METHODOLOGICAL AND COGNITIVE ASPECTS OF TRANSCRANIAL ELECTRICAL STIMULATION

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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 quoted in the text, contributions, references and acknowledgements.

Göttingen, 11th February 2015

Zsolt Turi

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Chapter 1: General Introduction

Throughout human history, there has been a continuous desire to understand how the human mind works. It might be surprising for us nowadays but the brain was not always in the main focus of philosophers when contemplating the human intellect. The overwhelming majority of antique philosophers, for instance, associated emotions, personality and certain cognitive functions with various internal organs, such as the liver, the kidney or the spleen. Aristotle himself assumed that the mind or the 'rational soul' as he called it, was predominantly controlled by the heart, and that the brain had only trivial roles in the body, such as cooling the blood (Gross, 1995). Through history, important progress had to be made before the concept of the antique anatomists, Herophilus and Erasistratus (i.e. that the brain controls intellect), became gradually accepted among scientists and grew into a fact generally agreed upon (Bay & Bay, 2010). Among other important anatomical and medical discoveries, well documented brain lesions and their observed behavioral consequences drew the attention of scientists in the early 19th century to the brain, but they were unable to investigate it exhaustively due to the lack of techniques.

In the 20th century, the technical progress in medicine accelerated exponentially, and a number of different approaches were introduced that now enable scientists to directly study processes in the human brain in a more refined fashion. Some of these approaches, such as electroencephalography (EEG; Berger, 1929), positron emission tomography (PET; Sweet, 1951; Wrenn, Good, & Handler, 1951), and functional magnetic resonance imaging (fMRI; Ogawa et al., 1992; Ogawa, Lee, & Kay, 1990) provide fascinating methods to observe the electrophysiological or brain activation correlates of ongoing brain processes. Other approaches are aimed at inducing perturbations in the brain and observing their functional consequences. This can be achieved by means of invasive (direct electrical stimulation;

Penfield, 1937) or non-invasive brain stimulation (NIBS) techniques, such as by transcranial magnetic stimulation (TMS; Barker, Jalinous, & Freeston, 1984) or transcranial direct current stimulation (tDCS; Nitsche & Paulus, 2000; Priori, Berardelli, Rona, Accornero, & Manfredi, 1998). Although the scientific relevance of invasive brain stimulation techniques both as clinical and research tools cannot be questioned (but see: Borchers, Himmelbach, Logothetis, & Karnath, 2011), they are less suitable methods for everyday research in healthy participants due to their invasiveness and expense.

Fortunately, NIBS techniques offer the potential to induce perturbations in the central or peripheral nervous system with a minimum of possible health risks, ethical concerns and cost (but see: Cohen Kadosh, Levy, O'Shea, Shea, & Savulescu, 2014). Externally controlled perturbations can be achieved non-invasively by electromagnetic induction, such as in TMS (Barker et al., 1984), while other techniques, such as transcranial electric stimulation (tES) pass a current between two or more electrodes attached on the scalp (Antal et al., 2008; Nitsche & Paulus, 2000; Priori et al., 1998).

1.1. Transcranial Electric Stimulation (tES)

Transcranial electrical stimulation (tES) techniques are based on the external application of low-intensity electrical current to the brain. The external current can modulate cortical excitability by depolarizing or hyperpolarizing resting membrane potentials, thereby modulating the spontaneous firing rate, as is the case with tDCS (Nitsche & Paulus, 2000). In transcranial alternating current stimulation (tACS), the externally applied alternating current is thought to entrain endogenous neural oscillations, possibly by increasing the power of the oscillations or the phase-locking index between the driving and the endogenous oscillations (Helfrich et al., 2014; Neuling, Rach, & Herrmann, 2013).

Evaluating the functional consequences of external manipulations makes tES techniques uniquely suitable for causal inference, an interpretation process intended to separately identify causes and consequences (Sober, 1998). The observation of the physiological or behavioral consequences of tES, e.g. on cortical excitability or on brain functions, provides an exceptional method to gain further insight into the functional role of a given brain region and into how brain processes emerge in anatomically distributed but functionally connected brain networks. Not surprisingly, tES is often combined with imaging methods such as PET, fMRI, or EEG in order to exploit the complementary advantages of the different approaches (see: Miniussi, Brignani, & Pellicciari, 2012; Catarina Saiote, Turi, Paulus, & Antal, 2013 for reviews). Importantly, scientists not only gain crucial information about their area of primary interest (e.g. brain processes) but, in addition, about the mechanism of action of tES itself. These two information sources are often combined to formulate and test new hypotheses.

1.2. Transcranial Direct current Stimulation (tDCS)

Transcranial direct current stimulation (tDCS) is the most frequently employed research tool in studies that use tES as a NIBS technique (for a review see: Nitsche et al., 2008). The application of tDCS requires a minimum of two electrodes; one surface positive electrode (anode) and one surface negative electrode (cathode). The typical tDCS stimulus current is 1.0 mA, but the generated electric field in the brain is reduced due to the shunting effect of the scalp. It is estimated to be approximately 1.0 mV/mm for 1.0 mA applied externally (Datta et al., 2009; Reato, Rahman, Bikson, & Parra, 2010).

There is evidence at the cellular level that anodal and cathodal tDCS affects different cellular compartments with different polarities (Radman, Ramos, Brumberg, & Bikson, 2009; Rahman et al., 2013). Brain slice experiments suggest that anodal tDCS hyperpolarizes the membrane potential in the apical dendritic regions and depolarizes it in the somatic region,

while the cathodal electrode has an opposite effect (e.g., Radman et al., 2009). The stimulation effect on cortical excitability is usually quantified by measuring the amplitude of the motor-evoked-potentials (MEPs) induced by single-pulse TMS. Physiological studies involving the motor cortex showed that cortical excitability increased after approximately 10 minutes of anodal stimulation and decreased after cathodal stimulation (Nitsche & Paulus, 2001). Thus, the after-effects measured by TMS-evoked MEPs seem to reflect somatic depolarization and hyperpolarization, respectively, although the currently accepted hypothesis of the mechanism of action at the cellular level requires further confirmation (Radman et al., 2009; Rahman et al., 2013).

The on-line effects, i.e. those during stimulation, and the long-lasting after-effects, i.e. those after stimulation, induced by tDCS are typically evaluated by pharmacological studies combined with TMS evoked MEP measures (for a review see: Stagg & Nitsche, 2011). Earlier studies have shown that the ongoing effects of tDCS are based on the activity of voltage-dependent calcium and sodium channels (Nitsche et al., 2003). Evidence also suggests that the immediate effects of tDCS may not involve synaptic plasticity processes, since blocking glutamatergic or gamma-aminobutyric acid (GABA) activity had no influence on the modulatory effect on cortical excitability induced by tDCS (Nitsche et al., 2003; Nitsche, Liebetanz, et al., 2004). Studies demonstrated that the long-lasting after-effects of tDCS primarily involve glutamatergic activity (Nitsche et al., 2003). The N-methyl-D-aspartate (NMDA) receptor antagonist dextromethorphan blocked the long-lasting after-effects induced by both anodal and cathodal tDCS (Nitsche et al., 2003). In addition, D-cycloserine, an NMDA receptor agonist, prolonged the effects of tDCS on cortical excitability (Nitsche, Jaussi, et al., 2004). Subsequent studies confirmed that neuromodulators, such as dopamine or serotonin also influence the long-lasting after-effects of tDCS (Nitsche et al., 2006, 2009).

Considering the effects of tDCS at the neurotransmitter level, magnetic resonance spectroscopy (MRS) studies, with which one can measure local cortical GABA concentrations have shown that the primary physiological effect of anodal tDCS may be, at least partially, driven by a decrease in local cortical GABA concentrations (e.g., Stagg et al., 2009). Anodal tDCS significantly decreases GABA concentrations, whereas the Glx signal, the signal that does not differentiate between glutamate and glutamine, remains unchanged. On the other hand, the inhibitory cathodal tDCS effect appears to be driven by a reduction in excitatory glutamatergic signaling, due to a decreased conversion of glutamine to glutamate. Hence, cathodal tDCS leads to a significant decrease in glutamate, with a corresponding decrease in GABA (Stagg et al., 2009).

The above-mentioned evidence indicates that the long-lasting after-effects of tDCS involve changes in synaptic plasticity. Synaptic plasticity refers to the capacity of the brain to modify the efficacy of information transmission between synapses in an experience-dependent manner (for a review see: Citri & Malenka, 2008). Synaptic plasticity has been proposed to play a crucial role in forming long-term memory traces and therefore plays a key role in multiple learning processes (Pastalkova et al., 2006; Whitlock, Heynen, Shuler, & Bear, 2006). Long-term potentiation (LTP) and long-term depression (LTD) are two forms of long-term synaptic plasticity (Citri & Malenka, 2008). NMDA or GABA receptors play an important role in long-term plasticity (for an overview see: Malenka & Bear, 2004), similar to the after-effects induced by tDCS (Nitsche et al., 2003). Based on the similarities between LTP/LTD and the neurobiological mechanisms involved in the long-lasting after-effects of tDCS, it was proposed that the after-effects of tDCS are mediated by LTP- and LTD-like plasticity. From this it follows that tDCS is a potentially interesting tool for modulating learning-related processes in the motor and cognitive domains.

The functional effects of tDCS have been demonstrated in the motor, visual and cognitive domains (for reviews see: Jacobson, Koslowsky, & Lavidor, 2012; Reis et al., 2008). Polarity-specific after-effects of tDCS were observed in the motor domain, where anodal tDCS led to improved motor learning and cathodal tDCS to impaired motor function (Reis et al., 2008). On the other hand, the polarity-specific effects of tDCS in the cognitive domain seem to be less consistent (Jacobson et al., 2012), although it is generally assumed that anodal tDCS improves and cathodal tDCS decreases cognitive abilities. The enhancing effect of anodal tDCS was demonstrated on various cognitive functions including working memory (e.g., Sandrini, Fertonani, Cohen, & Miniussi, 2012; Zaehle, Sandmann, Thorne, Jäncke, & Herrmann, 2011), executive functions (e.g., Dockery, Hueckel-Weng, Birbaumer, & Plewnia, 2009), declarative memory (e.g., Javadi & Walsh, 2012) and implicit learning (e.g., de Vries et al., 2009). But, on the other hand, anodal tDCS has also been shown to impair categorization (e.g., Ambrus, Zimmer, et al., 2011). Likewise, cathodal tDCS has been shown to decrease performance of working memory (e.g., Berryhill, Wencil, Branch Coslett, & Olson, 2010; Marshall, Mölle, Siebner, & Born, 2005) and verbal fluency, (Iyer et al., 2005), but to enhance executive functions (Dockery et al., 2009) and complex motion perception (Antal et al., 2004). Some experiments have found that cathodal tDCS had no effect (e.g., Cerruti & Schlaug, 2008), whereas in other studies it even led to behavioral improvement (e.g., Antal et al., 2004).

The reasons for the relatively large variability of the results in the cognitive domain compared to the motor domain are far from understood (but see: López-Alonso, Cheeran, Río-Rodríguez, & Fernández-Del-Olmo, 2014; Wiethoff, Hamada, & Rothwell, 2014 for variability on the motor domain). So far, two main hypotheses have been proposed to explain the inconsistent results of tDCS on the behavioral level and both of them concentrate on the effect of cathodal tDCS.

According to one hypothesis, one reason for the inconsistent results might be that the polarity effects of tDCS on the cognitive domain are further modulated by the neural state of the region being stimulated (Jacobson et al., 2012). This might be because the stimulated region is already activated by the cognitive task and the application of the low-intensity cathodal current might not generate sufficient inhibition that would lead to decreased cognitive performance (Jacobson et al., 2012).

The alternative proposal focuses on the enhancing effect of cathodal tDCS (Antal et al., 2004). It proposes that the behavioral effects could depend on the complex relationship between some features of the task and the induced activity pattern in the associated neural regions (Antal et al., 2004). In a coherent motion detection task, moving dots are presented with coherent motion (e.g. 40 % of the dots move in the same direction) or incoherent or random motion (e.g. 60 % of the dots move in random directions). It is assumed that this task evokes a complex activity pattern in V5 that represents the different directions with different degrees of activation-levels. For the random dots, this activity pattern is assumed to be reduced compared to the coherently moving dots. Cathodal stimulation may improve motion detection performance possibly by sufficiently inhibiting those activity patterns that are suboptimal, i.e. that encode the incoherent motion, which may in turn improve the motion detection of the coherent dots (Antal et al., 2004). Nevertheless, both of these explanations are unspecific in nature and additional investigations are needed to develop a conceptual framework that links cortical excitability changes caused by tDCS to the behavioral level and provide testable hypotheses.

1.3. Transcranial Alternating Current Stimulation (tACS)

The existence of a link between cortical oscillations and behavior, i.e. motor or cognitive performance, was discovered long ago (for a review see: Engel, Fries, & Singer, 2001), but the idea of interfering non-invasively with the physiologically relevant, ongoing oscillation via repetitive TMS (rTMS; Thut et al., 2003) or tACS (Antal et al., 2008) has only recently been introduced into human neuroscience. Due to its ability to modulate the power of oscillation or the oscillation synchrony of a group of neurons in a frequency-specific manner, tACS has the potential to be used in the study of basic but intriguing scientific questions, such as whether brain oscillations are only mere epiphenomena or are causally related to behavior (for a review see: Herrmann, Rach, Neuling, & Strüber, 2013).

The physiological mechanisms by which tACS acts are less well understood than those of tDCS, but studies so far have shown that tACS applied at a 1.0 mA peak-to-peak amplitude can entrain neural oscillations. This effect may be achieved by increasing the phase-locking values between the endogenous activity and the external stimulation (Helfrich et al., 2014). tACS was shown to increase the amplitude of a specific EEG frequency by applying an external frequency closely matched to the rhythm of the endogenous oscillation (Neuling et al., 2013). This is in accordance with the results of neocortical slice preparation experiments (Fröhlich & McCormick, 2010; Schmidt, Iyengar, Foulser, Boyle, & Fröhlich, 2014).

The amplitude of endogenous EEG oscillations were found to be increased in a frequency-specific and brain state-dependent manner (Helfrich et al., 2014; Neuling et al., 2013). Computational network simulation studies combined with in vitro experiments have demonstrated the possibility of entraining neural oscillations by applying external electric fields of relatively low amplitudes (minimum estimated cortical electric field of 0.2 mV/mm), if the externally applied electric field closely matched the intrinsic frequency (Fröhlich & McCormick, 2010; Reato et al., 2010; Schmidt et al., 2014). In accordance with these

findings, human EEG experiments provide further support for neural entrainment by demonstrating that individual alpha frequency (10 ± 2 Hz) tACS increased the EEG alpha amplitude after 10 minutes of stimulation (Zaehle, Rach, & Herrmann, 2010). Another study showed that the after-effects of tACS is brain state dependent (Neuling et al., 2013). It was only effective when the externally applied alpha amplitude exceeded the endogenous alpha oscillation amplitude. More recently, neural entrainment by tACS was demonstrated for the first time by simultaneously recording EEG during stimulation (Helfrich et al., 2014). This study showed that the ongoing oscillatory power was increased over pre-stimulation levels during 10 Hz tACS specifically in the alpha, but not in the theta or beta band. The increase in alpha power after tACS lasted longer than the stimulation. Phase-locking values between intrinsic and driving frequency were increased in the alpha band but not in the other bands (Helfrich et al., 2014).

The examples given above consistently indicate that it is possible to entrain the endogenous neural oscillations with tACS, provided that the externally applied frequency closely matches the endogenous frequency. The induced oscillation changes were found to have frequency-specific effects, that is, the after-effects were restricted to the alpha frequency band and did not influence neighboring theta and beta bands. Hence, tACS is a potentially relevant tool for studies investigating the effect of neural oscillations on cognition and motor functions.

1.4. Limitations of tES Techniques

Up to this point, I have highlighted the considerable potential that tES has to offer for advancing research in neuroscience. However, it is important to recognize its limitations and their various sources. Two main sources of limitations will be described in the following

summary, both of which are related to the causal inference property of tES. One important source of limitation is conceptual in nature and general in the sense that it arises from the interpretative framework of the brain when functional inferences are derived from the perturbing effects of tES (Sober, 1998). Strictly speaking, this is not a limitation of the tES technique itself but a limitation arising from an inadequate application of the inference strategy per se. In this thesis I will put less emphasis on this limitation and describe it only in passing.

A second limitation is methodological in nature and is related to the application of tES in an experimental context. It is essential that both investigator and participant are unaware of, i.e. are blinded to the experimental set-up, a precaution referred to as double-blinding. When applying tES, an important factor is how to ensure blinding in face of the fact that stimulation can cause cutaneous (e.g., Ambrus et al., 2012) and visual (i.e., phosphenes; only with tACS) sensations (e.g., Schutter & Hortensius, 2010). These procedural sensations present a challenge in designing tES studies that meet the requirements for a randomized, placebo controlled double-blind study design. This limitation is directly related to tES and will form the main focus of this chapter.

Regarding the first limitation mentioned above, it is widely assumed that brain processes are non-linear, as the brain is a complex hierarchical system with multiple, temporarily nested anatomically and/or functionally interconnected systems and subsystems (Engel et al., 2001; Roux & Buzsáki, 2014). Therefore, perturbing one region of a system will most possibly also affect other, functionally connected areas. Accumulating evidence from neuroimaging studies suggests that tES and TMS alter neural activity beyond the boundaries of the directly stimulated areas. This is known as the network-effect of tDCS (Antal, Polania, Schmidt-Samoa, Dechent, & Paulus, 2011). Stimulating the primary motor cortex, for example, can influence the activity of the supplementary motor area or of even remotely connected

subcortical regions (Antal et al., 2011; Polanía, Paulus, & Nitsche, 2012). The consequence is that inferring functionality of a given brain region in an isolated manner without considering its elicited network effects might be difficult or even impossible to accomplish using tES (similarly to TMS; O'Shea, Thut, & Bestmann, 2012).

Related to this but as a separate argument, recent realistic finite element models (FEM) raise similar concerns about the possibility of 'directly' stimulating a given region because the current flow profile of tDCS at the macroscopic level seems to not be restricted to the area directly underneath the electrodes but rather spreads around this area and into the neural tissue between the electrodes (Miranda, Mekonnen, Salvador, & Ruffini, 2013; Ruffini, Fox, Ripolles, Miranda, & Pascual-Leone, 2014). Moreover, the phrase 'directly stimulate' is vague because the principal mechanism of action of tDCS or tACS at the cellular level is still elusive. It is not exactly known which morphological part of principal and interneurons, and which layers of these, are 'directly stimulated' by tES (Radman et al., 2009). Future experimental work may focus on improving our understanding of the stimulated morphological structures, as well as the effects on various brain regions.

As mentioned above, a second source of the limitations is related to the tES techniques themselves and is associated with the fact that tDCS and tACS elicit cutaneous sensations. Cutaneous sensations, such as itching, tingling or burning mostly occur during and after the application of current at the electrode-skin interface (Ambrus, Paulus, & Antal, 2010; Poreisz, Boros, Antal, & Paulus, 2007). Cutaneous sensations have been identified as a major impediment for effective blinding in many tDCS and tACS studies (O'Connell et al., 2012; Schutter & Hortensius, 2010). In addition, tACS not only induces cutaneous sensations but also visual phenomena known as phosphenes (e.g., Kanai, Chaieb, Antal, Walsh, & Paulus, 2008). Phosphenes are visual flickering experiences that are most probably due to an unintended stimulation of the retina by the alternating current as a result of the current-

distribution effect of tACS (Kar & Krekelberg, 2012; Laakso & Hirata, 2013). These can be an additional problem for blinding tACS experiments (Raco, Bauer, Olenik, Brkic, & Gharabaghi, 2014; Schutter & Hortensius, 2010).

The term "blinding" refers to the methodological endeavor to control for psychological mechanisms, including the effects of expectations on part of both the investigator and the participants (for a comprehensive review see: Price, Finniss, & Benedetti, 2008). That is, knowledge on the part of the participants about the delivery and the type of a treatment could evoke some measurable physiological response (Price et al., 2008). The standard solution for evaluating the efficacy of a treatment is based on study designs that effectively control for this expectation-effect (Price et al., 2008). This becomes clear when interpreting the experimental situation as a complex psychobiological event, which includes the application of an intervention such as tDCS in a specific psychosocial context, e.g. the interaction of the investigator and the participant (Finniss, Kaptchuk, Miller, & Benedetti, 2010). Strong evidence exists that the participants' responses following an intervention not only reflect the effect of the intervention itself, but also dynamic psychological mechanisms such as expectations (Finniss et al., 2010).

