangular geometry.
*Methods:* Twelve subjects were tested for tDCS and tRNS skin perception characteristics in the intensity range of 200–2000 μA using round and rectangular electrode configurations.
*Results:* We have not found any substantial differences between detection thresholds, detection rates, false positive rates or consistent alterations in the sites of perceived stimulation.
*Conclusion:* We conclude that there is no difference between the round and the rectangular electrode configurations regarding their blinding potentials.
*Significance:* The results of this investigation indicate that the altering of the electrode geometry to a round configuration is unwarranted for better blinding purposes in future studies using tDCS and tRNS.


## 1. Introduction

Noninvasive transcranial electrical stimulation techniques are increasingly used in neuroscience research and the potentials of their clinical applications are also being investigated. Transcranial direct current stimulation (tDCS) and transcranial random noise stimulation (tRNS) have been shown to be able to cause changes in cortical excitability, with the effect potentially overlasting the duration of the stimulation (Terney et al., 2008; Nitsche et al., 2008). In both cases the stimulation is usually applied using two rubber electrodes mounted onto the surface of the skull using sponges as electrode wrappers and elastic rubber bands to fix the montage. Among the main parameters influencing the effects of stimulation are current density (applied current/electrode surface area), duration of stimulation and electrode geometry (Nitsche et al., 2008; Datta et al., 2009).

The most prominent phenomenon associated with electrical stimulation techniques is the itching or tingling of the skin surface under the electrode (Poreisz et al., 2007); other effects include

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1388-2457/$36.00 
doi:10.1016/j.clinph.2010.08.023

burning sensations, redness of skin and light flashes at the beginning/end of stimulation (Poreisz et al., 2007; Antal et al., 2010; for an overview see Nitsche et al., 2008).

Most studies employ rectangle-shaped electrodes. Simulation models have shown that currents tend to be concentrated in the vicinity of the regions surrounding the edges of the electrodes (Miranda et al., 2006; Gilad et al., 2007; Wagner et al., 2007; Nitsche et al., 2008; Datta et al., 2009), which might lead to local skin irritation. The basis of our investigation was the assumption that applying the same current density more uniformly to the stimulated surface may reduce the sensations associated with the procedure.

Acquiring information about the perception thresholds of electrical stimulation methods might help to design better placebo-controlled studies in two ways. First, by evaluating the response rates of verum and sham stimulation the effectiveness of subject blinding can be assessed. Any method that would reduce this response rate to a given type of stimulation and intensity would lead to better blinding compared to a non-stimulation placebo condition. Second, it can be considered to use a physiologically inert, but still perceivable intensity as placebo stimulation. The importance of both approaches is especially apparent considering studies using short stimulation durations.

In a previous study (Ambrus et al., 2010) we have determined the cutaneous perception characteristics of tDCS and tRNS at various intensities using the conventional, rectangular electrode geometry. In this present study we used the same experimental design with two types of electrode configurations. The aim of our investigation was to determine, whether the elimination of corners and reduction of perimeter length by using circle-shaped electrode wrappers is associated with different perception characteristics, e.g. lower perception thresholds, a lower rate detection rates, differences in sites of perception, when compared to the widely used, rectangle-shaped wrappers, keeping the nominal current density constant across conditions. Finding such differences would mean that the round electrode configuration could be considered as a superior solution regarding tolerance of procedure and blinding issues, a cornerstone of clinical investigations.

## 2. Methods

### 2.1. Subjects

Twelve healthy volunteers participated in the study (six male; age range between 20 and 27 years). Participants reported no previous history of neurological or psychological disorders, drug or alcohol abuse, and had no metal implants. They were not taking regular medication relevant to the study. All subjects gave written, informed consent before participating. The study was conducted with the approval of the ethics committee of the University of Göttingen and in accordance with the guidelines of the Declaration of Helsinki.

### 2.2. Stimulation

The electrical stimulation has been delivered by a battery-driven constant current stimulator (neuroConn GmbH, Ilmenau, Germany). The current was transferred by a pair of standard carbon rubber electrodes (Physiomed Elektromedizin AG, Schnaittach, Germany) placed in viscose sponge wrappers soaked in isotonic sodium chloride solution. Depending on the experimental session, either round (circle, $d = 6.6755$ cm), or a rectangle-shaped ($5 \times 7$ cm) sponge wrappers (both 35 $cm^2$ in area) have been used.

One electrode was placed over the left supraorbital area; the other electrode, to which polarity refers to, was placed contralaterally over the C3, the approximate location of the M1. The electrodes were fixed to the head with elastic rubber bands, seeking the best possible contact between the whole surface of the electrode and the scalp. The stimulator was triggered by a personal computer via parallel port connection. The tRNS stimulation has been administered using a 0.1–640 Hz noise signal frequency (see Terney et al., 2008).

No special skin surface preparation had been performed before the experiment. The rubber bands have been applied firmly so that the electrodes were not able to move, but with no extraneous pressure applied. Approximately 15 ml of fluid was applied to wet the sponge, with particular consideration given so that no solution was completely soaking the hair and scalp. The electrode montage was positioned throughout the duration of the experiment and careful consideration was given to ensure that the sponges remained consistently wet.

### 2.3. Experimental design and data acquisition

Participants were seated in a reclining chair and were given a keyboard. In order to reduce the observer-expectancy effect caused by communication between the investigator and the subject the computer, the stimulator and the investigator were placed outside the visual field of the participant.

Each subject participated in six sessions. Anodal tDCS, cathodal tDCS and tRNS stimulation has been applied using a pair of rectangle-shaped and round-shaped sponge coverings ($3 \times 2$ sessions). Half of the subjects (=6) started the study with the three stimulation sessions using the rectangular-shaped electrode wrapper. The order of sessions regarding the stimulation condition was randomized. At least 24 h elapsed between two consecutive sessions.

One experimental session consisted of 26 trials: 19 trials of verum stimulation with an intensity of 200–2000 μA, with 100 μA increments, and seven non-stimulation trials. The order of these trials was randomized in each session.

Participants were instructed to start the trial by pressing the space bar on the keyboard. They were told that in some trials they might not feel any effect or they are not going to receive any stimulation at all. They were instructed to press the space bar again if they perceived any skin sensation during the trial.

Each verum stimulation trial consisted of a random lead-in interval (0–5 s – no stimulation), a fade-in phase (8 s – increasing the intensity to the specified value); the stimulation with the specified intensity (15 s), and a fade-out phase (8 s – decreasing the intensity to zero). Non-stimulation trials lasted from 31 to 36 s, mimicking the random lead-in interval, fade intervals and the stimulation interval of the trials with verum stimulation. A trial was interrupted when the subject pressed the space bar again.

In the case of correct identification of the verum trial (“hit”) the reaction time has been measured between the actual start of the stimulation (beginning of the fade-in phase) and the button press. In the case of responses in non-stimulation trials (“false positive”) the reaction time has been measured between the starting button-press and the down push marking the beginning of the skin sensation. A “no-response” registry has been assigned to, and no reaction time has been measured in verum trials without a response (“miss”) and non-stimulation trials without a response (“correct rejection”). Approximately 30 s elapsed between consecutive trials.

### 2.4. Data analysis

The lowest intensity at which 50%+ of the subjects reported cutaneous perception has been identified as the perception threshold for a given shape and stimulation condition.

