Investigation of Methodological and Physiological Factors Influencing Non-Invasive Transcranial Electrical Brain Stimulation

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Abstract

Non-invasive transcranial electrical brain stimulation (tES) techniques, including transcranial direct current stimulation (tDCS), transcranial alternating current stimulation (tACS) and transcranial random noise stimulation (tRNS), can alter neuronal activity and related brain functions. However, tES effects seem to be modulated by various influencing factors, leading to high inter-individual variability in tES effects and often only low effect sizes, or even no effects. The present thesis therefore aimed to investigate methodological and physiological influencing factors of tDCS, tACS and tRNS that have not been sufficiently examined so far. A first study investigated the influence of montage and individual functional performance level on the effects of anodal tDCS over the left dorsolateral prefrontal cortex (DLPFC) in healthy adults. Compared with sham stimulation, a multichannel montage led to stronger effects than a bipolar montage. For both montages the effects of stimulation were dependent on the functional performance level of participants. A second study investigated the effects of multichannel tDCS over the left DLPFC in healthy children and adolescents, considering the influence of concurrent target task performance during stimulation and individual head anatomy. tDCS did not influence the target outcome but led to transfer effects on non-target task performance and neurophysiological activity, that were only partly influenced by task performance during stimulation. The individual head anatomy had no influence on stimulation effects. A third study investigated tACS and tRNS effects on motor cortex excitability in healthy children and adolescents in comparison to adults. The individual response to sham stimulation was investigated as marker for the individual physiological brain state. Motor cortex excitability was not modulated by age but by individual response to sham stimulation. All studies provide important insights into the modulatory factors of stimulation effects. Based on these results, future studies should aim at individualising tES application.

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List of Abbreviations

ADHD	Attention-Deficit/Hyperactivity Disorder
ADHS-E	ADHS-Screening für Erwachsene
AMT	active motor threshold
ANOVA	Analysis of Variance
BA	Brodmann area
BCM theory	Bienenstock–Cooper–Munro theory
BDI-II	Beck-Depressions-Inventar Revision
CBCL	Child Behavior Checklist
CFT 20-R	Grundintelligenztest Skala 2 - Revision
CPT	Continuous Performance Task
CSF	cerebrospinal fluid
DLPFC	dorsolateral prefrontal cortex
EEG	electroencephalography
E-field	electric field
E/I balance	excitation/inhibition balance
EMG	electromyography
ERP	event-related potential
FBB-ADHS	Fremdbeurteilungsbogen für Aufmerksamkeitsdefizit-/Hyperaktivitätsstörungen
fMRI	functional magnetic resonance imaging
FDI	first dorsal interosseus
FPN	fronto-parietal network
GABA	gamma-Aminobutyric acid
ISI	inter-stimulus interval
LME	linear mixed effects model
LTD	long-term depression
LTP	long-term potentiation
MCE	motor cortex excitability
MEP	motor evoked potential
MRI	magnetic resonance imaging
MST	Mnemonic Similarity Task
NMDA	N-methyl-D-aspartate
NTBS	non-invasive transcranial brain stimulation
RMT	resting motor threshold
ROI	region of interest
RT	reaction time
RTM	regression to the mean
SI	stimulus intensity
SRS	Social Responsiveness Scale
tACS	transcranial alternating current stimulation
tDCS	transcranial direct current stimulation
tES	transcranial electrical stimulation
TFR	time-frequency representations
TMS	transcranial magnetic stimulation
tRNS	transcranial random noise stimulation
WM	working memory

1 Introduction

How does the human brain work? And how can we influence this functioning? These questions and the search for their answers have fascinated countless researchers throughout history. Non-invasive transcranial electrical brain stimulation (tES) techniques can help to answer these questions by offering the possibility to investigate causal relationships of brain activity and cognitive functions and behaviour in vivo. tES is aimed at altering brain functions by applying a low electrical current (≤ 4 mA) to the brain via two or more electrodes placed on the head (for a review see Reed & Cohen Kadosh, 2018). By inducing an electric field (E-field) measured in Volts per meter (V/m) in the brain, tES is able to influence neuronal activity (Merton & Morton, 1980). Two main methods for tES exist; transcranial direct current stimulation (tDCS), which applies a constant current between at least one anode and one cathode (Gebodh et al., 2019) and transcranial alternating current stimulation (tACS), which applies sinusoidal current of a specified frequency to the brain (Antal & Herrmann, 2016). An addition method, transcranial random noise stimulation (tRNS), is essentially a specialised version of tACS, that uses an alternating current (Antal & Herrmann, 2016) which varies randomly in both frequency and intensity within a pre-defined range.

tES has been demonstrated to influence various cognitive functions (Dedoncker, Brunoni, Baeken, & Vanderhasselt, 2016; Meiron & Lavidor, 2014; Snowball et al., 2013). Based on these results, the use of tES as a treatment for neuropsychiatric diseases in children and adults is the vision of many researchers and clinicians. Indeed, there are promising results in the investigation of tES in clinical cohorts (Ciullo et al., 2020; Lee, Kenney-Jung, Blacker, Doruk Camsari, & Lewis, 2019).