A double-blinded study design is used to control for this phenomenon. In the typical case, two or more treatment conditions are utilized with seemingly identical treatment characteristics but with different mechanism of actions (Price et al., 2008). Usually, one condition is an inert condition serving as a control or baseline (often called placebo or sham stimulation in NIBS literature), whereas the other condition or conditions constitute the main focus of the investigation (known as active or real stimulation). The essential criterion for such a design is that neither the participant nor the investigator knows the difference between the conditions (i.e. they are both blind) and importantly, neither of them is able to detect during the experiment which condition was applied (effective blinding). But blinding is

compromised when the participants can reliably perceive the difference between the conditions (O'Connell et al., 2012). This can lead to a situation in which the perceived differences in discomfort between the conditions will unintentionally influence not only the participants but also the behavior of the investigator. As a consequence, this can potentially lead to incorrect conclusions regarding the efficacy of tES, misinterpreting the effect caused by expectation as one evoked by the stimulation. Therefore, developing thoughtful sham/placebo tES protocols and formally validating their efficacy is a reasonable objective of all NIBS studies (Ambrus et al., 2012; O'Connell et al., 2012) because the effects of tES cannot be meaningfully evaluated without an appropriate study design. In the following chapter I will present two experimental projects focusing on this topic.

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Chapter 2: Cutaneous Sensation and Visual Phenomena (Phosphenes) during the Application of Transcranial Direct Current Stimulation and Transcranial Alternating Current Stimulation

Studies employing tES demonstrate great variability in stimulation duration, intensity and other stimulation parameters. Most of the studies apply the stimulation for 10 minutes, but there are a number of experiments that used a stimulation duration of 30 minutes (e.g., Clark, Coffman, Trumbo, & Gasparovic, 2011). Likewise, the most commonly used stimulation intensity is 1.0 mA, but many researchers apply a stimulation current of up to 2.0 mA (e.g., Batsikadze, Moliadze, Paulus, Kuo, & Nitsche, 2013; Russo, Wallace, Fitzgerald, & Cooper, 2013), especially for clinical purposes (e.g. Fregni, Boggio, et al., 2006; Fregni, Gimenes, et al., 2006; Khedr et al., 2014).

Despite the great variability in the stimulation parameters, only few studies have investigated and formally validated the blinding potential of the various factors or their interaction, which is necessary when performing randomized, double-blind studies (Ambrus et al., 2012; Gandiga, Hummel, & Cohen, 2006; O'Connell et al., 2012). The most frequently applied placebo stimulation protocol is the fade-in, short stimulation, fade-out protocol (Siebner et al., 2004). This protocol consists of three consecutive blocks (see Figure 1). In the first block, the current is gradually increased over an interval of several seconds from zero to the maximum intended intensity (e.g. 1.0 mA). This is followed by a short stimulation at the maximum intensity, typically for 30 seconds, after which the stimulation current is reduced to zero over several seconds (Figure 1). Earlier physiological studies confirmed that such a short stimulation does not affect cortical excitability (Nitsche & Paulus, 2001) but does induce cutaneous sensations resembling those of real stimulation protocols (Gandiga et al., 2006). Subsequent studies also established that this placebo stimulation protocol effectively blinds

participants and experimenters up to 1.0 mA and 20 minutes (Ambrus et al., 2012; Gandiga et al., 2006) because the cutaneous sensation in the placebo stimulation condition persists for several minutes after the cessation of the stimulation (Ambrus et al., 2012). However, in the case of tDCS many studies plan to increase the stimulation current to 2.0 mA with the intention to further enhance the magnitude of the tDCS after-effects. There is evidence that this important change in the stimulation protocol impairs the efficacy of the blinding strategy (O'Connell et al., 2012). In addition, major concerns have been also raised about the applicability of placebo protocols in case of tACS studies (Raco et al., 2014; Schutter & Hortensius, 2010), since alternating current evokes phosphenes during the entire stimulation period (Raco et al., 2014). In the following, I shall present two projects that are related to the blinding potential of tDCS and tACS, respectively.

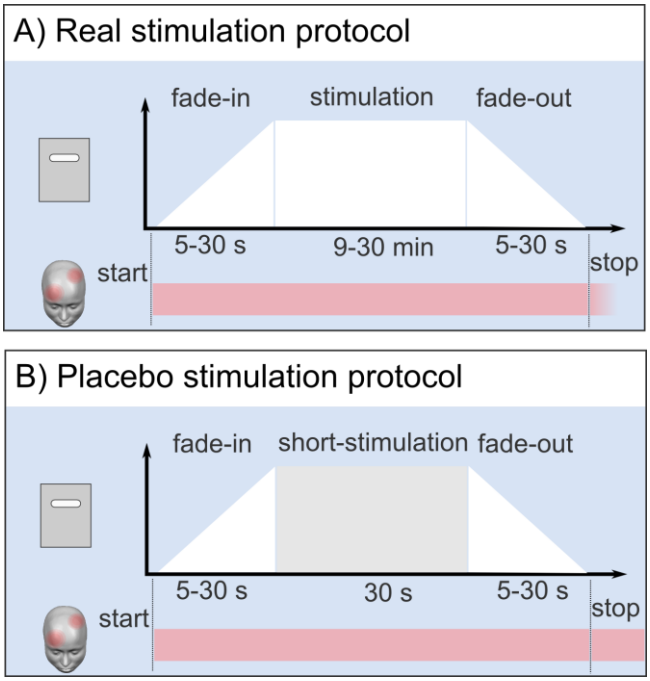

Figure 1. The fade-in, stimulation, fade-out protocol in the real stimulation condition (A) and the fade-in, short-stimulation, fade-out protocol in the placebo stimulation condition (B). In both stimulation protocols, participants report cutaneous discomfort at the beginning of the stimulation, and the cutaneous perception

outlasts the stimulation duration even in the placebo stimulation. For both figures, the top parts represent the course of the current strength, while the bottom parts show the time-course of the evoked cutaneous discomfort.

2.1. 1. The Role of Electrode Size in Evoking Cutaneous Sensations during tDCS

A prior study in 100 participants showed that the cutaneous discomfort due to stimulation plays an important role when blinding is not effectively maintained (O'Connell et al., 2012). In order to reduce the stimulation-related discomfort, an electrode size of 35 cm^2 is commonly used (Nitsche et al., 2008), in part because it is assumed that with a larger electrode the current density will still be effective but low enough that the stimulation will be more tolerable than when using electrodes with a smaller area.

On the one hand, the stimulation protocol should meet the criteria for an effective double-blind design by reducing the stimulation-related discomfort. But it is also essential to use a stimulation protocol that will allow researchers to control the spatial distribution of the stimulation as far as possible. This latter aim can be achieved by using smaller electrodes together with the suggestions of computational FEM or physiological studies (Datta et al., 2009; Dmochowski, Datta, Bikson, Su, & Parra, 2011; Faria, Hallett, & Miranda, 2011; Ruffini et al., 2014), but it is in conflict with the general requirement to keep the current density at a low level.

Since many research groups are trying to increase the focality of tDCS (Dmochowski et al., 2011; Faria et al., 2011; Ruffini et al., 2014), the objective of the present work was to investigate the effect of electrode size on the self-reported intensity and perceived spatial dimension of stimulation-related cutaneous discomfort. We asked whether increasing the electrode size would indeed lead to better tolerability as the traditional view holds. Although the current density is decreased by using larger electrodes, more nociceptors are stimulated at the same time. Therefore, we also considered the possibility that using smaller electrodes could reduce rather than increase discomfort. This second, and maybe counter-intuitive, hypothesis was taken into consideration based on previous evidence that a smaller stimulated

area recruits the response of fewer nociceptors (Martinsen, Grimnes, & Piltan, 2004; Price, Mchaffie, Larson, & Larson, 1989), and as a result, increases the perceptual threshold. Thus, with smaller electrodes fewer nerve endings might be affected, which would be reflected by the subjectively lower level of cutaneous discomfort during tDCS (Martinsen et al., 2004).

Our data support the latter hypothesis, which stated that participants would perceive greater discomfort with the larger electrodes (35 cm^2) than with the smaller ones (16 cm^2), even though the current density was kept constant. According to our interpretation, this pattern of findings can be explained by the spatial summation effect; that is, participants are more likely to perceive discomfort or indicate a greater degree of discomfort when the stimulated region is increased because more cutaneous nerve endings are stimulated, that which are spatially summed. Our results suggest that it may be possible to simultaneously increase the spatial focus of the stimulation and to decrease cutaneous discomfort induced by tDCS.

2.1. 2. Original Publication of Data of Chapter 2: The Role of Electrode Size in Evoking Cutaneous Sensations during tDCS[1]

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When Size Matters: Large Electrodes Induce Greater Stimulation-related Cutaneous Discomfort Than Smaller Electrodes at Equivalent Current Density

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Cutaneous discomfort
Electrode size

ABSTRACT

Background: Cutaneous discomfort is typically reported during transcranial direct current stimulation (tDCS), restricting the current intensity and duration at which tDCS can be applied. It is commonly thought that current density is associated with the intensity of perceived cutaneous perception such that larger electrodes with a lower current density results in milder cutaneous sensations.
Objective: The present study examined the relationship between current density, current intensity and cutaneous sensations perceived during tDCS.
Methods: Two experiments were performed. In the first control experiment, the cutaneous sensations induced by varying current intensities (0.025, 0.5, 1.0 and 1.5 mA) were examined up to 10 min. These data were used for optimizing inter-stimulation intervals in the second main experiment, where participants rated the intensity, spatial size and location of the cutaneous sensations experienced during tDCS using two electrodes sizes (16 cm^2 and 35 cm^2). In the *equivalent current density* condition, the current density was kept constant under both electrodes (0.014, 0.029 and 0.043 mA/cm^2), whereas in the *equal current intensity* condition (0.5, 1.0 and 1.5 mA) the same intensities were used for the two electrode sizes.
Results: Large electrodes were associated with greater cutaneous discomfort when compared to smaller electrodes at a given current density. Further, levels of cutaneous perception were similar for small and large electrodes when current intensity was kept constant.
Conclusion: Cutaneous sensations during stimulation can be minimized by reducing the electrode size from 35 cm^2 to 16 cm^2.

Introduction

Transcranial direct current stimulation (tDCS) is currently one of the most frequently investigated Non-invasive Brain Stimulation (NIBS) techniques (e.g., Ref. [1]). It is capable of inducing cortical excitability changes [1] and brain activity changes in the underlying and remote neocortical areas [2,3], which are often associated with behavioral changes in the motor [4] or cognitive domain, including categorization [5], working memory [6] and declarative memory [7]. tDCS involves the use of a battery driven stimulator that passes a direct electric current to the brain through electrodes placed on the scalp.

For safety reasons, such as to avoid skin burns [8] and for adequate blinding, the applied current intensity is generally low, usually ranging between 1.0 and 2.0 mA [4,9–11]. Despite the low current intensity, most participants perceive cutaneous sensations during and after the stimulation [12]. The most commonly reported sensations are a slight to mild tingling, itching, burning and/or prickling sensation [12–14]. Thus, the perceived discomfort restricts the extent to which stimulation parameters can be increased.

These cutaneous sensations are thought to be induced by electrochemical reactions, in which electrons are transferred between the electrode and the stimulated tissue [15]. Nevertheless, multiple additional factors contribute to cutaneous perceptions, such as the concentration of the saline solution [14], the electrode position [14,16] and differences in skin-microstructure [17].

Much effort is being taken to minimize the frequency and intensity of the stimulation-induced discomfort such as (1) including

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a fade-in and fade-out phase at the beginning and end of the stimulation [11], (2) reducing the impedance by using a conductive medium [1] and (3) by using relatively large electrodes to maintain a low level of current density (e.g., Refs. [10,11,18]). While from a safety point of view it is beneficial to reduce the current density by increasing the electrode size, in doing so, the spatial focality of the stimulation is also reduced [19].

Most tDCS studies typically utilize a current intensity of 1.0 or 2.0 mA but use different electrode sizes (between 16 cm^2 and 35 cm^2), thus apply varying current densities [20]. Although larger electrodes have lower current densities than smaller electrodes at any given current intensity, more cutaneous receptors are affected due to the extended electrode-skin interface [21]. On the other hand, while smaller electrodes have a higher current density, fewer receptors are stimulated but with a stronger intensity [21].

At present, there is little research investigating tDCS-related cutaneous discomfort as a function of electrode size and current density (e.g., Ref. [21]). It is commonly thought that larger electrodes, with a lower current density result in milder cutaneous perceptions [22], though this idea has not been empirically tested for tDCS. In contrast, previous research by Martinsen and colleagues [21] suggested that the cutaneous perceptual threshold is primarily dependent on the current intensity rather than on the current density (during constant current stimulation over the hand area), since the perceptual threshold decreased as electrode size increased. They posit that this was due to a spatial summation effect [23,24]. As electrode size increased, the number of stimulated nerve endings also increased [25], and the perception from these receptors is then spatially summated, resulting in a lowered perceptual threshold.

Many groups working with transcranial electrical stimulation techniques seek to apply more focal stimulation possibly by reducing the electrode size. Therefore, we investigated the effect of electrode size and current density on self-reported intensity and perceived spatial dimension of stimulation-induced cutaneous discomfort during tDCS. Two independent experiments were conducted. In Experiment 1, the time-course (i.e., the lasting effect) of the perceived intensity and the spatial dimension of the discomfort induced by short-duration stimulation was studied. In Experiment 2, the optimized time-course data from Experiment 1 were used to separate stimulation trials to minimize the carry-over effects that may potentially originate from the preceding stimulation trial. The aim of Experiment 2 was to investigate the effect of the electrode size on the intensity and the spatial dimension of the perceived discomfort. Keeping the current density constant under the large and small electrode pairs, the subjective rating of the cutaneous discomfort was examined. Consistent with the idea of spatial summation [21], it was hypothesized that decreasing the electrode size while keeping the current density constant would decrease the level of subjective cutaneous discomfort. The results of this study can potentially be used to inform the design of future experiments in such a way as to minimize stimulation-related cutaneous discomfort while increasing the spatial focality of the stimulation.

Method

Participants

Experiment 1

Ten healthy participants (4 females, mean age: 24.9 ± 1.9 years) took part in the experiment. Exclusion criteria included the presence of metal implants in the head, neck and heart (e.g., pacemaker), all known neurological or psychiatric disorders, history of epilepsy and drug and/or alcohol addiction. Participants were evaluated by a neurologist prior the experiment. The study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethical committee.

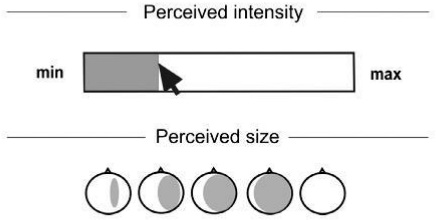

Figure 1. Screenshot of the cutaneous rating system to rate the perceived intensity and spatial magnitude of the tDCS-induced sensations in Experiment 1. The five possible categorical rating options for the spatial magnitude from left to right are: (1) underneath the electrodes only, (2) left/right side extensively – right/left side no sensation perceived, (3) left/right side dominantly – right/left side partially, (4) left/right side dominantly – right/left side extensively and (5) no sensation perceived). This example shows right-side electrode positioning only.

Experiment 2

Twenty healthy participants (10 females, mean age: 25.1 ± 5.9 years) took part in Experiment 2. Only naïve participants with no prior experience with NIBS techniques were recruited, thus participants who had participated in Experiment 1 were ineligible to participate. The same exclusion criteria for Experiment 1 were used.

Stimulation

Experiment 1

A CE-certified medical device (neuroConn DC-STIMULATOR PLUS, neuroConn, Ilmenau, Germany) was used to deliver the DC current through a pair of electrodes placed on the scalp. The electrodes were positioned according the 10/20 international EEG system either on the left (F3 for anode and C3 for cathode) or right hemisphere (F4 for anode and C4 for cathode). Participants were randomized to either receive stimulation on the left or right hemisphere. The electrodes were 5 × 7 cm in size and attached to the head using conductive paste. The electrodes were aligned vertically. No specific skin preparations were performed to avoid abrasion of the skin, which was found to increase the risk of tDCS-induced skin burns [8].

Participants received four stimulation trials with varying current intensities including 0.025, 0.5, 1.0 and 1.5 mA, the order of which was randomized for each participant. Each stimulation trial lasted for 31 s which consisted of an 8 s fade-in/fade-out period and a 15 s period of stimulation (identical to the protocol of [26]).

Experiment 2

A CE-certificated medical device (multichannel DC-STIMULATOR MC, neuroConn, Ilmenau, Germany) with two independent channels of micro-processor-controlled constant current sources was used (http://www.neuroconn.de/dc-stimulator_mc_en/). The DC current was delivered through two pairs of conductive rubber electrodes (large: 5 × 7; small: 4 × 4 cm). The large electrodes were aligned vertically.

One electrode-pair was positioned on the left side of the head (F3 for anode and C3 for cathode) and the other pair was placed on the right side (F4 for anode and C4 for cathode). As displayed in Fig. 3A, the positioning of the electrodes was counterbalanced such that half the participants had the large electrodes on the left and the small electrodes on the right side of the head and half the

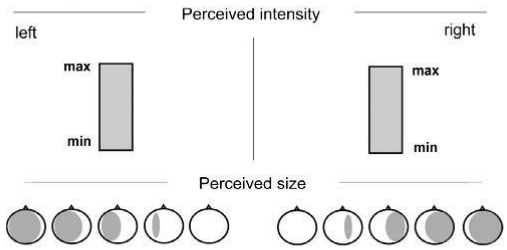

Figure 2. The perceived intensity and the perceived spatial dimension rating scale in Experiment 2.

participants had the small electrodes on the left and the large electrodes on the right side of the head.

Rating of cutaneous perceptions

Experiment 1

Participants were asked to rate two different aspects of the stimulation-induced discomfort: (1) the perceived intensity of the discomfort and (2) the perceived spatial dimension of the discomfort. The intensity of the perceived discomfort was assessed by a horizontal visual analog scale (VAS) [27]. The VAS was anchored by the words *min* and *max* on the left and right end respectively, where maximum referred to the perceived intensity at which participants thought they could no longer tolerate (see Fig. 1). For the subsequent data analysis, the VAS values were automatically converted into numbers ranging from 1 to 100.

The perceived spatial dimension of the discomfort was rated using five schematic head figures corresponding to five possible combinations of temporal location and size. The figures either depicted perceived sensation on the left or right side of the head, depending on the electrode placement. The magnitude of the discomfort was depicted by gray ellipses of varying sizes as seen in Fig. 1.

Experiment 2

The intensity and magnitude of the discomfort was rated similarly to Experiment 1; two vertical VASs were used, one for the left and one for the right side of the head. Both VASs were anchored by the words *max* and *min* at the top and bottom of the scale respectively. Figure 2 displays the rating scales used.

In order to minimize the attentional demand of the task, participants were given verbal cues by the experimenter when they were required to rate the intensity and spatial magnitude of the cutaneous sensations. Participants were always asked to first rate

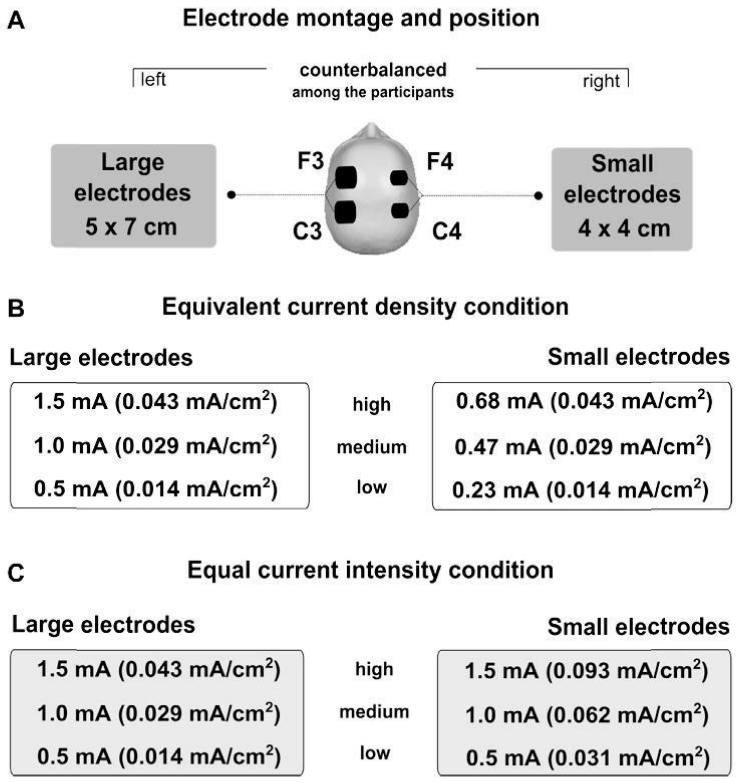

Figure 3. A) The electrode montage and position. B) Current intensities and densities in the *Equivalent current density condition*. C) Current intensities and densities for the *Equal current intensity condition*.

the perceived intensity of the stimulation and then the perceived spatial magnitude. Participants were given 7 s to respond. Two steps were taken to optimize the participants' evaluations: (1) participants were trained before the start of the experiment to ensure that they can respond within 7 s and (2) at the end of each trial (i.e., after the end of the second trial of stimulation), participants had unlimited time to change their response if necessary.