Detection rates have been calculated in each condition for every individual. These values have been averaged for every condition (wrapper shape and stimulation type) and have been compared using repeated measures ANOVA. Repeated measures ANOVA has been used to compare false positive rates, which have been obtained by averaging the individuals' false positive rates in each condition. The assessment of perception sites has been conducted using repeated measures ANOVA with the perception sites as categories.

All statistical comparisons have been conducted with a significance level of 5% ($p < 0.05$).

## 3. Results

All of the subjects tolerated tDCS and tRNS stimulation and reported no side-effects during or after the experimental sessions. None of the participants have indicated skin perception during the initial non-stimulation lead-in intervals of the verum stimulation trials.

### 3.1. 50% Thresholds

In general, tRNS detection thresholds were higher than the thresholds for tDCS in both shape conditions. In the case of anodal stimulation, both the rectangular and the round condition resulted in a 300 μA cutaneous perception threshold, with a 600 μA threshold for the ramp-up phase, except for the round-cathodal condition, where the ramp-up threshold was at 500 μA. The tRNS detection threshold in the rectangular condition was at 1200 and 1100 μA in the round condition. The ramp-up detection rate has only reached the 50% limit at the intensity of 1900 μA in the round-tRNS condition.

### 3.2. Detection rates

Table 1 summarizes the detection rates and the 50% thresholds for the shape and stimulation conditions. Generally it can be said that in both wrapper-shape conditions the tRNS detection rates were lower than that of tDCS.

In the case of the anodal, rectangular condition, the participants have reached an 86.84% (198 responses/228 trials) perception accuracy, in the anodal, round condition this value was 87.28% (199/228). The cathodal, rectangular condition yielded an 85.53% (195/228) detection rate, this value was 82.89% (189/228) in the round condition. The tRNS stimulation condition yielded the lowest response rates, in the rectangular condition this value was 39.04% (89/228), and in the round condition, 39.47% (90/228). Regarding the detection rates, repeated measures ANOVA demonstrated that there was no effect of electrode wrapper shape ($F = 0.03$; $p = 0.862$; df = 1) while there was an effect of stimulation type ($F = 26.53$; $p = 0.000$; df = 2). Paired samples $t$-test revealed differences between the tDCS and tRNS conditions regardless of electrode wrapper shape (anodal $tDCS_{rect}$ vs. $tRNS_{rect}$: $t = 4.748$, $p = 0.000$; cathodal $tDCS_{rect}$ vs. $tRNS_{rect}$: 4.840, $p = 0.000$; anodal $tDCS_{round}$ vs. $tRNS_{round}$: $t = 5.291$, $p = 0.000$; cathodal $tDCS_{round}$ vs. $tRNS_{round}$: $t = 6.246$, $p = 0.000$). There were no significant differences between any of the tDCS conditions or between the tRNS conditions in detection rates.

### 3.3. Reaction times

To visualize the reaction time characteristics of the stimulation-type and wrapper-shape as well as the non-stimulation trials, a cumulative curve of the percentage of positive responses relative to the actual number of positive responses for each second of the

**Table 1**
Percentage of subject responding to stimulation intensities. This table shows the percentage of positive responses given by the subjects in different stimulation groups in the different verum stimulation trials. The percentages of positive responses during the initial ramping phase are also presented here.

| Electrode | Stimulation | Phase | Intensity (μA) | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 200 | 300 | 400 | 500 | 600 | 700 | 800 | 900 | 1000 | 1100 | 1200 | 1300 | 1400 | 1500 | 1600 | 1700 | 1800 | 1900 | 2000 |
| *Rectangular* | | | | | | | | | | | | | | | | | | | | | |
| | tRNS | All | 16.7 | 8.33 | 16.7 | 33.33 | 25 | 8.33 | 33.3 | 16.67 | 41.7 | 41.7 | 50 | 50 | 50 | 58.3 | 50 | 50 | 66.67 | 66.7 | 58.33 |
| | | Ramp-up | 0 | 0 | 0 | 8333 | 0 | 0 | 16.7 | 0 | 8.33 | 8.33 | 25 | 16.67 | 16.7 | 16.7 | 16.67 | 25 | 33.33 | 41.7 | 41.67 |
| | Anodal | All | 25 | 50 | 50 | 75 | 66.7 | 100 | 91.7 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 91.67 |
| | | Ramp-up | 16.7 | 8.33 | 33.3 | 41.67 | 50 | 75 | 66.7 | 75 | 75 | 66.7 | 66.7 | 83.33 | 91.7 | 91.7 | 100 | 91.7 | 91.67 | 100 | 83.33 |
| | Cathodal | All | 25 | 50 | 58.3 | 91.67 | 83.3 | 83.3 | 91.7 | 83.33 | 91.7 | 100 | 91.7 | 91.67 | 100 | 100 | 91.67 | 100 | 100 | 91.7 | 100 |
| | | Ramp-up | 8.33 | 8.33 | 25 | 50 | 33.3 | 58.3 | 75 | 75 | 91.7 | 83.3 | 91.7 | 66.67 | 100 | 83.3 | 91.67 | 100 | 91.67 | 83.3 | 100 |
| *Round* | | | | | | | | | | | | | | | | | | | | | |
| | tRNS | All | 8.33 | 16.7 | 16.7 | 8333 | 25 | 33.3 | 25 | 33.33 | 41.7 | 50 | 33.3 | 50 | 41.7 | 66.7 | 50 | 58.3 | 66.67 | 58.3 | 66.67 |
| | | Ramp-up | 0 | 0 | 16.7 | 0 | 8.33 | 8.33 | 0 | 0 | 0 | 16..7 | 25 | 33.33 | 33.3 | 33.3 | 41.67 | 33.3 | 33.33 | 50 | 41.67 |
| | Anodal | All | 33.3 | 58.3 | 58.3 | 66.67 | 83.3 | 83.3 | 91.7 | 91.67 | 100 | 91.7 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | Ramp-up | 0 | 0 | 33.3 | 41.67 | 58.3 | 66.7 | 66.7 | 75 | 75 | 91.7 | 83.3 | 83.33 | 91.7 | 83.3 | 91.67 | 91.7 | 91.67 | 91.7 | 91.67 |
| | Cathodal | All | 33.3 | 50 | 58.3 | 66.67 | 66.7 | 75 | 83.3 | 75 | 83.3 | 100 | 83.3 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | Ramp-up | 0 | 8.33 | 16.7 | 25 | 50 | 41.7 | 58.3 | 58.33 | 58.3 | 91.7 | 75 | 75 | 75 | 83.3 | 83.33 | 83.3 | 100 | 83.3 | 91.67 |

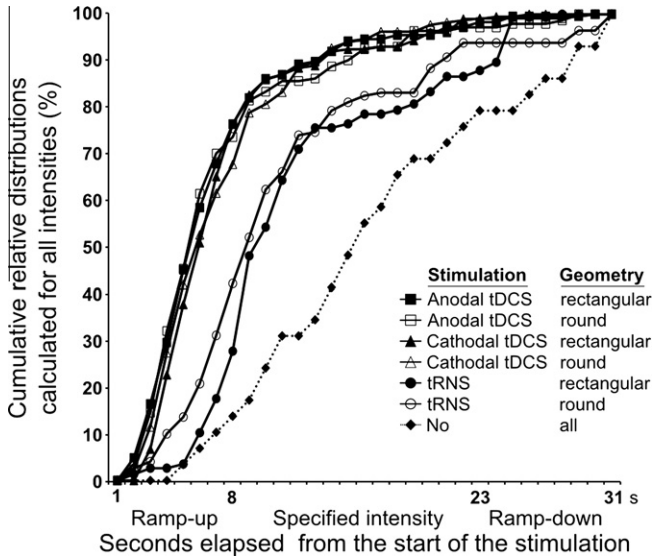


**Fig. 1.** Cumulative relative distribution of reaction times of positive answers in verum trials in different stimulation and electrode-shape conditions based on the possible number of positive responses.

