Aus der Klinik für Klinische Neurophysiologie.
(Prof. Dr. med. W. Paulus)
der Medizinischen Fakultät der Universität Göttingen

Influence of electrode placement on the efficacy of transcranial direct current stimulation

INAUGURAL-DISSERTATION

zur Erlangung des Doktorgrades
der Medizinischen Fakultät der
Georg-August-Universität zu Göttingen

vorgelegt von

Dona Kyprianou

aus

Nikosia / Zypern

Göttingen 2017

Dekan:	Prof. Dr. rer. nat. H.K. Kroemer
Referentin	Prof. Dr. Andrea Antal
Ko-Referent:	Prof. Dr. Jochen Staiger

Datum der mündlichen Prüfung: 23.11.2017

Hiermit erkläre ich, die Dissertation mit dem Titel " Influence of electrode placement on the efficacy of transcranial direct current stimulation" eigenständig angefertigt und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben.

Göttingen, den …… ……

(Unterschrift)

Table of Contents

Abbreviations

A	Ampere
Ag	Silver
AgCl	Silver Chloride
ANOVA	Analysis of Variance
AV	Average
cAMP	Cyclic Adenosine Monophosphate
cm	Centimeter
cm^2	Square Centimeter
FDI	First Dorsal Interosseus
Hz	Hertz
iTBS	Intermittent Theta-burst stimulation
LTD	Long Term Depression
LTP	Long Term Potentiation
M1	Primary Motor Cortex
mA	Milliampere
MEP	Motor Evoked Potentials
min	Minutes
MRI	Magnetic Resonance Imaging
mV	Millivolt
NIBS	Non-Invasive Brain Stimulation
NMDA	N-Methyl-D-Aspartate
PAS	Paired Associative Stimulation
PET	Positron Emission Tomography
rTMS	Repetitive Transcranial Magnetic Stimulation
SD	Standard Deviation
tDCS	Transcranial Direct Current Stimulation
TES	Transcranial Electrical Stimulation
TMS	Transcranial Magnetic Stimulation

1. Introduction

1.1 Neuroplasticity

The brain has the potential to change and develop throughout our lives, depending on our experiences and our learning processes (Elbert et al. 1995; Rossini and Pauri 2000). Neuroplasticity is the phenomena of forming new connections, fading old ones and reorganising the brain (Berlucchi and Buchtel 2009; Trachtenberg et al. 2002). The brain adjusts to everyday activities, to changes in the living environment, and even to the exposure to new situations (Mattar et al. 2011). For example, learning a new language or trying out a new hobby leads to the creation of new connections. Also, even after severe injuries, if there are enough intact neurons, the brain is able to compensate the loss and form new connections (Nudo 2013). This dynamic behaviour leads research to a new level, to search for better methods and new applications of neuroplasticity in the real life.

This impressive property of the brain was first described in 1793 from Vincenzo Malacarne, who worked with trained animals and observed changes in the size of their brain and related them to their cognitive capabilities (Rosenzweig 2007). In the 1870s Ferrier, Fritsch and Hitzig, were able to identify the relationship of the neuronal signal transmission and the electrical activity, after observing the functioning of the brain (Carlson and Devinsky 2009; Ferrier 1874; Fishman 1995; Fitsch and Hitzig 1870). Almost a century later, in the 1960s, Paul Bach-y-Rita managed to teach congenitally blind people, how to identify a picture of supermodel Twiggy; new signals were inputted to the brain and the participants were able to convert tactile information to visual (Bach-Y-Rita et al. 1969). In the 1980s Barbara Arrowsmith-Young, having herself severe learning disabilities, created brain exercises to enhance her cognitive ability (Arrowsmith-Young 2012). This “workout”, as described above, is one way to achieve neuroplasticity. Another way is the external stimulation of the brain. With regard to the external stimulation several trials were conducted, mainly using electrical stimulation (e.g. (Leyton and Sherrington 1917; Penfield and Boldrey 1937)). This approach was not elaborated until the 1980s when Merton and Morton contrived the Transcranial Electrical Stimulation (TES) method (Merton and Morton 1980), which involved the non invasive application of a high-intensity electrical current on the cerebral cortex. However, it was painful for the subjects and therefore the utilisation of TES was limited. Barker et al. introduced in 1985 the Transcranial Magnetic Stimulation (TMS) method, that had similar effects compared to TES but minimised the discomfort of the procedure (Barker 1991).

Transcranial Direct Current Stimulation (tDCS) is another way of inducing neuroplasticity non-invasively, which uses currents of much lower intensity than TES, thus causing no pain and probably acting on the neuronal level by modulating the membrane resting potential (Bindman et al. 1964; Nitsche and Paulus 2000; Nitsche et al. 2003c; Nitsche et al. 2008; Purpura and McMurtry 1965).

1.2 TMS

As mentioned above, Barker et al. introduced in 1985 the TMS method (Barker 1991). With this method, a stimulation coil is employed which generates transient magnetic fields that penetrate the intact skull. This fast changing magnetic field produces electric currents in the brain. The neurons underneath the stimulation coil are triggered by the electromagnetic field and this can initiate action potentials, when an adequate amount of stimulation intensity is used (Barker 1991; Rossini et al. 1991; Schubert 1997).

TMS is a suitable device to measure the excitability alterations induced by tDCS. These changes are reflected by the amplitude modulations of the Motor Evoked Potentials (MEPs) (Landau et al. 1964; Nitsche and Paulus 2000; Priori et al. 1998). It is suggested that the amplitudes of the produced MEPs correspond to the excitability of the primary motor cortex (M1) (Nitsche and Paulus 2000); anodal tDCS increases and cathodal tDCS decreases the peak-to-peak amplitudes of the MEPs.

1.3 tDCS

1.3.1 tDCS history

tDCS is a method for non-invasive brain stimulation (Nitsche and Paulus 2000). In the sixties Bindman et al. applied weak direct current, using intracerebral or epidural electrodes, on anesthetised rats, and showed that it had an influence on the excitability of the cortex (Bindman et al. 1964). Particularly, anodal direct current stimulation over the M1 increased the firing rates of the neurons and cathodal stimulation reduced it (Bindman et al. 1962; Purpura and McMurtry 1965). Costain et al. 1964 and Carney 1969, examined the application of tDCS in the treatment

of psychiatric patients and found positive results (Carney 1969; Costain et al. 1964). However, the replication of these clinical results was not successful in subsequent studies (Lolas 1977). After almost twenty years, Nitsche and Paulus investigated the position of the electrodes that could lead to an effective stimulation condition in healthy subjects (Nitsche and Paulus 2000), and this technique was again re-discovered and explored. The following montage was tested and approved for stimulation of the M1: the "active" electrode placed over the cortical representation of the tested muscle in the M1 - and the return or "passive" electrode, over the contralateral orbitofrontal cortex. Here it should be stated, however, that the terms "active" and "passive" are not completely appropriate, since both electrodes determine equally the amount of current passing through the brain. Nevertheless, the stimulation polarity is often named after the active electrode (anodal or cathodal stimulation), that is, the electrode over the region of interest.

tDCS was also proved in clinical trials to be a useful tool for many patients with neurological and mental disorders. This is supported by the following later studies: in 2005 Cohen, Hummel, Fregni et al. performed clinical trials on stroke patients using anodal tDCS over the affected hemisphere and cathodal on the opposite site and improved their motor skills (Fregni et al. 2005; Hummel and Cohen 2005). Also patients with major depression can profit from tDCS. For instance, Boggio et al. stimulated 40 patients with depression using anodal tDCS and proved, using depression-scores, the effectiveness of the method (Boggio et al. 2008). tDCS is in the meantime increasingly used in clinical trials and is a good support in the treatment of various neurological disorders i.e. Alzheimer's disease (Boggio et al. 2009; Ferrucci et al. 2008), chronic neuropathic pain (Mori et al. 2010; Soler et al. 2010), treatment of depression (Ferrucci et al. 2009), migraine (Antal et al. 2011; Dasilva et al. 2012), stroke recovery (Baker et al. 2010; Boggio et al. 2007; Rocha et al. 2015), Parkinson's disease (Benninger et al. 2010; Boggio et al. 2006; Fregni et al. 2006) etc. However, the basic neuronal mechanisms of tDCS are yet not fully understood.

1.3.2 Possible neuronal operating principles of tDCS

As it was mentioned above, it is suggested that tDCS operates by altering the polarisation of the neuronal membranes and thus inducing excitability changes of the cortex (Nitsche and Paulus 2000). Membranes can either depolarise - this leads to an increased excitability of the neurons – or hyperpolarise – neuronal excitability decreases (Bindman et al. 1962; Purpura and McMurtry

1965). tDCS cannot trigger action potentials like TMS, but has probably a neuromodulating effect.

Several studies suggested that anodal tDCS over the M1, in a duration of 5-13 minutes and a minimum intensity of 1 mA, could enhance cortical excitability whereas cathodal could reduce it (e.g. (Nitsche and Paulus 2000; Nitsche et al. 2007)). It is assumed that long term excitability changes caused by tDCS can be explained by the concept of long term potentiation (LTP) and long term depression (LTD) (Hattori et al. 1990; Hunter et al. 2013; Islam et al. 1995; Moriwaki 1991; Nitsche and Paulus 2000). These two terms were first described by Terje Lomo in 1966 (Lomo 2003). LTP defines the dynamic of intensifying the connection joining two neurons, due to long lasting and iterated stimulation (Bliss and Collingridge 1993; Cooke and Bliss 2006; Teyler and DiScenna 1987). On the contrary LTD is the procedure of diminishing the power of a connection between two neurons (Ito 1989). It is suggested that the tDCS effects might emerge through shifts of the intracellular cAMP (cyclic Adenosine Monophosphate) and calcium levels and are protein synthesis dependent (Gartside 1968; Hattori et al. 1990; Islam et al. 1995; Malenka and Nicoll 1999; Moriwaki 1991). It is also believed that the NMDA (N-Methyl-D-Aspartate) Glutamate receptor subtype is involved in the cortical excitability changes generated by tDCS (Hunter et al. 2013; Liebetanz et al. 2002; Nitsche et al. 2003a). Liebetanz et al. used in their study Dextromethorphan an NMDA receptor blocker and showed that the tDCS effects were suppressed both after anodal and cathodal stimulation. In this way an association of the NMDA receptor with the tDCS related changes is probable.

The changes of the cortical activity induced by direct current are stable and last up to an hour after the end of the stimulation (Bindman et al. 1964), by a stimulation duration of 5-13 minutes. In order to evaluate these alterations in plasticity after tDCS, an appropriate tool is needed that allows the assessment of the neuronal reaction to tDCS and that is the TMS, as mentioned above (Nitsche and Paulus 2000; Priori et al. 1998). The amplitude of the MEPs generated by TMS reflects these alterations of excitability (Gorman 1966; Landau et al. 1964): anodal tDCS enhancing and cathodal tDCS diminishing peak-to-peak amplitudes of MEPs (Nitsche and Paulus 2000, 2001; Nitsche et al. 2003b).

1.3.3 tDCS Physical parameters

Several stimulation parameters play an important role in the efficacy of tDCS. The key parameter is the polarity of the stimulation and therefore the direction of the current flow. The current flow

is determined by the electrodes arrangement and the neuroanatomy of the individual subject. As already mentioned above, anodal tDCS over the M1 enhanced and cathodal tDCS reduced the MEP size. The after effects are therefore polarity specific (Lang et al. 2005; Nitsche and Paulus 2000; Nitsche et al. 2007).
The current density is another important criterion and it defines the electrical field strength (Purpura and McMurtry 1965). The current density is the quotient of the current strength (at least 1 mA stimulation intensity should be used) and the electrode size. The larger the current density, the stronger are the effects of tDCS (Iyer et al. 2005; Nitsche and Paulus 2000). Several researchers use a current density between 0.029 and 0.08 mA/cm² (for a review see: (Nitsche et al. 2008)).

The duration of the stimulation is another parameter that, together with current density, is important for the strength and lasting of the after-effects of tDCS. The stimulation duration varied from a few seconds up to 20 minutes in most of the published studies (for a review see (Nitsche et al. 2008)). In general, the longer the stimulation duration, the longer the after-effects last (Bindman et al. 1964; Nitsche and Paulus 2000, 2001) but this was not always verified (Lopez-Alonso et al. 2014; Wiethoff et al. 2014).

The size of the electrodes used for tDCS plays an important role in the focality of the stimulation. Studies comparing the size of the electrodes showed that small electrodes with a size of 3.5 cm², over the M1, increased the focality of the stimulation, having a bigger effect on the alteration of the excitability in contrast to large electrodes with a size of 35 cm², which showed more dispersed results (Boros et al. 2008; Nitsche et al. 2007).

The efficacy of tDCS also depends on biological factors like age, genetics, and gender. For example cathodal tDCS over the M1 revealed prolonged excitability inhibition in women (Kuo, Paulus et al. 2006;), whereas anodal tDCS in the visual cortex showed elevated excitability in women compared to men (Chaieb et al. 2008; Kuo et al. 2006).

1.4 Safety aspects of tDCS

To be in a position to use tDCS in clinical trials as well as in the treatment of neurological diseases, it is of great importance to apply safety limits, in order to avoid causing any damage to the study volunteers and/or patients. Nitsche and Paulus described some stimulation paradigms in their protocols, that guide the tDCS users on how to operate the device in a safe way (Nitsche

and Paulus 2000, 2001; Nitsche et al. 2003b; Nitsche et al. 2008). A current density up to 0.029 mA/cm² and a stimulation length up to 13 minutes were tested in more than 2000 subjects and didn't lead to any severe side effects. Under these conditions, some subjects reported a slight itching or burning under the electrodes, headache, nausea and tiredness (Poreisz et al. 2007).
Some precautions should be taken into consideration when using tDCS, such as the electrode montages. No heart-nerve or brainstem stimulation should be attempted (Lippold and Redfearn 1964). The skin-electrode contact could lead to some skin irritation due to allergies or to toxins produced electrochemically in the contact area and due to electrode dissolution products (Nitsche et al. 2008). Consequently the subjects should be explicitly questioned for any dermatological diseases (Palm et al. 2008).

The exclusion criteria -applying for any electrical stimulation and therefore for studies with tDCS as well-, are explained in the chapter materials and methods of this study.

1.5 Aims of the study

It was generally assumed for a long time that peak current occurs underneath the stimulating electrodes (Nitsche and Paulus 2000, 2001; Nitsche et al. 2007). However, evidence from many previous and present studies suggest that this assumption may not be completely correct: e.g. Lang et al. observed a diffuse cortical excitability change after stimulating the prefrontal cortex with tDCS by conducting a positron emission tomography (PET) study (Lang et al. 2005). Opitz et al showed how anatomical factors, such as thickness of the cerebrospinal fluid and the skull, gyral depth and distance between the electrodes can affect the efficacy of tDCS depending on the way the electrodes are positioned on the skull (Opitz et al. 2015). Miranda and Mekonnen proposed in their study using computational models that the maximum peak current can be found at the edge of the electrodes (Miranda et al. 2013). According to this assumption the stimulation electrode should not be placed directly over the M1 but in the direct surrounding area of it, if we aim to have an optimal stimulation efficacy. That could induce a greater excitability change and consequently have more efficient neuroplasticity modulation for the users of tDCS.
Therefore, this study aimed to examine whether a local distribution of current at M1 or global current pathways elucidate the modifications in cortical excitability and in neuroplasticity. In order to do that, two electrode montages were compared: the 'classical montage' with the active electrode over M1 and the return electrode over the contralateral orbita and the 'shifted

montage' with the active electrode moved 3 cm posterior to the M1 and the return electrode kept constant. TMS-evoked MEPs were used to record the excitability changes after stimulating with both montages.

For each of the montages we had two separated sessions, in which anodal or cathodal stimulation were tested (a diagram of the study design can be found in the chapter materials and methods).