However, following an initial boom in interest for tES, disillusionment has set in. The usefulness and validity of tES has been questioned due to tES studies showing either a high inter-individual variability in tES effects, only low effect sizes, or even no effect (Héroux, Loo,

Taylor, & Gandevia, 2017; Horvath, Carter, & Forte, 2014). In this context, it is necessary and useful to conduct methodological studies to examine factors that have already been proven or are suspected to have an influence on the outcome of tES. Among these are tES montage specific effects (Miranda, Mekonnen, Salvador, & Ruffini, 2013). Additionally, tES was shown to be influenced by age of participants (Fresnoza et al., 2020; Moliadze et al., 2015), individual functional performance level (Fertonani & Miniussi, 2017) and functional and physiological state of the brain during stimulation (Antal, Terney, Poreisz, & Paulus, 2007; Kortuem, Kadish, Siniatchkin, & Moliadze, 2019). Although there is preliminary evidence on the influence of these factors on tES effects, many aspects related to these factors are insufficiently explored. To use tES effectively, and to be able to deal with the criticisms mentioned above, a sufficient understanding of these modulating factors is required. The aim of this thesis was therefore to investigate factors influencing the effects of tDCS, tACS and tRNS.

1.1 Mechanisms of Non-Invasive Transcranial Electrical Brain Stimulation (tES)

In order to investigate which factors influence the effects of tES and in what way, it is first necessary to understand the general mechanisms of action that are assumed for tES (Vosskuhl, Strüber, & Herrmann, 2018). All tES techniques have in common that they apply low-intensity current over a short (several seconds) or longer (typically 10-40 minutes) period of time via electrodes placed on the head (Paulus, Nitsche, & Antal, 2016). tES is usually applied by battery driven stimulators, which are able to adjust the voltage output depending on the impedance at the electrode-skin interface (Knotkova, Nitsche, Bikson, & Woods, 2019). Typically, a pair of circular saline-soaked surface sponge electrodes between 16 and 32 cm^2 are used as electrodes. In addition, montages with several small electrodes are increasingly being utilized, which are intended to enable greater focality (see Chapter 1.4.1). Montages using two electrodes usually define one active electrode that is placed over the target region. The second, often called reference or return electrode, is placed over an area of no interest. Importantly,

regardless of naming, the return electrode is also physiologically active, since the current flows from one electrode to the other (Paulus et al., 2016). The same principle applies to montages using more than two electrodes, whereby several electrodes at the same time can be active or reference.

Due to the transcranial application, a major amount of the current is shunted by the skin, skull and cerebrospinal fluid (CSF), while a small amount actually reaches the brain (Holdefer, Sadleir, & Russell, 2006). Because of these low current intensities inside the brain, tES does not trigger action potentials (Radman, Datta, Ramos, Brumberg, & Bikson, 2009). Instead, it is assumed that the induced E-field in the brain leads to a polarization of neuronal membranes and thus influences endogenous neuronal activity. The strength of the E-field depends on the intensity of the applied current, with approximately 1 m/V field strength per 2 mA of applied current strength, leading to maximal 0.2 mV membrane polarisation (Alekseichuk, Mantell, Shirinpour, & Opitz, 2019; Jackson et al., 2016). Generally, the effects of tES are divided into short-term, also named online effects, i.e. effects that occur during stimulation, and long-term, also named offline or after-effects, i.e. effects that develop after after several minutes or termination of stimulation. The following sections present an overview of the specific mechanisms of tDCS, tACS and tRNS and their assumed short- and long-term effects.