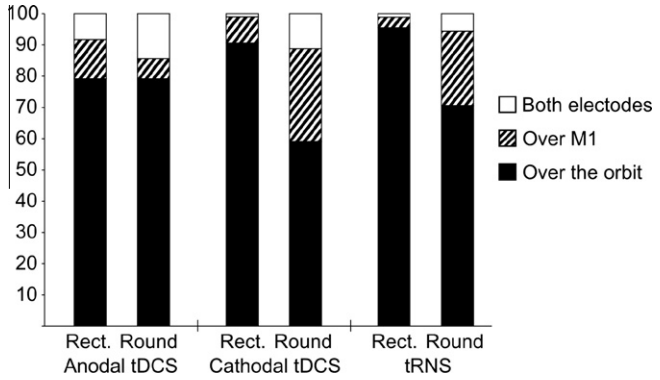


**Fig. 2.** Proportion of the different reported sites of perception in the stimulation and electrode-shape conditions in percantage.

trial for all stimulation and non-stimulation trials has been plotted (Fig. 1).

### 3.4. Sites of perception

At the end of each trial with a positive response subjects have been asked to identify the location where they perceived the stimulation. After collecting all the results, we have found that the subjects have named the three following sites: under the electrode over the motor cortex, under the electrode over the contralateral orbit and under both electrodes. Fig. 2 shows the ratio of the perceived sites of the stimulation in the stimulation and electrode-shape conditions. Repeated measures ANOVA revealed effects of stimulation site ($F = 76.40$, $p = 0.000$, df = 2), and interactions between stimulation site and electrode shape ($F = 9.50$, $p = 0.001$, df = 2) and between electrode shape, stimulation site, and type of stimulation ($F = 4.57$, $p = 0.003$, df = 4).

A Bonferroni post hoc test investigating the main effect of the sites of the perceived stimulation has revealed that the rate of responses naming the orbital electrode was significantly higher than that of the M1 and both electrodes (both comparisons: $p = 0.000$). No significant difference was observed between the M1 and the both electrodes comparison ($p = 0.447$) in that regard.

When investigating the interaction between the perceived site of stimulation and the shape of the electrodes, the Bonferroni post hoc test yielded a significant difference between the round and rectangular electrode-shape conditions in the rate of orbital responses ($p = 0.026$), the value being larger in the rectangular condition. The ratios of the orbital responses in both electrode conditions were significantly larger than those naming the M1 or both electrodes in the two electrode conditions (all comparisons: $p = 0.000$). This comparison has shown no further significant differences. While the rates of responses naming the orbit and of those naming the M1 and both electrodes have been shown to be significantly different in all stimulation conditions in the rectangular electrode shape condition (all comparisons: $p = 0.000$), in the round electrode condition comparing the ratios of the orbital responses and the M1 responses no significant differences have been found in the cathodal ($p = 1.000$) or in the tRNS ($p = 0.6710$) conditions. Furthermore, no difference has been observed comparing the ratios of the orbital responses and the rates of the responses reporting sensations from both electrodes in the round, cathodal condition ($p = 0.3782$).

The Bonferroni post hoc test yielded no differences between the shape conditions regarding the proportion of trials with reports of sensations from the orbital electrode, comparing the same stimulation conditions (anodal: $p = 1.000$; cathodal: $p = 0.0552$; tRNS: $p = 0.390$). There was no difference between the round and rectangular shape conditions regarding the proportion of responses naming both electrodes in any stimulation conditions (all comparisons: $p = 1.000$). Regarding the stimulation, using paired, between-shape-condition comparisons of the proportions of sensations over the M1 electrode, no differences have been found in either of the stimulation conditions ($p = 1.000$).

Generally, it can be concluded that the supraorbital region was the site most frequently identified in all verum stimulation and electrode-shape conditions.

### 3.5. False positive rates

The number of responders to non-stimulation trials and mean false positive rates in the rectangular condition were: anodal tDCS: 3, 0.0595 (SD: ±0.128); cathodal tDCS: 3, 0.0389 (SD: ±0.092); tRNS: 3, 0.0714 (SD: ±0.142). These values were the following in the round condition: anodal tDCS: 2, 0.0357 (SD: ±0.088); cathodal tDCS: 2, 0.0375 (SD: ±0.088); tRNS: 6, 0.1071 (SD: ±0.137). Repeated measures ANOVA on the false positive rates has revealed no effect of stimulation type ($F = 0.905$, $p = 0.420$, df = 2) or electrode wrapper shape ($F = 2.623$, $p = 0.136$, df = 1).

## 4. Discussion

In this study we aimed to investigate whether the reduction of perimeter length and the elimination of corners by using circle-shaped electrode geometry can lead to reduced skin sensations and altered perception characteristics when compared to the standard rectangle-shaped configuration, while keeping the nominal current density constant during the comparison.

For the purposes of this investigation we have used a pair of 6.7 cm diameter circle-shaped, round sponge wrappers, and the standard 5 × 7 cm electrode wrappers for comparison. Both configurations have had an area of 35 $cm^2$, thus the nominal current density was equal in both cases, while the same current intensity was applied, but the round electrode had smaller perimeter length (round wrapper: 20.97 cm; rectangular wrapper: 24 cm, difference: ~3 cm, ~4.29%) and had no salient corners where currents could potentially accumulate, assumingly leading to a more balanced current distribution. It is worth noting, however, that we

cannot rule out that fluid leaking from the sponges might change the contact area and shape. We consider this to be an inherent property of experimental designs using sponge electrodes. Other protocols, e.g. designs using electrode cream need additional, independent evaluation.

In a finite element method (FEM) simulation study comparing the non-uniformity of the current distribution of circular and square-shaped external defibrillation electrodes (area ~80 $cm^2$), Krasteva and Papazov (2002) have found that the round electrode has ~30% less non-uniformity, defined as the quotient of the maximum and the minimum current density.

Our results show no in-condition differences between the 50% perception thresholds of anodal and cathodal tDCS. This is consistent with our previous findings, with the note that in our present study the threshold was at 300 μA, while this value was at 400 μA in the previous experiment. The difference is most probably due to sample size ($n$ = 30, $n$ = 12). Furthermore, no differences between the thresholds for the tDCS conditions have been detected between the wrapper-shape conditions. The 50% detection thresholds for tRNS were substantially higher than that of both anodal and cathodal tDCS. In our previous study the threshold intensity for tRNS was at 1200 μA, and our current investigation also found this threshold at 1200 μA in the rectangular wrapper condition, while in the round condition the threshold was at 1100 μA.