To sum up 3 variables were tested in this study:

1) Electrode montage: classical versus shifted
2) Anodal and cathodal tDCS: excitability changes
3) The duration of the excitability changes induced by tDCS up to one hour after stimulation

2. Materials and Methods

2.1 Participants

This study was approved by the ethics-committee (8/9/12) of the University Medical Center, Goettingen. The study and all relevant experiments were conducted in the Department of Clinical Neurophysiology, Georg-August-University in Goettingen. Eleven (11) healthy right-handed (Oldfield 1971) subjects participated in the study -5 females, 6 males- with an average (AV) age + standard deviation (SD) : 25.08 +/- 2.87 years. Before commencing the experiments, all subjects received, in both written and oral form adequate information on the goals, the procedures and the risks involved and they all signed an informed consent (see appendix). The participants received a monetary reward and they had the right to withdraw from the experiments at anytime without giving any particular reason if they did not wish to continue.
The participant's selection was performed through a screening, where they were requested to inform the investigator if any of the following exclusion criteria applied to them - as provided in the safety guidelines of the TMS (Barker 1991; Brandt et al. 1997; Pascual-Leone et al. 1993; Wassermann 1998), the tDCS (Nitsche et al. 2008) and the MRI (Coskun 2011): history or evidence of chronic or residual neurological disease in the applicant or family history of the applicant, pacemaker or deep brain stimulation, metal implants, history of head injury with loss of consciousness, epilepsy, any serious medical conditions or psychiatric illness, pregnancy or breast-feeding, consumption of alcohol, medication before the planned experiments (apart from birth control pill) or drug addiction, local or global aphasia, age; < 18 or > 45 years old, participation in another scientific or clinical study within the last 6 weeks. In such case where the prospective subjects stated that none of the above criteria applied to them, they subsequently had to undergo a medical examination (neurological status). The screening was completed by the application of TMS, which confirmed their eligibility (finding the 'hotspot' and obtaining 20 MEPs with an AV of 0.8-1.2 mV and SD< AV/2, see below) for the study.

2.2 Experimental design

The study was double-blinded; neither the participants nor the investigator knew the type of tDCS (anodal or cathodal) applied in each of the sessions. The first session was a structural MRI, which was used in the following experimental sessions for neuronavigation (Herwig et al. 2001).

The sessions 2-5 were the measurement sessions in which tDCS was used and one of the two montages was applied either with anodal or with cathodal stimulation. Sessions 2-5 were performed in a randomised order (as shown in the diagram below) and they were separated by an interval of minimum 5 days as stated in the safety guidelines of the TMS and tDCS.

EXPERIMENTAL DESIGN			
1	Structural MRI		
2	tDCS + TMS	classical montage anodal	Double-blinded randomised order
3	tDCS + TMS	classical montage cathodal	
4	tDCS + TMS	shifted montage anodal	
5	tDCS + TMS	shifted montage cathodal	

Table 1: The experimental design consisted of 5 sessions. Session 1: Structural MRI. Sessions 2-5 tDCS and TMS experiments using a double-blinded randomised classical or shifted montage.

2.3 Structural MRI

The structural MRI was acquired in a 3-Tesla scanner (Magnetom TIM Trio, Siemens Healthcare Solutions, Erlangen/Germany) at the department of Cognitive Neurology in Goettingen. All participants had to complete a questionnaire (see appendix) as prescribed by the MRI safety guidelines (Coskun 2011). The MRI was performed by a trained employee of the department and included the following scans: structural T1, structural T2, and diffusion-weighted imaging. The images were transferred in the neuronavigation system so as to be used in the subsequent sessions.

2.4 Preparing the setting

The participants were seated in a comfortable dentist chair with head and arm rests. The head- and backrest were adjustable so the subjects were in a relaxed position. MEPs were recorded by placing Ag/AgCl bipolar surface electrodes on the right hand. The active electrode was positioned above the first dorsal interosseus (FDI), found by moving the forefinger to the left

and right inducing a muscle contraction and the reference electrode above the forefinger middle joint (articulation interphalangeal). The grounding electrode was placed over the forearm. Signals were sampled with 5 kHz and band-pass filtered between 2 Hz and 2000 Hz. The conversion from analog to digital form was performed with a micro 1401 AD converter (Cambridge Electronic Design, Cambridge, UK). Signals were viewed with "Signal 3" (Cambridge Electronic Design, v. 2.13) and saved on the computer for later analysis. See a diagram of the setting below (figure 1).

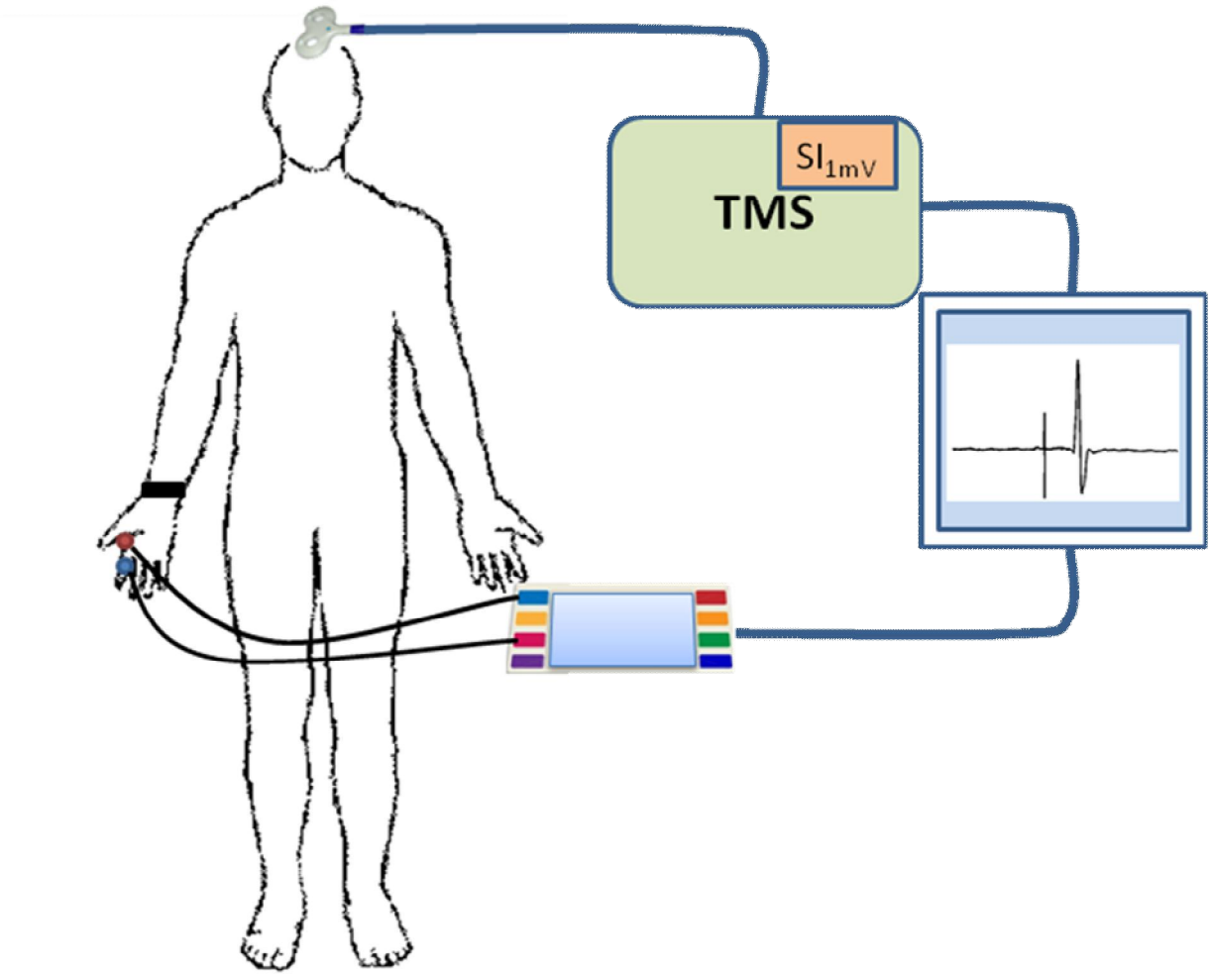

Figure 1: The experimental setting.

2.5 TMS ‘hotspot’ and baseline

After adjusting the hand electrodes the ‘hotspot’ was determined. The ‘hotspot’ was defined as the coil position that produced the largest MEPs of the FDI. This spot was identified by TMS using a MagPro X100 stimulator with a C-B60 coil (figure of eight coil, 35 mm inner diameter, 75 mm outer diameter, 11 mm winding height; MagVenture, Inc., Atlanta, Georgia USA). The TMS was set to give 1 pulse every 4 seconds with an external trigger, connected to the software “Signal 3”.

The coil was held in a 45 degrees angle tangential to the head (figure 2). To find the ‘hotspot’ the coil was moved over the M1-region of the left hemisphere. The TMS intensity was increased until a muscle activity was observed - MEPs and muscle contraction -. The best possible position of the coil was found at the place, where the MEPs were the biggest and the most stable. This spot was marked with a permanent waterproof marker. Then the stimulation intensity (SI_{1mV}) was adjusted to elicit single pulse MEPs with peak to peak amplitudes of an AV of 1 mV from twenty MEPs. That was stated as the individual threshold for each subject.

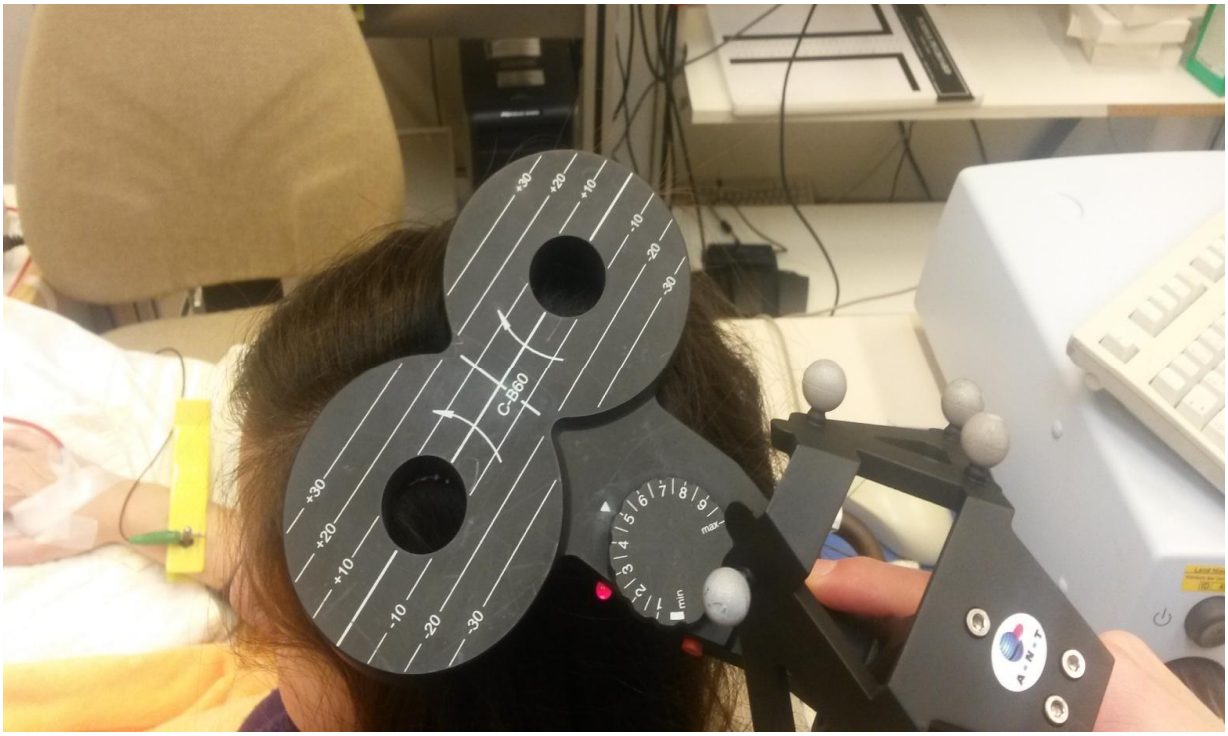

Figure 2: Coil positioning over the M1 during the TMS in a 45° angle tangential to the head of the participant.

After determining the threshold, the baseline was recorded over the marked 'hotspot' using the previously found SI_{1mV}. The MEPs were recorded and saved using the program "Signal 3". The baseline and the subsequent measurement blocks consisted of 20 single-pulse-stimuli.

2.6 Neuronavigation

A neuronavigation system (Visor2, ANT, Netherlands) was used, as described by Herwig and Schönfeldt, (Herwig et al. 2001) to determine the exact coil position in relation to the subjects brain in real time. In addition to that, taking into account the individual cortical anatomy of the participants, the exact 'hotspot' position and the electrodes placement for both montages were registered in the neuronavigation system (session 2). In the subsequent sessions the marked locations were used and the neuronavigation was performed before recording the baseline. In this way the same electrode and 'hotspot' positions could be applied for all the sessions (limitation with an accuracy of 1-3 mm (Herwig et al. 2001)).

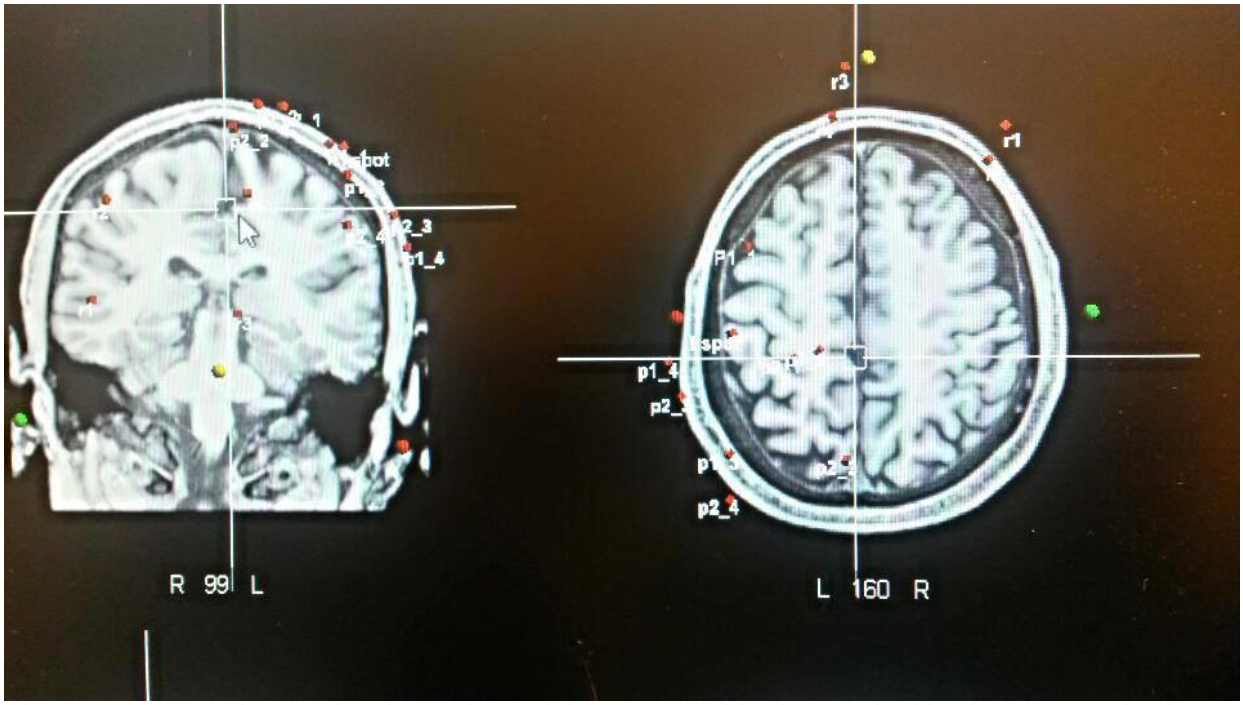

Figure 3: An overview of the electrodes and 'hotspot' positioning marked in the neuronavigation system. The yellow dot is the location of the nasion, the green dot the position of the right ear, and the biggest red dot the position of the left ear. The 'hotspot' and the electrode placements for both classical and shifted montage are also marked.