Experimental procedure

Experiment 1

Before the practice trials, participants were given written instructions explaining the task along with images of the rating scales used. Prior to stimulation, participants familiarized themselves with the program and using the rating scales in accordance with verbal cues from the assessor (related to intensity or the spatial dimension of the cutaneous sensation). To minimize incorrect ratings, verbal instructions on how to use the rating scales were also provided [28]. The experiment began once the assessor was confident in the participant's ability to understand the task and to use the program.

Each trial lasted 10 min, consisting of the stimulation, ratings and a washout period before the start of the next trial. Participants were asked to give their first rating 15 s after the stimulation started. Cues to perform the ratings were then given every 45 s, repeated 7 times. The entire block lasted 5.5 min upon which the program ended and participants waited 4.5 min before the next trial started.

Experiment 2

All participants took part in two stimulation conditions: the *Equivalent current density* condition and the *Equal current intensity* condition. Three different increments were assessed for both conditions (low, medium and high intensities), the details of which are displayed in Fig. 3B and C respectively. Note that for the large electrodes, the same intensities were used in both conditions. In total, six stimulation blocks were used: three blocks in the *Equivalent current density* condition and three blocks in the *Equal current intensity* condition. Each block contained two stimulation trials: one for the small and one for the large electrodes. Each stimulation block was repeated twice in order to counterbalance the order of the stimulation on the left and right side of the head.

Participants only received stimulation on one side of their head at a time in order to avoid potential confounding effects of the cutaneous perceptions spreading to the non-stimulated hemisphere. The order of the blocks in the two stimulation conditions was randomized for each participant.

Blinding

Experiment 1

In order to meet the criteria of a double-blinded design, the "study mode" of the stimulator was used. The four stimulation conditions, 0.025, 0.5, 1.0 and 1.5 mA were randomly encoded as A, B, C and D, and this randomized association between the different letters and intensities was unknown to the investigator who conducted the experiment. The study mode encoding was secured with a 5-digit code, which was only accessible to the principal investigator (A.A.) who was not involved in the data collection and analysis process.

Experiment 2

The DC stimulator was programmed by an independent member of the study not involved in the data collection and analysis process (G.G.A and T.S). Arbitrary codes (e.g., S01, S02) were assigned to the different trial conditions in the stimulator interface program, thus, maintaining blinding of the assessor to the stimulation parameters being given.

Moreover, the investigator conducting the experiment followed a written protocol regarding the interaction with the participants during the session. They were also trained to provide standardized responses to questions from participants. Where questions related to the stimulation parameters under investigation (e.g., regarding electrode size, current intensity, current density or stimulation order) arose, they were addressed at the end of the experiment.

Data management

Experiment 1

During Experiment 1, participants were asked to rate the presence of the cutaneous discomfort during (15 s after the start of the stimulation) and after the stimulation (up to 5.5 min) every 45 s. In order to assess whether the occurrence of cutaneous discomfort differed amongst the four stimulation conditions, participants' responses were merged into four blocks (*block 1*: 15 s–1 min; *block 2*: 1 min 45 s–2 min 30 s; *block 3*: 3 min 15 s–4 min; *block 4*: 4 min 45 s–5 min 30 s). For each block, participants' responses were coded from 0 to 2, where 0, 1 and 2 indicate no perception at both time points (e.g., at 15 s and 1 min), perception at one time point and perception at both time points, respectively.

Experiment 2

For the perceived intensity analysis, the two datasets (participants received each stimulation block twice) were collapsed by calculating the mean of the corresponding values. A Generalized Linear Model (GLM) was used for the statistical analysis by using the Generalized Estimating Equation Package ('geepack') developed for the statistical program R [29]. By defining the family name parameter as Poisson (since the data showed a Poisson distribution), the geeglm and anova functions were used to compute the analysis of deviance for GLM fit using a Chisquared-based estimate (for models with Poisson dispersion). Separate analyses were conducted for the equivalent current density and for the equal current intensity conditions. In both cases, there were two within-subject factors – *electrode size* (2 levels: small and large) and *current intensity* (3 levels: low, medium and high).

For the perceived occurrence analysis, the two datasets were collapsed and a dichotomous variable was created where 0 coded for perception in none of the stimulation blocks and 1 coded for the perception of sensation in at least one of the two stimulation trials. By defining the family name parameter as Binomial, the geeglm and anova functions were used to compute the analysis of deviance for GLM fit using a Chisquared-based estimate. Separate analyses were conducted for the equivalent current density and equal current intensity conditions. There were two within-subject factors – *electrode size* (2 levels: small and large) and *current intensity* (3 levels: low, medium and high).

For the perceived spatial dimension data, separate analyses were performed for the equivalent current density and for the equal current intensity conditions. Wilcoxon signed-rank test was used to compare the spatial dimension of the cutaneous sensation reported in the small and large electrodes (Bonferroni corrected $P = 0.016$). As mentioned above, participants were given five schematic head figures corresponding to five possible combinations of spatial location and size. Since each stimulation trial was repeated twice, the two datasets were collapsed by calculating the means across the two datasets. The values provided in the descriptive statistics represent the mean ± standard error of the mean (SEM) for parametric or the median for the non-parametric tests.

Table 1
The occurrence and intensity of the perceived cutaneous perception for the four stimulation conditions (0.025, 0.5, 1.0 and 1.5 mA) as a function of time (between 15 s and 5 min and 30 s). Occurrence indicates the number of participants who reported cutaneous discomfort (total $n = 10$). Intensity was rated on a scale of 0–100.

	Occurrence				Intensity			
	0.025 mA	0.5 mA	1.0 mA	1.5 mA	0.025 mA	0.5 mA	1.0 mA	1.5 mA
00 min 15 s	2	6	8	9	3	6.5	17.1	27.1
01 min 00 s	1	1	3	4	3	5	3	10.2
01 min 45 s	1	0	1	3	4	0	5	4.7
02 min 30 s	2	2	1	1	4.5	2	9	3
03 min 15 s	1	1	1	0	5	3	4	0
04 min 00 s	0	2	1	1	0	6	1	4
04 min 45 s	1	3	0	1	5	3.5	0	2
05 min 30 s	1	2	1	1	11	5.5	13	4

Results

Experiment 1

All participants tolerated the stimulation and no serious adverse effects (e.g., nausea and vomiting) were reported during and after the stimulation. Table 1 summarizes the occurrence (i.e., the number of participants indicating discomfort) and the perceived intensity of the sensation as a function of time (up to 5.5 min) and stimulation intensity. In all of the four conditions less than 50% of the participants reported cutaneous discomfort 1 min after the start of the stimulation.

The non-parametric Friedman-test was used to compare the occurrence and intensity of the cutaneous discomfort in the four stimulation conditions. There was a significant difference between the four stimulation conditions in terms of occurrence for *block 1*, $\chi^2(3, N = 10) = 17.58$, $P = 0.001$), but not for the other blocks ($Ps > 0.096$). The results were similar for the perceived intensity such that there was a significant difference for *block 1*, $\chi^2(3, N = 10) = 21.94$, $P < 0.001$), but not for the other blocks ($Ps > 0.315$). Thus, participants displayed differences in the perceived intensity of the cutaneous perception between the four stimulation conditions in the first block but this difference dissipated as time progressed.

Follow-up pairwise comparisons were conducted on *block 1* with a Wilcoxon signed-rank test (Bonferroni corrected $P = 0.016$). In the case of occurrence, a significant difference was found between the 0.025 mA and 1.0 mA conditions ($Z = -2.46$, $P = 0.014$), and between the 0.025 mA and 1.5 mA conditions ($Z = 2.59$, $P = 0.01$). There was no significant difference between the 0.025 mA and 0.5 mA conditions ($P = 0.059$). The same pattern of findings was found for perceived intensity. Compared to the 0.025 mA condition, participants indicated greater discomfort at 1.0 mA ($Z = 2.52$, $P = 0.012$) and 1.5 mA ($Z = 2.67$, $P = 0.008$) but not at 0.5 mA ($P = 0.075$). In other words, for up to 1 min of stimulation, participants reported more frequently and more intense cutaneous discomfort at higher intensities (1.0–1.5 mA) than when compared to the lowest intensity (0.025 mA). However, this difference amongst stimulation conditions was abolished by 1.5 min after the *start* of the stimulation (i.e., 1 min after the end of the stimulation).

Overall, it was shown that even in the condition with the highest intensity (1.5 mA), the frequency and the perceived intensity decreased significantly 30 s after the end of the stimulation (see Fig. 4). Participants mostly experienced the cutaneous sensations immediately beneath the electrodes or on the side of the head which was stimulated. Only one participant reported feeling cutaneous discomfort on the whole head for all four conditions. Given these findings, a wash-out period of one minute was set between stimulation trials in order to avoid carry-over effects in Experiment 2.

Experiment 2

Impedance in the two electrode pairs

Impedance was measured at three time points (1st, 6th and 12th trial) for the two electrode sizes. A repeated measures two-way ANOVA (rm ANOVA) was performed, with *time* (3 levels: 1st, 6th and 12th trial) and *electrode size* (2 levels: small and large) as within subject factors and impedance as the dependent variable. The rm ANOVA revealed a significant main effect for *time* ($F_{2,38} = 34.42$, $P < 0.001$) but no significant main effect for *electrode size* ($F_{1,19} = 0.159$, $P = 0.694$). Thus, the impedance significantly increased during the time-course of the experiment (mean ± SEM; 1st trial = 5.75 kΩ ± 0.49 kΩ, 6th trial = 8.4 kΩ ± 0.37 kΩ and 12th trial = 9.47 kΩ ± 0.47 kΩ), however, there is no evidence to suggest that the discomfort ratings for the different electrode sizes were affected by differences in impedance (small = 7.93 kΩ ± 0.3 kΩ and large = 7.81 kΩ ± 0.46 kΩ).

Analysis of the perceived intensity

In the *equivalent density condition* there was a significant main effect of *electrode size* $\chi^2(1, N = 20) = 9.76$, $P = 0.0018$ and of *current intensity* $\chi^2(2, N = 20) = 24.97$, $P < 0.001$. In other words, the

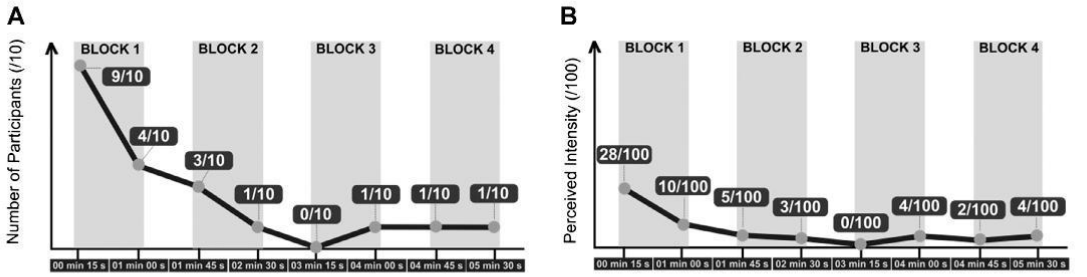

Figure 4. A) The number of participants who reported cutaneous discomfort during and after stimulation at 1.5 mA. B) The perceived intensity of the cutaneous discomfort during and after stimulation at 1.5 mA on a scale of 1–100.

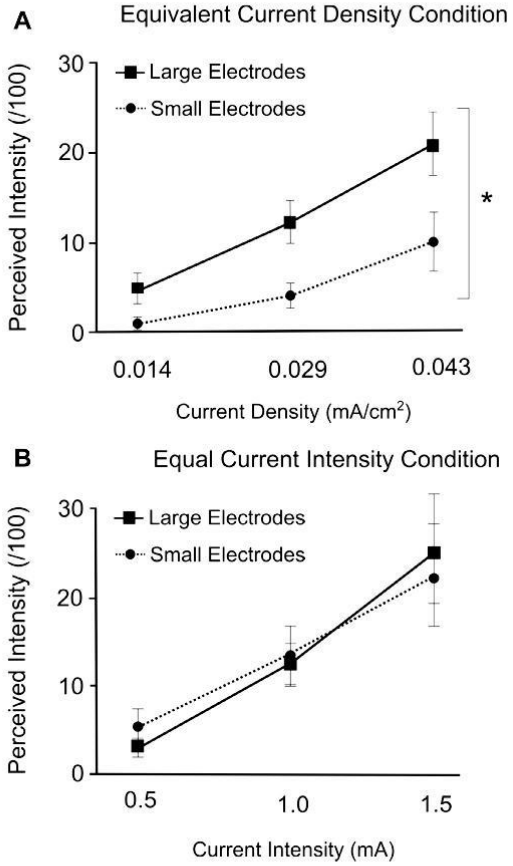

Figure 5. The perceived cutaneous discomfort for the large and small electrodes for (A) the equivalent current density condition and (B) the equal current intensity condition. Asterisk corresponds to a significant difference. Error bars represent standard error of the mean.

intensity ratings for small electrodes were significantly lower (4.93 ± 2.23) than those for large electrodes (12.3 ± 3.0) when the current density was kept constant. When stimulation intensity increased, the perceived intensity of discomfort also increased (low = 2.7 ± 1.32; medium = 7.97 ± 2.12, high = 15.2 ± 3.6) (see Fig. 5A).

In the *equal intensity condition* there was no significant main effect of *electrode size* ($P = 0.95$). This indicates that there was no significant difference in subjective discomfort between the small (13.4 ± 4.27) and large electrodes (13.2 ± 4.32). However, there was a significant main effect of *current intensity* $\chi^2(2, N = 20) = 29.8$, $P < 0.001$ such that participants experienced greater discomfort when stimulus intensity increased (low = 3.73 ± 1.56, medium = 12.5 ± 2.86, high = 23.6 ± 5.93) (see Fig. 5B).

Analysis of the occurrence of the discomfort

For the *equivalent density condition* there was a significant main effect of *electrode size* $\chi^2(1, N = 20) = 8.2$, $P = 0.0042$ and *current intensity* $\chi^2(2, N = 20) = 22.1$, $P < 0.001$. The results indicate that smaller electrodes induced cutaneous discomfort less frequently (total = 33/60) than large electrodes (total = 48/60), when the current density was kept constant. When the stimulation intensity increased, the occurrence of the evoked discomfort also increased (low_{total} = 15/40; $medium_{total}$ = 32/40; $high_{total}$ = 34/40).

In the *equal intensity condition* there was no significant main effect of *electrode size* ($P = 0.24$), but a significant main effect for *current intensity* $\chi^2(2, N = 20) = 16.09$, $P < 0.001$. In other words, the incidence of perception was the same for the small (total = 46/60) and larger electrodes (total = 51/60). Furthermore, the occurrence of the discomfort increased as current intensity increased (low_{total} = 23/40; $medium_{total}$ = 35/40; $high_{total}$ = 39/40).

Analysis of the spatial dimension of the cutaneous discomfort

In the *equivalent current density* condition, there were no differences between the large and small electrodes in the low and moderate current intensity conditions (both $Ps > 0.145$). However, a significant difference was observed in the high intensity condition ($Z = -2.848$, $P = 0.004$). In the *equal current intensity* condition, there was no significant difference between the small and large electrodes for all current intensities (all $Ps > 0.03$). In other words, participants did not report differences in the spatial dimension of the sensations perceived for the two electrode sizes for low to moderate current intensities. However, more extended cutaneous perception was indicated in the *equivalent current density* condition at the highest intensity (I_{large} = 1.5 mA, I_{small} = 0.68 mA) for the large (median = 2) compared to the small (median = 1.25) electrode pair. This confirms that the cutaneous sensations induced by the stimulation did not spread beyond the surface area of the electrodes.

General discussion

The present study investigated the role of the electrode size and stimulation intensity on the cutaneous discomfort evoked by tDCS. Two experiments were conducted. In the first calibration experiment, the cutaneous sensations induced by varying current intensities (0.025, 0.5, 1.0 and 1.5 mA) were examined up to 10 min. It was found that a 1 min wash-out period was sufficient for separating two stimulation trials, as the cutaneous discomfort was largely reduced 30 s after the stimulation ended. It was also found that the spatial dimension of the cutaneous discomfort was predominantly perceived beneath the electrodes. These data were used for optimizing inter-stimulation intervals in the second, main experiment, where participants rated the intensity, spatial size and location of the cutaneous sensations experienced during tDCS using two electrodes sizes.

In the second experiment, it was found that both the occurrence rate and the intensity of the cutaneous discomfort was equal under the small and large electrodes, when current intensity was kept constant. This is contrary to the generally accepted notion that the cutaneous discomfort diminishes as the current density is reduced either by increasing the electrode size or by reducing the current intensity [22]. In addition, there was significantly less discomfort reported for the smaller electrodes when current density remained constant. The results of this study indicate that for current intensities between 0.5 and 1.5 mA, decreasing the electrode size from 35 cm^2 to 16 cm^2 leads to a reduction of cutaneous discomfort in tDCS. Thus, these findings can potentially be used to inform the design of future experiments in such a way as to reduce stimulation-related cutaneous discomfort. Further, previous studies have found that when the electrode size is reduced, the spatial focality of the stimulation can also be increased [19]. The findings of the present study are consistent with those of Martinsen and colleagues [21], indicating that current *intensity* and not current *density* determines the perceptual threshold for direct currents. It is posited that larger

electrodes may induce greater cutaneous sensations, irrespective of the lower current density, due to a spatial summation effect [21]. That is, participants are more likely to experience discomfort during stimulation when the surface area of the electrode is increased and more cutaneous receptors are stimulated.

The phenomenon of spatial summation of sensory information is well documented in the human somatosensory system [23]. Spatial summation can be evoked by various nociceptive stimuli including mechanical [30], thermal [24] and electrical stimuli [21]. In general, there are two types of neural mechanisms that are both thought to account for spatial summation of cutaneous discomfort [24]. From the local integration point of view, the afferent inputs from varying sizes of stimulus areas are integrated within the receptive fields of individual nociceptive neurons. On the other hand, the neuronal recruitment account holds that when the nociceptive stimulus area is extended, the total number of activated nociceptive neurons increases as well [24]. Accordingly, the findings of the present study may be due to the fact that the larger electrodes stimulated larger areas of the receptive fields of individual neurons, and/or activated more nociceptive neurons.

While it was found that discomfort levels could be reduced through the use of smaller electrodes, how this was affected by stimulation duration, was not explored. The use of a short-stimulation paradigm limits the applicability of the current findings as the results cannot be directly extrapolated to longer-lasting stimulation protocols. A recent study compared the cutaneous discomfort induced by small and large electrodes in a large sample of 149 volunteers [31]. In a double-blinded study, participants received 2 mA tDCS for 30 min using electrodes that were either 25 cm^2 or 35 cm^2 in area. While participants reported similar levels of cutaneous perception for the two electrode sizes, subjective sensations (e.g., tingling, itching) were more frequently reported with smaller electrodes. Interestingly, these findings suggest that while electrode size has no effect on the level of perceived discomfort, it may affect the number of stimulation-related sensations. However, it must be noted that the effect of static and dynamic impedance was not examined, a critical factor to consider for longer stimulation protocols. Therefore, the effect of changes in dynamic impedance cannot be ruled out; it is likely that the smaller saline-soaked sponge electrodes dried out more quickly than the larger electrodes, possibly leading to an increased level of impedance. It must be noted that impedance may have a proportional effect on skin irritation [32]. Nevertheless, an important limitation of this present study is the lack of information about the subjective symptoms during and after the stimulation.

The effect of electrode size on the physiological after-effects of tDCS was also not considered in this study. Previous studies using transcranial magnetic stimulation (TMS) measuring cortical excitability have found that the spatial focality of tDCS can be improved by using smaller electrodes [19,33]. By using 3.5 cm^2 and 35 cm^2 stimulating electrodes while keeping the current density constant, it was shown that the functional efficacy of tDCS was maintained despite the decreased electrode size [19]. In other words, it was found that the spatial focality of tDCS was improved by decreasing the electrode size. In another study, Bastani and Jaberzadeh [33] found that anodal tDCS resulted in a greater increase in cortical excitability when smaller electrodes were used (12 cm^2 vs. 24 cm^2 or 35 cm^2), keeping current density constant (0.029 mA/cm^2). In contrast to the conventional electrode montage using one pair of stimulating electrodes, "High-Definition" (HD) tDCS involves the use of small, gel-based electrodes ($\sim$25 cm^2 of total area) in a ring configuration (i.e., 4 $\times$ 1 electrode montage) [34]. Similar to conventional tDCS, HD-tDCS induced polarity-specific changes in motor cortical excitability following 10 min stimulation at 2.0 mA, but producing a longer lasting after-effect than the conventional montage [35]. Thus, so far there is no evidence indicating that smaller electrodes (e.g., 12 cm^2) are less effective than larger electrodes (e.g., 35 cm^2). In fact, Bastani and Jaberzadeh [33] and Kuo and colleagues [35] both found that the magnitude or the time-course of the after-effect was the largest for the small electrodes, though further systematic investigation is needed.

The effect of perimeter length and electrode geometry was not addressed in the present study. Modeling studies have shown a non-uniform current distribution underneath the traditional, rectangular electrodes, where the current density is increased toward the edges [36]. Although a previous study found no evidence that reducing the perimeter length and eliminating the corners (by using circle-shaped electrodes) affected the intensity of the cutaneous discomfort [16], the effect of current geometry cannot be ruled out in the present experiment.