Detection rates in the present study are comparable to those in our previous investigation. As it has been observed before, anodal and cathodal tDCS detection rates did not differ from each other significantly. tDCS detection rates in this present experiment did not differ between the wrapper-shape conditions either. Both in our previous experiment and in our present investigation the detection rates were significantly lower for tRNS than for both tDCS conditions. This was also observed in our present investigation in both the wrapper-shape conditions, while tRNS detection rates did not differ significantly.

In line with our previous findings, no differences in false positive rates have been observed between stimulation type conditions, and, in the case of our current results, this was also true for the comparison between the two wrapper-shape conditions.

The reports on the sites of the perceived stimulation, as in our previous experiment, named the orbit most frequently in all stimulation conditions. While the most frequent responses reporting the site of sensation in both electrode-shape conditions were those naming the orbital electrode, the application of round electrodes seems to reduce this effect, observed in the cathodal and tRNS stimulation conditions, when the response rates from the orbital site are compared to other sites of perception. As this tendency is completely missing in the anodal stimulation condition, we cannot rule out the influence of random effects due to the relatively small sample size used in this present investigation.

From these data we can conclude that the difference in the electrode geometry did not change the overall perception characteristics consistently in either tDCS or tRNS. Thus, this far there is no experimental data from transcranial electrical stimulation studies that would indicate that the round electrode configuration has superior blinding properties compared to the rectangle-shaped electrode solution.

Further methods have been suggested to reduce the non-uniformity of current distribution, and thus procedural discomfort associated with various forms of electrical interventions. These measures include the usage of a conductive gel layer between the electrode and the stimulated surface (Gilad et al., 2007), with increasing resistivity towards the periphery (Krasteva and Papazov, 2002). An additional barrier with higher resistivity around the electrode has also been proposed (Gilad et al., 2007; Krasteva and Papazov, 2002). Placing the electrode into a cone-shaped casing filled with electrode paste has also been suggested (Gilad et al., 2007). The effectiveness of these methods remains to be tested with regard to their application using transcranial electrical stimulation methods.

Perception (at least in the initial phase of the stimulation, as tested here and in our previous study) tends to concentrate under the forehead electrode in both electrode-shape conditions. This finding underscores the expedience of the usage of a larger forehead electrode. Since a larger surface area implies a lower current density, which is associated with a decreased skin perception, it can lead to better blinding properties. A larger electrode can also have the advantage that it reduces the physiological effectiveness and can render the supraorbital electrode inert (Nitsche et al., 2007). The exact relationship between physiological effectiveness, cutaneous perception and current density needs to be the subject of future investigations. The decrease of the rate of orbital sensations relative to other sites in the cathodal and tRNS stimulation conditions using the round electrode configuration also needs additional verification.

## Funding

This study was initiated and funded by an unrestricted grant awarded by the Rose Foundation and by the Bernstein Center for Computational Neuroscience Göttingen.

## Conflicts of interest

The authors declare no conflicts of interest.

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Brain Stimulation xxx (2012) 1–6

Contents lists available at SciVerse ScienceDirect

Brain Stimulation

journal homepage: www.brainstimjrnl.com

Original Research

# The fade-in – Short stimulation – Fade out approach to sham tDCS – Reliable at 1 mA for naïve and experienced subjects, but not investigators

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ARTICLE INFO

*Article history:*
Received 19 October 2011
Received in revised form
1 December 2011
Accepted 9 December 2011
Available online xxx

*Keywords:*
tDCS
Sham stimulation
Cutaneous perception
Motor cortex

ABSTRACT

*Objective:* Slowly ramping down initial current intensity after a minimal interval of stimulation is the de facto standard for sham stimulation in transcranial electrical stimulation research. The aim of this study is to further investigate the effectiveness of this method of blinding.
*Methods:* We have investigated the time course of the cutaneous perception during 10 min of anodal, cathodal, and sham transcranial direct current stimulation, probing the perceived strength and site of the perceived sensation. We have also utilized post-stimulation assessment and measurements of sleepiness prior to and after the intervention. Previous exposure to tDCS has also been taken into account: the experiment has been repeated in naïve and experienced subject groups, and a group consisting of investigators who use tDCS as a research tool.
*Results:* Although we have observed a general reduction in the perceived strength of the stimulation with time, we have not found the complete disappearance of the cutaneous perception during either the verum or the sham conditions. Experienced subjects were more likely to be able to differentiate between trials with stimulation and non-stimulation trials and to correctly identify sham and verum stimulation conditions.
*Conclusion:* When taking only naïve and experienced subjects into account, there was no significant difference between the strength of the perceived stimulation in the verum and sham conditions. The fade-in – short stimulation – fade-out sham stimulation can be indistinguishable from verum stimulation, but not because it mimics the disappearance of the cutaneous sensations associated with the verum stimulation, but because these sensations persist also in the sham stimulation. The significance of this finding with potential confounding factors and limitations are discussed.


## Introduction

Transcranial direct current stimulation (tDCS) is a non-invasive method that induces changes in cortical excitability outlasting the duration of the stimulation in a spatially restricted and reversible manner [1]. Among the most widely reported phenomena associated with the application of stimulation are the itching and tingling sensations under the electrodes [2]. Few reports of headache and burning sensations also exist [3]. It is widely assumed in the literature that the cutaneous perception associated is restricted to the initial few seconds of the stimulation [4–6].

The standard method of administering the sham intervention is therefore the *fade in*, *short stimulation*, *fade out* approach, in which the stimulation intensity is slowly ramped down after a few seconds of actual stimulation, mimicking the initial sensations associated with the stimulation. It has been shown by Gandiga et al. [6] that this method is reliable method of sham stimulation and is suitable for double-blind experiments. This approach is now a generally accepted procedure for blinding purposes, but its underlying assumption, that in the case of verum stimulation the sensations are only perceived during the initial phase of the stimulation, has not been quantitatively examined so far. Thus, it can be speculated that blinding, using this method, is achieved not by the mimicking of the complete disappearance of the sensations after the initial phase of the stimulation, but by inducing a subjective sensation that can still be perceived after the intensity has been ramped down, and this subjective sensation is not easily distinguishable from the perceptions associated with the verum stimulation in quality and quantity.

That this speculation might have merit, is hinted at by Dundas et al. [4], who have found that their subjects perceived the sensations even 1 min after the beginning of the stimulation. As this

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doi:10.1016/j.brs.2011.12.001

study only assessed perception at one time point (1 min after the stimulation onset), did not control for sham and had not reported whether or not an initial ramping up of the stimulation intensity had been performed, further investigation is deemed necessary.

Whether the subject perceives sensations associated with the sham and/or verum stimulation only initially or throughout the duration of the experiment, may have important consequences, as they can elevate arousal, focus, or divert attention (depending on task difficulty, see: Yerkes and Dodson [7]; Diamond et al. [8]), thus, potentially compromising the validity of the acquired results.

In previous blinding-related studies we have explored the cutaneous perception characteristics of tES. We have probed the cutaneous perception thresholds of tDCS using short stimulation durations in the current intensity range of 200–2000 μA [9], and have probed the blinding potentials of rectangular and circle-shaped electrode configurations [10]. In this present study we aim to investigate the characteristics of the cutaneous perception during the entire course of the stimulation.