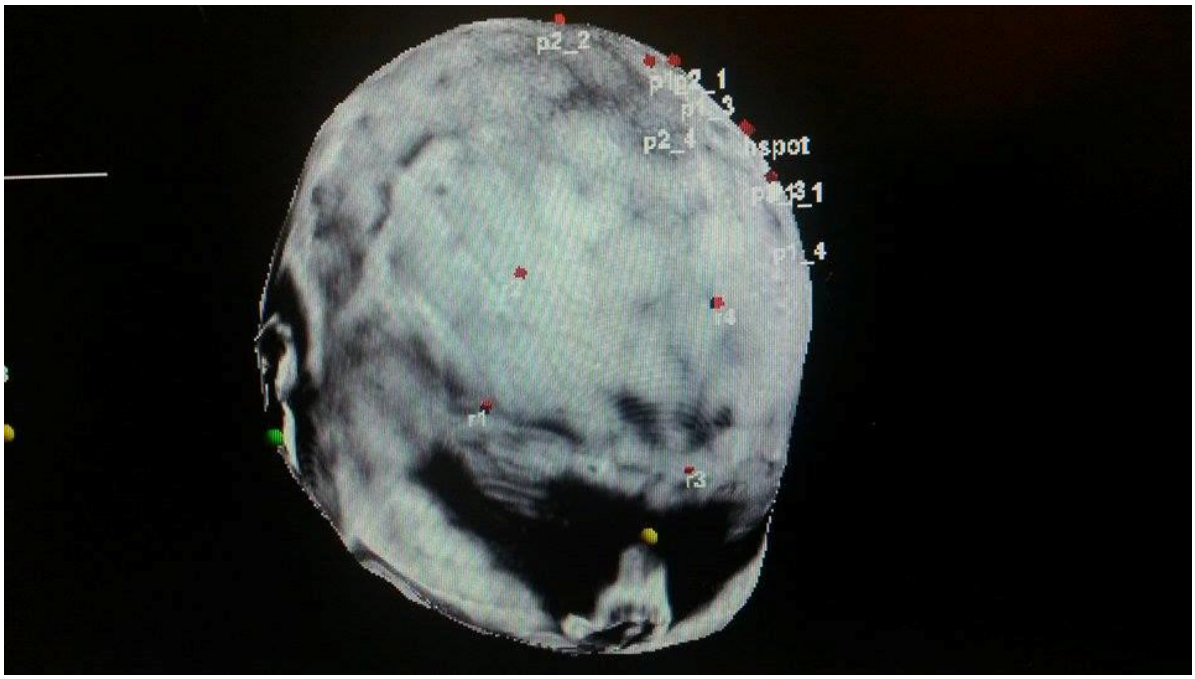

Figure 4: In this picture the marked orbitofrontal electrode is shown (points r1-r4). The yellow dot is the position of the nasion, the green dot the position of the right ear.

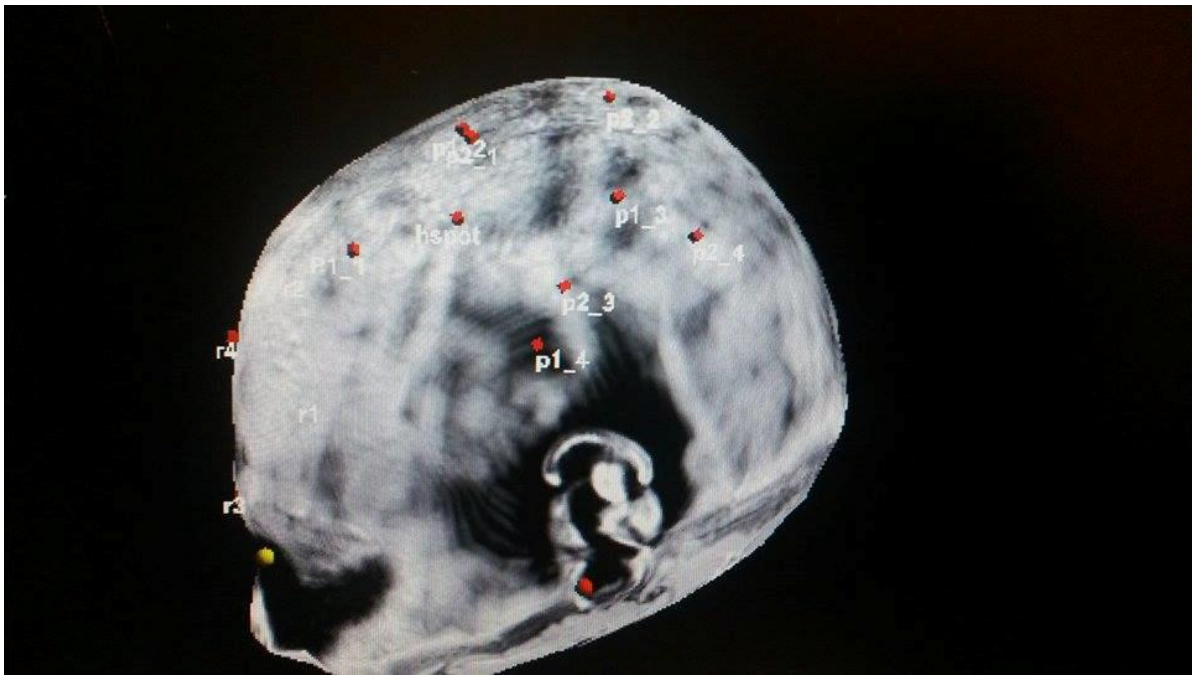

Figure 5: The 'hotspot' is marked with hspot and is located in the middle of the dots marked for the classical montage (p1_1-p1_4). The shifted electrode montage is positioned 3 cm posterior to that (p2_1-p2_4). The yellow dot is the position of the nasion, the biggest red dot the position of the left ear.

2.7 tDCS

tDCS was applied using a battery-driven constant current stimulator (version DC-Stimulator-Plus NeuroConn GmbH, Ilmenau, Germany) through conductive rubber electrodes (35 cm^2). To adjust the electrodes over the head an electrode paste (Ten 20 conductive neurodiagnostic electrode paste, USA) was used. Two montages were tested. The order of the montages and the art of stimulation (anodal or cathodal) were randomised. For the classical montage, the stimulation electrode was fixed over the left M1 representing the right FDI, as determined in the neuronavigation from session 2. The reference electrode was positioned on the forehead above the contralateral orbita (Nitsche and Paulus 2000). For the shifted montage, the M1 electrode was fixed 3 cm posterior to that used in the classical montage, and the reference electrode position was the same (see figure 6).

The stimulation duration was 10 minutes. The current was ramped up and down to and from 1 mA over 8 seconds, as recommended to prevent current transients (Nitsche et al. 2008). Most of the subjects felt tingling, burning or itching during the stimulation.

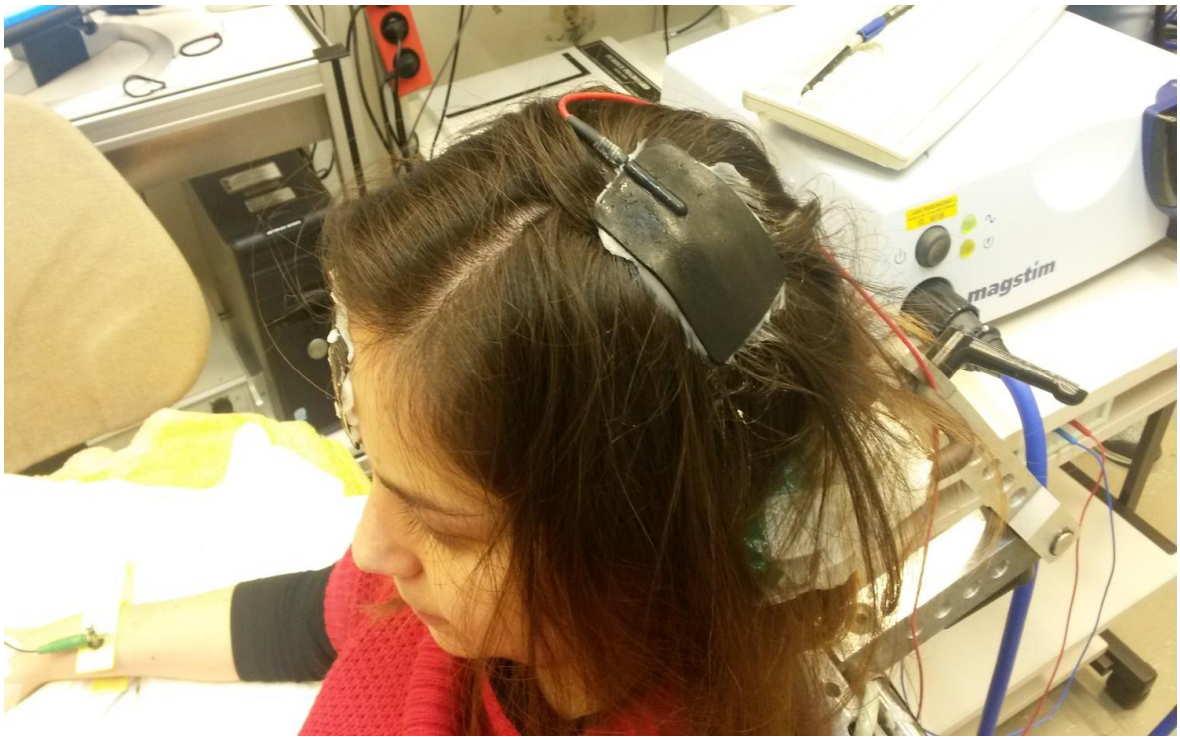

Figure 6: Shifted montage electrode placement: the stimulation electrode was fixed 3 cm posterior of the left M1 and the reference electrode was fixed on the forehead above the contralateral orbita.

2.8 Measurements

Immediately after finishing the stimulation and removing the tDCS electrodes 20 single pulse MEPs were obtained using TMS at an intensity of SI_{1mV}. That was repeated every 10 minutes up to 1 hour so overall 6 measurement blocks were recorded (figure 7).

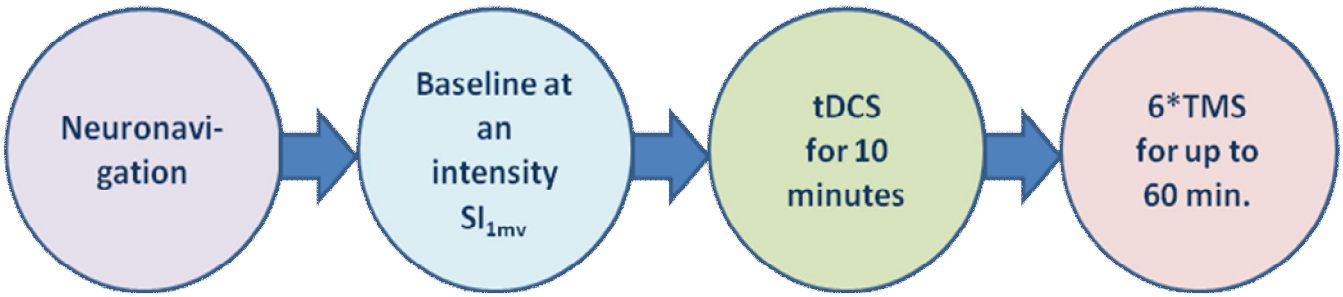

Figure 7: Design of an experimental session.

2.9 Data analysis

The MEPs were recorded using the "Signal 3" software and saved for analysis. First using the software "NuCursor", the AV of the peak-to-peak amplitudes and corresponding SDs of the 20 MEPs of each block were calculated. The desirable mean was 1 mV - values between 0.8 – 1.2 mV were accepted - and the SD should be smaller than the half of the mean (SD< Mean/2).
The statistical analysis was performed using Matlab (version 2011). The raw MEP amplitudes of each time point were divided by the baseline amplitude for normalization. A maximum of five MEPs per block (block= 20 MEPs) with pre-activations were excluded. If pre-activations occurred in more than one block per session, then the affected session was repeated.

We first investigated the data separately for each condition (anodal classical, anodal shifted, cathodal classical, cathodal shifted) to visualize individual variability at each time point. After that, an analysis of variance (ANOVA) was performed with condition (anodal classical, anodal shifted, cathodal classical, cathodal shifted) and time (baseline, 0 min 10 min, 20 min, 30 min, 40 min and 50 min) as factors, with the α-Value set <.05.

3. Results

3.1 Side effects of tDCS

All of the participants tolerated the stimulation well. None of them interrupted the study due to adverse effects. They reported, nevertheless, mild side effects due to tDCS that were divided in three groups: side effects during stimulation, after stimulation and both during and after stimulation. Nine out of eleven participants reported during the stimulation a tingling and burning sensation under the electrodes. Four of them said that the stimulation was slightly painful, and one participant mentioned flashes at the beginning of the stimulation. Five participants felt itchy under the electrodes throughout and after tDCS.
After stimulation nearly everyone (ten out of eleven) had skin redness at the location of the electrodes. The redness disappeared after a few minutes. Two participants mentioned mild headaches after tDCS that lasted a few hours. One participant reported twitching of the little finger after his last tDCS that ceased after 2 weeks. Both during, but mainly after stimulation the majority of the volunteers (ten of them) reported moderate sleepiness.

3.2 Averaged MEPs: overall trend

Figure 8 shows the MEP amplitudes for all four tDCS conditions. The classical montage did not clearly confirm the previously reported changes of the MEPs - anodal tDCS: MEP increase; cathodal tDCS: MEP decrease - (Nitsche et al. 2008). Furthermore, the MEP responses for the shifted montage did not significantly differ to those of the classical montage. Both anodal stimulations - classical and shifted - showed a big fluctuation of the MEP amplitudes, no consistent trend could be identified. The classical cathodal montage and the shifted cathodal montage showed both a non significant decrease of the MEP amplitudes.

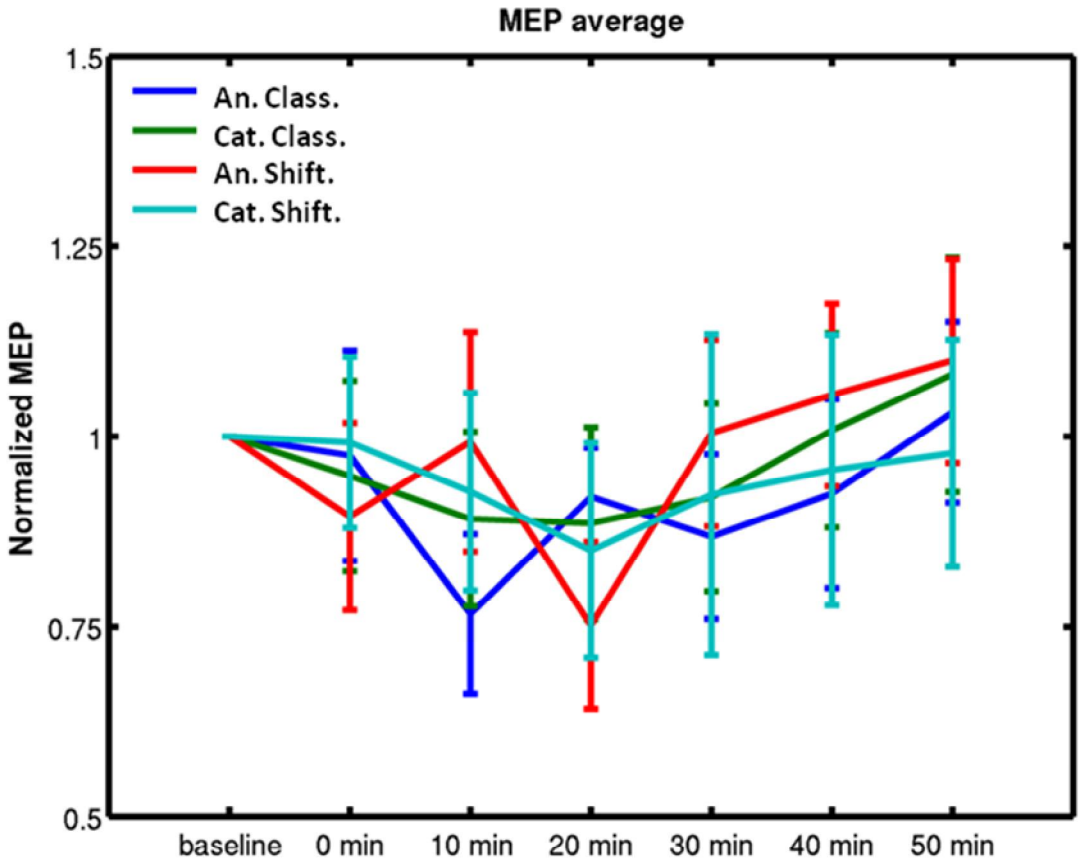

Figure 8: MEPs recorded before and after stimulation: all conditions. Each colour represents one condition. Blue: anodal classical montage; green: cathodal classical montage; red: anodal shifted montage; turquoise: cathodal shifted montage. The error bars marked for each stimulation time and each condition reflect the SD.