1.1.1 Transcranial Direct Current Stimulation (tDCS)

tDCS applies a direct current between electrodes (Gebodh et al., 2019). The anode has a positive, while the cathode has a negative voltage (see Figure 1-1). Regarding the short-term effects of tDCS, it is assumed that during stimulation the induced current leads to a polarisation of neuronal membranes and thereby modulates excitability (Bikson et al., 2004; Creutzfeldt, Fromm, & Kapp, 1962). This means, tDCS affects the spontaneous firing rate of neurons by increasing or decreasing the resting membrane potential. Simplified, it is assumed that this stimulation effect is polarity dependent: The inward directed current flow under the anode leads to a depolarization of cortical pyramidal neurons and increased firing rate, while the outward

directed current flow under the cathode leads to a hyperpolarization and decreased firing rate (Bindman, Lippold, & Redfearn, 1962; Jackson et al., 2016; see Figure 1-2). However, it must be considered that the neurons are not de- or hyperpolarized as a whole, but individual sections are influenced differently depending on the current flow (Chan, Hounsgaard, & Nicholson, 1988). Thus, for cortical neurons and radial current flow under the anode it can be assumed that the soma is depolarized while the apical dendrite is hyperpolarized (Jefferys, 1981; Radman et al., 2009). Accordingly, under the cathode the soma is hyperpolarized, and the apical dendrite depolarized. These polarity specific tDCS effects have also been confirmed by studies in humans (Jacobson, Koslowsky, & Lavidor, 2012; Nitsche & Paulus, 2000). However, repeatedly studies are not able to demonstrate a polarity dependent tDCS effect (Bestmann, Berker, & Bonaiuto, 2015; Fertonani & Miniussi, 2017; Wörsching et al., 2018), which implies an oversimplification of the polarity dependent assumption.

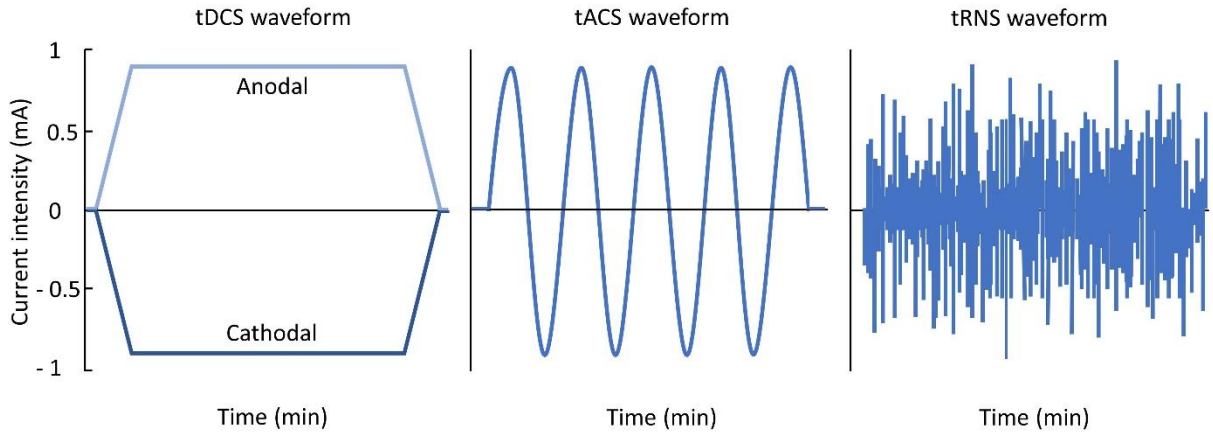

Figure 1-1. tES current waveforms. From left to right: tDCS waveform for anodal and cathodal stimulation, oscillatory tACS and tRNS (Modified after Reed & Cohen Kadosh, 2018).

If applied for several minutes, long-term tDCS effects in form of altered cortical excitability can be induced, lasting from minutes up to several hours after stimulation (Monte-Silva et al., 2013; Nitsche & Paulus, 2001; for a review see Stagg, Antal, & Nitsche, 2018). These changes are assumed to be based on long-term potentiation (LTP) and depression (LTD) like effects (Fritsch et al., 2010; Jackson et al., 2016). Just as for short-term effects, these after-

effects can be polarity specific, with anodal stimulation increasing, and cathodal stimulation decreasing, cortical excitability (Stagg et al., 2018). The stimulation induced plasticity seem to be based on calcium-dependent changes in the glutamatergic system, changing the concentrations of inhibitory (gamma-Aminobutyric acid, GABA) and excitatory (glutamate) neurotransmitters (Liebetanz, Nitsche, Tergau, & Paulus, 2002; Nitsche et al., 2003; Stagg & Johansen-Berg, 2013).