It would be of interest for future studies to investigate the polarity-specific effect of tDCS on cutaneous sensation. While this issue was not investigated in the present study, a previous study by McFadden and colleagues [13] suggests that subjective levels of intolerability and sharp sensations were greater under the cathode than anode when 2.0 mA tDCS was applied. However, other studies failed to find such differences between the polarities when lower intensities, such as 1.0 mA were used (e.g., Refs. [10,26]). Further research is necessary to establish the potential interaction between the electrode size and electrode polarity.

To our knowledge, this is the first study that investigates the effects of current density and electrode size on cutaneous perceptions during tDCS. It is commonly thought that the application of larger electrodes with a lower current density results in reduced cutaneous perception compared to smaller electrodes with a higher current density. In contrast to this, the present study found that smaller electrodes induced less cutaneous perception than larger electrodes, when current density was kept constant. Further, smaller electrodes were also associated with milder cutaneous perceptions when compared to larger electrodes even when the current density was higher. While further studies are necessary to extend the generalizability of the findings to different stimulation parameters (e.g., electrode size, current intensity), future studies could consider decreasing the size of the electrodes to reduce cutaneous discomfort and to increase the spatial focality of the stimulation [19].

Acknowledgments

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2.2. 1. The Role of Stimulation Frequency in Evoking Cutaneous Sensations and Phosphenes during tACS

Many motor and cognitive processes are tightly linked to specific brain oscillations (for reviews see: Buzsáki & Moser, 2013; Fell & Axmacher, 2011) that cannot be selectively entrained by applying a constant current. One of the main advantages of tACS over tDCS is that it has the potential to interfere with physiologically relevant brain oscillations (Helfrich et al., 2014). For instance in the motor domain, tACS with a frequency in the beta frequency range (20 Hz) slowed voluntary reaching movement (Pogosyan, Gaynor, Eusebio, & Brown, 2009) while it improved implicit motor learning when a frequency of 10 Hz was used (Antal et al., 2008). In the cognitive domain, tACS with a frequency in the theta range (4-8 Hz) increased risk taking behavior (Sela, Kilim, & Lavidor, 2012) and improved motion sensitivity when the frequency was in the alpha frequency range at 10 Hz (Kar & Krekelberg, 2014).

However, similar to tDCS (Poreisz et al., 2007), tACS also induces cutaneous sensations, such as tingling or itching and in addition, it also evokes phosphenes (Ambrus, Antal, & Paulus, 2011; Kanai et al., 2008; Schutter & Hortensius, 2010). Phosphenes are visual flickering effects of retinal and, presumably, cortical origin that can be detected during the entire application of tACS even at lower stimulation intensities (Kanai et al., 2008; Raco et al., 2014; Schutter & Hortensius, 2010). Although the exact source of the phosphenes evoked during tACS is still debated, they are most probably induced by retinal stimulation as computational modeling studies of the current flow path suggest (Kar & Krekelberg, 2012; Laakso & Hirata, 2013). It was seen that a portion of the AC can reach the retina by passing through the eyes, due to the current spread effect, and that this is sufficiently strong to induce phosphenes (Laakso & Hirata, 2013). The phosphenes evoked during tACS may compromise the present placebo-controlled protocols, as participants can easily detect the difference

between the placebo and real tACS due to the presence or absence of evoked phosphenes (Figure 2).

The objective of the present study was to investigate the effects of stimulation frequencies between 2 and 250 Hz on the subjectively perceived intensity of cutaneous sensations and phosphenes. We recruited 20 naive participants who were requested to rate the subjective intensity of procedural sensations using a computerized visual analog scale in a placebo-controlled single-blind study. We demonstrated that both cutaneous sensations and phosphenes were evoked by tACS in a frequency-specific manner although the former exhibited less frequency specificity than the latter. According to our results, tACS in the alpha, beta and gamma frequency range poses challenges for studies employing a single- or double-blind study design.

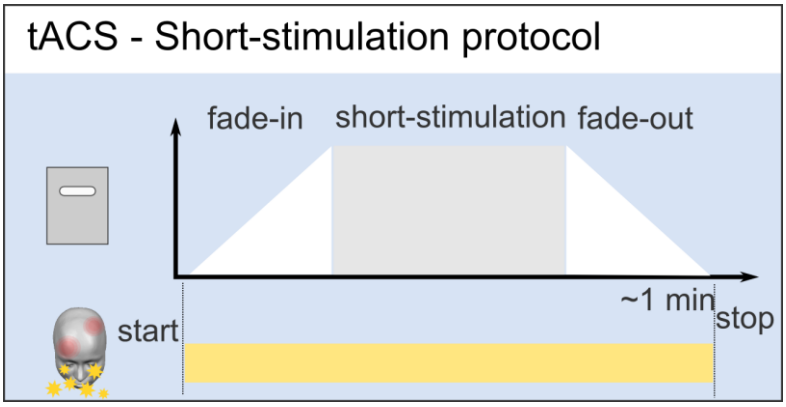

Figure 2. The fade-in, short-stimulation, fade-out protocol in the placebo stimulation condition. Unlike the cutaneous discomfort in tDCS placebo protocols, the phosphenes are not perceived after the stimulation ceases, as indicated by the yellow horizontal bar.

2.2. 2. Original Publication of Data of Chapter 2: The Role of Stimulation Frequency in Evoking Cutaneous Sensations and Phosphenes during tACS[2]

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Both the cutaneous sensation and phosphene perception are modulated in a frequency-specific manner during transcranial alternating current stimulation

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Abstract.
Purpose: Transcranial alternating current stimulation (tACS) is a non-invasive stimulation technique for shaping neuroplastic processes and possibly entraining ongoing neural oscillations in humans. Despite the growing number of studies using tACS, we know little about the procedural sensations caused by stimulation. In order to fill this gap, we explored the cutaneous sensation and phosphene perception during tACS.
Methods: Twenty healthy participants took part in a randomized, single-blinded, sham-controlled study, where volunteers received short duration stimulation at 1.0 mA intensity between 2 to 250 Hz using the standard left motor cortex – contralateral supraorbital montage. We recorded the perception onset latency and the strength of the sensations assessed by visual rating scale as dependent variables.
Results: We found that tACS evoked both cutaneous sensation and phosphene perception in a frequency-dependent manner. Our results show that the most perceptible procedural sensations were induced in the beta and gamma frequency range, especially at 20 Hz, whereas minimal procedural sensations were indicated in the ripple range (140 and 250 Hz).
Conclusions: We believe that our results provide a relevant insight into the procedural sensations caused by oscillatory currents, and will offer a basis for developing more sophisticated stimulation protocols and study designs for future investigations.

Keywords: Oscillatory current, transcranial alternating current stimulation (tACS), procedural sensations, cutaneous sensation, phosphene perception, motor cortex

1. Introduction

Transcranial alternating current stimulation (tACS) is a relatively new technique for inducing neuroplastic changes in humans non-invasively (Antal et al., 2008). It can be used to effectively change a wide range

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of behavioral measures in both the motor and cognitive domains in a frequency-dependent manner (Antal et al., 2008; Chaieb, Antal, & Paulus, 2011; Kanai, Paulus, & Walsh, 2010; Pogosyan, Gaynor, Eusebio, & Brown, 2009; Sela, Kilim, & Lavidor, 2012; Zaghi, Acar, Hultgren, Boggio, & Fregni, 2010).

Alternating currents are generated by a battery-driven stimulator and delivered to the head via a pair of rubber conductive electrodes, similar to other types of transcranial electrical stimulation methods, such as transcranial direct current stimulation (tDCS) and transcranial random noise stimulation (tRNS). These electrodes are typically inserted into a sponge saturated with saline solution to minimize procedural sensations (i.e. cutaneous sensation) caused by stimulation. Even so, cutaneous sensations and phosphenes, which are flashing sensations in the visual field, can be perceived during tACS (Antal et al., 2008).

Similar to tACS, skin-related procedural sensations and adverse-effects also occur during and after tDCS (Poreisz, Boros, Antal, & Paulus, 2007). The most common cutaneous sensations associated with tDCS were mild itching, tingling and burning sensations. Interestingly, Ambrus and colleagues found that tDCS had a markedly lower detection threshold (0.4 mA) than transcranial random noise stimulation (tRNS) (1.2 mA), the latter transmitting randomly distributed currents between 0.1–600 Hz (Ambrus, Paulus & Antal, 2010).

For tACS, however, the stimulation-related cutaneous sensations have not yet been explored experimentally. Generally, in non-invasive brain stimulation (NiBS) studies, the presence of stimulation-related cutaneous sensations was considered as a negligible, non-relevant factor for a long time and relatively few studies addressed this issue previously (Ambrus et al., 2010, 2012; Gandiga, Hummel, & Cohen, 2006). The studies investigating phosphene perception (Kanai, Chaieb, Antal, Walsh, & Paulus, 2008; Kar & Krekelberg, 2012; Paulus, 2010; Schutter & Hortensius, 2010) concentrated on the origin of the phosphene, rather than on the effect of stimulation frequency in evoking the phosphene perception itself. Here, we aim to explore and clarify the effect of stimulation frequency on (1) cutaneous sensation, and (2) on phosphene perception evoked by tACS, giving us methodical insights into the frequency-dependent effects of these phenomena. This information might be useful for developing and optimizing stimulation protocols specifically for future studies using oscillating currents, by taking the frequency-dependent procedural sensations into account.

2. Methods

2.1. Participants

Twenty healthy volunteers (7 male; age range: 21–29 years; mean age: 24.9 years) took part in the experiment. Participants had no previous history of neurological or psychiatric disorders, and they had no metal implants in the head or neck area. Participants gave written and verbal informed consent before participating. The experiment was in accordance with the guidelines of Declaration of Helsinki, and it was approved by the ethics committee of the University of Göttingen.

2.2. Stimulation

tACS was generated by a battery-driven electrical stimulator (NeuroConn GmbH, Illmenau) through a pair of conductive rubber electrodes with a current intensity of 1.0 mA (peak-to-peak). One electrode was placed over the left motor cortex, whereas the other electrode was placed over the contralateral supraorbital region. The size of the stimulating electrodes was 3 cm × 3.5 cm, which were inserted in electrode sponge wrappers (5 cm × 7 cm) (Fig. 1, A). Twenty two stimulation frequencies between 2 and 250 Hz were applied and compared to seven no-stimulation trials in order to assess most accurately the minimum and maximum points of the frequency range in which phosphene perception could be evoked (Fig. 1, B). The selection of the stimulation frequencies was predominantly based on previous tACS studies using stimulation frequencies at theta (5–6.5 Hz by Feurra, Bianco, Santarnecchi, Del Testa, Rossi & Rossi, 2011; Sela et al., 2012), alpha (10 Hz by Wach, Krause, Moliadze, Paulus, Schnitzler & Pollok, 2012), beta (20–30 Hz by Antal et al., 2008) gamma (50–80 Hz by Feurra, Paulus, Walsh & Kanai, 2011) and at the ripple range (140 Hz by Moliadze, Atalay, Antal & Paulus, 2012). The duration of the stimulation was 31 seconds for each trial.

2.3. Procedure

The experimental procedure was identical to that used in a previous study: see Ambrus and colleagues

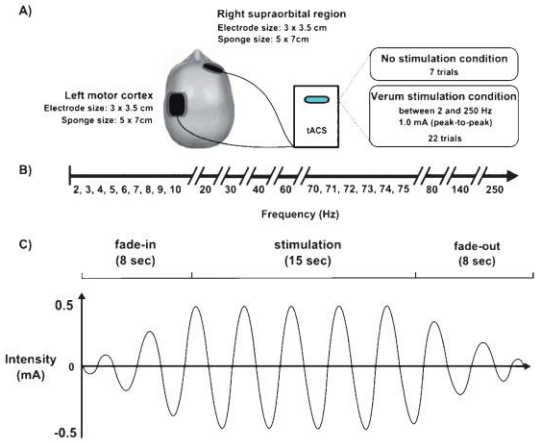

Fig. 1. A) The stimulation montage and the stimulation conditions. B) The non-linear arrangement of stimulation frequencies between 2 and 250 Hz. C) The stimulation protocol for the verum stimulation trials. The stimulation intensity gradually increased from zero to 1.0 mA in the fade-in phase and was decreased to zero after 15 seconds of stimulation with maximal intensity.

(2010). Briefly, participants were seated in a dimly illuminated room and in a reclining chair placed in front of the investigators in order to minimize the observer-expectancy effect. The entire experiment consisted of one session lasting approximately 90 minutes. The experimental session contained 29 trials, which were divided into 22 verum and 7 no-stimulation trials (similar to Ambrus et al., 2010). Thus, participants received each verum stimulation trial only once, whereas they received the no-stimulation trial 7 times. The order of the verum and no stimulation trials were individually randomized.

Participants started each trial by pressing the SPACE button on the keyboard. Each verum stimulation trial consisted of a fade-in period (8 sec), stimulation period with maximum intensity (15 sec) and fade-out period (8 sec). In the fade-in period, the stimulation intensity was progressively ramped-up from zero to 1 mA intensity. In the fade-out period the stimulation intensity was gradually ramped-down from 1.0 mA to zero again (Fig. 1, C). In the case of the no-stimulation trials, the stimulator was unplugged and disconnected from the trigger cable, and no stimulation was applied. All the other parameters were identical to those in the verum stimulation condition.

After self-initiating the stimulation trial by the button press, participants were required to indicate the presence of (1) cutaneous sensations, (2) phosphenes, or (3) the occurrence of both percepts via one of the three possible response buttons. The selected option(s) and the perception onset latency between the start of the stimulation trial and the response were stored for off-line analysis. Subsequently, participants were presented with a visual rating scale for indicating the intensity of the perceived procedural sensations (*i.e.*, for cutaneous sensations, for phosphenes, or for both). Participants were instructed to indicate the subjective intensity of the perceived sensation by changing the position of the mouse along a continuous line anywhere between the two endpoints. They were told

that the left point represents the lowest point at which the procedural sensation can be detected, whereas the right endpoint indicates the maximum intensity beyond which the sensation is no longer bearable. The chosen intensity was then also stored for later analysis. In the next step, participants were asked whether they felt (1) a tingling sensation, (2) an itching sensation, and/or (3) a burning sensation during the stimulation, and they were also requested to provide the degree of the perceived intensity in a numerical rating scale between 0 and 100 in the case of confirmatory answers (Williamson & Hoggart, 2005). In order to reduce the possible carry-over effects between the experimental trials, the next trial was only started when the participants clearly indicated the end of the perceived procedural sensations arising from the previous trial.

2.4. Questionnaire

After the end of the experiment, participants filled out a post-experimental questionnaire taken, in a modified form, from Poreisz and colleagues (2007). The questionnaire assessed whether participants experienced fatigue, nervousness, anxiety and headache before and after the entire experiment using a numerical rating scale from 0 to 100, where 0 represented the absence of the sensation. Participants completed the questionnaire under the supervision of the experimenter.

2.5. Statistical analysis

The calculation of perception onset latency is described in a previous paper by Ambrus and colleagues (2010). In brief, cumulative perception onset latency for each stimulation condition was computed, which was then averaged to the number of positive answers.

The assessment of the normality of data was performed using the Shapiro-Wilk test. Since the data significantly deviated from a normal distribution ($p < 0.05$), non-parametric tests were used for the analysis. Wilcoxon's matched pairs test was used to compare the intensity of the cutaneous sensation between each verum stimulation trial (22 trials) and the average of the 7 no-stimulation trials (*no-stimulation condition*). To test whether the proportion of the reported procedural sensations (i.e. positive answers for each stimulation frequency) was different from the value reported in the *no-stimulation condition*, a binominal test was used. The proportion value for the binominal test was set according to the averaged reported value in the *no-stimulation condition* (9.29 for cutaneous sensation and 4.29 for phosphene perception). In the case of multiple comparisons, we used the Bonferroni correction (Bender & Lange, 2001). Null hypotheses below the alpha-value of 0.0022 were rejected. Trends were considered above the alpha-value of 0.0022 and below of 0.1.

In the case of the side of phosphene perception, we analyzed data from 16 participants due to the failure in data collection for the first 4 participants.

3. Results

In order to explore the procedural sensations during tACS between 2–250 Hz, we measured participants' perception onset latency and intensity of the sensations by using a visual rating scale. All participants tolerated the stimulation; no serious adverse-effects were reported by the volunteers.

3.1. Cutaneous sensation

Between 30% (at 250 Hz) to 70% (at 20 Hz) of the participants reported cutaneous sensation in the *verum stimulation condition* and ~10% of them in the *no-stimulation condition* (Fig. 2). A binominal test was used to determine whether the proportion of the positive answers for cutaneous sensations in the verum stimulation frequencies was different from 9.29%, the average reported value correspond to the *no-stimulation condition*. We found a significant difference in all stimulation trials ($ps \leq 0.0015$) except for 250 Hz, where the alpha-value reached only a trend ($p = 0.008$). Thus, at all stimulation frequencies with the exception of 250 Hz, participants reported cutaneous sensations significantly more frequently than in the *no-stimulation condition*.

The intensity of the cutaneous sensation assessed using the 100-point visual rating scale ranged between 2 and 19.4 points, where the minimum was at 250 Hz and the maximum at 20 Hz (Fig. 3). The Wilcoxon's matched pairs test revealed that the intensity for cutaneous sensation was significantly different from the *no-stimulation condition* (median = 0) at 7 Hz (median = 4.5), 20 Hz (median = 7), 30 Hz (median = 11) and 73 Hz (median = 3.5) (all $ps \leq 0.0018$).

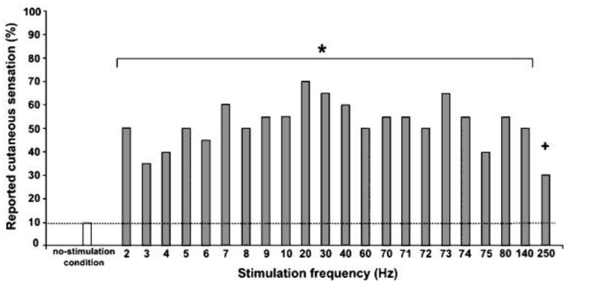

Fig. 2. The percentages of the participants who reported cutaneous sensation during the various stimulation trials. The horizontal axis represents the stimulation frequencies (Hz). During the *no-stimulation condition*, there was no current flowing through the stimulation electrodes. Asterisk represents significant differences in the cutaneous sensation reported during the verum stimulation trials compared to the proportion value (9.29%) measured in the *no-stimulation condition* ($ps \leq 0.0015$) (binominal test), whereas '+' shows a trend toward significance ($p = 0.008$).

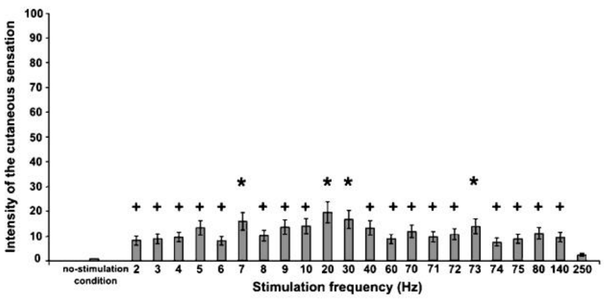

Fig. 3. The intensity of the reported cutaneous sensations during the stimulation trials assessed using a visual rating scale. The horizontal axis represents the stimulation frequencies (Hz). During the *no-stimulation condition*, there was no current flowing through the stimulation electrodes. Error bars indicate standard error of mean. Asterisk represents significant differences ($p \leq 0.0018$) compared to the *no-stimulation condition* (Wilcoxon's matched pairs test), whereas ' + ' shows a trend toward significance ($0.0028 \geq ps \leq 0.0406$).

All the remaining stimulation trials (except for 250 Hz) showed a trend towards significance ($0.0028 \geq ps \leq 0.0406$). In other words, participants indicated significantly more intense cutaneous sensations when they received tACS at 7, 20, 30 and 73 Hz. On the other hand, at 250 Hz the reported intensity was statistically not different from the *no-stimulation condition*. In the case of the remaining frequencies the participants have demonstrated a trend towards reporting more intense cutaneous sensations.

The intensity of itching, tingling and burning sensations was assessed by using a 100-point numerical rating scale. Volunteers reported itching sensations with a maximum intensity of 15.7 points (at 30 Hz) and a minimum of 2.5 points (at 250 Hz). Wilcoxon's matched pairs test between the *no-stimulation condition* (median = 0) and verum stimulation trials revealed only one significant difference at 30 Hz (median = 8) ($Z = -3{,}059$, $p < 0.0022$). At 3, 4, 5, 7, 9, 10, 20, 40, 60, and between 70 and 80 Hz, the analysis revealed a trend toward significance ($0.0022 \geq ps \leq 0.0506$).