The ANOVA did not reveal significant changes for stimulation condition ($F_{(3,30)}$=.047, p=.99) or for time ($F_{(6,60)}$=2.0, P=.08). Also, the interaction between time and stimulation condition was not significant ($F_{(18,180)}$=.533, p=.94).

3.3 Condition: Anodal classical montage

The individual MEPs for each measurement block, for anodal stimulation with the classical montage are shown in figure 9. The trend of the averaged MEPs shows an initial decrease of the amplitudes 0 and 10 minutes after anodal stimulation compared to the baseline. An increase of the MEP amplitude concerning the whole group after anodal tDCS, as reported in many studies

using tDCS, could not be reproduced. Moreover, a big individual variability in the MEP-responses after stimulation is observed for each participant (see different colours).

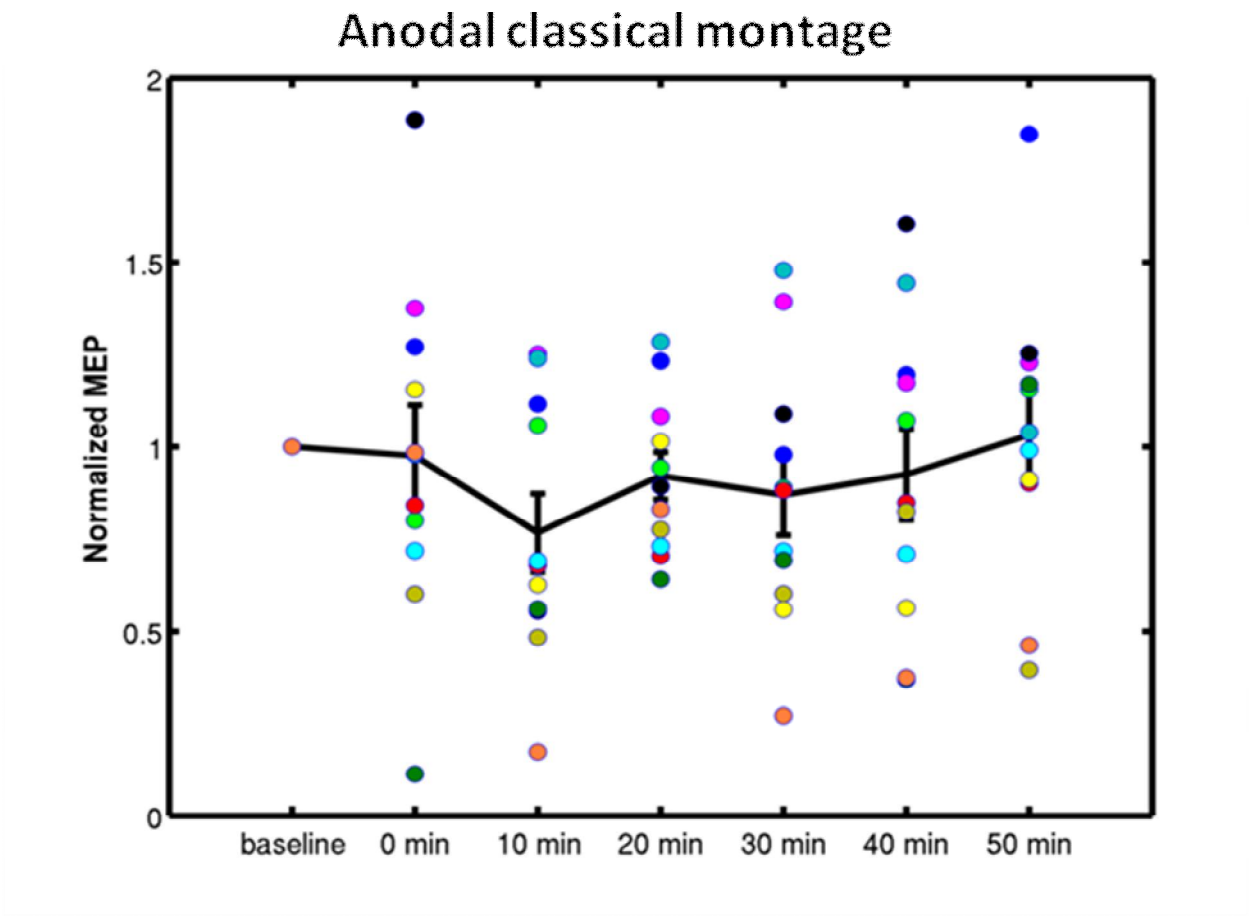

Figure 9: MEPs recorded before and after anodal tDCS, classical montage. Each colour represents one participant. The black line shows the AV of the MEP-amplitudes for each measurement block. The error bars marked for each stimulation time reflect the SD.

3.4 Condition: Anodal shifted montage

Figure 10 shows the change of the MEPs using the anodal shifted montage. The MEPs before and after stimulation didn't show a significant increase as expected. Furthermore a small decrease is observed comparing the baseline MEP-amplitudes and the MEP-amplitudes in 20 minutes after stimulation.

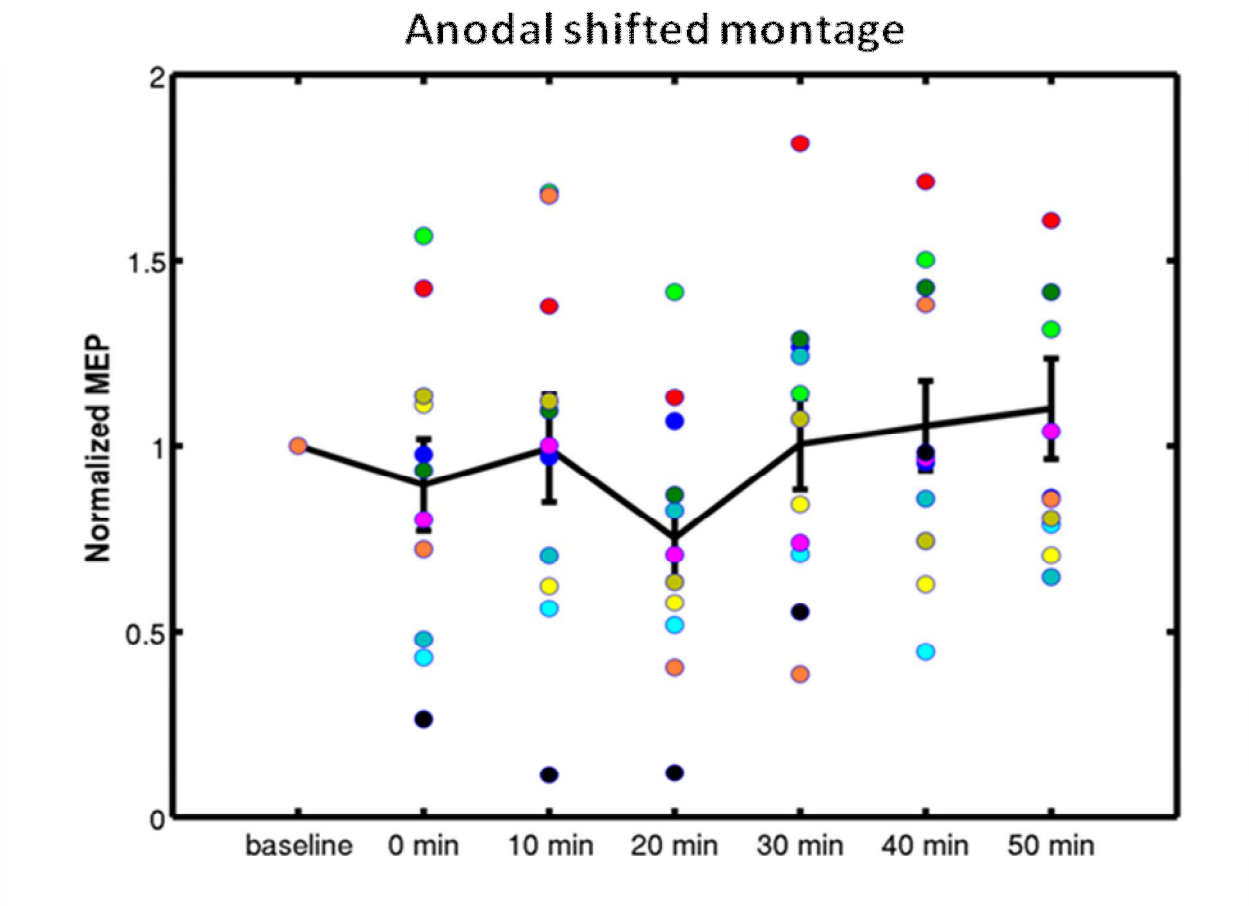

Figure 10: MEPs recorded before and after anodal tDCS, shifted montage. Each colour represents one participant. The black line shows the AV of the MEP-amplitudes for each measurement block. The error bars marked for each stimulation time reflect the SD.

3.5 Condition: Cathodal classical montage

Figure number 11 illustrates the alteration of the MEPs using the cathodal classical montage. Former studies suggest a decrease of the MEP amplitude of about 30% comparing baseline and immediately after cathodal stimulation (e.g. (Nitsche and Paulus 2000)). These effects could not be observed in our study, considering the whole group: Some of the participants show a decrease and some of them show an increase of the MEP sizes.

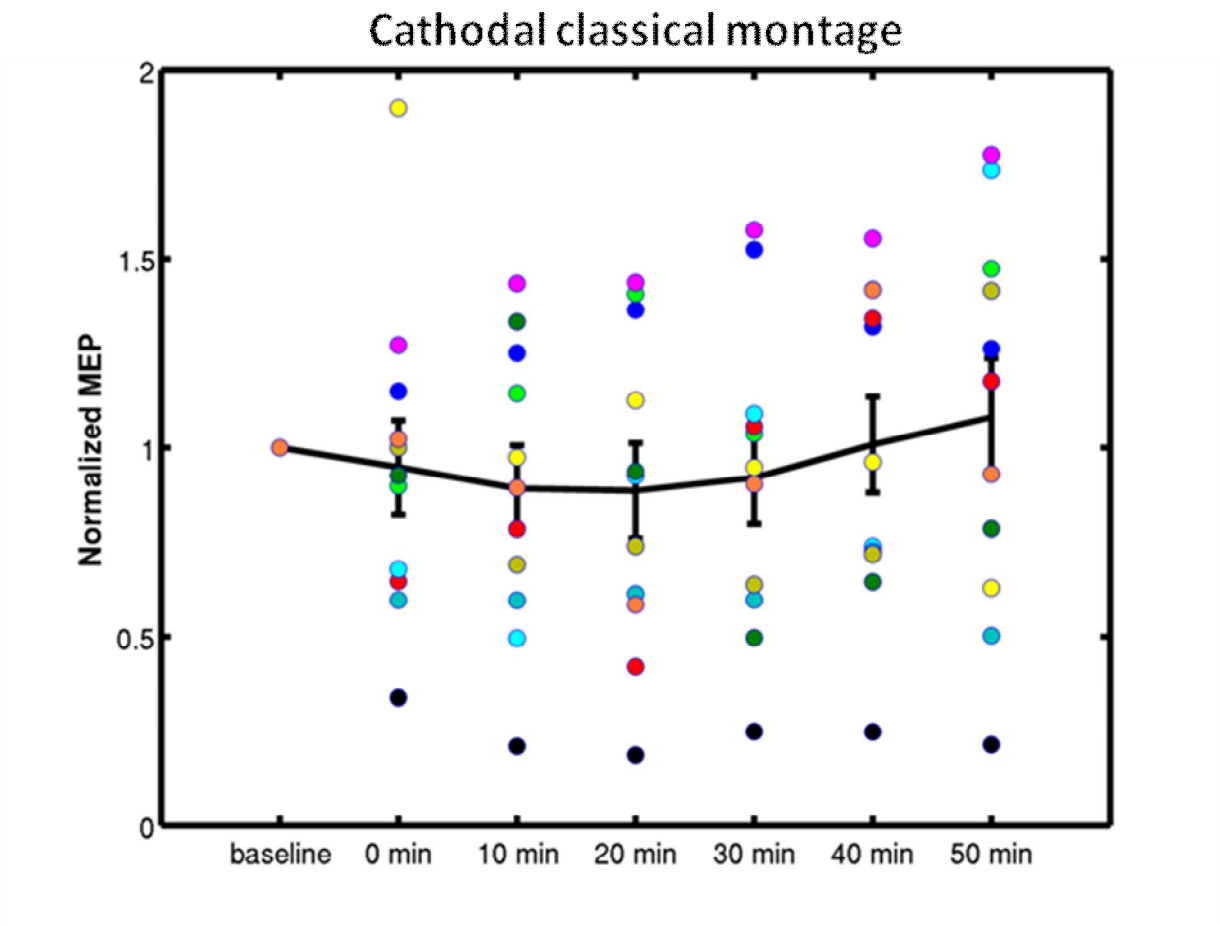

Figure 11: MEPs recorded before and after cathodal tDCS, classical montage. Each colour represents one participant. The black line shows the AV of the MEP-amplitudes for each measurement block. The error bars marked for each stimulation time reflect the SD.

3.6 Condition: Cathodal shifted montage

Figure 12 presents the variation of the MEPs using the shifted cathodal montage. A decrease of the MEP amplitudes averaged for all the participants of about 15% for 20 minutes after stimulation is observed in the graph. After that, the MEPs returned to the starting baseline values. Again the diagram shows though a large individual variation of the MEP amplitudes in all the time points that are measured.

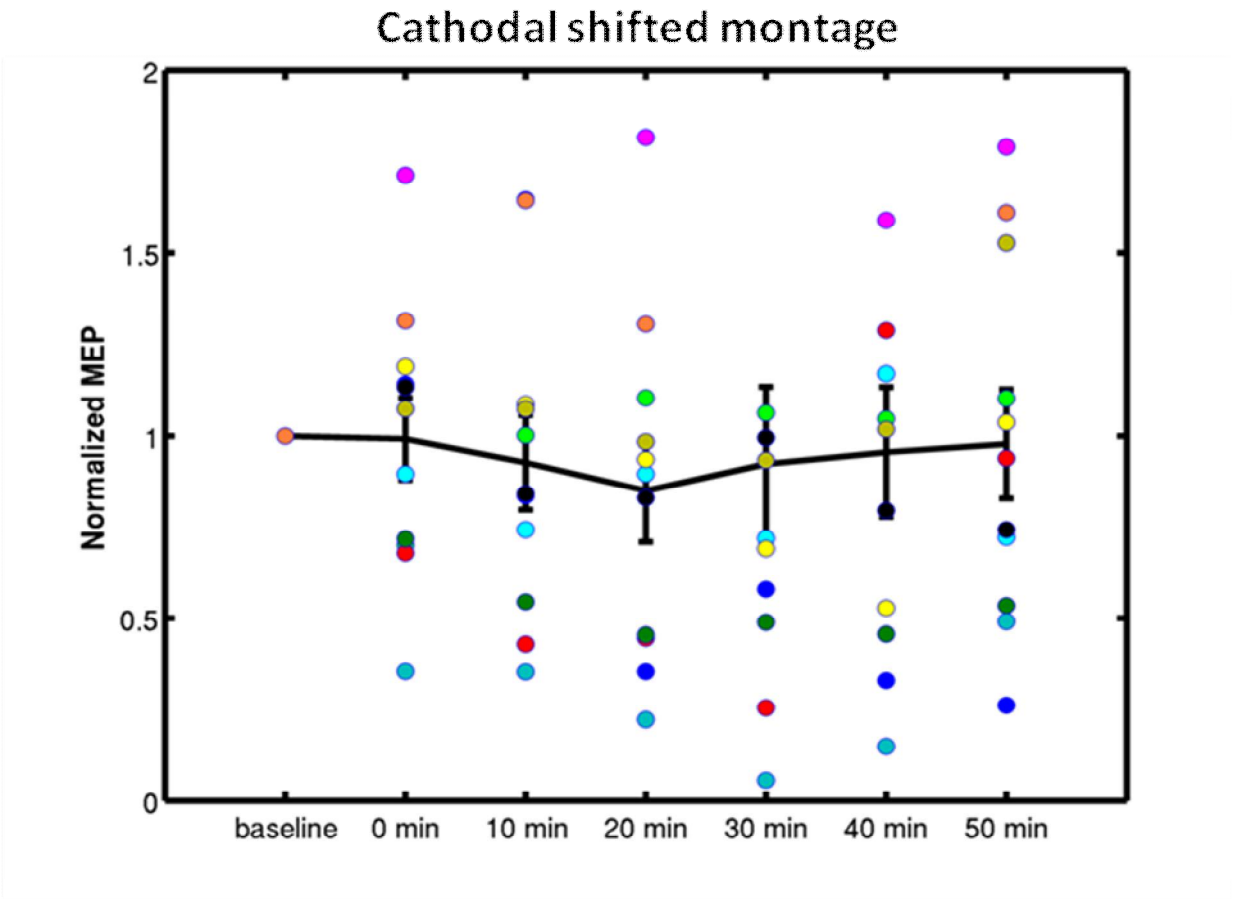

Figure 12: MEPs recorded before and after cathodal tDCS, shifted montage. Each colour represents one participant. The black line shows the AV of the MEP-amplitudes for each measurement block. The error bars marked for each stimulation time reflect the SD.