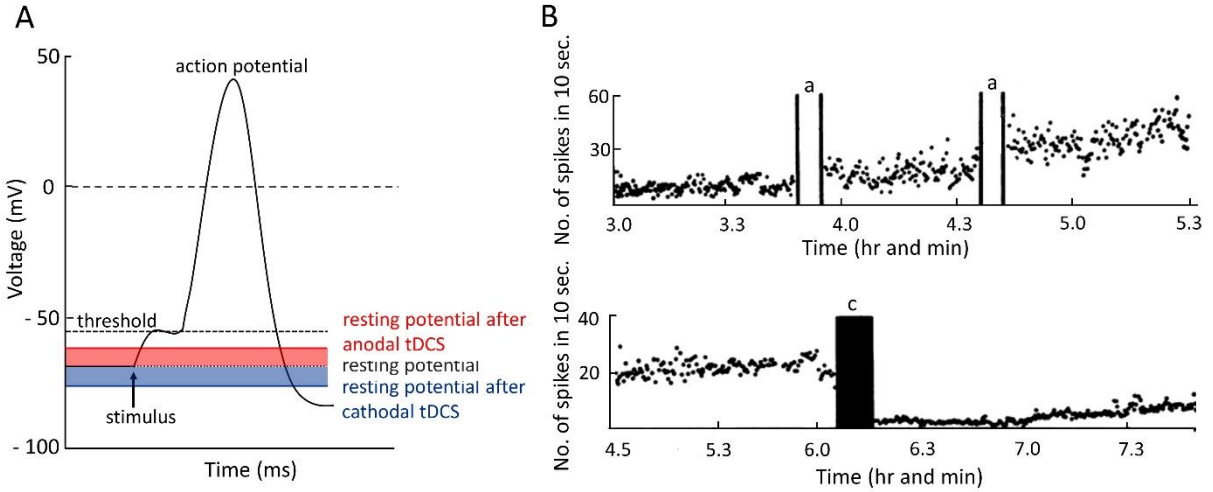

Figure 1-2. Effects of anodal and cathodal stimulation. (A) Schematic representation of resting membrane potential modulation through anodal and cathodal tDCS (adapted from Caytak, Shapiro, Borisenko, and Bolic 2015). (B) Changes in spike activity following anodal (top) and cathodal (bottom) stimulation in the rat cerebral cortex. Anodal stimulation was applied at timpoints a, cathodal stimulation at timepoint c (adapted from Bindman, Lippold and Redfearn, 1964).

1.1.2 Transcranial Alternating Current Stimulation (tACS)

In tACS a sinusoidal current with a defined frequency is applied to the brain (Antal et al., 2008; for a review see Antal & Herrmann, 2016). It has been shown that effects of tACS differ depending on the current intensity and frequency (Paulus et al., 2016). Previous studies in humans used frequencies inside the conventional EEG range (0.1 – 80 Hz) or in the "ripple" range (≥ 140 Hz; Moliadze, Antal, & Paulus, 2010; Paulus et al., 2016). Due to the current's oscillatory nature, the stimulation electrodes are not static anodes or cathodes. Instead, each electrode serves as anode for one half of a circle and as cathode for the other half of the circle, with increasing and decreasing current strength throughout the circle. The specification of the

current intensity usually refers to the peak-to-peak intensity. tACS applied with 2 mA intensity therefore implies that the intensity oscillates between -1 and +1 mA (see Figure 1-1).

The aim of tACS is to influence ongoing oscillations in the brain. The endogenous oscillations can be influenced on a short-term or long-term scale (for a review see Vosskuhl et al., 2018). During stimulation, tACS can lead to neural entrainment, i.e. an endogenous brain oscillation synchronized to the external rhythmic driving force (Thut, Schyns, & Gross, 2011; Vosskuhl et al., 2018). The more the endogenous oscillation and the external stimulation differ in frequency, the higher the intensity of the external force must be to achieve entrainment. This relationship is called *Arnold Tongue* (Pikovskij, Rosenblum, & Kurths, 2003). As for tDCS, tACS after-effects are thought to reflect plasticity like mechanisms. In humans it has been shown that these after-effects can influence the amplitude (Neuling, Rach, & Herrmann, 2013) or frequency (Helfrich et al., 2014) of endogenous oscillations.

1.1.3 Transcranial Random Noise Stimulation (tRNS)

tRNS uses an alternating current for stimulation and can be classed as a specialised type of tACS. However, unlike tACS, the intensity and frequency of the tRNS current vary randomly within a fixed range (see Figure 1-1). The frequency usually fluctuates within a full (0.1 – 640 Hz), low (0.1 – 100 Hz) or high spectrum (101 – 640 Hz; Terney, Chaieb, Moliadze, Antal, & Paulus, 2008). For every sample that is generated by the stimulator, a random current level is generated. Overall, the current probability function follows a normal distribution (Antal & Herrmann, 2016; Terney et al., 2008). This means, for application of 1 mA tRNS 99% of all current levels lies between -0.5 and 0.5 mA. The effects and mechanisms of tRNS have not been investigated as much as for tDCS and tACS. The effects of tRNS might be based on repetitive opening of Na^+ channels, leading to an inward sodium flow and a depolarisation of the neural membrane (Schoen & Fromherz, 2008; Terney et al., 2008). Besides, it is often assumed that tRNS effects might be based on *stochastic resonance* (Stacey & Durand, 2000). Stochastic resonance describes the phenomenon that the detection of a weak stimuli or signal

can be enhanced by adding random noise (Moss, Ward, & Sannita, 2004). tRNS might be able to add neural noise to subthreshold neural oscillations in the brain, amplifying the endogenous neural activity (Antal & Herrmann, 2016; Pavan et al., 2019; van der Groen & Wenderoth, 2016).