Tingling sensations were rated between 8.4 points (at 7 Hz) and 1 point (at 250 Hz). Wilcoxon's matched pairs test revealed no significant difference between any of the verum stimulation trials and the *no-stimulation condition* (all $ps \geq 0.01$). At 2, 3, 4, 5, 6, 7, 8, 9, 20, 30, 40, 60, 70, 71, 72, 73, 74, 80 and at 140 Hz, we found a trend toward significance ($0.01 \geq ps \leq 0.092$).

The maximum value for burning sensations was 8.7 points (at 9 Hz) and the minimum value was 1.7 points at 40 Hz. No burning sensations were reported at 250 Hz. Wilcoxon's matched pairs test showed no significant difference between any of the verum stimulation trials and the *no-stimulation condition* (all $ps > 0.041$). At 5, 7, 9, 20, 60, 73, 80 and at 140 Hz, the test showed a trend ($0.041 \geq ps \leq 0.067$). Thus, these three observations indicate that the reported intensity of the outcome measures (itching, tingling and burning sensations) was in no cases significantly different between the *no-stimulation* and verum stimulation trials (except one single case at 30 Hz in the itching sensation).

3.2. Phosphene perception

More than 50% of the participants indicated phosphene perception during stimulation trials between 7 and 60 Hz. At 20 Hz, 95% of the participants experienced a flashing light sensation, which was the highest percentage reported. Interestingly, 140 Hz and 250 Hz did not induce phosphenes in any of our volunteers (Fig. 4). Participants reported phosphene perception in 4.3% of *the no-stimulation condition* trials.

A binominal test was used to determine, whether the proportion of the positive answers indicated in the verum stimulation trials was different from that value indicated in the *no-stimulation condition* (4.29%). We found a significant difference between 2 and 75 Hz ($ps \leq 0.0013$) and a trend at 80 Hz ($p = 0.0095$). In other words, participants reported significantly more frequently phosphene perceptions between 2 and 75 Hz than in the *no-stimulation condition*. This difference at 80 Hz was reduced to a trend.

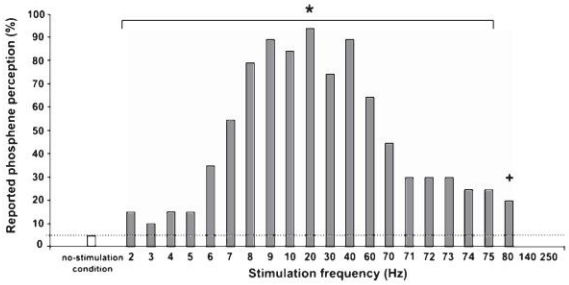

Fig. 4. The percentages of participants who reported phosphene perception during the stimulation trials. The horizontal axis represents the stimulation frequencies (Hz). During the *no-stimulation condition*, there was no current flowing through the stimulation electrodes. Asterisk represents significant differences in phosphene detection reported during the verum stimulation trials compared to the proportion value (4.29%) measured in the *no-stimulation condition* ($ps \leq 0.0013$) (binominal test), whereas + shows a trend toward significance ($p = 0.0095$).

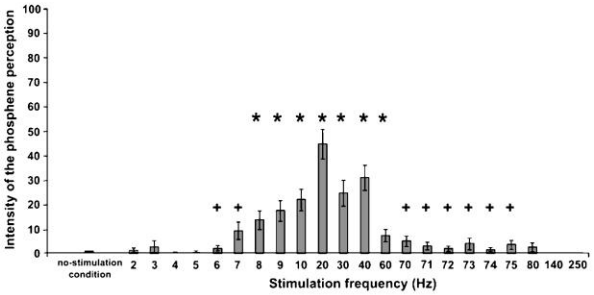

Fig. 5. The intensity of the reported phosphene perception during the stimulation trials assessed using a visual rating scale. The horizontal axis represents the stimulation frequencies (Hz). During the *no-stimulation condition*, there was no current flowing through the stimulation electrodes. Error bars indicate standard error of mean. Asterisk represents significant differences ($ps \leq 0.0012$) compared to the *no-stimulation condition* (Wilcoxon's matched pairs test), + shows a trend toward significance ($0.0028 \geq ps \leq 0.0463$).

The reported intensity for phosphene perception was assessed using a 100-point visual rating scale. Between 8 and 60 Hz, the indicated intensity was over 10 points, with a maximum intensity of 44.75 points at 20 Hz (Fig. 5). Wilcoxon's matched pairs test demonstrated that the intensity for phosphene perception was significantly different (all $ps \leq 0.0012$) from the *no-stimulation condition* (median = 0) at 8 Hz (median = 6.5), 9 Hz (median = 12.5), 10 Hz (median = 18), 20 Hz (median = 39.5), 30 Hz (median = 16), 40 Hz (median = 30.5) and 60 Hz (median = 3). At 6, 7 and between 70 and 75 Hz, the test revealed a trend toward significance ($0.0028 \geq ps \leq 0.0463$). Thus, participants indicated significantly more intense phosphene perception between 8 and 60 Hz compared to the *no-stimulation condition*. Above 75 Hz, the intensity for phosphene sensation was statistically indistinguishable from the *no-stimulation condition*.

In the case of phosphene perception, the right or both eyes were indicated in 59.1% and 38.6% of the trials. Participants observed phosphenes in the left eye only in 2.3% of the cases. Wilcoxon's matched pairs test showed a significant difference between the right and left eye ($Z = -3.443$, $p = 0.001$), between both eyes and the left eye ($Z = -3.047$, $p = 0.002$), but not between the right and both eyes (Bonferroni corrected alpha-value = 0.016). Thus, participants perceived more phosphenes on the right eye alone, or on both eyes but not on the left eye alone. It appears to be that the evoked phosphene perception heavily depends on the position of the electrodes. Volunteers tend to report more phosphene perceptions for the electrode that is closer to the retina (i.e., right supraorbital region) than for the electrode that is on the motor cortex (left side).

3.3. Perception onset latency

At 5 and 8 Hz, all responding participants reported *cutaneous sensations* during the fade-in phase of the stimulation (Fig. 6). In the case of phosphene perception, participants indicated *phosphenes* in the fade-in phase at 20 and 30 Hz. If the response was less than 8 seconds, volunteers perceived the procedural sensations even in the fade-in period, where the current intensity has not yet reached the maximum value (which is 1.0 mA).

3.4. Questionnaire

After the experiment, participants filled out a questionnaire with regards to nervousness, fatigue, headache and anxiety. *Before* the experiment, 30% of the participants reported nervousness with an average intensity of 3.2 out of 100 (3.2/100), 60% experienced

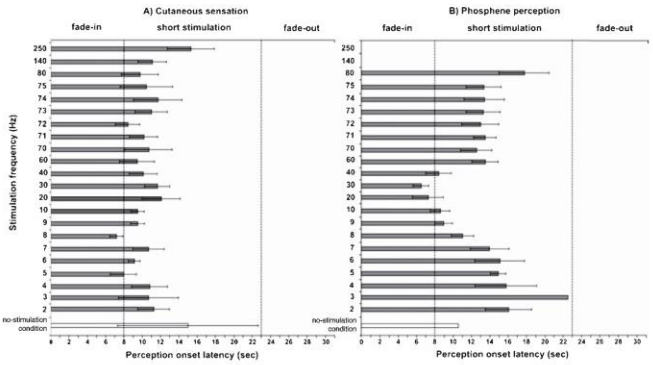

Fig. 6. The averaged perception onset latency for the positive answers for cutaneous sensations (A) and for phosphene perception (B). The vertical axis represents the stimulation frequencies (Hz). During the *no-stimulation condition*, there was no current flowing through the stimulation electrodes. The horizontal axis represents the perception onset latency in seconds for each stimulation trial. The maximum time for the stimulation trials is 31 seconds, which includes 8 seconds of fade-in phase, 15 seconds short stimulation with maximum intensity of 1.0 mA (peak-to-peak) and 8 seconds if fade-out phase. Error bars indicate standard error of mean.

fatigue (17.7/100), 30% headache (12.6/100) and none of them had reported feelings of anxiety. *After* the experiment, none of them reported nervousness, 85% of the participants indicated fatigue (25.7/100) and 45% reported headache (11.6/100).

The Wilcoxon's matched pairs test revealed that before and after the experiment the difference in the indicated intensity for nervousness ($Z = -2.207$, $p = 0.024$), fatigue ($Z = -2.253$, $p = 0.027$) and headache ($Z = -0.564$, $p = 0.573$) was not significant (Bonferroni corrected alpha-value = 0.016). Thus, although none of the participants indicated nervousness at the end of the experiment, the decrease has not reached a level of significance. The intensities for fatigue and headache were unchanged from before to after the experiment.

4. Discussion

The main purpose of this exploratory study was to provide basic information about the cutaneous sensation and phosphene perception evoked by tACS at different stimulation frequencies between 2 and 250 Hz. By keeping the stimulation intensity constant, we found that the procedural sensations were modulated in a frequency-dependent manner, but in different ways: We found a clear and consistent pattern of frequency-dependency for phosphene perception in both outcome measures, while in the case of cutaneous sensations, these differences were less pronounced, but still present in terms of perceived intensity.

Our research was also motivated by the implications of the well-known placebo/nocebo effect, which was explored with regard to tDCS by several previous studies (Ambrus et al., 2010, 2012; Antal, Terney, Kühnl, & Paulus, 2010). The placebo/nocebo effect derives from highly active psychological mechanisms such as expectation and conditioning (de la Fuente-Fernández et al., 2001), which can induce a significant bias in the response to a therapy or intervention (Enck, Benedetti, & Schedlowski, 2008). So far, only limited information was available concerning the tACS-evoked procedural sensations, information which might be crucial in experiments using a placebo-controlled, double-blinded study-design.

The presence of cutaneous sensations have often been regarded as a relatively non-influential and negligible procedural concomitants for NiBS methods, such as for tDCS (Gandiga, Hummel, & Cohen, 2006; Nitsche et al., 2008). However, experimental evidence demonstrated that the tDCS-mediated neuroplastic changes were surprisingly task-specific, possibly modulated -at least partly- by the attentional involvement of the participants (e.g. Antal, Terney, Poreisz, & Paulus, 2007). In this current experiment, 30 to 70% of the participants reported perceiving cutaneous sensations in all of the stimulation trials. These values were significantly higher at each stimulation frequency (except for 250 Hz) compared to the value reported in the *no-stimulation condition*. It might be argued that the cutaneous sensations evoked by tACS modify the alertness of the volunteers and are possibly capable of altering attentional processes as well. Therefore, the role of the skin sensations in tACS have to be taken into consideration in future studies. Future studies might also study the location of the cutaneous sensation on the scalp and whether or not the location is modulated by the different frequencies.

The indicated intensity for cutaneous sensation was frequency-dependent and generally low. We found significant differences mainly at the beta band frequencies (20 and 30 Hz), whereas cutaneous sensations in a high oscillating frequency (250 Hz) were almost unnoticeable. Recently, Jelinek and McIntyre (2010) have also found a modulatory effect of the stimulation frequency of electrocutaneous stimulation on the perceived magnitude of cutaneous sensation. By using squared-wave, low-intensity electrocutaneous stimulation over the anterior forearm between 20 and 200 Hz at suprathreshold intensity (10% above the detection threshold), Jelinek and McIntyre showed that the higher the stimulation frequency was, the higher the reported magnitude of sensation was indicated, with a plateau at 120 Hz. Importantly, we also found an effect of stimulation frequency on the magnitude of the perceived sensation, however, in a different way. Our results showed that the perceived intensity of cutaneous sensations gradually increased as a function of stimulation frequency up to the beta frequency range, and did not show a trend for further increase beyond the beta range. On the contrary, the lowest cutaneous sensation was indicated at the highest intensity. It is difficult to explain the discrepancy between the two studies, but the different waveform (sinusoid vs. squared-wave), electrode placement (head vs. forearm), current intensity (predetermined at 1.0 mA vs. individually adjusted) may all contribute to the divergent findings.

Phosphene perception evoked by tACS has recently received much attention with NIBS research. The main debate was on the possibility that tACS is capable of entraining ongoing brain oscillatory mechanisms and evoke phosphene perception in a frequency-dependent manner (Kanai et al., 2008), or the possibility that the phosphene perception could be partially explained by retinal contribution due to the volume-conduction effect (Kar & Krekelberg, 2012; Schutter & Hortensius, 2010).

Similar to the cutaneous sensation results, the most frequently reported and the most intense *phosphene perception* was also observed at 20 Hz, but in general, the phosphene perception reached the significance level at each stimulation frequency between 2 and 75 Hz. The strength of the phosphene perception also differed significantly from the *no-stimulation condition* between 8 and 60 Hz. In other words, we found a bell-shaped relationship between the stimulation frequency and the evoked magnitude of phosphene perception. Frequencies beyond 8 Hz produced increased phosphene perception up to the maximum at 20 Hz while perception declined dramatically from 60 Hz onward.

The averaged cumulative perception onset latency for phosphene perception was the lowest between 9 and 40 Hz, thus, participants responded numerically faster for the stimulation frequencies that evoked the most intense visual flickering percepts (please note that this result was not statistically verified; see Results). This result might be due to the fact that participants were required to simultaneously attend to two different procedural sensations (cutaneous sensations and phosphene perceptions), which might have increased the perception onset latency for reporting cutaneous sensations. Importantly, participants were always provided with a subsequent self-paced screen with visual analog scales for both procedural sensations *(i.e.,* irrespectively of indicating only one of the sensations initially). Accordingly, participants had the possibility to indicate the other procedural sensation for each experimental trial using the visual analog scale. Therefore, we believe that multisensory interactions or split attention processes minimally affected our main results.

In this study, none of the participants reported any serious adverse-effects during or after the stimulation.

Nevertheless, 30% of the participants reported minimal nervousness (3.2 out of 100) before the experiment, which reduced to zero after the testing. Contrary to a previous paper investigating applications of tDCS during and after stimulation (Poreisz et al., 2007), the subjective intensity of headache and fatigue did not differ significantly before and after the experiment according to the self-reports of the participants in this current study. The baseline amount of fatigue and headache (with relatively low intensities in both cases), however, was relatively high. The reason for this elevated amount is unknown to us, but one speculative reason would be that most of the participants were university students, and these values reflect their regular, daily routines. Interestingly, most of the previous studies assessed the status of the participants during or after but not before the intervention, therefore, we have no previous comparisons in this regard.

In summary, beta (13–30 Hz) and gamma (>30 Hz) frequencies induced the most intense and frequent procedural sensations during stimulation, but the procedural sensations were perceivable between 7 and 80 Hz in general. The role of beta and gamma frequency ranges have been reported to be associated with motor and cognitive processes (Donner & Siegel, 2011; Engel & Fries, 2010), and there is already an increasing interest in the tACS literature for the beta-range (Kanai et al., 2008; Pogosyan et al., 2009; Zaghi et al., 2010). Future studies using the standard electrode montage at beta and gamma frequency ranges might consider the possibility that *the magnitude and the frequency of* the phosphenes and the cutaneous sensations could influence the outcome of the stimulation in both motor and cognitive functions. Our data suggests that future studies are needed to optimize sham stimulation protocol for tACS, especially in the beta-range.

5. Limitations

(1) One of the main limitations of our study was that only some selected frequencies were investigated within the range of 2 and 250 Hz. Thus, this study offers only limited information about the cutaneous sensations and phosphene perception at higher frequencies (i.e.>80 Hz). (2) Another key concern might be the short stimulation duration used in our study. Importantly, the aim of our exploratory research was to investigate a broad range of the frequency spectrum during tACS, which limited the stimulation duration because of the length of the entire experiment. By considering the data presented in this report, future studies might aim to focus on the temporal development of the evoked procedural sensations in different frequency ranges. (3) Future studies might aim to employ repeated trials for each stimulation frequencies to increase the intra-subject validity of the reported procedural sensations. (4) Our results are also montage- and intensity- specific. It is generally accepted that the stimulation-related cutaneous sensations significantly depend on the current density (Ambrus et al., 2010; Brunoni et al., 2011) and different montages possibly evoke procedural sensations differently (Poreisz et al., 2007).

6. Conclusion

In this study we have demonstrated that both cutaneous sensations and phosphene perception is evoked by tACS in a frequency-dependent manner, mostly in the beta and gamma frequencies. The better understanding of the stimulation-related procedural sensations might form a basis for developing more optimized stimulation protocols and study designs for paradigms using oscillatory currents in the future.

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Chapter 3: The Effect of Transcranial Direct Current Stimulation on Instrumental Learning

In order to investigate the cognitive effects of tDCS, the third project assessed the effect of anodal tDCS on instrumental learning. Instrumental learning describes the elementary capability to learn to choose actions that will lead to a greater reward and to avoid those actions that are less rewarding, non-rewarding or even aversive (for reviews see: Dolan & Dayan, 2013; Schultz, 1997, 2007). This complex behavior is achieved by learning to exploit the most rewarding action and to avoid the less rewarding ones by evaluating the outcome of each action provided by the environment (Frank, Seeberger, & O'Reilly, 2004).

In everyday situations, the environment rewards actions stochastically, thus, the individual is required to generate predictions and constantly test them in a non-deterministic or dynamically changing environment. This procedure involves two complementary and conflicting decisional strategies; exploration and exploitation (Daw, O'Doherty, Dayan, Seymour, & Dolan, 2006; Frank, Doll, Oas-Terpstra, & Moreno, 2009). Whereas the former is required for discovering the value of appropriate actions, the latter is needed to make use of the benefit of the action that appears to be the best, thereby maximizing the total amount of reward (Daw et al., 2006). In other words, exploitation is based on choosing the most rewarding action (or the action, which is *assumed* to be the most rewarding one). On the other hand, exploration prompts the individual to shift from the most rewarding option to alternatives with the intention to ensure that the exploited action is indeed the right one, adapting to a dynamically changing environment or finding better ones (Frank, Moustafa, Haughey, Curran, & Hutchison, 2007). Adaptive behavior requires the precise synchronization of both strategies, since reward cannot be maximized by the exclusive practice of either exploitation and exploration strategies. When only exploitation is employed, i.e. when the same decision is made in all cases, the individual might exploit the

inappropriate option, whereas only exploring the action values, i.e. changing the decision from trial-to-trial, prevents the individual from exploiting the most rewarding alternative.

There is strong evidence for the assumption that individuals create models about the expected values of each action (predictions) and constantly test these predictions by comparing the expected and the actual outcome, also known as the prediction error (Schultz, 1997; Steinberg et al., 2013; Tobler, Fiorillo, & Schultz, 2005). Reinforcement learning models allow scientists to infer information about these processes by estimating key parameters from behavioral performance (Frank et al., 2007). The classical reinforcement learning model we employed in our study comprises two such parameters: The learning-rate parameter α describes how the prediction error is used to update the estimated expected value of the actions (Frank et al., 2007; Jocham, Hunt, Near, & Behrens, 2012; Jocham, Klein, & Ullsperger, 2011; Rutledge et al., 2009). In other words, it provides information on the extent to which past experience affects current decisions. It can be used to gradually modify the expected value of an action, that is, prior experience exerts a greater influence on action value estimation. Alternatively the prediction error can be used to overwrite the entire accumulated reinforcement history, thus, past experience plays a lesser role in estimating expected values (Jocham et al., 2011).

The second estimated parameter is called the noise or temperature parameter β, which describes to which extent the estimated value of an action biases decisions (Beeler, Daw, Frazier, & Zhuang, 2010; Frank et al., 2007; Jocham et al., 2011). In a deterministic environment, it is unreasonable to allow that decisions are not always selected according to the highest expected value because this would indicate that actions that are less rewarding or even aversive might be intentionally selected by the individual. However, in a dynamic environment the individual needs to constantly test the action values and sometimes is required to shift the decision from the action that is assumed to be the most rewarding one to

an alternative. The noise parameter β describes the extent to which this strategy is employed by the individual (Beeler et al., 2010; Jocham et al., 2011).

From a neural point of view, the complex capacity of instrumental learning emerges from the functional interaction of multiple, hierarchically organized brain systems consisting of striatal and prefrontal components (Badre & Frank, 2012; Balleine & O'Doherty, 2010; Dolan & Dayan, 2013). These components show different functional characteristics in agreement with the assumption that they presumably serve different computational roles (Gläscher, Daw, Dayan, & O'Doherty, 2010). The striatal component is characterized by computational rigidity but requires minimal computational effort (Dayan & Balleine, 2002). A large number of studies indicate that its operation is based on the prediction error (Daw, Niv, & Dayan, 2005; Steinberg et al., 2013). On the other hand, the prefrontal component has greater computational flexibility combined with high computational requirements, and is hence limited in capacity (Collins & Frank, 2012; Daw et al., 2005). Studies suggested multiple roles for the prefrontal cortex. One of the proposed roles of the prefrontal system is to actively maintain reinforcement history in a way that renders it utilizable for working memory (Collins & Frank, 2012). Keeping the reinforcement history accessible in working memory allows the individual to adjust its behavior in a fast and flexible manner following negative outcomes (Frank et al., 2007).