3.7 Synopsis of the MEP responses

We divided our participants in four groups, depending on their responses after tDCS, as it was previously done by Wiethoff et al. (Wiethoff et al. 2014): anodal excitatory - cathodal inhibitory, anodal inhibitory - cathodal excitatory, both inhibitory, both excitatory. In order to do that, we averaged the MEP size from all the measurement points after stimulation - 0 min. after tDCS, 10 min. after tDCS, 20 min., 30 min., 40 min. and 50 min - and performed the data analysis for each participant and both montages.

The first pie chart (figure 13) presents the MEP size averaged from all the measurement points for each participant in respect to the classical montage. 18% of the subjects responded in a canonical manner (Nitsche et al. 2008), 18% responded with decrease after anodal stimulation

and increase of the averaged MEPs after cathodal stimulation and another 18% after both anodal and cathodal stimulation with increase of the mean MEP size. Interestingly 46% responded with an average decrease of the MEP sizes after both anodal and cathodal tDCS.

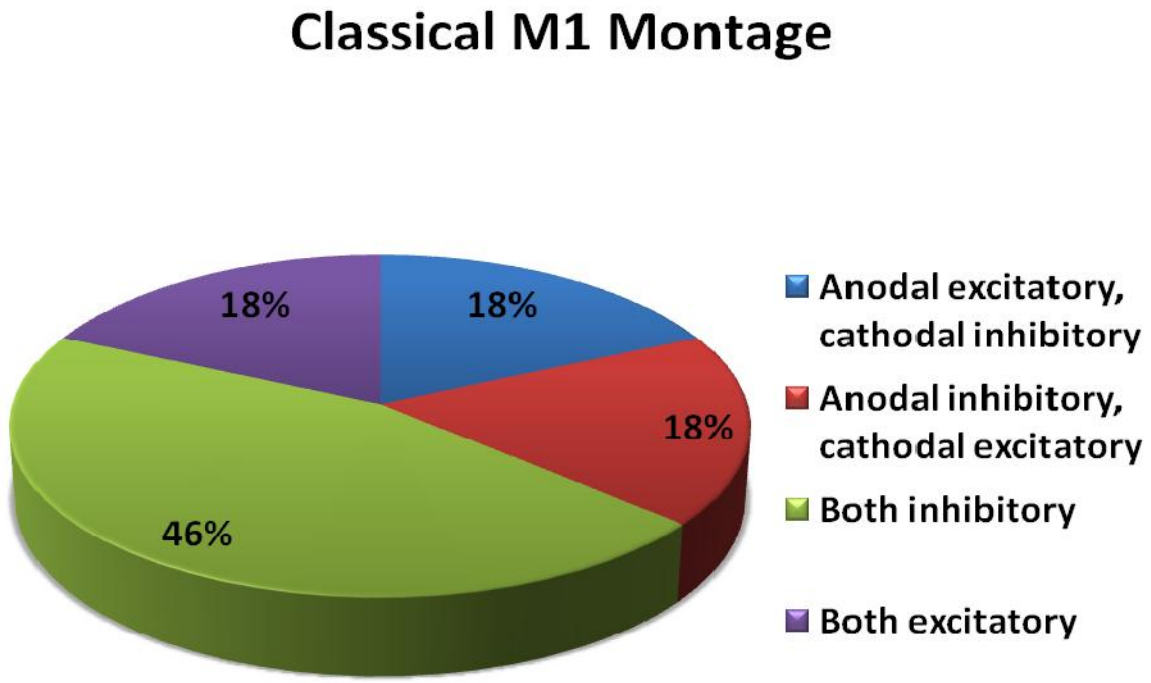

Figure 13: Classical M1 montage. MEP size averaged for all the measurement points and summarised for all the participants, divided in 4 sectors.

The second pie chart (figure 14) shows the MEP size averaged from all the measurement points for each participant in respect to the shifted M1 montage. The proportions for each kind of response are very similar to those of pie chart 1, whereas almost 1/3 responded in a canonical manner and 1/3 in exactly the opposite way. Still, 37% of the participants, and thus the greatest proportion responded in both anodal and cathodal stimulation with decrease of the average MEP size.

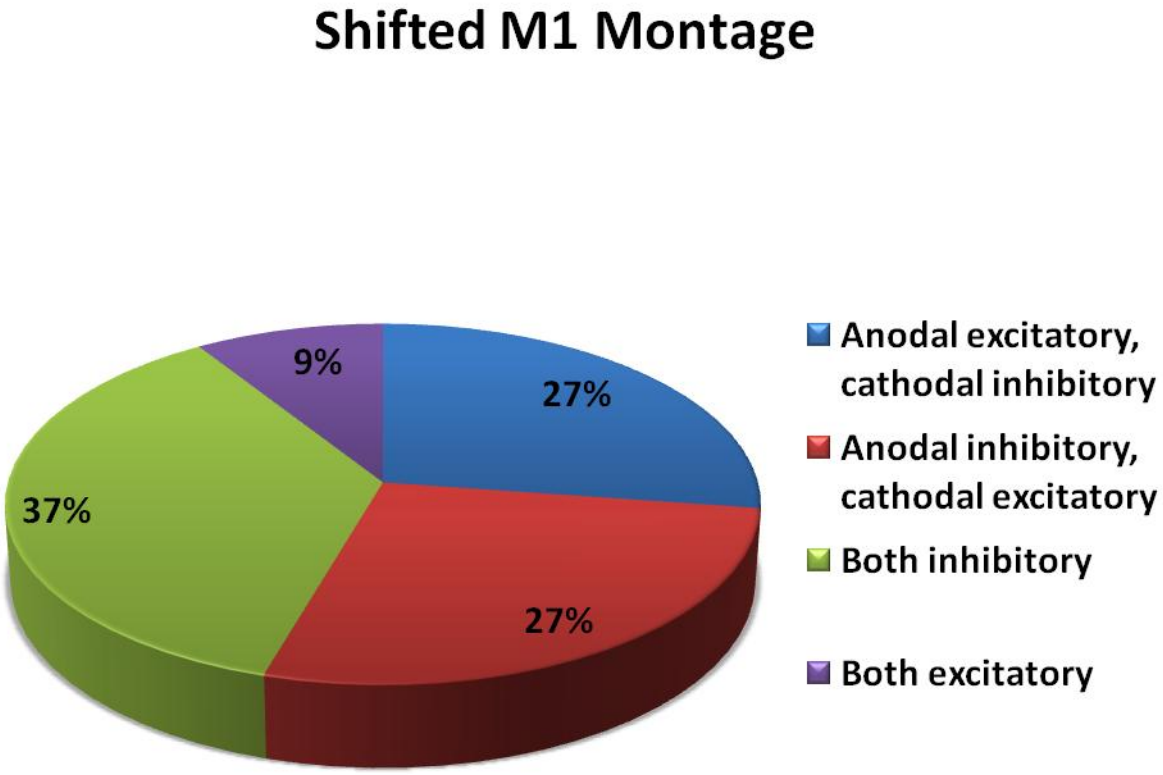

Figure 14: Shifted M1 montage. MEP size averaged for all the measurement points and summarised for all the participants, divided in 4 sectors.

4. Discussion

4.1 Hypothesis and findings

In this study we tested the effects of anodal and cathodal tDCS and analysed the after-effects in relation to the time by recording MEPs up to one hour after the stimulation. We compared the efficacy of two electrode montages for tDCS: the classical with the "active" electrode placed over the M1 and the return or "reference" electrode, over the contralateral orbitofrontal cortex (Nitsche et al. 2008) and the shifted montage with the "active" electrode moved 3 cm posterior of the M1 and the return electrode at the same position.

Surprisingly, we could not reproduce the expected after-effects after tDCS (e.g. (Nitsche and Paulus 2000; Nitsche et al. 2003c; Nitsche et al. 2007)). Our participants responded to anodal and cathodal tDCS using the classical montage incredibly variable, making it impossible to find an overall trend of the MEP alterations. The data collected after stimulating with the shifted montage was very similar to that of the classical montage. Therefore no significant improvement or reduction of the outcome i.e. the MEP alterations could be noted.

We also categorised the responses after tDCS for each participant individually depending on the averaged MEPs for each condition and montage, as it was previously done by Wiethoff et al. (Wiethoff et al. 2014). We observed that 27%, almost 1/3 of the volunteers responded in a canonical manner after tDCS with the shifted montage, whereas after tDCS with the classical montage only 18% responded that way. To our surprise in both classical and shifted montage the highest percentage reacted with an average decrease of the MEP sizes after both anodal and cathodal stimulation. Nevertheless, these findings should be further evaluated with a bigger sample size.

4.2 Factors explaining the variability

The excitability changes of the brain are under influence of a lot of factors that cannot be completely controlled. Several reasons could explain the different tDCS responses between the subjects. We categorised the reasons in three main groups: subject-, investigator- and setting-associated.

To the subject-associated factors we firstly added the biological factors, such as age, gender and genetics. Aging can for example lead to delayed tDCS responses, or even affect the MEP size alterations after non-invasive brain stimulation (NIBS) (Fujiyama et al. 2014; Muller-Dahlhaus et al. 2008). Gender differences concerning the effectiveness of tDCS have also been reported in various studies e.g. (Chaieb et al. 2008; Kuo et al. 2006). Furthermore, the genetics of a subject, such as different polymorphisms may also play an important role in the way he/she responds to plasticity inducing protocols (Antal et al. 2010; Cheeran et al. 2008; Hasan et al. 2013; Plewnia et al. 2013). For example Cheeran et Talleli and Antal et Chaieb demonstrate in their studies how BDNF polymorphisms (brain derived neurotropic factor gene) i.e. homozygote Val66Val or heterozygote Val66Met respond differently to NIBS according to the stimulation method used.

Another significant factor is the cortical thickness. As Conde et al. described in their study from 2012, the subject's cortical thickness should be considered as an important modulating factor of the brain stimulation's after-effects (Conde et al. 2012). The individual head anatomy of the participants (thickness of skull and cerebrospinal fluid, gyral depth, distance between the electrodes), the hair (could alter the conduction), but also not anatomical factors, such as emotions like stress, worry, sadness, contentment, loss of concentration, agitation, sleepiness and hunger are also possible confounders that could lead to a variability in the MEP responses (Antal et al. 2007; Nitsche et al. 2008; Opitz et al. 2015). In their study Antal et Terney tested the same sample under three circumstances: resting position, whilst doing a cognitive- and whilst doing a motor task and they showed this way, that the induced plasticity varied according to the state of the subject during stimulation.

Among the investigator-associated factors is the correct usage of the neuroplasticity inducing tools - like tDCS and TMS - as well as the orientation of the TMS coil. Hamada et al reported a dependency of the MEP responses to the interneuron networks recruited by the TMS stimulation, which can be directly affected by the coil orientation (Hamada et al. 2013). Moreover, the concentration, stability of the hand, correct electrode positioning and last but not least even emotional factors of the investigator could intrude in his/her capability of correct usage and positioning of the coil. Furthermore, the expectations of the investigator for a certain kind of reaction after tDCS (e.g. anodal stimulation MEP size increase and cathodal stimulation MEP size decrease) could lead to an unintended manipulation of the coil position, so as to obtain the expected results. In order to overcome this source of inaccuracies we underwent a double-blinded study design (as explained in the materials and methods part).

As mentioned above the third group is the setting-associated factors that could cause variability of the results. Background noises, that cannot always be eliminated, alter the concentration of the participant and therefore interfere with the excitability of the brain. Also the time of the day that the experiment was performed is a parameter that influences the plasticity response, due to circadian rhythms and variation of cortisol levels (Ridding and Ziemann 2010; Sale et al. 2008). Low cortisol levels lead to an increased preparedness to induce excitability changes after NIBS. Ridding et al. stimulated their subjects once in the morning when the cortisol levels in the blood are high and once in the evening when the cortisol levels fall (due to circadian secretion of cortisol). In the evening the MEPs increased significantly after Paired Associative Stimulation (PAS), whereas in the morning they didn't.

4.3 Supporting studies

As Wiethoff et al. report in their study about the variability after tDCS (Wiethoff et al. 2014), its cause is multifactorial. They divide the factors in two groups: extrinsic and intrinsic. Extrinsic factors are modifiable such as: motor 'hotspot' detection, steadiness of holding the coil and concentration of the subjects. Intrinsic factors are not modifiable for example age, gender, and genetics of the subject. Their findings are in agreement with our data and support the fact that the MEP responses after tDCS can be highly variable. Therefore, the tDCS protocols should be further optimised in order to be able to eliminate confounders.

In a recent study, where the interindividual variability in response to NIBS methods was tested, a significant variability between individuals was confirmed in all three protocols tested - PAS, anodal tDCS, iTBS (Intermittent Theta-Burst Stimulation) - (Lopez-Alonso et al. 2014). The excitability alterations reported after performing NIBS were not significant for all of the participants. Lopez-Alonso et al. divided therefore the subjects in responders and non-responders depending on whether the MEP responses were as expected (i.e. as stated in protocols (Nitsche et al. 2008)) or not. Only 12.5% of the participants responded in a canonical manner in all three protocols, whereas 25% had an unpredicted response in all three of them. In this way Lopez-Alonso et al. proved that a significant response to one protocol does not suggest a significant response to other stimulation methods.

Interestingly, Nettekoven et al. suggested in their study that the variability to multiple blocks of Repetitive Transcranial Magnetic Stimulation (rTMS) depends on the preinterventional network

connectivity of the stimulated area (here iTBS, a form of rTMS was used) (Nettekoven et al. 2015). It is still unknown, if these findings could also be extrapolated to other forms of transcranial stimulation like tDCS. However, the baseline connectivity and the state of the brain prior to stimulation could also play an important role to the interindividual variability of the results and could be added to the factors explaining our findings as well.

In addition to the above stated paradigms, Opitz et al. conducted a study about the factors influencing the electric field during tDCS and observed how individual anatomic factors such as: the skull thickness, the thickness of the cerebrospinal fluid, the gyral depth and the distance to the electrodes can affect and alter the responses after tDCS (Opitz et al. 2015). They used two realistic finite element head models and stimulated with tDCS in various electrode positions over the M1, and showed alterations of the electric field distribution as a result of the divergent individual anatomy of the skull, the gyral depth, the thickness of the cerebrospinal fluid, and the distance to anode and cathode as mentioned above. They also demonstrated that due to those factors some "negative hotspots" can result, which can be partly resistant to the chosen electrode placement. Therefore these anatomical factors could be another explanation for the interindividual and the subject to subject variability found in our study as well.

There are some more studies reporting variability and inconsistency of the MEP alterations after NIBS, such as PAS, for example: Muller-Dahlhaus et al. showed in their study that the resting motor threshold, the individual SI_{1mV} and the age of each participant can determine the PAS effects (Muller-Dahlhaus et al. 2008). In their study Fratello et al. could on the one hand show a stability of the effects after PAS in the group measure, but also found an inter- and intraindividual variability from session to session after PAS (Fratello et al. 2006). Stefan et al. observed how the attention of the subject during the experiment can alter the response to PAS (Stefan et al. 2004). These findings underline the fact that future studies might have to undergo stricter protocols or even adjust the pre-selection criteria (see materials and methods) for participants in order to respond to NIBS in the desired way.

4.4 Contradicting studies

On the contrary numerous studies support the fact that consistent and significant MEP alterations can be induced after tDCS. Nitsche and Paulus reported in their study about excitability changes of up to 40% after tDCS that lasted for several minutes (Nitsche and Paulus

2000). In 2006 Kuo and Paulus analysed the data of 118 participants and also revealed steady changes in the MEP responses (Kuo et al. 2006). Many other studies found similar results after tDCS i.e. (Monte-Silva et al. 2010; Nitsche et al. 2003b; Nitsche et al. 2003c; Nitsche et al. 2007). Nevertheless, 90% of these studies were not double-, even not single-blinded only one electrode position was tested without anatomical MR data.