1.2 Target Regions of tES

The mechanisms of action just presented explain how tES can produce effects in general. However, the actual stimulation effects differ, depending on which part of the brain is targeted by the stimulation. The basic hypothesis in a standard tES experiment is that the current flow in the target region is sufficiently strong to affect the endogenous activity in this specific region. At the same time, it can be assumed that remote tES effects occur. They arise when functionally and structurally connected neural populations are affected by the altered activity in the target region (Di Luft, Pereda, Banissy, & Bhattacharya, 2014; Fertonani & Miniussi, 2017; Knotkova, Nitsche, & Polania, 2019; Wörsching et al., 2016) . Thus, although tES is designed to stimulate a fixed region, the alterations in the target region and associated functions are not necessarily due to the isolated action of tES in this area. However, the majority of tES studies defines a certain target region that is stimulated and hypothesise about tES effects on associated functions.

Two popular tES target regions are the motor cortex and the DLPFC. These regions were used as targets regions for the studies included in this thesis and are considered in more detail below.

1.2.1 The Motor Cortex

Most studies in humans investigating the general mechanisms of action and physiological effects of tES use the primary motor cortex as target region (Dissanayaka, Zoghi, Farrell, Egan, & Jaberzadeh, 2017; Knotkova, Nitsche, & Polania, 2019). But what is it that makes this brain area so attractive as a target region? Firstly, this region is easy to reach due to

its location close to the surface. Secondly, stimulation-related physiological changes can be scaled with comparatively objective methods. One very popular method to measure tES effects is provided by transcranial magnetic stimulation (TMS). Unlike tES, TMS is capable of triggering action potentials in the brain (Bergmann, Karabanov, Hartwigsen, Thielscher, & Siebner, 2016). When TMS is applied over the primary motor cortex (M1), MEPs can be generated. These electrical signals can be measured from descending motor pathway or muscles using electromyography (EMG; Legatt, 2014). MEPs allow quantitative statements about the excitability of the motor cortex. Usually, the size of a single MEP is defined as a peak-to-peak amplitude (Rossini et al., 2015). Besides single pulse TMS protocols, short interval intracortical inhibition (SICI) and intracortical facilitation (ICF) can be used to determine motor cortex excitability. These are paired pulse TMS protocols, i.e. two TMS pulses, a conditioning stimulus and a test stimulus, are delivered in short sequence. In this way, intracortical inhibition and facilitation can be measured. An inter-stimulus interval (ISI) of 1 – 6 ms leads to SICI, demonstrated in a reduced test pulse amplitude, which expresses cortical inhibition. A longer ISI of 6 - 30 ms leads to ICF, which is reflected in an increased amplitude of the test pulse and represents cortical facilitation (Rossini et al., 2015; Ziemann, Rothwell, & Ridding, 1996).

A polarity dependence of tDCS effects (increased excitability after anodal, and decreased excitability after cathodal stimulation) has been demonstrated in numerous studies, using different motor behaviour outcomes, including MEPs (Dissanayaka et al., 2017; Jacobson et al., 2012; Nitsche & Paulus, 2000, 2001). Influences on motor cortex excitability could also be demonstrated for tACS (for a review see Dissanayaka et al., 2017). These effects have been shown to be dependent on frequency of tACS. It was shown that higher frequencies (≥ 140 Hz) lead to excitatory effects on motor cortex excitability, while lower frequencies show inconsistent effects (Chaieb, Antal, & Paulus, 2011; Dissanayaka et al., 2017; Moliadze et al., 2010). Specifically, tACS in the beta range (20 Hz) over the motor cortex has been shown to slow voluntary movement (Pogosyan, Gaynor, Eusebio, & Brown, 2009; Wach et al., 2013).