According to competition by mutual inhibition theory the prefrontal system is involved in instrumental learning by representing the available actions. These options are dynamically inhibited by each other and the final decision is based on the active remaining one (Hunt et al., 2012; Jocham et al., 2012). The model assumes that the degree of inhibition relative to excitation in the network is crucial. In fact, a previous study has shown that high GABA and low glutamate levels were associated with a higher value of the inverse temperature parameter (Jocham et al., 2012). A high value in this parameter indicates that the

participant is capable of deciding correctly between the alternatives despite the small difference between the two choices; in other words, the decision process is less noisy. MRS (a method to non-invasively measure cortical GABA levels) studies indicate that tDCS may be capable of perturbing the balance between excitation and inhibition, as the primary neurophysiological effect of anodal tDCS is driven by local decrease of cortical GABA concentration (Stagg et al., 2009; Stagg, Bachtiar, & Johansen-Berg, 2011).

In order to investigate the cognitive effects of tDCS on instrumental learning, the third project assessed the effect of anodal tDCS over the left dorsolateral prefrontal cortex (DLPFC) optimized using an FEM model derived from magnetic resonance imaging (Windhoff, Opitz, & Thielscher, 2013). The left DLPFC is considered to be a central region of a network involved in controlling both working memory (e.g. McNab & Klingberg, 2008) and decision making processes (Collins & Frank, 2012; Daw et al., 2005; Frank et al., 2009). There is evidence from previous tDCS studies that anodal tDCS improved working-memory performance in healthy individuals (Andrews, Hoy, Enticott, Daskalakis, & Fitzgerald, 2011; Zaehle et al., 2011). In accordance with these prior findings, participants in the anodal tDCS session were expected to demonstrate an increased amount of trial-to-trial behavioral adjustment after negative but not after positive outcomes compared to the placebo session, which might be due to the increased capacity of working-memory processes (Frank et al., 2007). We also expected to observe an increased learning-rate parameter. As an alternative prediction, we anticipated that participants would show a greater amount of behavioral shifts and increased noise parameter in accordance with the mutual inhibition theory of decision making (Hunt et al., 2012; Jocham et al., 2012).

Sixteen healthy male participants (Dreher et al., 2007) were asked to perform an instrumental learning paradigm while receiving anodal and placebo tDCS using a randomized, counter-balanced, cross-over and double-blind study design. The position of the

electrodes was based on a pre-computed montage calculated by MR-derived finite element model-based computation approach of the current flow (Windhoff et al., 2013). The increased amount of behavioral shifts both after positive and negative outcomes, as well as the greater value of the noise parameter congruently indicate that anodal tDCS increased randomness in choices relative to the placebo session. According to the interpretation of the present results, our findings do not support the hypothesis that anodal tDCS influenced instrumental learning by modulating working memory. Instead, our results are in agreement with the mutual inhibition theory of decision-making.

3.1. Original Publication of Data of Chapter 3: The Effect of Transcranial Direct Current Stimulation on Instrumental Learning[3]

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Research report

Transcranial direct current stimulation over the left prefrontal cortex increases randomness of choice in instrumental learning

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ABSTRACT

Introduction: There is growing evidence from neuro-computational studies that instrumental learning involves the dynamic interaction of a computationally rigid, low-level striatal and a more flexible, high-level prefrontal component.
Methods: To evaluate the role of the prefrontal cortex in instrumental learning, we applied anodal transcranial direct current stimulation (tDCS) optimized for the left dorsolateral prefrontal cortex, by using realistic MR-derived finite element model-based electric field simulations. In a study with a double-blind, sham-controlled, repeated-measures design, sixteen male participants performed a probabilistic learning task while receiving anodal and sham tDCS in a counterbalanced order.
Results: Compared to sham tDCS, anodal tDCS significantly increased the amount of maladaptive shifting behavior after optimal outcomes during learning when reward probabilities were highly dissociable. Derived parameters of the Q-learning computational model further revealed a significantly increased model parameter that was sensitive to random action selection in the anodal compared to the sham tDCS session, whereas the learning rate parameter was not influenced significantly by tDCS.
Conclusion: These results congruently indicate that prefrontal tDCS during instrumental learning increased randomness of choice, possibly reflecting the influence of the cognitive prefrontal component.

1. Introduction

In most everyday situations, we constantly have to adapt and optimize our behavior to cope with various, often conflicting, demands and constraints posed by each specific environment. An important aspect of adaptive behavior is the capability of choosing those actions that lead to a high amount of cumulative reward. One way to achieve this goal is by successively

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generating predictions about the consequences of each action. Generating and using these predictions to guide behavior is known as instrumental learning (Dayan & Balleine, 2002).

Instrumental learning in humans recruits multiple, functionally interacting and parallel brain systems (for reviews see Dolan & Dayan, 2013; Samson, Frank, & Fellous, 2010); these involve a striatal reinforcement learning (RL) component and a cognitive, prefrontal control component (Collins & Frank, 2012; Daw, Niv, & Dayan, 2005; Daw, O'Doherty, Dayan, Seymour, & Dolan, 2006), also known respectively as the model-free and model-based controls of instrumental learning (Daw, Gershman, Seymour, Dayan, & Dolan, 2011; Daw et al., 2005; Wunderlich, Smittenaar, & Dolan, 2012). The low-level RL (or model-free) component is characterized by computational rigidity and it requires a large number of learning trials to gradually integrate the long-term probability of reinforcement values in response to probabilistic reward associations (Frank, Moustafa, Haughey, Curran, & Hutchison, 2007).

The high-level cognitive (or model-based) component, driven by the prefrontal system, has greater computational flexibility as it dynamically computes the policy to optimize behavior by evaluating the instrumental requirements of the decision situation (Daw et al., 2006). On the one hand, this is achieved by actively maintaining the reinforcement history in working memory (WM) which permits fast goal-directed decisions, albeit with the restriction of a limited capacity (Collins & Frank, 2012; Frank, et al., 2007). On the other hand, functional neuroimaging evidence also suggests that the prefrontal system controls adaptive exploration (Daw et al., 2006). Further evidence also indicates the role of prefrontal involvement specifically, as individual genetic differences in regulating prefrontal dopamine (DA) Catechol-O-methyltransferase (COMT) rs4680 single nucleotide polymorphism has an impact on exploratory behavior but not on the level of striatal DA (Frank, Doll, Oas-Terpstra, & Moreno, 2009).

Nevertheless, genetic studies are correlational in nature and a more direct demonstration of the involvement of the prefrontal component in cognitive control in instrumental learning requires a focal interference with prefrontal regions. Transcranial direct current stimulation (tDCS) has the potential to temporarily shift neuronal membrane potentials of a given neuronal population by passing a low-intensity electrical current through the brain (Nitsche & Paulus, 2000). These physiological effects have been linked to changes in a wide range of cognitive functions, including those that are related to the prefrontal cortex, such as WM (e.g., Zaehle, Sandmann, Thorne, Jäncke, & Herrmann, 2011) or prototype learning (Ambrus et al., 2011).

Modeling studies investigating the tDCS-induced current profile characteristics indicate that the effect of tDCS, at least from electrodes in close spatial proximity, is primarily limited to the neocortex (Datta, Elwassif, Battaglia, & Bikson, 2008; Faria, Hallett, & Miranda, 2011), although tDCS may have the ability to remotely activate deeper brain structures, such as the striatal system (Chib, Yun, Takahashi, & Shimojo, 2013). The common notion that anodal tDCS leads to an increase and cathodal tDCS leads to a decrease in neuronal excitability in the brain area underneath the electrode have been challenged by recent evidence (Reato et al., 2013). First, the electric field induced by tDCS can both de- and hyperpolarize within the same gyrus (Reato et al., 2013) and second, different types of neurons are differentially modulated depending on their morphology and axonal orientation (Radman, Ramos, Brumberg, & Bikson, 2009). Hence, a simple mechanistic relation between polarity and expected behavioral changes may be difficult to establish. Indeed, recent evidence suggests that tDCS has less consistency in polarity effects in cognitive tasks compared to basic motor functions (Jacobson, Koslowsky, & Lavidor, 2012).

The aim of the present experimental work has been to study, which component of instrumental learning was influenced by prefrontal tDCS by evaluating the effect of anodal tDCS on behavior as measured by accuracy and computational model parameters. Advances in computational modeling of RL using Q-learning algorithms allow distinct processes to be modeled in detail. This entails the ability to derive information about how performance is affected by specific behavioral influences or strategies by fitting the RL model to behavioral data (e.g., Frank et al., 2009).

In the classical model we employed in this study (Jocham, Klein, & Ullsperger, 2011), the learning rate parameter α reflects the impact of the prediction error (i.e., the difference between the previous outcome estimate and the actual estimate after a certain action). Larger α values reflect trial-to-trial fluctuations (a recency effect), whereas lower values indicate a gradual value integration and more stable value estimation (Frank et al., 2007). If prefrontal anodal tDCS biases participants to rely more on the WM component, we expected to observe a trial-to-trial behavioral adjustment (i.e., change of decision after negative response) during learning and an increased α value. In contrast, if anodal tDCS compels participants to rely less on the WM component, then a lower α value and less trial-to-trial behavioral adjustment will be observed – which would increase outcome-dependent exploitation of the better symbol. In addition, the β parameter, also known as the "temperature" or "noise" parameter, reflects the learners' bias towards either exploitation (i.e., choosing the better option in case of lower β values) or exploration (i.e., choosing the items more randomly; higher β values) (Frank et al., 2007; Jocham, et al., 2011). This model is designed to capture behavior in a probabilistic environment where not only the expected value (determined by integrating past outcomes with learning rate α) determines the decision, but choices are also characterized by intrinsic randomness, reflected in the noise parameter β (Beeler, Daw, Frazier, & Zhuang, 2010). If anodal tDCS affects exploration and induces randomness in choices, participants will demonstrate increased shifting behavior (i.e., a tendency to change, rather than repeat a response to the same stimulus) and a decreased preference for symbols that are associated with the higher reward probability, reflected by higher β values.

2. Material and methods

2.1. Participants

Sixteen right-handed, healthy, native German-speaking participants took part in the study (mean age of 22.9 ± 2.2 years). In order to avoid menstrual cycle-dependent level changes of

the gonadal steroid hormones and their neurofunctional modulation of the reward system, only male participants were included in the study (Dreher et al., 2007; Jocham et al., 2011). All participants gave written informed consent. The study was conducted in accordance with the Declaration of Helsinki and it was approved by the local ethics committee.

2.2. Stimulation

A battery-driven CE-certified medical device (DC-Stimulator-Plus, NeuroConn GmbH, Ilmenau, Germany) was used to deliver the direct current to the head. Two rubber electrodes (3 × 3.5 cm) were covered with conductive paste and positioned on the scalp using the standard 64 channel 10/20 EEG caps in different sizes (small, medium and large; ANT-waveguard: https://www.ant-neuro.com/products/waveguard). The vertex was identified as the intercept of the half-way distance between the nasion and inion and the half-way distance between the pre-auricular points. The Cz electrode location of the EEG cap was placed over the vertex and this position was re-measured after the EEG cap was fitted to the participant's head. The electrode montage was based on electric field simulations using a realistic MR-derived finite element model (Opitz, Windhoff, Heidemann, Turner, & Thielscher, 2011) employing SimNibs (Windhoff, Opitz, & Thielscher, 2013). In total, 136 different electrode montages were simulated. Two circular-shaped electrodes with a diameter of 32 mm were used in each of the simulations. Electrodes were placed such that coverage of almost any location in the brain could be achieved in at least one montage. Out of all combinations, the electrode montage was selected that maximized absolute electric field strength in the dorsolateral prefrontal cortex, as determined based on anatomical landmarks (Mylius et al., 2013).

The anodal electrode was adjusted to the F3 location corresponding to the left dorsolateral prefrontal cortex (DLPFC) by moving it in the anterior and superior directions, such that the F3 location was in the lower-right corner of the vertically aligned electrode (see Fig. 1). The cathodal electrode was placed over the temporal cortex, where the middle point of the horizontally aligned electrode was exactly located over the T7 position.

Two stimulation protocols were used; one for the anodal tDCS and one for the sham tDCS condition. In the anodal tDCS condition, the stimulation was administered for 16 min, comprising a 30 sec fade-in/fade-out period and 15 min of stimulation at 1.0 mA intensity. In the sham tDCS condition, the stimulation protocol was identical to the anodal stimulation, except the stimulation duration, which lasted for only 30 sec (Ambrus et al., 2012). Although the stimulation duration in the real session was 4 min shorter than the learning phase, tDCS studies conducted on the motor cortex showed that the excitability changes following anodal or cathodal stimulation outlasts the stimulation duration by an hour, provided the stimulation duration is about 10 min or longer (Nitsche & Paulus, 2001).

2.3. Experimental design

The study employed a double-blind, placebo-controlled, repeated-measures experimental design. Subjects attended two separate experimental sessions, in which they completed two versions of the behavioral task (see later), which used two different sets of stimuli. Both the order of the version of the task presented first, as well as the order of the stimulation conditions (tDCS *vs* sham), were randomized for each participant and counterbalanced such that half of the participants

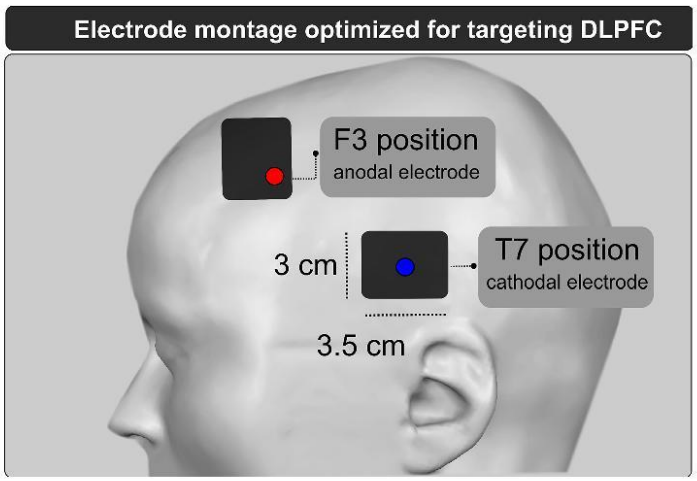

Fig. 1 – The electrode montage was optimized by using a realistic MR-derived finite element computational model in order to maximize the current flow in the DLPFC. The anodal electrode was shifted in the anterior and superior direction and aligned vertically, whereas the cathodal electrode was placed over the temporal cortex and aligned horizontally. DLPFC: dorsolateral prefrontal cortex.

started with anodal tDCS and half with sham stimulation, and half with task version 1 and half with task version 2.

In order to meet the criteria of a double-blind design, the "study mode" of the stimulator was used; that is, the two stimulation conditions, anodal tDCS *vs* sham tDCS, were randomly encoded as A or B modes, respectively. For each session, the investigator selected the stimulation mode according to a predetermined randomized list. The association between modes and stimulation conditions was unknown to the investigator who conducted the experiment. The study mode encoding was secured with a 5-digit code that was only accessible to the principal investigator (A.A.), who was not involved in the data collection and analysis process. The study mode was further advanced by the so called "pseudo-stimulation" mode, which resulted in identical display information (i.e., stimulation duration and impedance information) for the anodal tDCS and sham condition. In order to maintain the participant's unawareness of whether tDCS or sham stimulation was used, the standard "fade-in/short stimulation/fade-out" procedure was used in the sham condition (Ambrus et al., 2012), which is effective at 1.0 mA for up to 20 min (Gandiga, Hummel, & Cohen, 2006). In addition, participants filled out a short questionnaire after each session in order to discover whether adequate blinding was in fact maintained.

2.4. *RL and choice task*

The experimental task was adapted from Jocham et al. (2011), originally developed by Frank, Seeberger, and O'Reilly (2004). The task consisted of a learning and a test phase. In the learning phase (see Fig. 2), participants saw three pairs of symbols (labeled AB, CD and EF for reference), one pair at a time. Each symbol was probabilistically associated with a reward, which followed an inverse relationship within a pair (.8/.2, .7/.3 and .6/.4 for A/B, C/D and E/F, respectively). For example, symbol A was 80% correct and 20% incorrect, whereas symbol B was 20% correct and 80% incorrect. The task of the participants was to select the "better" symbol from the pair (i.e., the one with higher reward probability). The value of the reward probability was unknown to the participants. The learning phase consisted of 6 learning blocks, where each symbol pair was presented 20 times, resulting in 120 presentations of each symbol pair during the entire learning phase (360 presentation trials in total). For each symbol pair, the location of each symbol (left or right) was counterbalanced. The total trial duration was 3.3 sec. The sequence of events within a trial was similar to the study by Jocham et al. (2011): Each trial started with the presentation of a fixation cross for a duration of either 200, 500 and 800 msec (randomly chosen) followed by the symbol pair until a response was given. If no response was made after 1700 msec, the trial was canceled. Finally, the selected symbol was highlighted for 200 msec and feedback was displayed for 200 msec. The feedback was either a 'happy' or a 'sad' emoticon (i.e., a meta-communicative pictorial representation of facial expressions in Western style) for the positive or for the negative feedbacks, respectively. An additional 'confused face' emoticon was used in case of no answer.

In the transfer phase, participants were randomly presented with all possible combinations of the symbols (3 learned combinations plus 12 new combinations; 15 in total) repeated 12 times each. To prevent the participants from additional learning in the transfer phase, no feedback was provided at this time.

Before the start of the experiment, participants were given written instructions about the learning and the transfer phase

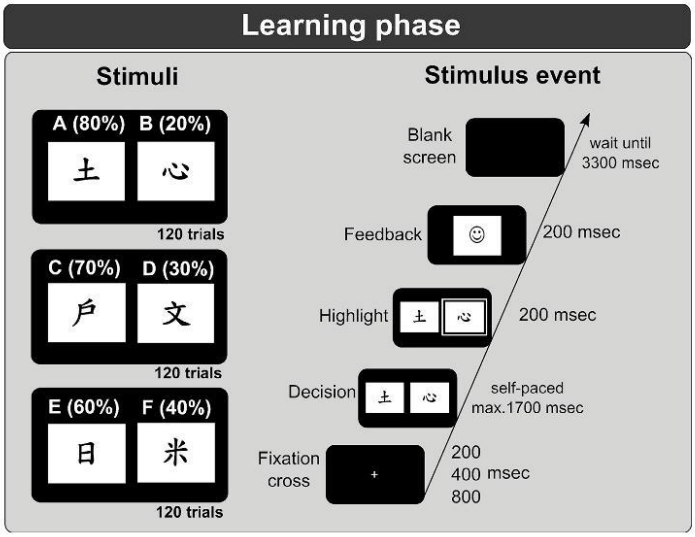

Fig. 2 – The learning phase consisted of 3 symbol pairs (Chinese characters; left), each of which probabilistically associated with the reward (see text for details).

(translated to German from Frank et al., 2007). Then, participants were asked to perform a training session of 13 trials, to ensure that they were comfortable with the experimental setup. Before the start of the experiment proper, participants were shown the 6 individual symbols twice, presented separately for 5 sec, to familiarize them with the stimuli.

2.5. Analysis of the RL and choice task

In the learning phase, our main interest focused on whether the participants' decisions following a positive or negative feedback were influenced by the stimulation. After receiving positive (win) or negative (lose) feedback to their decisions, participants could choose the previously chosen symbol (stay) or select the alternative symbol (shift) in the subsequent trial containing the same symbol pair (note that the three symbol pairs were randomly intermixed, each learning block containing 20 trials for each symbol pair). Therefore, win-stay behavior was defined when participants chose the same symbol after having received positive feedback on the previous trial in which the same pair had been presented. Win-shift behavior was defined when participants chose the alternative symbol even though they had previously received a positive feedback for this choice. Lose-stay and lose-shift behavior were described respectively as staying on the previous symbol or shifting the decision to the alternative symbol after having received a negative feedback. Each trial was assigned to one of these four categories, however, only win-stay and lose-stay behaviors were included into the analysis, as stay and shift behavior complement each other and add up to the total percentages rewarded.

In the transfer phase, we analyzed the accuracy according to the standard "choose A – avoid B" classification scheme (Frank et al., 2004). The percentages of correct choices were separately calculated for "choose A" trials (AC, AD, AE, and AF) and for "avoid B" trials (BC, BD, BE, and BF).