4.5 Strengths of the study

The experimental sessions including tDCS (sessions 2-5) were all conducted under the same conditions: i.e. in the same laboratory with the same lighting, the same chair and the same devices were used for all the participants. The investigator was instructed and trained for the correct usage of the devices so as to minimise any technique-related problems. Furthermore a proper screening of the participants was undertaken. The screening included a questionnaire with the exclusion criteria (see appendix) as stated in the tDCS state of the art, a medical examination and a TMS response test, in order to identify those eligible for the study (20 MEPs with AV 0.8-1.2 mV and SD< AV/2). Only the participants who successfully completed all three stages of the screening were allowed to take part in the study. In addition to that in the first session of our study a structural MRI scan was performed and read on the neuronavigation system by qualified personal, who excluded subjects if the MRI scans showed that the position of the M1 (precentral gyrus) was difficult to reach with the TMS coil due to their head anatomy/scull formation.

Our study was double-blinded, both investigator and participants were unaware of the type of the stimulation (anodal or cathodal) and the sessions were performed in randomised order. In this way neither the participant nor the investigator had any expectations of the measured MEP changes. Therefore any manipulation of the coil positioning in order to collect the expected results could be excluded.

In the first measurement session the 'hotspot' position was marked with a permanent marker and then using the neuronavigation system the 'hotspot' was also marked on the structural MRI of the participant. In the following sessions the marked 'hotspot' was used. In that way we limited the chances of using different spots in every experimental session.

Due to our experimental design with 5 sessions for each subject we also tested the tDCS responses in repetition. Thus, we showed that the variability found is not just a matter of

unconscious bias (like external or setting associated factors) that can randomly occur during a session, but also results from a variation between individuals.

Last but not least, a session was excluded if more than five MEPs with pre-activations in one block, or even if pre-activations occurred in more than one block (e.g. when the subjects were not able to relax). The session was then repeated on another day.

4.6 Limitations of the study

We have divided the study limitations in 4 groups in order to have a better overview.

The first group is the experimental design limitations. In our study we only tested a group of healthy right handed subjects (Oldfield 1971). Consequently an extrapolation of our results to left handed subjects or even to patients cannot be done, based only on these data. It should also be mentioned that due to our experimental design (composed with 5 measurement sessions and a screening session for each subject), a higher dropout rate than that expected, was observed because of incompliance. To minimise the dropout rate the participants were well informed about the length of the study and any possible side effects. They also had to successfully complete the three stages of the screening and sign an informed consent to be able to enrol in the study (see appendix).

Another group is the cluster limitation. We tested a group of just 11 participants all in similar age groups (AV age and SD 25.08 +/- 2.87 years). In order to validate these results prospect studies need a larger group of participants from different age groups.

Furthermore we also have the outcome-limitations. We only tested tDCS for the conditions (intensity, duration) stated in the chapter materials and methods of this manuscript; subsequently it must be tested in future studies if these results are applicable for other conditions i.e. classical and shifted montages with a tDCS intensity >1 mA or a stimulation duration longer than 10 minutes.

Finally we have general limitations that might occur in every study using tDCS or TMS. The emotional state of each participant before the stimulation i.e.: being angry or stressed, having had too much coffee or if they became sleepy or bored during the experiment, is not known in advance. Nevertheless, this emotional state can interfere with the MEP responses after stimulation. To overcome this limitation, each participant was informed about his/her behaviour

24 h prior to the measurement sessions i.e. sleeping well, no coffee or alcohol, eating enough, relax during the measurements.

As already stated above the investigator was well trained and educated about the usage of TMS and tDCS. The fact that the study was double-blinded and the utilisation of the neuronavigation system to mark and find the 'hotspot' underlines the accuracy of the data collected.

Even though the results of our study suggest variability after tDCS and agree with the above mentioned findings of several recent studies in the field of the brain stimulation, it should be mentioned that the data found applies to the conditions used for this experiments (i.e. intensity, stimulation duration, electrode montage) and should carefully be deducted to any future studies.

4.7 Suggestions for future studies

In order to validate the above stated results and to overcome the limitations, we would suggest that future studies should test a bigger sample size, including left- and right-handers. As mentioned above a parallel test including healthy volunteers in various age groups would lead in obtaining more accurate data for a bigger population.

To make the results more robust, stricter protocols are needed in order to control the "emotional condition" of the volunteers. Another idea would be the development of a TMS- coil holding/positioning machine (Robot) connected to the Neuronavigation system where the 'hotspot' is marked, so as to minimise any movements or misplacements of the coil during the experiment.

The study setting could be further optimised in prospective studies by using a soundproof room with day light lamps. That way interference due to different time of the day in each session or noise distractions could be eliminated.

4.8 Why is it important to continue the studies in this field?

tDCS offers several advantages when compared to other neuroplasticity inducing tools e.g. TMS. First, it is easy to use, as it is a mobile device with a buyer friendly size. Moreover, the users can move their hands and heads during the stimulation, which makes it more comfortable particularly for long lasting stimulations. This is very helpful when stimulating agitated patients

which can't stay still over a long time. The handling of tDCS is easy to learn and doesn't require a medical doctor (but a qualified person) to be present during the stimulation. In addition to that, tDCS is comparatively economical.

Various studies reported the positive effects of tDCS on patients and healthy subjects - e.g. (Kuo et al. 2006; Lopez-Alonso et al. 2014; Monte-Silva et al. 2010; Nettekoven et al. 2015; Nitsche and Paulus 2000). A range of studies performed on patients after having a stroke, or suffering from Parkinson's and Alzheimer's disease, depression or even migraine show an improvement from the non-invasive brain stimulation (Antal et al. 2011; Baker et al. 2010; Benninger et al. 2010; Boggio et al. 2006; Boggio et al. 2007; Boggio et al. 2009; Dasilva et al. 2012; Ferrucci et al. 2008; Ferrucci et al. 2009; Fregni et al. 2005; Fregni et al. 2006; Li et al. 2015; Rocha et al. 2015). tDCS is even a promising tool on healthy subjects improving cognitive performance (Andrews et al. 2011).

In order to optimise the usage of the tDCS, be able to identify the responders in advance and minimise the costs of the extended utilisation from non responders, it is highly needed to improve the tDCS protocols. Furthermore, additional investigation needs to be undertaken in this field to extend the findings and the parameters of the usage for a bigger population.

5. Summary

tDCS is a method for non-invasive brain stimulation that can induce neuroplasticity. Many patients with neurological and mental disorders use tDCS in order to improve their condition. In many previous studies, anodal tDCS has shown to increase the amplitude of the TMS-evoked MEPs leading in an increased excitability, whereas cathodal tDCS has shown to reduce the excitability. In this study we aimed to examine whether a local distribution of current at M1 or global current pathways elucidate the modifications in cortical excitability and in neuroplasticity after tDCS. Two electrode montages were compared: the 'classical montage' with the active electrode over M1 and the return electrode over the contralateral orbita and the 'shifted montage' with the active electrode moved 3 cm posterior to the M1 and the return electrode kept constant. TMS-evoked MEPs were used to record the excitability changes up to one hour after stimulation.

The study was consisted of five sessions and eleven healthy right-handed participants took part. In the first session a structural MRI was performed, which was used in the following sessions with a neuronavigation system to determine the exact coil position and mark the 'hotspot'. Sessions two to five were double-blinded. A ten minute tDCS was performed with one of the two montages, anodal or cathodal stimulation and then TMS-evoked MEPs were recorded up to one hour after stimulation.

Our results revealed that the classical montage did not confirm the previously reported MEP alterations after anodal and cathodal tDCS. The responses to the shifted montage did not significantly differ to those of the classical montage. A big fluctuation of the MEP amplitudes was recorded and a consistent trend for the majority of the participants could not be identified. Furthermore most of the participants responded to anodal and cathodal stimulation after stimulating with both montages with a decrease of the average MEP size.

Therefore no significant improvement or reduction of the outcome i.e. the MEP alterations could be noted. We categorised the reasons for the variability of the responses in three main groups: subject-, investigator- and setting-associated. To the subject-associated factors we added the age, gender, genetics and cortical thickness of the participant. To the investigator-associated factors we added most importantly the correct usage of the neuroplasticity inducing tools and the correct orientation of the TMS coil. The third group is the setting-associated factors for example background noises and time of the day that the stimulation is undertaken.

As a conclusion, the tDCS protocols should be further optimised in order to be able to eliminate confounders. A bigger sample size and volunteers in various age groups should be tested and a way to identify the responders prior to stimulation should be elaborated.

6. Appendix

6.1 Inclusion criteria

Subjects should be right-handed (Oldfield 1971), capacity for free-willing consent; subjects who are already familiar with tDCS are preferred.

6.2 Exclusion criteria

1. History or evidence of chronic or residual neurological disease in the applicant or family history of the applicant.
2. Pacemaker or deep brain stimulation.
3. Metal implants in head or neck area (e.g. postoperative clips after intracerebral aneurysm; arterial aneurysm in the vascular system, implantation of an artificial hearing aid.
4. Age; < 18 or > 45 years old.
5. Left-handedness (Oldfield 1971).
6. History of bleeding.
7. Prior evidence of epileptic seizures, history of epilepsy.
8. History of head injury with loss of consciousness.
9. Any serious medical conditions (disease of the internal organs) or psychiatric illness, including schizophrenia, mania or depression.
10. Pregnancy or breast-feeding.
11. Alcohol, medication or drug addiction.
12. Local or global aphasia.
13. Any legal reason why the candidate cannot participate.
14. Participation in another scientific or clinical study within the last 6 weeks.

6.3 Informed consent/ Einverständniserklärung

Klinische Neurophysiologie

Leiter der Forschungsgruppe: Prof. Dr. med. Walter Paulus

Einverständniserklärung zur Untersuchung:

Einfluss von Elektrodenplatzierung auf Motorkortex Exzitabilität und funktioneller Konnektivität

Name, Vorname:.....

Ich,……..., wurde von einem Mitarbeiter der Abteilung Klinische Neurophysiologie vollständig über Wesen, Bedeutung und Tragweite der Magnetresonanz-Untersuchung sowie der transkraniellen Gleichstromstimulation und transkraniellen Magnetstimulation aufgeklärt. Ich habe den Aufklärungstext gelesen und verstanden. Ich hatte die Möglichkeit, Fragen zu stellen, und habe die Antworten verstanden und akzeptiere sie. Ein Mitarbeiter der Abteilung Klinische Neurophysiologie hat mich über die mit der Teilnahme an der Untersuchung verbundenen Risiken und den möglichen Nutzen informiert.

Ich hatte ausreichend Zeit, mich zur Teilnahme an dieser Untersuchung zu entscheiden und weiß, dass die Teilnahme freiwillig ist. Ich weiß, dass ich jederzeit und ohne Angaben von Gründen diese Zustimmung widerrufen kann, ohne dass sich dieser Entschluss nachteilig auf eventuell spätere ärztliche Behandlungen auswirken wird.

Mir ist bekannt, dass meine persönlichen Daten in verschlüsselter Form gespeichert werden. Mir ist bekannt, dass mein Name, mein Geburtsdatum, mein Gewicht, mein Geschlecht, meine Telefonnummer und meine Adresse in einer Kartei der Abteilung Klinische Neurophysiologie der Georg-August-Universität Göttingen gespeichert werden. Die Messdaten werden getrennt hiervon aufbewahrt. Ihre Verwendung erfolgt in namentlich nicht kenntlicher Form.

Obwohl die durchgeführte Untersuchung keine diagnostische Untersuchung ist, besteht die Möglichkeit, dass pathologische Befunde entdeckt werden (Zufallsfund). Sie haben die

Wahlmöglichkeit, ob Sie in einem solchen Fall über den Zufallsfund informiert werden möchten oder nicht. Bitte kreuzen Sie entsprechend an:

Über einen Hinweis auf einen Zufallsfund möchte ich informiert werden. JA

NEIN

Bilddaten, welche auf einer eventuell ausgehändigten CD gespeichert sind, dürfen nicht für diagnostische Zwecke genutzt werden!

Die personenbezogenen Daten werden mindestens 10 Jahre aufbewahrt. Mir ist bekannt, dass ich Auskunft über die gespeicherten Daten erhalten kann und dass ich mein Einverständnis zur Speicherung der personenbezogenen Daten jederzeit widerrufen kann. Im Falle des Widerrufs werden alle gespeicherten personenbezogenen Daten gelöscht.

Auf Wunsch erhalte ich eine Kopie des Informationsblattes und dieser Einwilligungserklärung. Ich erkläre hiermit meine freiwillige Teilnahme an dieser Untersuchung.

Anschrift: ……..

Telefon: ……..

Beruf: ……..

Größe: ……..

Gewicht: ……..

Geburtsdatum: ……..

Ort/Datum:…… Unterschrift (Testperson):……..……

Ort/Datum:…… Unterschrift (Untersucher):……

6.4 Information for participants

Abteilung Klinische Neurophysiologie, Medizinische Fakultät, Universität Göttingen

Information für Probanden über die Untersuchung:

Einfluss von Elektrodenplatzierung auf Motorkortex Exzitabilität bei der transkraniellen Gleichstromstimulation

Sehr geehrte Damen und Herren!

Wir bedanken uns für Ihr Interesse an der o.g. Studie. Ziel dieser Studie ist es, mit einer Kombination von nicht-invasiven (= nicht in den Körper eingreifenden) neurophysiologischen Stimulationsverfahren zu untersuchen, welche Einfluss der elektrodenort bei der Gleichstromstimulation (tDCS) auf die motorische Erregbarkeit im Gehirn hat. Im Folgenden möchten wir Ihnen den Ablauf und die Ziele der Untersuchung näher erläutern.

Bei der Untersuchung handelt es sich um eine wissenschaftliche Studie. Ihre Teilnahme daran ist freiwillig. Dieser Informationsbogen beinhaltet Informationen zu den Untersuchungsmethoden transkranielle Gleichstromstimulation (tDCS), transkranielle Magnetstimulation (TMS) und Magnetresonanztomografie (MRT), die im Rahmen dieser Studie zum Einsatz kommen sollen. Darüber hinaus möchten wir Sie informieren, wie die Untersuchungsmethoden bei dieser Studie eingesetzt werden sollen.

Die eingesetzten Untersuchungstechniken sind prinzipiell unbedenklich, solange Personen damit untersucht werden, bei denen keine sogenannte „Kontraindikationen“ für die Untersuchung vorliegen. Die Kontraindikation ist ein Umstand, der die Anwendung eines diagnostischen oder therapeutischen Verfahrens bei an sich gegebener Indikation in jedem Fall verbietet oder nur unter strenger Abwägung sich dadurch ergebender Risiken zulässt. Sollte eine Kontraindikation vorliegen, kann es zu schweren Gesundheitsschäden kommen. Um sicher zu gehen, dass dies bei Ihnen nicht der Fall ist, Sie also gefahrlos untersucht werden können, bitten wir Sie, den Fragebogen (s.u.) sorgfältig zu lesen und zu beantworten. Falls Ihnen etwas unklar bleibt, wenden Sie sich bitte an uns!

Im Vorfeld der Untersuchungen werden Sie bei einem persönlichen Gespräch hinsichtlich der Ziele und des Ablaufs der Studie informiert. Hier haben Sie die Möglichkeit Fragen zu stellen, falls Ihnen etwas unklar sein sollte. Diese Studie dient der Grundlagenforschung, persönliche Nutzen sind durch diese Untersuchung nicht zu erwarten.

Transkranielle Gleichstromstimulation (tDCS)

Bei der tDCS handelt sich um eine Untersuchung, bei der mittels durch die Kopfhaut und den Schädel (transkraniell) gegebenen schwachen Stroms die Erregbarkeit des Gehirns beeinflusst werden kann. Die tDCS ist eine nicht-invasive (= nicht in den Körper eingreifende) Stimulationsmethode. Die Stimulation erfolgt mittels zweier Elektroden, die auf Ihrem Kopf aufgelegt werden. Über diese Elektroden fließt während der Untersuchung ein schwacher Strom. Die Stromstimulation ist für Sie nicht oder allenfalls sehr geringfügig wahrnehmbar.

Im Rahmen dieser Studie wollen wir insbesondere untersuchen, inwieweit sich Ihre Gehirnaktivität im Ruhezustand kurzfristig ändert.