## Methods

### *Subjects*

Thirty-six healthy volunteers, students and employees of the University of Göttingen, participated in the study (17 male; age 25.80 ± 4.28). Participants reported no previous history of neurological or psychological disorders, drug or alcohol abuse, and had no metal implants. None of the subjects were taking any chronic or acute medication at the time of the study. All subjects gave written informed consent before participating. The study was conducted with the approval of the ethics committee of the University of Göttingen and in accordance with the guidelines of the Declaration of Helsinki.

As in our previous investigation [9], we have given special attention to the participants' prior knowledge of and exposure to tES stimulation. We have created three experimental groups:

*Group 1. Naïve subjects* ($n$ = 12; 6 male; mean age = 24.66 ± 2.34). Newly recruited participants with no previous experience with tES methods.

*Group 2. Experienced subjects* ($n$ = 12; 6 male; mean age = 26.16 ± 4.60). Participants who took part in at least one study involving tES prior to this current experiment.

*Group 3. Investigators* ($n$ = 12; 5 male; mean age = 26.58 ± 5.43). Investigators at the Department of Clinical Neurophysiology, University of Göttingen, who took part, and also, have conducted experiments involving tES methods.

### *Transcranial direct current stimulation*

Stimulation was delivered by a battery-driven constant current stimulator (neuroConn GmbH, Ilmenau, Germany). The current was transferred by a pair of standard carbon rubber electrodes (Physiomed Elektromedizin AG, Schnaittach, Germany) placed in viscose sponge wrappers soaked in isotonic sodium chloride solution. One electrode was placed over the left supraorbital area; the other electrode, to which polarity refers to, was placed contralaterally, over the C3, the approximate location of the M1. The electrodes were fixed to the head with elastic rubber bands. The stimulator was triggered by a personal computer via parallel port connection.

No special skin surface preparation was performed before the experiment. The rubber bands have been fixed firmly so that the electrodes were not able to move, but no extraneous pressure was applied. Approximately 15 ml of NaCl solution was applied to wet the sponge.

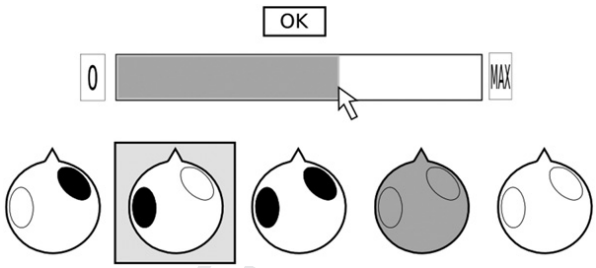


**Fig. 1.** The computerized form used for entering the perceived stimulation strength and the site of the cutaneous perception.

### *Experimental design and data acquisition*

The experiment was conducted according to a sham controlled, double blind, repeated measures design. Each subject participated in three sessions (anodal, cathodal and sham tDCS), in a randomized and counterbalanced order. Two consecutive sessions were separated by an interval of at least four days.

As the standard procedure in our lab requires, a written information sheet and consent form was handed to the participants. It is important to note that this sheet informs the participants that during the stimulation they might perceive a slight itching sensation under the electrodes, and that in rare cases a light and transient headache or skin irritation may occur.

During the experiment participants were seated in front of a computer display and were given a mouse. At the beginning of each session, the investigator and the subject reviewed the experimental workflow. The subject started the stimulation by pressing the space bar. The trial lasted for approximately 10 min, during which real or sham (placebo) stimulation is applied continuously (the nature of the sham stimulation is not disclosed at this point). The task of the subject is, when prompted, to report the perceived strength and site of the cutaneous sensation associated with the stimulation. The subject enters these parameters using a computerized form (Fig. 1), where a horizontal slider and two additional buttons (*no sensation*; *extreme discomfort*) represents the perceived strength, and five buttons represent the site of the sensation. The subject finalizes the form by pressing the OK button, after which the form disappears.

The choices for sites of perception were based on the findings of our previous experiments investigating cutaneous perceptions associated with tES methods, where subjects reported having perceived the sensation either form the orbital electrode, the M1 electrode, from both electrodes, or from the whole scalp surface [9,10].

After reviewing the objectives the investigator fixed the electrodes and tested the devices to ensure normal functioning of the setup. A second investigator in a separate room then programmed the stimulator to give anodal, cathodal, or sham stimulation, according to a randomized, balanced schedule. The first investigator took a position outside the visual field of the participant for the rest of the session, connected the stimulator to the computer, and instructed the subject to start the experiment. During the course of the study the first investigator had no access to the session/stimulation condition schedule, and the second investigator did not come into contact with the subjects during the experimental sessions.

The parameters for the verum and sham stimulation are as follows.

#### *Verum stimulation*

In the case of the verum (anodal and cathodal) stimulation trials, the stimulation intensity was ramped up from 0 to 1 mA in 20 s. The

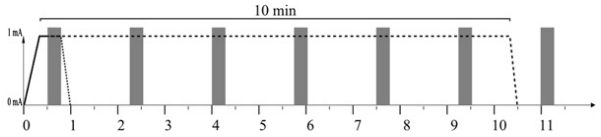


**Fig. 2.** The time course of the sham and verum stimulation. Both conditions begun with a 20 s fade-in phase; in the case of sham stimulation the current was ramped down in 10 s after 30 s of stimulation, in the verum case the stimulation lasted for 10 min, after which it was ramped down in 10 s. During the course of the session the participant was propmted to report the cutaneous perception (grey bars).

current intensity then remained constant for another 10 min (600 s), after which it was ramped down in 10 s.

*Sham stimulation*

In the case of sham stimulation the initial ramp-up phase was also 20 s, after which 30 s of stimulation followed with 1 mA intensity. The current then was ramped down during the following 10 s. The polarity of the sham stimulation was randomized and counterbalanced across groups and conditions.

The participants were prompted to input the cutaneous perception parameters consecutively every 1.75 min; seven times during the verum stimulation interval, starting from 30 s after the beginning of the trial, and once after the verum stimulation had ceased, at 11 min (see Fig. 2).

The double blind procedure, the use of a computer interface to report the sensations, and locating the investigator outside the visual field of the participant during the course of the trial were all conscious measures taken in order to avoid any observer-expectancy biases and verbal or non-verbal cues potentially capable of influencing the subjects' responses.

Before and after the stimulation we have assessed the wakefulness of the subjects using the Stanford Sleepiness Scale (SSS). Furthermore, in a post-stimulation questionnaire we have asked the subjects to describe the perceived sensations during the experiment in their own words. We have also asked them whether the sensations during the stimulation were painful at any time, and if the answer was yes, they had to report the painfulness on an analog scale, ranging from "not painful at all" to "intolerably painful". Then, we have asked them whether they have felt itching, light flashes or headache during, and/or after the stimulation. Finally, we have asked them if, in their opinion, they have received real, or placebo stimulation, and to rate their confidence in this decision on an analog scale ranging from "not sure at all" to "I am certain".

*Data acquisition and analysis*

To assess the effects and interactions of stimulation type, experience and the factor of time on the perceived stimulation strength, we have used a repeated measures analysis of variance (rmANOVA). For each subject in every condition, the ratios of reports identifying the different sites of perception have been calculated. These values have been analyzed using rmANOVA. Greenhouse-Geisser corrections have been applied, where the assumption of sphericity has been violated. Bonferroni-corrected post-hoc analyses have been used. The subjects' groupwise assessments of stimulation conditions were analyzed using Cochrane's Q test.