2.6. RL model

We used the RL model described in (Jocham et al., 2011). In brief, action values Q(A) through Q(F) for each item A through F were estimated based on the individual history of sequence of choices and the corresponding feedback experienced after each decision during the learning phase. The action values for each item were initialized to zero and were gradually updated using a modified version of the Rescorla–Wagner algorithm: $Q_{t+1}(i) = Q_t(i) + \alpha(r_t - Q_t(i))$ for $i \in \{A, B, C, D, E, F\}$ and t the trial number. The prediction error defined as $r_t - Q_t(i)$ is the difference between the actual and the expected feedback, where r_t represents the received reward on trial t (either 0 or 1 for negative and positive feedbacks, respectively). The learning rate parameter α reflects the impact of the prediction error; lower α-values indicate that the Q-values are integrated gradually over multiple-trials, whereas higher α-values reflect the recency effect (Frank et al., 2007). The probability of choosing one item over the other from a given pair was calculated using the soft-max rule. Thus, the probability of choosing A when AB was presented was calculated using the following rule: $P_t(A) = \exp(Q_t(A)/\beta)/[\exp(Q_t(A)/\beta) + \exp(Q_t(B)/\beta)]$. The parameter β reflects the participant's bias towards either exploration or exploitation: lower β-values indicate that the participant exploits the decision (i.e., choosing the better option with higher probability), whereas higher β-values reflect exploration (i.e., choosing the items more randomly) (Frank et al., 2007; Jocham et al., 2011). The maximum-likelihood (ML) parameter estimate (MLE) was selected by choosing parameters α, β that maximized the log-likelihood $l(d|\alpha, \beta) = \sum_{t=1}^{n} \log P_t(d_t)$, where $d_t \in \{A, B, C, D, E, F\}$ is the participant's decision on trial t. We maximized the parameters for each participant separately using the Nelder–Mead simplex algorithm (Nelder & Mead, 1965). The optimization algorithm was run 100 times for each subject from randomly generated starting points in the interval [0,1] for α and [0,3] for β to ensure uniqueness of the solution.

2.7. Statistical analysis

For each symbol and participant, we calculated the percentages (i.e., the percentage of choosing a symbol), accuracy and reaction time (RT). The percentage values for choosing the symbols were calculated relative to the total number of decisions corrected for the missing values. Accuracy was defined as "correct", when the statistically better symbol (i.e., A, C and E) was chosen from a given pair. Therefore, when participants received negative feedback after choosing the better option (e.g., A), the decision is still considered to be an accurate decision. Similarly, when participants chose B (e.g., suboptimal) and received positive feedback, the decision is considered incorrect. The probability to stay after positive, p(stay|win), or negative feedback, p(stay|lose), was calculated as the number of stays after positive or negative feedbacks, divided by the total number of positive or negative feedbacks.

A Shapiro–Wilk test was performed, which indicated that in the case of accuracy in the "choose A – avoid B" classification scheme data, the assumption of normality was violated (all $ps < .004$); therefore, an arcsine square root transformation was applied on these data such that the assumptions for the ensuring parametric tests were fulfilled (all $ps > .05$). Data were analyzed using repeated-measures Analyses of Variance (ANOVA). The assumption of sphericity was tested using the Mauchly test. If there was violation of sphericity, a Huynh-Feldt correction was applied that adjusts the p-values and degrees of freedom, and the latter values were rounded up to the first decimal place. Statistical analyses were conducted using a significance level of $p < .05$. If significant interactions occurred, post-hoc multiple comparisons were performed, where the p-value was always adjusted for multiple comparisons using the Bonferroni–Holm method (Holm, 1979).

In the learning phase, the within-subject factors were stimulation (2 levels: sham and anodal tDCS), block (6 levels: 1–6 blocks), block part (2 levels: first 40 decisions and last 80 decisions) and behavioral shifting (2 levels: win-stay and lose-stay). In the transfer phase, within-subject factors were stimulation (2 levels: sham and anodal tDCS), feedback learning (2 levels: choose A and avoid B classification) and symbols for the final Q-values (6 levels: for A, B, C, D, E and F symbols).

3. Results

3.1. Analysis of the learning phase

3.1.1. General accuracy and RT

During the course of the experiment, participants learned to reliably choose the statistically better symbol from the pairs in both stimulation conditions, evidenced by a significant increase in the arcsine square root transformed accuracy across blocks ($F_{3.4,51.1} = 15.417$, $p < .001$, $\eta_p^2 = .507$). The general learning performance was not influenced by tDCS. There was neither a main effect of stimulation nor a stimulation × block interaction (all $ps > .189$). The RT data revealed the same pattern of results, that is, the significant main effect of block indicates that participants became faster ($F_{5,75} = 49.519$, $p < .001$, $\eta_p^2 = .768$), but the lack of a significant main effect of stimulation and the stimulation × block interaction suggests that in general, RT was not modulated by the stimulation ($F < 1$) (see Table 1).

3.1.2. Analysis of the stay and shift behavior

When analyzing the amount of stay behavior separately for the symbol pairs, we found a significant main effect of stimulation for the AB pair ($F_{1,15} = 5.09$, $p = .04$, $\eta^2 = .07$) (see Fig. 3). Neither the main effect of stay type ($F_{1,15} = 2.07$, $p = .17$, $\eta^2 = .06$), nor the stimulation by stay type interaction ($F_{1,15} = .04$, $p = .84$, $\eta^2 = .0000$) reached a level of significance (see Table 2).

For the CD and the EF pair, the analysis revealed neither main effect for stimulation, nor a stimulation × behavioral shifting interaction (all $ps > .336$; all $Fs < 1$) (see Table 2 for descriptive statistics).

3.2. Analysis of the transfer phase

3.2.1. General accuracy and RT

General accuracy in the anodal tDCS and sham tDCS session was compared with paired *t*-tests, which revealed that participants performed significantly better when receiving sham tDCS compared to anodal tDCS [$t(15) = 2.887$, $p = .012$]: $M_{sham} = .82$, $SEM_{sham} = .02$; $M_{anodal} = .75$, $SEM_{anodal} = .03$. RT was not affected by the stimulation [$t(15) = 1.232$, $p = .237$].

Table 1 – Mean (untransformed) accuracy (ACC) and reaction time (RT) in the sham and the anodal tDCS sessions in the six learning blocks. SEM: standard error of mean.

Block number	Mean ACC ± SEM		Mean RT ± SEM (msec)	
	Sham	Anodal	Sham	Anodal
1	.69 ± .04	.69 ± .04	948.3 ± 41.0	951.0 ± 49.0
2	.79 ± .04	.75 ± .04	858.0 ± 45.9	862.1 ± 57.1
3	.82 ± .04	.81 ± .04	785.2 ± 48.7	816.8 ± 37.3
4	.85 ± .04	.81 ± .05	792.4 ± 38.5	778.0 ± 44.8
5	.85 ± .04	.80 ± .05	770.8 ± 47.2	751.8 ± 43.4
6	.88 ± .04	.83 ± .04	727.6 ± 42.3	734.0 ± 39.2
Mean ± SEM	.81 ± .04	.78 ± .04	813.7 ± 43.9	815.6 ± 45.1

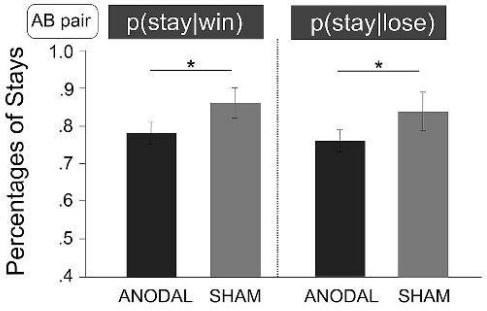

Fig. 3 – In the anodal tDCS session, participants stayed significantly less after receiving reward or punishment in the AB pair. Values represent mean percentages calculated for the six experimental blocks in the learning phase. Error bars represent standard error of mean. Asterisk indicates significant differences.

3.2.2. Analysis of the "choose A – avoid B" trial classification scheme

A repeated-measures ANOVA on the arcsine square root transformed accuracy measure revealed a significant main effect of stimulation ($F_{1,15} = 6.412$, $p = .023$, $\eta_p^2 = .299$) and significant stimulation × feedback learning ($F_{1,15} = 5.115$, $p = .039$, $\eta_p^2 = .254$). Post-hoc comparisons showed that in the anodal tDCS condition, participants performed less well on "choosing A" (calculated from the AC, AD, AE, and AF trials) when compared to the sham tDCS session [$t(15) = -3.017$, $p = .018$] (Fig. 4). No significant differences were found on the "avoid B" [$t(15) = -.691$, $p = .5$] (calculated from BC, BD, BE, and BF trials).

3.3. Analysis of the final Q-values at the end of the learning phase

The final Q-values showed a significant main effect ($F_{1,75} = 33.40$, $p = 2.0 \times 10^{-7}$, $\eta^2 = .338$) of symbol. However, none of these values were modulated by the stimulation ($F_{1,15} = .93$, $p = .35$, $\eta^2 = .017$) and stimulation by symbol interaction ($F_{1,75} = 1.19$, $p = .32$, $\eta^2 = .0008$). These results may indicate that the participants did not differ in the two stimulation conditions with regard to the ability to learn expected reward values for each symbol.

3.3.1. Analysis of the RL parameters at the end of the learning

In order to maximize the likelihood of each participant's trial-by-trial sequence individually for each participant, we fitted the two free parameters α and β to the data from the learning phase. Since the data were not normally distributed for either the α or β parameters even after the arcsine square root transformation procedure (Shapiro–Wilk test; all $ps < 4.7 \times 10^{-6}$), Wilcoxon signed rank test was performed on the untransformed values. The analysis revealed a significant difference in the β parameter [$Z(15) = 2.07$, $p = .04$] between the anodal (.16 ± .02) and sham (.11 ± .02) stimulation

Table 2 – The mean propensity to stay following positive or negative outcome calculated separately for the three different symbol pairs. SEM: standard error of mean.

	AB		CD		EF	
	Sham	Anodal	Sham	Anodal	Sham	Anodal
p(stay|win) ± SEM	.86 ± .03	.78 ± .04	.81 ± .03	.79 ± .04	.75 ± .04	.72 ± .04
p(stay|lose) ± SEM	.84 ± .03	.76 ± .05	.81 ± .04	.76 ± .05	.74 ± .04	.72 ± .04

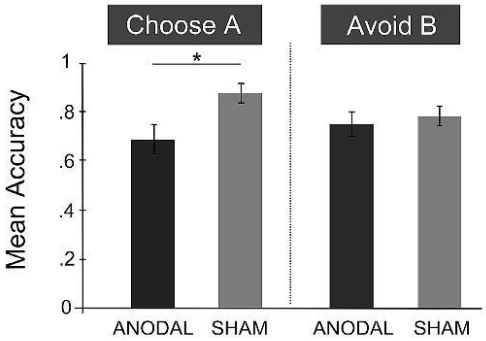

Fig. 4 – Participants performed significantly worse choosing A (calculated from AC, AD, AE, and AF pairs in the transfer phase) in the anodal tDCS compared to the sham tDCS condition, whereas avoid B (calculated from BC, BD, BE, and BF pairs in the transfer phase) performance was not influenced by the stimulation. Error bars represent standard error of mean. Asterisk indicates a significant difference.

conditions, but not in the α values [$Z(15) = -1.09$, $p = .3$] (anodal: .04 ± .01 and the sham .09 ± .06) (Fig. 5).

Because ML-based estimations sometimes have stability issues (parameter identifiability problems; Rutledge et al., 2009), we also ran a hierarchical Bayesian analysis as well as a model incorporating a perseverance parameter (Rutledge et al., 2009), for details on these analyses, see Supplemental Methods and Results.

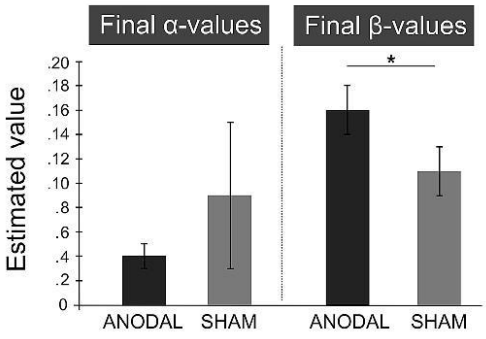

Fig. 5 – The mean final value for the β parameter was significantly higher in the anodal compared to the sham stimulation condition. The α parameter in the anodal and sham stimulation conditions was not significantly different. The vertical axis uses estimated values.

4. Discussion

The aim of the present study has been to investigate the role of the prefrontal high-level cognitive component of instrumental learning. Sixteen male participants were administered sham and anodal tDCS using a double-blind, sham-controlled, repeated-measures study design. Based on computer simulations of the electric current flow in the brain (Windhoff et al., 2013), we applied an electrode montage maximizing the current distribution over left DLPFC, a brain region playing a key role in high-level control of instrumental learning (Collins & Frank, 2012; Daw et al., 2006). During the learning phase, we observed a greater amount of behavioral shifting in the anodal tDCS as compared to the sham tDCS condition in the AB pair. In addition, fitting computational model parameters to the behavioral data also showed that participants were significantly impaired in exploiting the symbols associated with the higher reward probability as evidenced by increased β values (indicating increased randomness of choice) during learning and decreased accuracy for choosing the better option in the transfer phase in the anodal tDCS condition. Our findings complement previous computational, neuroimaging and genetic studies that investigated the role of the prefrontal component in instrumental learning (Collins & Frank, 2012; Daw et al., 2006; Frank et al., 2009; Frank et al., 2007) by means of a tDCS method.

In the anodal tDCS session, we observed more behavioral shifts (i.e., choosing the alternative symbol in next trial) relative to the sham tDCS session for the AB symbol pair during the learning phase. Anodal tDCS decreased the probability of win-stay and lose-stay behavior, in other words, participants shifted more often, both after positive and negative feedbacks. The pattern of these findings indicates that our participants showed increased shifting behavior, since they changed their decision both after positive and negative feedbacks. This was further supported by the increased β parameter in the anodal relative to the sham tDCS, which reflects increased randomness of choice.

A number of possible explanations can be provided for the observed pattern of results. TDCS might have affected instrumental learning through the WM. Previous experimental evidence suggests that the WM component provides the possibility for a flexible behavioral control of instrumental learning by actively maintaining the recently reinforced reward values (Collins & Frank, 2012). Genetic studies and computational modeling data indicate that in COMT Met individuals, the elevated PFC DA level may stabilize WM

representations and participants effectively use this ability to systematically adjust behavior on trial-to-trial basis following negative outcomes (Frank et al., 2007). Evidence also indicates that when participants performed in high and low WM-load conditions, COMT Met homozygotes performed better compared to Val carriers in the high (i.e., when the number of stimuli was higher), but not in the low WM-load conditions (i.e., when the number of stimuli was lower) (Collins & Frank, 2012). Further, a recent study also indicates left DLPFC involvement in the model-based control of decision-making via WM, as participants with low WM capacity (and possibly with low DA level) were impaired more after inhibitory continuous theta burst stimulation (cTBS) than individuals with high WM capacity (Smittenaar, FitzGerald, Romei, Wright, & Dolan, 2013). These findings congruently suggest that instrumental learning engages the prefrontal component via the WM.

An important aspect of the present data is that the behavioral effect was observed on the AB pair only even though it did manifest globally in the temperature parameter of the computational model. One possible explanation for this result would be that the low- and high-level components may be differently involved in instrumental learning based on reward probability. When the reward probability can be reliably separated within a pair (e.g., 80/20% as in the AB pair), the instrumental learning benefits more from WM system involvement by actively holding reinforcement outcomes in the WM. On the other hand, the active maintenance of the reinforcement history of the less reliable pairs might be beyond the capacity of the prefrontal system and therefore predominantly recruits the low-level components. In other words, prefrontal tDCS only interfered with the AB pair and not with the other pairs, since the reinforcement history of the AB pair may rely on the WM system, which was affected by tDCS. However, this account fails to explain the increased behavioral shift after both positive and negative feedback.

Although the present experiment employed an electrode montage that maximized the current distribution over the left DLPFC, we cannot claim that anodal tDCS impacted the WM component exclusively. The pattern of the present findings indicates that our participants showed increased shifting behavior, as they changed their decision after both positive and negative outcomes. This is contrary to a previous genetic study, where COMT Met carriers actively maintained recent negative reinforcement experiences and corrected their behavior on a trial-to-trial basis after negative outcomes (Frank et al., 2007). Further, previous studies investigating the left DLPFC found improved WM performance following anodal tDCS (Zaehle et al., 2011), which would lead to more adaptive trial-to-trial adjustment after negative outcomes and to an increased learning rate (α value), similar to COMT Met carriers. Interestingly, we observed only numerical differences in the learning rate parameter between the stimulation sessions by using the ML estimation technique (for the results of the hierarchical Bayesian modeling see Supplemental Fig. 2).

Alternatively, anodal tDCS may have affected the exploratory behavioral component of instrumental learning. Since we observed increased shifting behavior, i.e., an increased β parameter without a significant difference in the learning rate parameter, we conclude that a plausible explanation of the current findings is that anodal tDCS increases the randomness of choice. Current theory and experimental research on exploration suggest that exploration is accomplished by overriding an exploitative tendency of the striatal system by the prefrontal component (Daw et al., 2006). Intriguingly, the competition by mutual inhibition theory holds that decision-making is influenced by the relative degree of inhibition and excitation in the prefrontal cortex and consequently (Hunt et al., 2012), would partially depend on the balance between glutamatergic excitation and gamma-aminobutyric acid (GABA)ergic inhibition (Jocham, Hunt, Near, & Behrens, 2012). As anodal tDCS was shown to locally decrease the cortical GABA level (Stagg et al., 2009), we might speculate that our findings are the results of the decreased inhibitory GABA level in the frontal cortex, which may in turn increase choice randomness. Nevertheless, future neuroimaging experiments are needed to investigate this speculation directly.

Further, our findings are in line with a previous tDCS study, which applied anodal tDCS over the left DLPFC and observed suboptimal decision-making performance following anodal stimulation (Xue, Juan, Chang, Lu, & Dong, 2012). Although the experimental paradigm was somewhat different from that of the present experiment, the behavioral consequence of anodal tDCS was fundamentally equivalent in the two studies. Similar to our results, participants stayed less often after positive feedback, however, they also stayed more often after negative outcomes. Observing a brief and reversible decline in performance during anodal stimulation is not unprecedented in the literature (e.g., Ambrus et al., 2011), although it is commonly thought that anodal tDCS leads to an increase in neuronal excitability in the brain area underneath the electrode that should result in performance augmentation in a given task. However, it is hard to establish such a simple, linear and mechanistic relation between stimulation parameters, direction of the cortical excitability change and expected behavioral influence. In fact, this implicit assumption about the polarity effect of tDCS and its physiological consequences were recently questioned by a modeling study which showed that tDCS electric fields can de- and hyperpolarize within the same gyrus (Reato et al., 2013). Further, even in a homogeneous electric field, different types of neurons are differentially modulated based on their morphology and orientation (Radman et al., 2009). In line with the modeling studies, a meta-analysis on the effect of tDCS also supports the view that, compared to the motor domain, the polarity effect is less consistent on the cognitive domain (Jacobson et al., 2012).

An intriguing remaining question is whether tDCS increased randomness of choice by affecting the prefrontal or the striatal system. Positron emission tomography (PET) studies conducted on transcranial magnetic stimulation (TMS; another non-invasive brain stimulation technique) found that prefrontal TMS can have an impact on striatal DA release (Cho & Strafella, 2009; Ko et al., 2008; Strafella, Paus, Barrett, & Dagher, 2001). Unfortunately, the neurochemical effect of tDCS is still unexplored, and our experimental design did not allow us to directly answer this intriguing question as we lack PET/fMRI data. On purely speculative grounds however, we favor the view that the observed differences are mainly due to prefrontal rather than striatal changes. First, based on the present electrode montage, the computational model of electric current flow predicts that the electric field strengths in the

striatum are several orders of magnitude smaller than in the prefrontal cortex and are thus, very likely, not effective. In addition, the electric field estimation results are in line with previous fMRI findings showing local neurotransmitter change in the neocortex (Stagg et al., 2009). In addition, a previous functional neuroimaging study associated left DLPFC activity with maladaptive decision strategy, which was further influenced by anodal stimulation over the left DLPFC (Xue et al., 2012). Further, recent work indicates that the excitation-inhibition balance in the prefrontal cortex related to glutamatergic and GABAergic neurotransmitter balance (both of these are affected by tDCS) can itself explain value-based choice behavior variability (Jocham et al., 2012). Finally, although an animal study showed that tonic extracellular DA increase can influence exploration (i.e., the temperature or noise parameter) in rats (Beeler et al., 2010), the exact mechanism of how prefrontal tDCS could lead to altered striatal DA release in humans is unknown.

In summary, the present study tested the possible involvement of the prefrontal system in human instrumental learning by means of tDCS. DLPFC was targeted using an optimized montage based on computational electric field simulations (Windhoff et al., 2013). Stimulation with anodal tDCS increased behavioral shifting and decreased adaptive behavior compared to sham tDCS, possibly reflecting interference with the prefrontal system. The complexity of our results indicates that further studies are needed to investigate the interaction between the low-level and high-level components of instrumental learning.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cortex.2014.08.026.

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Chapter 4: Summary

The present thesis concentrated both on methodological and cognitive aspects of tES techniques because the cognitive effects of tES cannot be meaningfully evaluated without an appropriate and effective methodological study design. In the following I will first present a general discussion of the two methodological studies, which will be followed by the discussion of the cognitive effects of tDCS observed in our study.

4.1. Methodological Aspects of tDCS and tACS

tES techniques offer a fascinating opportunity to learn more about brain processes. They are interesting tools that can induce perturbations in the brain by delivering a current through external electrodes in a safe and non-invasive manner. However, in order to evaluate the efficacy of tES it is imperative to properly blind both the participants and the investigators to the applied stimulation protocols.