Beta oscillations are strongly connected to the motor system (Schmidt et al., 2019): At the same time, several other studies could not provide evidence of beta frequency tACS effects on motor cortex excitability (Rjosk et al., 2016) or demonstrated increased excitability (for a review see Wischnewski, Schutter, & Nitsche, 2019). For tRNS increased MEPs reflecting enhanced cortical excitability have been shown following stimulation over the motor cortex (Chaieb et al., 2011; Moliadze, Fritzsche, & Antal, 2014; Terney et al., 2008). These changes in excitability seem to be evoked by the high-frequency tRNS spectrum (100–640 Hz; Terney et al., 2008).

Taken together, studies in the motor cortex provide important information about the methods of action of tES and modulating factors. At the same time, it cannot be assumed that all results from the motor cortex can be easily transferred to other cortex areas. Differences in cortical architecture, receptor distribution and anatomical factors indicate that the effect of tES may vary depending on the targeted cortex area (Knotkova, Nitsche, & Polania, 2019).

1.2.2 The Dorsolateral Prefrontal Cortex

As already mentioned, tES can induce network effects. This is particularly important because cognitive functions involve large-scale brain networks (Bressler & Menon, 2010; Bressler & Tognoli, 2006). The DLPFC is part of such a network, the frontoparietal network (FPN; Ptak, 2012). As part of this network, the DLPFC is associated with a number of higher order cognitive processes. This includes the role of the FPN as a controller that directs the engagement of other regions (Bressler & Menon, 2010; Miller & Cohen, 2001). Specifically, this cognitive control system, and therefore also the DLPFC, is relevant for working memory (WM; Barbey, Koenigs, & Grafman, 2013). WM describes the ability to maintain information for a brief time interval in an active and easily accessible state (Baddeley & Della Sala, 1996; Chai, Abd Hamid, & Abdullah, 2018; Kane & Engle, 2002). WM integrates different components: the verbal working memory, the visual-spatial working memory and the central executive (Baddeley, 2010; Chai et al., 2018; D'Esposito et al., 1995). The central executive,

including the DLPFC and its role for executive control, is involved in overseeing manipulation, recall and processing of information for other cognitive functions, such as decision-making (Chai et al., 2018; D'Esposito et al., 1995; Rottschy et al., 2012). The aim of improving WM through tES is a common target of stimulation studies since a variety of mental disorders, such as schizophrenia (Galderisi et al., 2009) or attention-deficit/hyperactivity disorder (ADHD; Brennan & Arnsten, 2008), are associated with WM impairments.

Since the DLPFC is an important hub in the FPN, tES that targets activity in the DLPFC can potentially affect several functions (To, Ridder, Hart, & Vanneste, 2018). In the 2014 review of Tremblay et al. the authors were able to show that tDCS over the DLPFC can influence a number of cognitive functions, partly, however, with contradictory results between studies (Tremblay et al., 2014). For example, anodal tDCS over the left DLPFC resulted in increased or decreased WM performance, as well as increased positive emotion processing, pain thresholds, inhibition and problem solving. The authors concluded that "studies probing the same cognitive function using similar tDCS protocols can lead to opposite results" (Tremblay et al., 2014, p. 5). Indeed, the results on effects of tDCS targeting the DLPFC on higher cognitions are contradictory, which is well illustrated by the example of WM. While a review by Horvath, Forte, & Carter (2015) found no effect of tDCS on WM in healthy subjects, other reviews proved that tDCS over the DLPFC can enhance WM functions in healthy as well as clinical samples (Brunoni & Vanderhasselt, 2014; Dedoncker et al., 2016; Hill, Fitzgerald, & Hoy, 2016). However, Hill et al. (2016) limit that only low effect strengths and non-significant effects on outcomes were found. Similarly, Brunoni & Vanderhasselt (2014) could only demonstrate a DLPFC tDCS effect on reaction times in WM tasks.

The effects of tACS and tRNS over the DLPFC have also been investigated in previous studies, but to a much lesser extent than for tDCS. tACS in the theta range (4–6 Hz) over the DLPFC has been shown to improve WM performance (Meiron & Lavidor, 2014; Röhner et al., 2018) and cognitive control (Lehr, Henneberg, Nigam, Paulus, & Antal, 2019). However, in

other studies theta (Jaušovec & Jaušovec, 2014) and gamma tACS (Hoy, Whitty, Bailey, & Fitzgerald, 2016) over the DLPFC led to no effects on WM. Snowball et al. (2013) showed that tRNS in the high-frequency band (100 – 600 Hz) over the bilateral DLPFC improved arithmetic performance. However, in several studies no effect of tRNS over the DLPFC on higher cognitive functions could be confirmed (Brevet-Aeby, Mondino, Poulet, & Brunelin, 2019; Holmes, Byrne, Gathercole, & Ewbank, 2016; Mulquiney, Hoy, Daskalakis, & Fitzgerald, 2011).