For each participant, hit and false alarm rates were derived from the responses given during active (at the first trial time-point in the sham condition, when the stimulation was still present, and during all but the last trials in the active conditions) and non-stimulation (all but the first trial in the sham condition, and the last trials in the active conditions) trials. From these values, the d' sensitivity index was calculated for each subject, and a One-Way ANOVA was used to assess group level differences.

For the analysis of the SSS, the differences in the scores before and after the stimulation have been calculated for each participant in every stimulation condition. These values have been analyzed using a Friedman ANOVA.

All statistical comparisons have been conducted with a significance level of 5% ($p < 0.05$).

## Results

All of the subjects completed all three experimental sessions, tolerated the tDCS procedure and reported no side-effects (other than those discussed below) during or after the experimental sessions.

*Qualitative assessment of the sensations associated with the stimulation*

After the stimulation we asked the subjects to fill in both an open-ended questionnaire and a checklist of some of the commonly reported sensations (itching, pain, light flashes, headache) associated with tDCS. The reports of the sensations from both sources have been combined, and are presented in Table 1. The most prominent sensation was itching, reported in 85.1% of the cases. Pain was reported in 24% of the sessions. The strength of the pain sensation has also been assessed; see Fig. 3. Burning was reported in 11.1% of the sessions, while tingling was reported in 16.6% and headache in 9.2% of the sessions. Prickling was reported in 2.7% of the sessions. None of subjects reported seeing light flashes during the stimulation.

Cochrane's Q test did not show any difference between subject groups and stimulation conditions regarding *itching* (all groups: $Q = 1.555$; df $= 2$; $p < 0.459$; naïve group: $Q = 2$; df $= 2.000$; $p < 0.367$; experienced group: $Q = 2.666$, df $= 2$, $p < 0.263$; investigator group: $Q = 2.800$; df $= 2$; $p < 0.246$). Also, no such differences could be observed in the case of *pain* (all groups: $Q = 0.347$; df $= 2$; $p < 0.840$; naive group: $Q = 3.714$, df $= 2$; $p < 0.156$; experienced group: $Q = 0.222$; df $= 2$; $p < 0.894$; investigator group: $Q = 0.142$; df $= 2$; $p < 0.564$). No significant differences were observable regarding *headache* (all groups: $Q = 5.600$; df $= 2$; $p < 0.060$; naive group: $Q = 2.666$; df $= 2$; $p < 0.263$; experienced group: $Q = 3.500$; df $= 2$; $p < 0.173$; investigator group: $Q = 0.666$; df $= 2$; $p < 0.716$). Also, there was no difference regarding *burning sensation* (all groups: $Q = 4.800$; df $= 2$; $p < 0.090$; naive group: $Q = 2.000$; df $= 2$; $p < 0.367$; experienced group: $Q = 2.000$; df $= 2$; $p < 0.367$; investigator group: $Q = 2.666$; df $= 2$; $p < 0.263$).

*Differences in perceived discomfort between stimulation conditions*

The rmANOVA revealed a main effect of stimulation condition ($F\,[(2, 66] = 7.739$; $p = 0.001$). Bonferroni post-hoc tests revealed that the perceived strength of stimulation in the sham condition was significantly lower than it was in both verum stimulation conditions (anodal: $p = 0.002$; cathodal: $p = 0.006$), and that was no significant difference between the anodal and cathodal stimulation conditions in that regard ($p = 1.000$).

*Group level differences*

The repeated measures ANOVA has shown no main effect of subject group ($F\,[2, 33] = 0.254$; $p = 0.776$). The rmANOVA revealed an interaction between group and stimulation type ($F$ [4, 66] $= 2.501$, $p = 0.050$). A Bonferroni-corrected post-hoc analysis

**Table 1**
Reports of perceived phenomena associated with the stimulation. The table also shows the number and percentage of the subjects correctly identifying the given stimulation session as real or placebo ("identified"), and the number and percentage of subjects who identified all of the stimulation sessions correctly ("all correct").

| | | Itching | | Tingling | | Burning | | Pain | | Headache | | Prickling | | SSS difference | Identified | | All correct | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | n | % | n | % | n | % | n | % | n | % | n | % | Mean ± SD | n | % | n | % |
| Naive $n = 12$ | Anodal | 10 | 83.33 | 0 | 0.00 | 0 | 0.00 | 2 | 16.67 | 0 | 0.00 | 0 | 0.00 | 0.33 ± 0.89 | 11 | 91.67 | | |
| | Cathodal | 11 | 91.67 | 0 | 0.00 | 1 | 8.33 | 3 | 25.00 | 0 | 0.00 | 1 | 8.33 | 0.00 ± 0.43 | 10 | 83.33 | 1 | 8.33 |
| | Sham | 11 | 91.67 | 0 | 0.00 | 0 | 0.00 | 3 | 25.00 | 1 | 8.33 | 0 | 0.00 | −0.42 ± 0.79 | 2 | 16.67 | | |
| Experienced $n = 12$ | Anodal | 11 | 91.67 | 1 | 8.33 | 0 | 0.00 | 1 | 8.33 | 0 | 0.00 | 0 | 0.00 | −0.58 ± 0.89 | 10 | 83.33 | | |
| | Cathodal | 9 | 75.00 | 2 | 16.67 | 1 | 8.33 | 4 | 33.33 | 1 | 8.33 | 0 | 0.00 | −0.58 ± 2.07 | 9 | 75.00 | 2 | 16.67 |
| | Sham | 9 | 75.00 | 1 | 8.33 | 0 | 0.00 | 3 | 25.00 | 1 | 8.33 | 0 | 0.00 | −0.17 ± 1.85 | 2 | 16.67 | | |
| Investigator $n = 12$ | Anodal | 10 | 83.33 | 4 | 33.33 | 4 | 33.33 | 5 | 41.67 | 2 | 16.67 | 1 | 8.33 | 0.08 ± 0.79 | 11 | 91.67 | | |
| | Cathodal | 12 | 100.00 | 4 | 33.33 | 4 | 33.33 | 4 | 33.33 | 3 | 25.00 | 1 | 8.33 | −0.18 ± 0.40 | 11 | 91.67 | 8 | 66.67 |
| | Sham | 9 | 75.00 | 6 | 50.00 | 2 | 16.67 | 1 | 8.33 | 2 | 16.67 | 0 | 0.00 | −0.08 ± 0.51 | 10 | 83.33 | | |

has shown significant differences between the sham and verum stimulation conditions (anodal: $p = 0.002$; cathodal: $p = 0.004$) in the investigator group, with perceived stimulation strength in sham condition being markedly lower. No such differences have been observed in the naïve or the experienced group (all comparisons: $p = 1.000$).

*Sensitivity index*

Groupwise comparison of the d' values using a One-Way ANOVA revealed a significant main effect ($F$ [2, 33] = 7.067; $p = 0.002$). Bonferroni-corrected post-hoc comparisons have shown that the difference between the investigator group (mean = 2.168 ± 0.968) and the naïve (mean = 0.749 ± 1.153; $p = 0.013$) and the experienced (mean = 0.572 ± 0.1.278; $p = 0.004$) groups was significant, but no significant difference could be observed between the naive and the experienced groups ($p = 1.00$).