Studies using tES typically employ two stimulation conditions, one real and one placebo, and it is widely assumed that the blinding is properly maintained throughout the entire study by using the fade-in, short-stimulation, fade-out placebo protocol (Ambrus et al., 2012; Gandiga et al., 2006). However, ample experimental data exist showing that participants may indeed notice the difference between the two conditions, and that, in turn, may compromise the experimental work (O'Connell et al., 2012; Palm et al., 2013; Raco et al., 2014). This is most probably due to the perceived differences between the conditions, as both tES techniques induce cutaneous discomfort. In addition, tACS also evokes phosphenes (Kanai et al., 2008; Kar & Krekelberg, 2012; Laakso & Hirata, 2013; Raco et al., 2014; Schutter & Hortensius, 2010).

The first project concentrated on the role of electrode size in evoking cutaneous discomfort during tDCS. Participants received two blocks of stimulation in a counter-balanced order. In the equivalent current density condition, the current density through the two electrode pairs was kept at a constant level. In the equal current intensity condition, the current intensity was kept at constant level resulting larger current density through the smaller electrodes.

In the equal current density condition, it was found that both the occurrence rate and the intensity of the evoked discomfort were lower with the smaller electrodes. In the equal current intensity condition no significant difference was observed. Thus, the main finding of this study was that using smaller electrodes reduced the subjectively perceived cutaneous discomfort during tDCS. Our findings are in line with previous observations, namely, reducing the stimulated surface can reduce the stimulation-induced discomfort. This phenomenon is also known as the spatial summation effect, which was demonstrated for mechanical, thermal and electric nociceptive stimulations in earlier studies (Martinsen et al., 2004; Nielsen & Arendt-nielsen, 1997; Price et al., 1989). Our results replicated these prior observations by means of tDCS, as participants reported decreased amount of discomfort when smaller electrodes were employed.

Earlier works have shown that multiple factors may influence the tolerability of tDCS. At present, tDCS can be effectively blinded up to 20 minutes, when the stimulation is applied at 1.0 mA (Ambrus et al., 2012; Gandiga et al., 2006). When 2.0 mA is used, this blinding is compromised and both the participants and the investigator are able to reliably differentiate between the placebo and real stimulation sessions (O'Connell et al., 2012). So far, several methods of maintaining effective blinding have been tested. A previous study, for example, examined whether eliminating the corners by using round electrodes could reduce cutaneous discomfort as computational models of the current flow predicts higher current densities at

the corners of rectangular electrodes (Ambrus, Antal, et al., 2011; Minhas, Datta, & Bikson, 2011). This phenomenon may decrease cutaneous discomfort under local current accumulation points and, hence, participants may tolerate tDCS better than with rectangular electrodes (Ambrus, Antal, et al., 2011). However, participants reported no differences between the round and the rectangular electrodes, that is, electrode shape had no measurable effect on the tolerance of tDCS. Another approach involves the application of topical anesthesia. This did indeed successfully reduce cutaneous discomfort in the participants (McFadden, Borckardt, George, & Beam, 2011). Nevertheless, the anesthetic cream requires 20 minutes or longer for the analgesic to take effect. This prolongs the duration of the experiments, and the cream can therefore not be employed in certain studies. A more recent approach employed a different placebo protocol, which seemed to successfully maintain the blinding when higher intensity was used (Palm et al., 2013). Instead of using the fade-in, short-stimulation, fade-out protocol, this placebo protocol delivered short pulses of low intensity DC over a period of 10 minutes or longer (Palm et al., 2013). This can potentially continuously mimic the cutaneous discomfort perceived during the real stimulation. However, the physiological effect of such a placebo protocol is still not entirely established, and future work is required to ensure that it can be safely used as an inert placebo protocol.

The second project focused on the role of the stimulation frequency on cutaneous sensations and phosphenes during tACS. Participants received short-duration tACS using stimulation frequencies between 2 and 250 Hz and were requested to rate the subjective intensities of the procedural sensations. We found that the intensity of both cutaneous sensations and phosphenes was induced in a frequency-dependent manner, although this was more pronounced for phosphenes. According to our results, the alpha, beta and gamma frequency ranges (i.e., between 8 and 80 Hz) were the frequencies most affected. Our results may play an important role in designing placebo stimulation protocols for tACS, as the

current placebo stimulation protocols seem to be unable to effectively blind participants. The fade-in, short-stimulation, fade-out placebo stimulation protocol developed for tDCS can effectively mimic cutaneous discomfort because the procedural discomfort persists after the end of the placebo stimulation (Ambrus et al., 2012). However, our study and an additional subsequent investigation both indicated that phosphenes are not perceived by the participants as soon as the AC is turned off (Raco et al., 2014). Our findings suggest that future experimental work should concentrate on developing and optimizing tACS placebo protocols specifically for stimulation frequencies between the alpha and gamma ranges. Importantly, our results of the second project were replicated and further extended by a recent and independent study, which systematically investigated the combined effects of stimulation frequency, electrode montage, and current intensity on phosphenes (Raco et al., 2014). Similar to our results, they also found that close proximity of the electrodes to the eyes and a stimulation frequency in the beta range produces the most vivid visual percepts (Raco et al., 2014).

These above-mentioned works will hopefully contribute to the development of well-tolerated tDCS and tACS protocols with appropriate blinding characteristics that can be used in therapeutic applications (Palm, Ayache, Padberg, & Lefaucheur, 2014; Rothwell, 2012). TDCS has been used in neurorehabilitation of patients with various diseases that are characterized by abnormalities in neocortical activity. For example, anodal tDCS has been used to facilitate motor or speech recovery (e.g. naming ability) following stroke, (for a review see: Flöel, 2014), improve quality of life in patients with multiple sclerosis (Palm et al., 2014; C. Saiote et al., 2014) or enhance recognition memory in patients with Alzheimer's disease (e.g. Ferrucci et al., 2008). TACS may be utilized in the future to prevent epileptic seizures (e.g. Berényi, Belluscio, Mao, & Buzsáki, 2012) or restore visual functions by improving temporal processing of visual information in patients suffering from optic

neuropathy (Sabel et al., 2011). Although tDCS and tACS both are promising approaches in neurorehabilitation, their therapeutic efficacy still needs to be evaluated in large-sample, randomized, placebo-controlled, double-blinded clinical trials. Unfortunately, most of the studies investigating the clinical potentials of tES do not yet meet these criteria (O'Connell et al., 2012), hence, there is a need at the moment to improve stimulation protocols for future trials.

4.2. The Effect of tDCS on Instrumental Learning

Instrumental learning involves two major anatomical structures that work in parallel but are organized in a hierarchical structure. In humans, a wide range of evidence from clinical (Frank et al., 2004; Rutledge et al., 2009; Wrobe et al., 2011), pharmacological (Jocham et al., 2011; Pessiglione, Seymour, Flandin, Dolan, & Frith, 2006) and genetic studies (Frank et al., 2007; Klein et al., 2007) consistently supports the essential role of the striatal system in instrumental learning through the reward prediction error. In contrast, only few studies have so far investigated the high-level prefrontal component (Collins & Frank, 2012; Smittenaar, FitzGerald, Romei, Wright, & Dolan, 2013). The third project investigated the involvement of the prefrontal system in instrumental learning by using anodal DC stimulation.

Instrumental learning tasks typically require learning to select those actions that lead to a maximized reward. One of the proposed roles of the prefrontal cortex is that it represents the value of the different actions that are accessible by the working-memory system, enabling trial-to-trial behavioral corrections of choices following suboptimal outcomes (Collins & Frank, 2012; Frank et al., 2009, 2007). The prefrontal system represents various action values simultaneously, and it is suggested that these representations mutually inhibit each other until

one action "wins" this competition (Hunt et al., 2012; Jocham et al., 2012). In fact, earlier work has shown that inhibitory processes may play an important role in decision making, as the naturally occurring difference in GABA levels in the prefrontal cortex was associated with decision performance (Jocham et al., 2012). Thus, if the degree of inhibition and excitation influenced decision making performance, anodal tDCS may perturb this process by exerting its decreasing effect on the local neocortical GABA level (Stagg et al., 2009).

Indeed, our results clearly showed increased shifts in behavior after both positive and negative outcomes, and an increased value for the estimated noise parameter reflecting the degree of randomness in the decision making process. Therefore, both the behavioral and the estimated computational model parameters of the Q-learning algorithm congruently indicated increased randomness of choice during anodal compared to placebo tDCS, which is in accordance with the mutual inhibition hypothesis. The present study cannot rule out the role of working memory in instrumental learning, although participants did not show trial-to-trial adjustment following suboptimal decisions, which may indicate a working-memory effect. In summary, by applying anodal and placebo tDCS in a randomized, counterbalanced, double-blind, crossover study design, we demonstrated that changes in the excitation-inhibition balance in the prefrontal cortex influence instrumental learning. The pattern of our behavioral results are in agreement with the predicted consequences of previous MRS results performed over the motor cortex using anodal tDCS (Stagg et al., 2009), as well as with prior work highlighting the role of GABA on decision making (Jocham et al., 2012).

4.3. Future Directions in Research

In the first project we demonstrated for the first time that reducing the electrode size during tDCS reduces cutaneous discomfort. Based on these results, future studies may use

smaller electrodes in order to improve the blinding potential and the tolerance of tDCS, and, at the same time, to increase the focality of stimulation (Faria et al., 2011; Miranda, Faria, & Hallett, 2009). This study is, of course, not without limitations. Future experiments may address the question of whether the occurrence rate or the intensity of the reported cutaneous discomfort can be influenced by a longer stimulation duration. The present experiment employed 12 short-stimulation trials that lasted for 31 seconds each, in order to counterbalance the order of the various stimulation intensities and the sequence of stimulated side. Although there is evidence that short-stimulation protocols similar to the present one can be extrapolated to longer stimulation protocols (Ambrus et al., 2012; Raco et al., 2014), it would be of interest to investigate cutaneous discomfort when stimulation is applied for tens of minutes instead of seconds. For instance, it could be argued that smaller electrodes may dry out faster during prolonged stimulation, which could negatively influence cutaneous ratings. In addition, future work should concentrate on the possible qualitative differences in cutaneous discomfort (e.g. itching, tingling, burning sensation) between the various electrode sizes. Therefore, more data is needed in order to decide whether our results can be generalized to longer-stimulation protocols. Experiments are currently being conducted in our laboratory that are aimed at elucidating this issue by applying 10-minute-long stimulation protocols with three different electrode sizes (4, 16 and 35 cm^2).

The second project demonstrated that phosphenes are not just a negligible "side-effect" of tACS, as the overwhelming majority of our participants detected their presence. Therefore, there is a need to optimize placebo stimulation protocols for future tACS studies, since our data and those from an independent laboratory all suggest that participants perceive phosphenes when stimulation is applied (Raco et al., 2014; Turi et al., 2013). Some of the studies use individually determined stimulation intensities that are below the individual phosphene threshold (Neuling et al., 2013). This procedure not only significantly prolongs

the experimental time, but also raises some concerns about dosing tACS appropriately. Estimating the intensity of tACS may be based on more elaborate factors (e.g. bone thickness, skin properties or location of the target in the brain) rather than on the measurement of phosphene thresholds. One possible future solution for coping with phosphenes would be to apply additional electrodes in close proximity to the eyes (e.g. placed above them). This would stimulate the eyes with low intensity AC but reduce the stimulation of neocortical areas possibly by using current intensity based on FEM estimations (Figure 3). According to this idea, at least two electrode pairs would be employed; one pair would be placed above the neocortical target (to stimulate the region of interest) and the other pair directly above the eyes. The role of this second electrode pair would be to intentionally evoke phosphenes, even when the transcranial electrodes above the region of interest are turned off in the placebo condition. This placebo stimulation protocol would mimic the phosphenes caused by the real tACS, and participants would thus not be able to differentiate between the real and placebo conditions. Nevertheless, the ideal future solution would be to improve the control over the current flow and develop highly focal tACS montages that avoid leakage of the current to the retina, in order to prevent the occurrence of phosphenes.

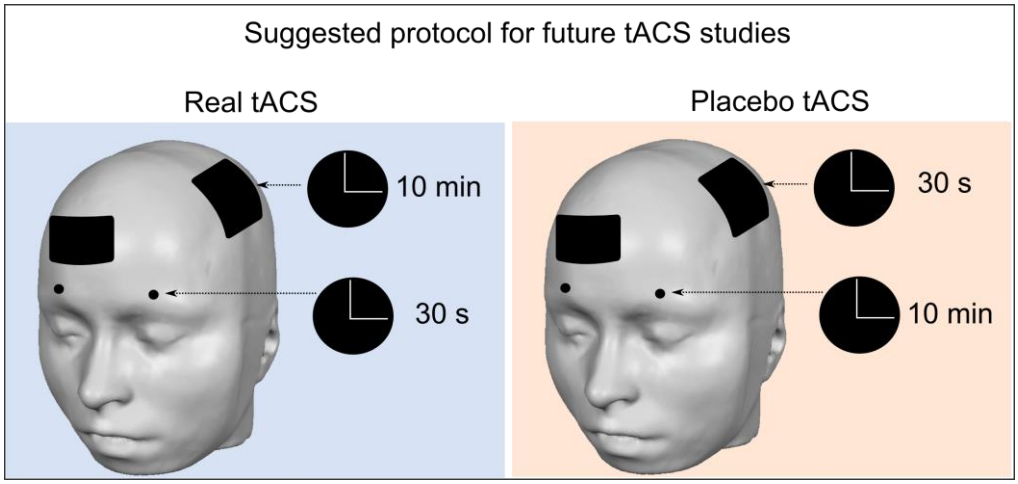

Figure 3. A suggested stimulation protocol for future studies using tACS. In addition to the original electrode pair (rectangular), one extra electrode pair is placed over the eyes to evoke phosphenes during the placebo tACS. Min: minutes, s: second.

Regarding my last project, future studies will be needed to replicate our behavioral findings, to unravel the molecular mechanism of tDCS and to identify the GABAergic circuits involved in its action (English et al., 2012). On the one hand, animal research will be needed to identify the various types of interneurons that are potentially involved in instrumental learning and affected by stimulation, possibly by combining optogenetic stimulation techniques with tDCS. On the other hand, future human research may focus on the combination of anodal tDCS with MRS to extrapolate prior findings of studies performed on the motor cortex (Stagg et al., 2009) to the prefrontal region. As a further step, establishing a causal link between anodal tDCS-decreased GABA levels and the associated behavioral consequences (e.g. shifting behavior) would further improve our understanding of the role of GABA in decision making, as well as of the working mechanism of tDCS itself. These results would provide information complementary to earlier studies that can be considered more correlative in nature (e.g. Jocham et al., 2012). At the present, studies are aimed at targeting only cortical structures due to the existing limitation of the tES technique, which does not allow it be focally delivered to subcortical structures. However, future work may consider indirectly targeting subcortical regions via targeting the cortical-subcortical network (Wang et al., 2014) or directly by using FEM optimized electrode montages, which is at present time not possible with tES techniques. New developments in tES techniques in combination with improved FEM estimations for current flow optimization may revolutionize the currently used stimulation montages and qualify tES as a suitable tool for non-invasively stimulating deep cortical structures.

Finally, I would like to mention an intense discussion about improving transparency and establishing new research and publication standards in future scientific works which may stimulate developments in our field as well. Replicating previous findings has recently received great interest in the neuroscience community when it was realized that some of the earlier findings in the field of biology or psychology could not be repeated (e.g. Begley, 2013; but see Bissell, 2013 opinion on this topic). In fact, in 2014 the journal Nature dedicated an online special to reproducibility issues in the biological sciences including neuroscience. This is available online at http://www.nature.com/nature/focus/reproducibility/. One approach would be to encourage code sharing between laboratories, which can increase transparency about how the statistical analyses were performed (Easterbrook, 2014). Another approach aims at developing new study validation mechanisms, at least for influential and/or high-profile papers that would preferably be performed by independent laboratories (Baker, 2012). Sharing the raw data in an anonymous way and making it accessible for scientific purposes (so called open-access repository) would also improve transparency (Baker, 2012). Our field would also benefit from implementing these above-mentioned suggestions, as there is a need to clarify recently discovered variability issues regarding earlier tDCS and repetitive TMS (rTMS) findings (Hamada, Murase, Hasan, Balaratnam, & Rothwell, 2013; Horvath, Carter, & Forte, 2014; López-Alonso et al., 2014; Wiethoff et al., 2014). Individual factors determining the size of the inter- and intra-individual variability of tDCS protocols are still elusive, and studies differ remarkably in this respect (for comparison see: Hamada et al., 2013; Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005). This might be partially due to different participant selection procedures, which are often poorly documented in NIBS studies. Not only the researchers themselves, but also scientific journals should encourage the authors to improve the quality of publication by requiring a more transparent and accurate documentation and by making it possible to submit supplemental materials, raw data and

code for statistical analyses (or alternatively, citable online code repositories for statistical analyses). My optimistic expectation is that in the next five years changes will be gradually implemented in our field similar to those in other fields in neuroscience.

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List of Abbreviations

AC – alternating current

DC – direct current

DLPFC - dorsolateral prefrontal cortex

EEG - electroencephalogram

FEM – finite element model

fMRI - functional magnetic resonance imaging

GABA – gamma aminobutyric acid

LTD - long-term depression

LTP - long-term potentiation

MR – magnetic resonance

MRI - magnetic resonance imaging

MRS – magnetic resonance spectroscopy

NIBS - non-invasive brain stimulation

NMDI - N-methyl-D-aspartate

PET – positron emission tomography

rTMS – repetitive transcranial magnetic stimulation

tACS - transcranial alternating current stimulation

tDCS - transcranial direct current stimulation

tES - transcranial electric stimulation

TMS - transcranial magnetic stimulation

Contributions to the Original Publications

Project 1

Zsolt Turi designed the experiment, partially performed the data collection (both for experiment one and two), performed the entire behavioral analysis (under the supervision of the Department of Medical Statistics, UMG), and significantly contributed writing the article (including introduction, methods, results and discussion).

Kerrie-Anne Ho partially performed the data collection (both for experiment one and two), significantly contributed writing the article (including introduction, methods, results and discussion).

Géza Gergely Ambrus programmed the experimental paradigm, contributed writing the article (including the introduction, methods, results and discussion parts).

Titas Sengupta contributed writing the introduction and discussion part of the article and contributed to double-blind data collection by blinding the stimulators and preparing the electrodes.

Walter Paulus contributed writing the article.

Andrea Antal contributed writing the article and supervised double-blind data collection.

Project 2

Zsolt Turi designed the experiment, supervised data collection, performed the entire behavioral analysis, and significantly contributed writing the article.

Géza Gergely Ambrus programmed the experimental paradigm, contributed to writing the article (including the introduction, methods, results and discussion parts).

Karolina Janacsek contributed to writing the article and supervised data analyses.

Kirsten Emmert performed data collection and contributed writing the article.

Leandre Hahn performed data collection and contributed writing the article.

Walter Paulus contributed writing the article.

Andrea Antal contributed to writing the article and supervised all aspects of the project.

Project 3

Zsolt Turi designed the experiment, programmed the experimental paradigm, supervised data collection, contributed performing the behavioral analysis (including Figure 1, 2, 3 and 4, Table 1 and 2), significantly contributed writing the article (including the introduction, methods, results and discussion parts) and contributed writing the supplementary materials.

Matthias Mittner performed the entire computational modelling analysis of the behavioral data (Figure 5), significantly contributed writing the article (including the introduction, methods, results and discussion parts) and significantly contributed writing the supplementary materials and performed all supplementary analyses and related figures.

Alexander Opitz performed the entire computational modeling of the current flow calculations for the electrode montage, contributed writing the methods and discussion parts, and contributed to writing the supplementary materials, methods part.

Miriam Popkes partially performed the data collection, translated the instruction to German, the questionnaires and contributed writing the article.

Walter Paulus contributed to writing the article.

Andrea Antal contributed to writing the article and supervised double-blind data collection.

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Publications

Original articles (in the topic of non-invasive brain stimulation)

Turi, Z., Mittner, M., Opitz, A., Popkes, M., Paulus, W. and Antal, A. (2015). Transcranial direct current stimulation over the left prefrontal cortex increases choice randomness in instrumental learning. *Cortex*, 63:145-154.

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Original articles (in the topic of psycholinguistics)

Nemeth, D., Janacsek, K., Turi, Z., Lukács, A., Peckham, D., Szanka, S., Gazso, D., Lovassy, N., Ullman, M. T. (accepted). The production of nominal and verbal inflection in an agglutinative language: Evidence from Hungarian. *PLoS ONE*.

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Review articles

Saiote, C., Turi, Z., Paulus, W., and Antal, A. (2013). Combining functional magnetic resonance imaging with transcranial electrical stimulation. *Frontiers in Human Neuroscience*, 7:435.

Turi, Z., Paulus, W., and Antal, A. (2012). Functional neuroimaging and transcranial electrical stimulation. *Clinical EEG and Neuroscience*, 43(3):200-208.

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