1.3 Neurophysiological Effects of tES

The aforementioned tES effects on higher cognitions, such as WM, are usually recorded with neuroscientific tasks. However, these tasks are operationalised very heterogeneously between studies. A further possibility to illustrate stimulation effects is provided by neurophysiological correlates recorded by EEG. They offer important insights into the mechanisms of action of tES by linking behavioural changes to fluctuations in the underlying neural activity. Owing to this, a growing number of tES studies (not limited to but often targeting the DLPFC), are investigating event-related potentials (ERPs), event-related oscillations or resting state oscillations.

One of the most common ERP is the P3 component. The P3 is represented by a positive amplitude deflection approximately between 250 and 500 ms post stimulus onset in parietal electrodes (Polich, 2007). The P3 amplitude is thought to reflect general attention and memory processes (Donchin, 1981; Polich, 2007). More specifically, it is also assumed that the P3 amplitude can picture WM-load for respective tasks (Scharinger, Soutschek, Schubert, & Gerjets, 2015; Watter, Geffen, & Geffen, 2001). Keeser et al. (2011) showed that anodal tDCS over the DLPFC increased performance in a 2-back WM task (Jonides et al., 1997; Kirchner, 1958), which was accompanied by increased P3 amplitudes. Dubreuil-Vall et al. (2019) as well found increased P3 amplitudes following anodal tDCS over the left DLPFC but in a Flanker

task (Eriksen & Eriksen, 1974), accompanied by decreased N2 amplitudes and improvement in reaction time. The N2 is a negative amplitude deflection (ca. 200 – 350 ms post stimulus onset) over anterior scalp sites, sensitive to mismatch and conflict stimuli (Folstein & van Petten, 2008; Luck, 2014).

Zaehle, Sandmann, Thorne, Jäncke, & Herrmann (2011) showed that anodal tDCS over the DLPFC modulated WM performance and increased event-related theta and alpha power following stimulation. The increase in alpha power might reflect an inhibition of non-task related areas, while theta increase might be based on enhanced memory encoding and retrieval (Klimesch, 1996; Pesonen, Hämäläinen, & Krause, 2007; Schmiedt-Fehr, Mathes, & Basar-Eroglu, 2009). Besides task related oscillations, changes in resting state oscillatory power following left DLPFC anodal tDCS have been observed in a study by Keeser, Padberg et al. (2011). Boonstra, Nikolin, Meisener, Martin, & Loo (2016) used anodal tDCS over the left DLPFC which resulted in a general slowing of resting state EEG. However, several studies were not able to prove an effect of tDCS over the left DLPFC on resting state oscillatory power (Gordon et al., 2018; Hill, Rogasch, Fitzgerald, & Hoy, 2019; Horvath et al., 2015).

1.4 Methodological and Physiological Factors Influencing tES

The results summarised in the preceding chapters show that the effects of tES are heterogeneous and often do not occur as expected, or do not occur at all. Hence, criticism of tES has increasingly developed, focusing on low effects, high variability, or a lack of reproducibility of tES results (Bland & Sale, 2019; Filmer, Mattingley, & Dux, 2020; Héroux et al., 2017; Horvath et al., 2014; Lafon et al., 2017; Medina & Cason, 2017). Clearly, tES effects are not uniform, but appear to be influenced by a variety of methodological and physiological factors (Polanía, Nitsche, & Ruff, 2018; Ridding & Ziemann, 2010). To understand the effects of tES, and to be able to use tES effectively (possibly even as a treatment method) these influencing factors must be investigated. An increasing number of studies aim at

identifying and systematically investigating factors influencing tES effects. However, many questions regarding these factors are still unresolved. It has been shown that tES montage in connection with the individual anatomy influences the density and distribution of electrical currents, and thereby the effects of tES (Albizu et al., 2020; Kasten, Duecker, Maack, Meiser, & Herrmann, 2019; Opitz, Paulus, Will, Antunes, & Thielscher, 2015). Previous studies have also shown a dependency of tES effects on individual age (Fresnoza et al., 2020; Moliadze et al., 2015), individual functional performance level (Gözenman & Berryhill, 2016; Learmonth, Thut, Benwell, & Harvey, 2015), functional state of the brain (Friehs & Frings, 2019), as well as physiological state of the brain (Kortuem et al., 2019; Krause & Cohen Kadosh, 2014) . In addition to these factors, there is range of other influencing factors (e.g. gender, genetic polymorphisms or pharmacology; Polanía et al., 2018). However, these will not be considered further here, as they would exceed the scope of this thesis. The following section will therefore focus on the factors just mentioned and present the findings to date on their influence on tES.