Transkranielle Magnetstimulation (TMS)

Während dieses Experiments sitzen Sie in einem unserer neurophysiologischen Labore auf einem bequemen Stuhl mit Armlehnen und Kopfstütze. An einem Handmuskel werden Oberflächenelektroden aufgeklebt und die elektrische Muskelaktivität gemessen. Dann wird mittels einer an den Kopf gehaltenen Magnetspule, von der ein kurzer Impuls ausgeht, ein Handmuskel erregt. Wichtig dabei ist, dass die optimale Spulenposition ermittelt wird und im weiteren Verlauf nicht verändert wird. Dazu wird diese mit einem Stift auf der Kopfhaut markiert. Diese Markierung wird nach dem Experiment wieder entfernt.

Ist die Reizung mit der Magnetspule stark genug, verspüren Sie eine leichte unwillkürliche Zuckung in einigen Muskeln der Hand. Wie stark die Erregung ist, können wir mit Hilfe der auf dem Muskel angebrachten Oberflächenelektroden quantitativ darstellen. Die Form und die Größe der Signale zeigen dabei die Erregbarkeit der verantwortlichen Gehirnbereiche. Aus statistischen Gründen müssen die Reize ca. 20 mal wiederholt werden. Aus den 20 Werten wird dann ein Mittelwert berechnet. Dieser dient als Basiswert vor der Stimulation.

Nach Berechnung des Basiswertes folgt eine 10 minütige tDCS. Nachkommend werden wir die kortikale Erregbarkeit noch 6mal bestimmen. Die Untersuchung wird eine Dauer von 120 Minuten nicht überschreiten. Für die Studie ist es allerdings notwendig diese Stimulation mehrmals zu wiederholen. Insgesamt wird es notwendig sein, an 4 Tagen die Stimulation durchzuführen. Zwischen den einzelnen Untersuchungen wird ein Abstand von mindestens 5 Tage eingehalten. Die Reihenfolge der Stimulationen wird randomisiert, das bedeutet, dass bei diesem Prozess die Versuchspersonen mit dem Zufallsprinzip den Stimulationsarten zugeteilt

werden. An den unterschiedlichen Einzeluntersuchungen werden wir die Elektrodenplatzierung und die Polarität variieren.

Magnetresonanztomografie (MRT)

Mit der MRT werden wir Abbildungen von Ihrem Gehirn anfertigen. Bei der MRT befindet sich Ihr Körper in einem Magnetfeld. Radiowellenimpulse (UKW-Frequenz) erzeugen Echosignale, die von empfindlichen Spulen aufgefangen werden. Ein Computer errechnet hieraus Schnittbilder Ihres Gehirns. Seit der Einführung der MRT vor über 20 Jahren sind nunmehr mehrere Millionen Untersuchungen weltweit durchgeführt worden; es haben sich keine nachteiligen Neben- oder Nachwirkungen gezeigt. Nach dem Stand unseres Wissens sind bei der angewandten Feldstärke (3 Tesla) schädigende Wirkungen theoretisch auch nicht zu erwarten. Die Untersuchung wird in einem speziellen Raum durchgeführt. Sie liegen bequem auf einer Liege, die sich in eine etwa 65 cm große Öffnung des Gerätes bewegt. Von der Untersuchung selbst ist nichts zu spüren. Sie sollten ruhig und entspannt liegen; Sie sollten sich nicht bewegen! Während der gesamten Untersuchung haben Sie Sprechkontakt mit dem Bedienungspersonal, das sie auch sehen kann. Über einen Alarmknopf können Sie uns jederzeit mitteilen, dass wir die Messung abbrechen sollen. Ihren Puls werden wir über einen kleinen Clip am Finger kontrollieren.

Im Rahmen der MR-Untersuchung werden wir in eine anatomische Messung durchführen.

Um den benötigten Zeitaufwand besser einschätzen zu können finden Sie nachfolgend eine tabellarische Übersicht über die einzelnen Untersuchungstermine:

Terminnummer	Art des Termins	Dauer des Termins
1	Anatomische MR-Untersuchung	1 x 40 Minuten
2-5	TMS-Untersuchung	4 x 1,5 Stunden

Aufwandsentschädigung

Sie erhalten für die Teilnahme an dieser Studie eine Aufwandserstattung in Höhe von 9- Euro pro Stunde.

Welche Risiken sind mit der Teilnahme an der Studie verbunden?

Die bereits vorliegenden, umfangreichen Erfahrungen haben gezeigt, dass die Stromstimulation risiko- und nebenwirkungsarm ist, wenn die Ausschlusskriterien beachtet werden. Belastungen sind gering. Allenfalls in seltenen Fällen ist mit Auftreten von Müdigkeit, Kopfschmerzen und durch das Aufkleben von Oberflächenelektroden mit dem Auftreten von Jucken oder Hautreizungen zu rechnen. Zu den allgemeinen Risiken des MRT gehören Auswirkungen, die durch Lärm, Enge oder langes Liegen erzeugt werden können. Schwerwiegende Nebenwirkungen sind bei Beachtung der Ausschlusskriterien ebenfalls nicht zu erwarten. Bitte teilen Sie den Mitarbeitern der Prüfstelle alle Beschwerden, Erkrankungen oder Verletzungen umgehend, ggf. telefonisch mit, die im Verlauf der Studie auftreten.

Zu den Risiken des MRT gehören schwere Verletzungen, falls seitens der Probanden oder des Personals die Regeln nicht beachtet werden. Vor Betreten des Untersuchungsraumes ist es unbedingt erforderlich, alle Metallteile wie z.B. Geldmünzen, Kugelschreiber, Schlüssel, Haarspangen, Uhren, Schmuck und Hörgeräte abzulegen. Bitte beachten Sie auch, dass Scheckkarten mit Magnetstreifen draußen bleiben müssen, da sie sonst gelöscht werden. Personen mit Metallteilen im Körper können nur mit Einschränkungen untersucht werden. Die Untersuchung selbst ist ein völlig ungefährliches Verfahren. Für gewisse Risikogruppen, z.B. Personen mit Metallteilen im Körper (Implantaten), mit stark angegriffenem Herz-Kreislaufsystem oder unter dem Einfluss bestimmter Medikamente, birgt sie jedoch z. T. erhebliche Gefahren. So können beispielsweise im Magnetfeld Knochenschrauben verdreht oder Gefäßclips gelöst werden oder eine Überlastung des Herz-Kreislaufsystems auftreten. Damit wir eine Gefährdung für Sie ausschließen können, lesen Sie bitte den unten folgenden Fragebogen gründlich durch und füllen Sie ihn gewissenhaft aus. Alle im Rahmen dieser Studie verwendeten Geräte sind speziell für den Einsatz in einem 3 Tesla MR-Tomografen entwickelt und zugelassen.

Obwohl die durchgeführte Untersuchung keine diagnostische Untersuchung ist, besteht die Möglichkeit, dass pathologische Befunde entdeckt werden (Zufallsfund). Sie haben in der Einverständniserklärung die Wahlmöglichkeit, ob Sie in einem solchen Fall über den Zufallsfund informiert werden möchten oder nicht. Die Bilddaten werden im Falle eines Zufallsfunds, insofern Sie der Weitergabe zugestimmt haben, an einen Neuroradiologen weitergegeben.

Datenschutz, Datenauswertung, Rücktrittsmöglichkeit, Widerruf

Ihre Daten werden in der MR-Forschung auf zwei verschiedene Arten gespeichert: Personenbezogene Daten (Name, Geburtsdatum, Gewicht, Geschlecht, Telefonnummer und Adresse) werden in einer Kartei der MR-Forschung gespeichert. Die Daten ihrer Untersuchung werden getrennt davon aufbewahrt. Diese werden pseudonymisiert gespeichert, d.h. ohne Namensnennung, sondern nur mit einer Nummer codiert. Die Zuordnung der Daten zu einer Person ist nur möglich, wenn hierfür der Schlüssel eingesetzt wird, mit dem die Daten pseudonymisiert wurden. Eine Entschlüsselung ist nur durch die verantwortlichen Studienleiter möglich. Dritte erhalten keinen Einblick in Ihre Originalunterlagen.

Die personenbezogenen Daten werden 10 Jahre aufbewahrt und danach vernichtet. Selbstverständlich ist es Ihnen jederzeit möglich, ohne Angaben von Gründen und ohne dass Ihnen hieraus Nachteile entstehen, von der Teilnahme an dieser Studie zurückzutreten. Die Aufwandserstattung wird Ihnen dann anteilig ausgezahlt. Sie haben die Möglichkeit, Auskunft über die gespeicherten Daten zu erhalten. Sie können Ihr Einverständnis zur Speicherung personenbezogener Daten jederzeit widerrufen. Im Falle des Widerrufs werden alle gespeicherten personenbezogenen Daten gelöscht.

6.5 Questionnaire for the participants

Fragebogen zu Studie: Einfluss von Elektrodenplatzierung auf Motorkortex Exzitabilität bei der transkraniellen Gleichstromstimulation

Lesen Sie sich zu Ihrer eigenen Sicherheit diesen Fragebogen gründlich durch und beantworten Sie gewissenhaft alle Fragen. Wenn Sie sich nicht sicher sind oder eine Frage nicht verstehen, wenden Sie sich bitte an einen unserer Mitarbeiter. Unterschreiben Sie anschließend den Fragebogen und lassen Sie sich von einem Mitarbeiter einweisen, bevor Sie den Magnet-Bereich betreten.

Wichtig: Aufgrund des sehr starken Magnetfeldes dürfen keinerlei Gegenstände oder Geräte, die aus Metall sind oder Metall enthalten könnten, mit in den Untersuchungsraum genommen werden. Legen Sie solche Gegenstände und Geräte (z.B. Mobiltelefone, Münzen, Kugelschreiber, Schlüssel, Haarspangen, Uhren, Schmuck, Brillen, Gürtel, Hörgeräte, Funkrufempfänger) unbedingt vorher ab!

Achtung: der Magnet ist immer an!

Betreten Sie den Untersuchungsraum nur nach Aufforderung durch das Personal!

Bitte Zutreffendes ankreuzen:

Fragen zur Magnetresonanz-Tomografie:

1	Sind Sie Träger eines Herzschrittmachers, Defibrillators, Hörgeräts, Medikamentenpumpe (Insulin?), Neurostimulators, Implantat mit Magnetventil (z.B. künstlicher Darmausgang)? Wenn ja, welche? ……..……	Ja Nein
2	Wurden Sie schon einmal an Kopf oder Herz operiert? Wenn ja, warum? ……	Ja Nein
3	Befinden sich an oder auf Ihrem Körper Metallteile oder metallhaltige Geräte (z.B. Beinprothesen, Elektroden, Katheter, Langzeit-EKG, Bestrahlungsquellen, Akupunkturnadeln, Piercing)? Wenn ja, welche? ……abnehmbar? Ja Nein	Ja Nein

4	Befinden sich in Ihrem Körper Metallteile oder Implantate, die z.B. bei einer Operation oder Verletzung mit einem metallischen Fremdkörper in Ihren Körper gelangt sind (z.B. Hüftprothesen, künstliche Gelenke, Herzklappen, Gefäßverschlüsse oder -erweiterungen, chirurgische Clips, Knochenschrauben oder -platten, Spirale, Shunts, Katheter, Elektroden, Spulen, Bestrahlungsquellen, Granatsplitter, Projektile, Stents)? Wenn ja, welche?.....	Ja Nein
5	Tragen Sie magnetisch fixierte Implantate (z. B. Zahnprothesen, Glasauge)?	Ja Nein
6	Haben Sie beruflich oder privat mit der Verarbeitung von Metallen zu tun?	Ja Nein
7	Tragen Sie (außer Amalgam-Füllungen) Zahnersatz, Brücken oder Zahnklammern/-spangen? Wenn ja: welche?.....abnehmbar? Ja Nein	Ja Nein
8	Leiden Sie unter einer schweren Erkrankung der Atemwege, des Herz-Kreislaufsystems oder des Bewegungssystems (z.B. Asthma, Herzschwäche, Herzrhythmusstörungen, Lähmungen)? Wenn ja, welche?.....	Ja Nein
9	Leiden Sie unter Diabetes oder einem Anfallsleiden (z.B. Epilepsie)?	Ja Nein
10	Neigen Sie zu Klaustrophobie, Schwindel- oder Panikanfällen?	Ja Nein
11	Sind Sie tätowiert oder haben Sie ein permanentes Make-up?	Ja Nein
12	Leiden Sie unter anderen Allergien? wenn ja, welche?.....	Ja Nein
13	Nehmen Sie zurzeit regelmäßig Medikamente ein? wenn ja, welche?.....	Ja Nein
14	Haben Sie in den letzten 24 Stunden Medikamente oder Alkohol zu sich genommen?	Ja Nein

Zusätzliche Fragen zur tDCS:

15	Wurden Sie innerhalb der letzten zwei Monate operiert? Wenn ja, woran?.....	Ja Nein

16	Haben Sie in den letzten 5 Tagen an einer MRT-, tACS-, tDCS- oder TMS-Untersuchung teilgenommen?	Ja Nein
17	Haben Sie Herzrhythmusstörungen?	Ja Nein
18	Ist bei Ihnen ein Anfallsleiden (Epilepsie, inkl. kindlicher Absencen) bekannt?	Ja Nein
19	Hatten Sie in der Kindheit jemals einen Fieberkrampf erlitten?	Ja Nein
20	Ist in Ihrer unmittelbaren Familie (Eltern, Geschwister) eine Epilepsie bekannt?	Ja Nein
21	Sind bei Ihnen andere neurologische oder psychiatrische Erkrankungen (inklusive Alkoholabhängigkeit oder –mißbrauch) bekannt? Wenn ja, welche?.....	Ja Nein
22	Wurde bei Ihnen je zu diagnostischen Zwecken ein EEG angefertigt?	Ja Nein
23	Hatten Sie je behandlungsbedürftige Kopfverletzungen?	Ja Nein
24	Leiden Sie regelmäßig an Kopfschmerzen?	Ja Nein
25	Leiden Sie an Schlafstörungen?	Ja Nein

Nur von Frauen auszufüllen:

26	Besteht die Möglichkeit, dass Sie schwanger sind?	Ja Nein
27	Tragen Sie eine Kupferspirale?	Ja Nein

– wird vom Personal ausgefüllt –

Untersuchung unbedenklich……..

KEINE Untersuchung möglich ……

Bemerkungen:.....

Datum:.....Unterschrift:.....