*Subjects' assessments of stimulation type*

Taken the whole sample into consideration, Cochrane's Q yielded a significant difference in the subjects' assessment of stimulation type ($Q = 8.10$; df = 2; $p < 0.017$). Group-level analysis of the responses did not find any differences regarding the naïve ($Q = 0.50$; df = 2; $p < 0.77$) or the experienced ($Q = 0.33$; df = 2; $p < 0.84$) group. In the investigator group, however, we have found that the "placebo" answers in the sham condition significantly outweighed those in the in the verum conditions ($Q = 16.20$; df = 2; $p < 0.000$).

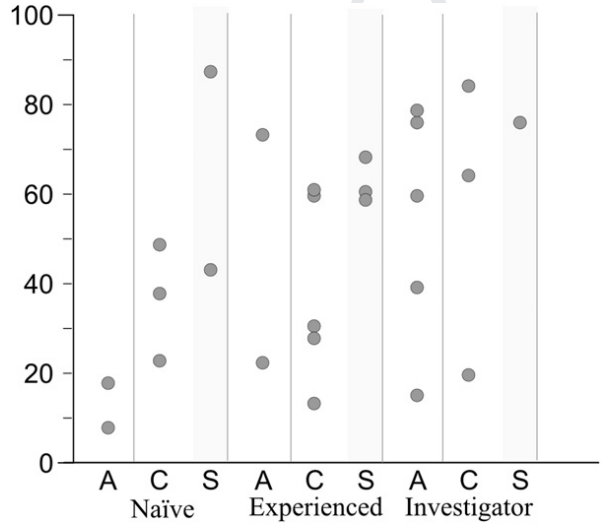


**Fig. 3.** Strength of perceived pain. The figure shows individual data for all subject groups and stimulation conditions (A: anodal, B: cathodal, S: sham).

Investigating how sure participants were in their assessment of the type of stimulation, rmANOVA did not show a significant main effect of group ($F$ [2, 33] = 1.243; $p = 0.301$), type of the received stimulation ($F$ [2, 66] = 0.342; $p = 0.711$), or an interaction of group and stimulation type ($F$ [4, 66] = 0.241; $p = 0.913$). Table 1 shows the number of subjects in each group and stimulation condition who have identified the stimulation session correctly as real or placebo stimulation.

*Time-course of the reported perception of stimulation strength*

The rmANOVA, corrected for sphericity (Mauchly's test: $\chi^2$ [22] = 136.48; $p = 0.000$), using a Greenhouse-Geisser correction ($\varepsilon = 0.37$), revealed a significant main effect of time ($F$ [2.24, 74.04] = 38.063; $p = 0.000$), and the Bonferroni-corrected post-hoc analysis revealed consecutive drops in perceived strength in the first three trials (4 min, $p < 0.007$); the change in perceived strength in the remaining trials was not shown to be significant ($p > 0.9$).

The time course of the reported strength of the stimulation in the different stimulation conditions has been found to be significantly different. An rmANOVA, corrected for sphericity (Mauchly's test: $\chi^2$ [77] = 184.06; $p = 0.000$) using the Greenhouse-Geisser correction ($\varepsilon = 0.53$) found a significant interaction between stimulation type and time ($F$ [8.71, 287.61] = 1984; $p = 0.042$). A post-hoc Bonferroni test was used to explore the differences in and between the stimulation conditions. No significant differences have been observed between the stimulation conditions during the first time-point (all comparisons: $p = 1.000$). In the case of sham stimulation condition, the reported strength of the stimulation dropped significantly in the subsequent trials (from the 2.25$^{th}$ minute, all comparisons: $p = 0.000$). In the case of the anodal condition, the drop in the perceived stimulation strength reached significance form the third (4 min, all comparisons: $p = 0.000$), and in the cathodal condition, the fourth time-point (5.75 min, all comparisons: $p = 0.000$). The time course of the perception of stimulation strength in all conditions and groups is shown in Fig. 4.

As investigators have shown to be more capable of discriminating between sham and active trials, and as investigators are generally not assumed to form a significant part of an experimental sample, we have also performed a second rmANOVA on a dataset containing only the responses of naïve and experienced subjects. This analysis yielded only a significant main effect of time (Greenhouse-Geisser [$\varepsilon = 0.36$] corrected, $F$ [2.162, 47.582] = 15.021;

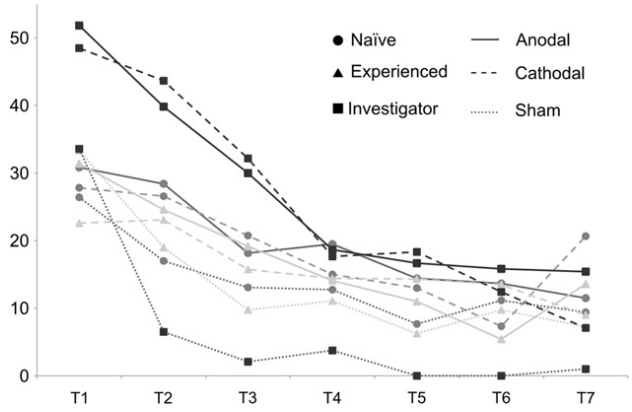


**Fig. 4.** The time course of the perceived stimulation strength for all subject groups and stimulation conditions.

$p = 0.000$), with post-hoc Bonferroni analysis showing a significant decline in perceived strength from the third trial (4 min), when compared to the first trial (all comparisons: $p = 0.000$). No main effects of stimulation type (Greenhouse-Geisser [$\varepsilon = 0.931$] corrected, $F$ (1.862, 40.984) = 1.241; $p = 0.297$) or subject group ($F$ [1, 22] = 0.084; $p = 0.773$) were observed, furthermore, no interactions between stimulation type and subject group ($F$ [2, 44] = 0.108; $p = 0.897$), subject group and time ($F$ [6, 13] = 0.166; $p = 0.985$), stimulation type and time (Greenhouse-Geisser [$\varepsilon = 0.513$] corrected, $F$ [6.164, 135.616] = 0.943; $p = 0.467$), or stimulation type, time, and subject group (Greenhouse-Geisser [$\varepsilon = 0.513$] corrected, $F$ [6.164, 135.616] = 0.967; $p = 0.451$), have been observed.

*Reported sites of cutaneous perception*

Analyzing the ratios of the sites of perception, the rmANOVA has shown a main effect of stimulation site (Greenhouse-Geisser [$\varepsilon = 0.734$] corrected, $F$ [2.202, 72.684] = 13.561; $p = 0.000$) and an interaction between the stimulation condition and the stimulation site (Greenhouse-Geisser [$\varepsilon = 0.595$] corrected, $F$ [3.573, 117.916] = 2.321; $p = 0.002$). Bonferroni-corrected post-hoc test have shown that the orbit was significantly more frequently identified as the site of stimulation than the other locations ($p < 0.03$), the M1 and both electrodes was more frequently reported than the whole scalp ($p = 0.003$ and $p = 0.006$, respectively), and that there was no difference in the rate of M1 and both electrodes responses ($p = 1.000$).

*Sleepiness scale*

Friedman's ANOVA did not reveal any significant differences between the changes in SSS scores before and after the stimulation between the stimulation conditions in any subject group ($\chi^2$ [2] = 2.065; $p = 0.356$).