7. References

Andrews SC, Hoy KE, Enticott PG, Daskalakis ZJ, Fitzgerald PB (2011): Improving working memory: the effect of combining cognitive activity and anodal transcranial direct current stimulation to the left dorsolateral prefrontal cortex. Brain Stimul 4, 84-89

Antal A, Terney D, Poreisz C, Paulus W (2007): Towards unravelling task-related modulations of neuroplastic changes induced in the human motor cortex. Eur J Neurosci 26, 2687-2691

Antal A, Chaieb L, Moliadze V, Monte-Silva K, Poreisz C, Thirugnanasambandam N, Nitsche MA, Shoukier M, Ludwig H, Paulus W (2010): Brain-derived neurotrophic factor (BDNF) gene polymorphisms shape cortical plasticity in humans. Brain Stimul 3, 230-237

Antal A, Kriener N, Lang N, Boros K, Paulus W (2011): Cathodal transcranial direct current stimulation of the visual cortex in the prophylactic treatment of migraine. Cephalalgia 31, 820-828

Arrowsmith-Young B: The woman who changed her brain: And other inspiring stories of pioneering brain transformation. Simon and Schuster, New York 2012

Bach-Y-Rita P, Collins CC, Saunders FA, White B, Scadden L (1969): Vision substitution by tactile image projection. Nature 221, 963-964

Baker JM, Rorden C, Fridriksson J (2010): Using transcranial direct-current stimulation to treat stroke patients with aphasia. Stroke 41, 1229-1236

Barker AT (1991): An introduction to the basic principles of magnetic nerve stimulation. J Clin Neurophysiol 8, 26-37

Benninger DH, Lomarev M, Lopez G, Wassermann EM, Li X, Considine E, Hallett M (2010): Transcranial direct current stimulation for the treatment of Parkinson's disease. J Neurol Neurosurg Psychiatry 81, 1105-1111

Berlucchi G, Buchtel HA (2009): Neuronal plasticity: historical roots and evolution of meaning. Exp Brain Res 192, 307-319

Bindman LJ, Lippold OC, Redfearn JW (1962): Long-lasting changes in the level of the electrical activity of the cerebral cortex produced bypolarizing currents. Nature 196, 584-585

Bindman LJ, Lippold OC, Redfearn JW (1964): The Action of Brief Polarizing Currents on the Cerebral Cortex of the Rat (1) during Current Flow and (2) in the Production of Long-Lasting after-Effects. J Physiol 172, 369-382

Bliss TV, Collingridge GL (1993): A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361, 31-39

Boggio PS, Ferrucci R, Rigonatti SP, Covre P, Nitsche M, Pascual-Leone A, Fregni F (2006): Effects of transcranial direct current stimulation on working memory in patients with Parkinson's disease. J Neurol Sci 249, 31-38

Boggio PS, Nunes A, Rigonatti SP, Nitsche MA, Pascual-Leone A, Fregni F (2007): Repeated sessions of noninvasive brain DC stimulation is associated with motor function improvement in stroke patients. Restor Neurol Neurosci 25, 123-129

Boggio PS, Rigonatti SP, Ribeiro RB, Myczkowski ML, Nitsche MA, Pascual-Leone A, Fregni F (2008): A randomized, double-blind clinical trial on the efficacy of cortical direct current stimulation for the treatment of major depression. Int J Neuropsychopharmacol 11, 249-254

Boggio PS, Khoury LP, Martins DC, Martins OE, de Macedo EC, Fregni F (2009): Temporal cortex direct current stimulation enhances performance on a visual recognition memory task in Alzheimer disease. J Neurol Neurosurg Psychiatry 80, 444-447

Boros K, Poreisz C, Munchau A, Paulus W, Nitsche MA (2008): Premotor transcranial direct current stimulation (tDCS) affects primary motor excitability in humans. Eur J Neurosci 27, 1292-1300

Brandt SA, Ploner CJ, Meyer BU (1997): Repetitive transcranial magnetic stimulation. Possibilities, limits and safety aspects. Nervenarzt 68, 778-784

Carlson C, Devinsky O (2009): The excitable cerebral cortex Fritsch G, Hitzig E. Uber die elektrische Erregbarkeit des Grosshirns. Arch Anat Physiol Wissen 1870;37:300-32. Epilepsy Behav 15, 131-132

Carney MW (1969): Negative polarisation of the brain in the treatment of manic states. Ir J Med Sci 8, 133-135

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

Cheeran B, Talelli P, Mori F, Koch G, Suppa A, Edwards M, Houlden H, Bhatia K, Greenwood R, Rothwell JC (2008): A common polymorphism in the brain-derived neurotrophic factor gene (BDNF) modulates human cortical plasticity and the response to rTMS. J Physiol 586, 5717-5725

Conde V, Vollmann H, Sehm B, Taubert M, Villringer A, Ragert P (2012): Cortical thickness in primary sensorimotor cortex influences the effectiveness of paired associative stimulation. Neuroimage 60, 864-870

Cooke SF, Bliss TV (2006): Plasticity in the human central nervous system. Brain 129, 1659-1673

Coskun O (2011): Magnetic resonance imaging and safety aspects. Toxicol Ind Health 27, 307-313

Costain R, Redfearn JW, Lippold OC (1964): A Controlled Trial of the Therapeutic Effect of Polarization of the Brain in Depressive Illness. Br J Psychiatry 110, 786-799

Dasilva AF, Mendonca ME, Zaghi S, Lopes M, Dossantos MF, Spierings EL, Bajwa Z, Datta A, Bikson M, Fregni F (2012): tDCS-induced analgesia and electrical fields in pain-related neural networks in chronic migraine. Headache 52, 1283-1295

Elbert T, Pantev C, Wienbruch C, Rockstroh B, Taub E (1995): Increased cortical representation of the fingers of the left hand in string players. Science 270, 305-307

Ferrier D (1874): Experiments on the Brain of Monkeys.--No. I. Proc R Soc Lond [Biol] 23, 409-430

Ferrucci R, Mameli F, Guidi I, Mrakic-Sposta S, Vergari M, Marceglia S, Cogiamanian F, Barbieri S, Scarpini E, Priori A (2008): Transcranial direct current stimulation improves recognition memory in Alzheimer disease. Neurology 71, 493-498

Ferrucci R, Bortolomasi M, Vergari M, Tadini L, Salvoro B, Giacopuzzi M, Barbieri S, Priori A (2009): Transcranial direct current stimulation in severe, drug-resistant major depression. J Affect Disord 118, 215-219

Fishman RS (1995): Ferrier's mistake revisited, or when it comes to the brain, nothing is simple. Arch Neurol 52, 725-730

Fitsch G, Hitzig E (1870): The excitable cerebral cortex. Arch Anat Physiol Wissen 37, 300-332

Fratello F, Veniero D, Curcio G, Ferrara M, Marzano C, Moroni F, Pellicciari MC, Bertini M, Rossini PM, De Gennaro L (2006): Modulation of corticospinal excitability by paired associative stimulation: reproducibility of effects and intraindividual reliability. Clin Neurophysiol 117, 2667-2674

Fregni F, Boggio PS, Mansur CG, Wagner T, Ferreira MJ, Lima MC, Rigonatti SP, Marcolin MA, Freedman SD, Nitsche MA (2005): Transcranial direct current stimulation of the unaffected hemisphere in stroke patients. Neuroreport 16, 1551-1555

Fregni F, Boggio PS, Santos MC, Lima M, Vieira AL, Rigonatti SP, Silva MT, Barbosa ER, Nitsche MA, Pascual-Leone A (2006): Noninvasive cortical stimulation with transcranial direct current stimulation in Parkinson's disease. Mov Disord 21, 1693-1702

Fujiyama H, Hyde J, Hinder MR, Kim S-J, McCormack GH, Vickers JC, Summers JJ (2014): Delayed plastic responses to anodal tDCS in older adults. Front Aging Neurosci 6, 115

Gartside IB (1968): Mechanisms of sustained increases of firing rate of neurones in the rat cerebral cortex after polarization: role of protein synthesis. Nature 220, 383-384

Gorman AL (1966): Differential patterns of activation of the pyramidal system elicited by surface anodal and cathodal cortical stimulation. J Neurophysiol 29, 547-564

Hamada M, Murase N, Hasan A, Balaratnam M, Rothwell JC (2013): The role of interneuron networks in driving human motor cortical plasticity. Cereb Cortex 23, 1593-1605

Hasan A, Misewitsch K, Nitsche MA, Gruber O, Padberg F, Falkai P, Wobrock T (2013): Impaired motor cortex responses in non-psychotic first-degree relatives of schizophrenia patients: a cathodal tDCS pilot study. Brain Stimul 6, 821-829

Hattori Y, Moriwaki A, Hori Y (1990): Biphasic effects of polarizing current on adenosine-sensitive generation of cyclic AMP in rat cerebral cortex. Neurosci Lett 116, 320-324

Herwig U, Schönfeldt-Lecuona C, Wunderlich AP, von Tiesenhausen C, Thielscher A, Walter H, Spitzer M (2001): The navigation of transcranial magnetic stimulation. Psychiatry Res 108, 123-131

Hummel F, Cohen LG (2005): Improvement of motor function with noninvasive cortical stimulation in a patient with chronic stroke. Neurorehabil Neural Repair 19, 14-19

Hunter MA, Coffman B, Trumbo M, Clark V: Tracking the neuroplastic changes associated with transcranial direct current stimulation: A push for multimodal imaging. In: Shennan Aibel Weiss MB (ed.): Open questions on the mechanisms of neuromodulation with applied and endogenous electric fields. Front Hum Neurosci, Lausanne 2013, 45-48

Islam N, Aftabuddin M, Moriwaki A, Hattori Y, Hori Y (1995): Increase in the calcium level following anodal polarization in the rat brain. Brain Res 684, 206-208

Ito M (1989): Long-term depression. Annu Rev Neurosci 12, 85-102

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

Kuo MF, Paulus W, Nitsche MA (2006): Sex differences in cortical neuroplasticity in humans. Neuroreport 17, 1703-1707

Landau WM, Bishop GH, Clare M (1964): Analysis of the form and distribution of evoked cortical potentials under the influence of polarizing currents. J Neurophysiol 27, 788-813

Lang N, Siebner HR, Ward NS, Lee L, Nitsche MA, Paulus W, Rothwell JC, Lemon RN, Frackowiak RS (2005): How does transcranial DC stimulation of the primary motor cortex alter regional neuronal activity in the human brain? Eur J Neurosci 22, 495-504

Leyton AS, Sherrington CS (1917): Observations on the excitable cortex of the chimpanzee, orang-utan, and gorilla. Quart J Exp Physiol 11, 135-222

Li H, Lei X, Yan T, Huang B, Li L, Xu L, Liu L, Chen N, Lu L, Ma Y (2015): The temporary and accumulated effects of transcranial direct current stimulation for the treatment of advanced Parkinson's disease monkeys. Sci Rep 5, 1-10

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

Lippold OC, Redfearn JW (1964): Mental Changes Resulting from the Passage of Small Direct Currents through the Human Brain. Br J Psychiatry 110, 768-772

Lolas F (1977): Brain polarization: behavioral and therapeutic effects. Biol Psychiatry 12, 37-47

Lomo T (2003): The discovery of long-term potentiation. Philos Trans R Soc Lond B Biol Sci 358, 617-620

Lopez-Alonso V, Cheeran B, Rio-Rodriguez D, Fernandez-Del-Olmo M (2014): Inter-individual variability in response to non-invasive brain stimulation paradigms. Brain Stimul 7, 372-380

Malenka RC, Nicoll RA (1999): Long-term potentiation--a decade of progress? Science 285, 1870-1874

Mattar AAG, Nasir SM, Darainy M, Ostry DJ: Sensory change following motor learning. In: Green A, Chapman CE, Kalaska JF, Lepore F (ed.): Enhancing performance for action and perception: multisensory integration, neuroplasticity & neuroprosthetics. Part 1. Elsevier, Amsterdam 2011, 31-44

Merton PA, Morton HB (1980): Stimulation of the cerebral cortex in the intact human subject. Nature 285, 227

Miranda PC, Mekonnen A, Salvador R, Ruffini G (2013): The electric field in the cortex during transcranial current stimulation. Neuroimage 70, 48-58

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

Mori F, Codeca C, Kusayanagi H, Monteleone F, Buttari F, Fiore S, Bernardi G, Koch G, Centonze D (2010): Effects of anodal transcranial direct current stimulation on chronic neuropathic pain in patients with multiple sclerosis. J Pain 11, 436-442

Moriwaki A (1991): Polarizing currents increase noradrenaline-elicited accumulation of cyclic AMP in rat cerebral cortex. Brain research 544, 248-252

Muller-Dahlhaus JF, Orekhov Y, Liu Y, Ziemann U (2008): Interindividual variability and age-dependency of motor cortical plasticity induced by paired associative stimulation. Exp Brain Res 187, 467-475

Nettekoven C, Volz LJ, Leimbach M, Pool EM, Rehme AK, Eickhoff SB, Fink GR, Grefkes C (2015): Inter-individual variability in cortical excitability and motor network connectivity following multiple blocks of rTMS. Neuroimage 118, 209-218

Nitsche MA, Paulus W (2000): Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol 527, 633-639

Nitsche MA, Paulus W (2001): Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology 57, 1899-1901

Nitsche MA, Fricke K, Henschke U, Schlitterlau A, Liebetanz D, Lang N, Henning S, Tergau F, Paulus W (2003a): Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. J Physiol 553, 293-301

Nitsche MA, Nitsche MS, Klein CC, Tergau F, Rothwell JC, Paulus W (2003b): Level of action of cathodal DC polarisation induced inhibition of the human motor cortex. Clin Neurophysiol 114, 600-604

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

Nitsche MA, Doemkes S, Karakose T, Antal A, Liebetanz D, Lang N, Tergau F, Paulus W (2007): Shaping the effects of transcranial direct current stimulation of the human motor cortex. J Neurophysiol 97, 3109-3117

Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, Paulus W, Hummel F, Boggio PS, Fregni F (2008): Transcranial direct current stimulation: State of the art 2008. Brain Stimul 1, 206-223

Nudo RJ (2013): Recovery after brain injury: mechanisms and principles. Neuroplasticity and Neurorehabilitation. Front Hum Neurosci 7, 887

Oldfield RC (1971): The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9, 97-113

Opitz A, Paulus W, Will S, Antunes A, Thielscher A (2015): Determinants of the electric field during transcranial direct current stimulation. Neuroimage 109, 140-150

Palm U, Keeser D, Schiller C, Fintescu Z, Reisinger E, Padberg F, Nitsche M (2008): Skin lesions after treatment with transcranial direct current stimulation (tDCS). Brain Stimul 1, 386-387

Pascual-Leone A, Houser CM, Reese K, Shotland LI, Grafman J, Sato S, Valls-Sole J, Brasil-Neto JP, Wassermann EM, Cohen LG (1993): Safety of rapid-rate transcranial magnetic stimulation in normal volunteers. Electroencephalogr Clin Neurophysiol 89, 120-130

Penfield W, Boldrey E (1937): Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. Brain 60, 389-443

Plewnia C, Zwissler B, Längst I, Maurer B, Giel K, Krüger R (2013): Effects of transcranial direct current stimulation (tDCS) on executive functions: influence of COMT Val/Met polymorphism. Cortex 49, 1801-1807

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

Priori A, Berardelli A, Rona S, Accornero N, Manfredi M (1998): Polarization of the human motor cortex through the scalp. Neuroreport 9, 2257-2260

Purpura DP, McMurtry JG (1965): Intracellular Activities and Evoked Potential Changes during Polarization of Motor Cortex. J Neurophysiol 28, 166-185

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

Rocha S, Silva E, Foerster A, Wiesiolek C, Chagas AP, Machado G, Baltar A, Monte-Silva K (2015): The impact of transcranial direct current stimulation (tDCS) combined with modified constraint-induced movement therapy (mCIMT) on upper limb function in chronic stroke: a double-blind randomized controlled trial. Disabil Rehabil 10, 1-8

Rosenzweig MR: Modification of Brain Circuits through Experience. In: Bermúdez-Rattoni F (ed.): Neural plasticity and memory: from genes to brain imaging. CRC Press, Boca Raton 2007, 67-95

Rossini PM, Desiato MT, Lavaroni F, Caramia MD (1991): Brain excitability and electroencephalographic activation: non-invasive evaluation in healthy humans via transcranial magnetic stimulation. Brain Res 567, 111-119

Rossini PM, Pauri F (2000): Neuromagnetic integrated methods tracking human brain mechanisms of sensorimotor areas 'plastic' reorganisation. Brain Res Brain Res Rev 33, 131-154

Sale MV, Ridding MC, Nordstrom MA (2008): Cortisol inhibits neuroplasticity induction in human motor cortex. J. Neurosci. 28, 8285-8293

Schubert M (1997): Klinische und experimentelle Aspekte der Magnetstimulation. Klin Neurophysiol 28, 114-119

Soler MD, Kumru H, Pelayo R, Vidal J, Tormos JM, Fregni F, Navarro X, Pascual-Leone A (2010): Effectiveness of transcranial direct current stimulation and visual illusion on neuropathic pain in spinal cord injury. Brain 133, 2565-2577

Stefan K, Wycislo M, Classen J (2004): Modulation of associative human motor cortical plasticity by attention. J Neurophysiol 92, 66-72

Teyler T, DiScenna P (1987): Long-term potentiation. Annu Rev Neurosci 10, 131-161

Trachtenberg JT, Chen BE, Knott GW, Feng G, Sanes JR, Welker E, Svoboda K (2002): Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. Nature 420, 788-794

Wassermann EM (1998): Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5-7, 1996. Electroencephalogr Clin Neurophysiol 108, 1-16

Wiethoff S, Hamada M, Rothwell JC (2014): Variability in response to transcranial direct current stimulation of the motor cortex. Brain Stimul 7, 468-475

Acknowledgments

I would firstly like to express my thanks to the department of Clinical Neurophysiology of the Georg-August-University-Goettingen, under the direction of Prof. Dr. med. Paulus, for the research facilities and giving me the opportunity to conduct this study. I am also very thankful for the great assistance from my supervisors Dr. Alexander Opitz and Dr. Catarina Saiote, teaching and coaching me throughout the experimental sessions. I would also like to express my gratitude to Professor Dr. Antal for her wise guidance and help through the writing procedure. My sincere thanks also to all the volunteers taking part in my study and making it happen.