## Discussion

In this study we have assessed the cutaneous perception characteristics of verum and sham tDCS stimulation during a 10 min interval.

Regarding the site of the perceived stimulation, our results have shown that the orbit is the most frequently identified location. This finding is in agreement with our previous data [9], and with those of Dundas et al. [4] and may be related to greater skin sensibility of the forehead compared to the piliferous skin under the M1 electrode.

In our study, itching was the most often reported sensation associated with the stimulation. We have however found no significant differences in the case of the rate of responses regarding itching, pain, and headache.

Our results indicate that in the case of naive and experienced subjects, sham stimulation is indistinguishable from verum stimulation regarding both perception of stimulation strength and assessment of stimulation type. In the case of investigators, however, sham stimulation significantly more often identified as "placebo", and it also differed in perceived stimulation strength.

In the light of these results, we can still consider this method of blinding efficient, but not because the sham fade-out phase mimics the presumed disappearance of the sensations in the verum stimulation conditions, instead, the cutaneous sensations associated with the sham stimulation persist after the ramp-down phase.

The fact that the sensations associated with both verum and sham stimulation can be perceived throughout the experiment may have methodological implications. As first described by Yerkes and Dodson [7] in mice, for every task there exists an optimal level of arousal, above and beyond which performance is going to decline. This optimal level of arousal changes with the type of task in question; it is lower in tasks that burden attention and cognitive resources, while it is higher in tasks that are less demanding.

Possible correlates of the interaction between arousal/stress levels and task performance in the prefrontal areas are extensively discussed in a review article by Diamond and colleagues [8], who propose that if the task performance relies on the PFC, a higher level of arousal is more likely to have a detrimental effect on performance, while a task less reliant on PFC-mediated cognitive processes benefit from increased arousal. Our initial results measuring wakefulness using the SSS did not show a difference between any of the stimulation conditions in any of the groups. This result is consistent with the findings reported by Gandiga and colleagues [6], who found that ratings of attention have not shown to be significantly altered by stimulation. It is conceivable that these kinds of self-reported questionnaires are not sensitive enough to show changes in attention and arousal *during* stimulation. Further behavioral studies, such as measurements of vigilance using varying levels of difficulty may tell more about the influence of the stimulation-related procedural discomfort on task performance.

## Limitations

In an experiment by van Laarhoven et al. [11] it has been shown that verbal suggestions can enhance the nocebo effect regarding itching and pain: when participants are told that most people experience itching or pain when exposed to a procedure, they are more likely to report an itching/pain sensation when exposed to the stimulus in question, compared to the participants who have been given the information that only a minority of the subjects experience itching/pain. Our consent form/information sheet informed the subjects that they might experience an itching sensation under the electrode; an information that may elevate their expectations, and thereby the actual number of the reports in both the verum and the sham stimulation conditions, regarding itching. It has to be noted that it is required that the participants must be informed about the circumstances and the potential adverse effects of the intervention, thus the potential expectation effects should also be present in the all studies using tDCS.

Similarly to experiments investigating the effects of electrical stimulation on the motor system, our subjects during this experiment were idle during the stimulation. In other experiments, such as many of those assessing the influence of stimulation on aspects

of cognition, the stimulation is applied during task performance. In that setting, it can be argued that the execution of a task may help the habituation to the sensations associated with the stimulation by diverting attention.

Also, during our experiment the participants have been instructed to pay attention to and report the sensations and this might have hindered habituation to the associated sensations, elevating the level of the perceived sensations.

Another factor confounding our results may be the acquiescence bias, that is, the participants' tendency to confirm with the questions asked or to indicate a positive connotation. We cannot rule out the possibility that the acquiescence bias can elevate the reported level of procedural discomfort, both in the verum and in the sham stimulation condition.

## Conclusion

We have found that investigator participants could more easily distinguish between verum and sham trials; this finding has to be taken into account when investigators are used in e.g. pilot studies.

Our investigation supports the use of the fade-in – short stimulation – fade-out approach to sham stimulation. It should be noted, however, that the reported strength of the perception decreased significantly with time, contrary to the observations reported previously, as most naïve and experienced subjects did not perceive the disappearance of the sensations after the initial phase of the verum stimulation procedure, and the sensations associated with the stimulation also persisted in the sham stimulation condition.

## Funding

This study was initiated and funded by an unrestricted grant awarded by the Rose Foundation and by the German Ministry for Education and Science via the Bernstein Centre for Computational Neuroscience (BCCN), Göttingen (Grant No. 01GQ0432).

## Conflicts of interest

The authors declare no conflicts of interest.

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# Glossary

**BDNF**: Brain-derived neurotrophic factor

**DLPFC**: Dorsolateral Prefrontal Cortex (also, lDLPFC, rDLPFC: left and right DLPFC, respectively)

**EMLA**: Eutectic mixture of local anesthetics

**FiSsFo**: Fade-in – Short duration stimulation – Fade-out; the most widely used method of sham stimulation in tES research.

**NIBS**: Non-invasive brain stimulation, e.g. TMS, TES, tES, transcranial ultrasound stimulation, transcranial static magnetic stimulation

**M1**: Primary motor cortex

**MEP**: Motor evoked potential.

**SNP**: Single nucleotide polymorphism

**tACS**: Transcranial Alternating Current Stimulation

**tDCS**: Transcranial Direct Current Stimulation

**tES**: Low-intensity transcranial electrical stimulation, e.g. tDCS, tACS, tRNS. (See also: TES)

**TBS**: Theta burst stimulation (also, cTBS, iTBS: continuous and intermittent TBS. respectively)

**TES**: Supra-threshold transcranial electrical stimulation

**TMS**: Transcranial Magnetic Stimulation (also, rTMS: repetitive TMS)

**tRNS**: Transcranial Random Noise Stimulation

# Index of Images and Tables

# Acknowledgements

I would like to express my deepest gratitude to my advisor, Prof. Dr. Andrea Antal. Without her constant support and guidance this thesis would not have been possible. I am also greatly indebted to Prof. Dr. Walter Paulus, who has been an invaluable source of inspiration, encouragement and constructive criticism throughout my studies.

I would like to thank Prof. Dr. Uwe Mattler and Prof. Dr. Michael Waldmann, members of my thesis committee, for their support and guidance during my work.

It is a pleasure to thank Giorgi Batsikadze, Dr. Klára Boros, Dr. Leila Chaieb, Dr. Shane Fresnoza, Dr. Olga Gamboa, Dr. Jessica Grundey, Manuel Hewitt, Dr. Katalin Jánossy, Dr. Min-Fang Kuo, Dr. Bence Laczó, Dr. Kátia Machado, Dr. Vera Moliadze, Dr. Nicole Neef, Dr. Rafael Polanía, Dr. Csaba Poreisz, Dr. Holger Rothkegel, Catarina Saiote, Dr. Nivethida Thirugnanasambandam, and Zsolt Turi, my colleagues and friends at the Department of Clinical Neurophysiology.

I also would like to thank my students, Hanan Al-Moyed, Christin Fritsche, Lauren Haag, Viktória Kállay, Maike Pelgrim, Lena Sarp, and Roman Stilling, for their

enthusiastic and truly outstanding performance. I have greatly enjoyed working with all of you.

I owe my deepest gratitude to my family and Ágnes Dekker for their patience, encouragement and untiring support.

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# Publications

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