1.4.1 Electrode Montage

It is plausible to assume that the tES montage has an influence on the tES effects. At the same time one has to keep in mind that the montage itself is made up of various factors, including the number, size and material of electrodes, their arrangement, as well as the general current intensity and its distribution across the individual electrodes, which influence the current flow (Miranda et al., 2013; Saturnino, Antunes, & Thielscher, 2015). Thus, differences with respect to these variables across studies may contribute in part to heterogeneity in tES effects. For example, Opitz et al. (2018) showed that for a reliable application of tES across sessions, electrode placement has to have an accuracy of < 1 cm. That being said, by design, arrangements of electrodes and current intensities often differ between studies, even though they target the same brain region and functions, leading inevitably to differences in tES effects (Polanía et al., 2018). Regardless of this variability between studies, a general disadvantage of tES is its low focality, especially considering the aim to stimulate specific brain regions

(Karabanov, Saturnino, Thielscher, & Siebner, 2019). This focality problem is due to the transcranial application of stimulation but can be influenced to some extent by the montage used. tES studies most commonly use bipolar montages with circular or rectangular saline-soaked surface sponge electrodes. These montages lead to a rather diffuse E-field distribution and therefore poor spatial targeting, according to computation modelling studies (Laakso et al., 2016; Miranda et al., 2013). Therefore, it is not clear whether the effects of stimulation are due to isolated changes in the target region or whether activity in adjacent structures is modulated as well and contributes to the effects (Karabanov et al., 2019). A recently developed alternative approach, that enables focused but also intense stimulation of a target region, is multichannel tES (Salvador et al., 2021; Saturnino, Siebner, Thielscher, & Madsen, 2019). These montages are based on automatic algorithms that optimise the induced E-field. Optimisation of the E-field is performed based upon a predefined target E-field map by adjusting the number, current intensity and spatial location of electrodes (Miranda et al., 2013; Ruffini, Wendling, Sanchez-Todo, & Santarnecchi, 2018). The effectiveness of multichannel tDCS has already been demonstrated for motor cortex excitability (Fischer et al., 2017). Here, multichannel tDCS led to increased effects on excitability compared to bipolar stimulation. However, the effects of optimised multichannel tES targeting other brain areas have not been investigated so far.

1.4.2 Age of Participants

Another known influencing factor of tES, which is still insufficiently studied, is the individual age of participants. tDCS studies targeting the motor cortex have shown that children respond differently to stimulation than adults (Moliadze et al., 2015; Moliadze et al., 2018). This discrepancy might be due to different age-related anatomical and functional features of the head and brain. Compared to adults, children show different conductivity of the skull tissue, different white and gray matter content and CSF volume as well as a smaller brain-scalp distance, all of which influence the E-field distribution (Beauchamp et al., 2011; Kessler et al.,

2013). Based on these findings, results from tES studies in adults cannot simply be assumed to be valid for children and adolescents.

In addition, childhood and adolescence is associated with a restructuring of the brain, including changes in white and gray matter and an increase in myelin, which contributes to improved connectivity between brain areas (Arain et al., 2013; Giedd, 2004). It has been shown that connections between network nodes change with development (Bressler & Menon, 2010; Bressler & Tognoli, 2006). Casey, Jones and Hare (2008) developed a neurobiological model of adolescent development which states that the influence of top-down control processes is weakened during adolescents, leading to suboptimal choice and risk behaviour. The authors concluded that this effect is partly due to protracted development of the prefrontal cortex. Therefore, stimulation in children and adolescents, especially targeting the DLPFC, will most probably be influenced by these factors.

An increasing number of studies have investigated the effects of tDCS in children and adolescents (Lee et al., 2019; Palm et al., 2016; Rivera-Urbina, Nitsche, Vicario, & Molero-Chamizo, 2017; Salehinejad et al., 2020). In most cases, these studies examine clinical samples. What is lacking, however, are systematic, methodological studies in children and adolescents to understand tDCS mechanisms of action and the influencing factors across this age group. Regarding tACS and tRNS, only a limited number of studies have been performed in children and adolescents. As such, little is known about mechanism of tACS and tRNS in this age group and age related differences in effects.

1.4.3 Individual Functional Performance Level

For tDCS it has been shown, that stimulation effects are dependent on the individual functional performance level of participants. Regarding higher cognitive functions, several tDCS studies proved a negative relationship between initial baseline performance and tDCS effects: the worse participants initially performed, the more they benefited from stimulation applied over the DLPFC (Habich et al., 2017; Rosen et al., 2016) or posterior-parietal cortex

(Gözenman & Berryhill, 2016). However, Jones & Berryhill (2012) found that while initial high performers showed an improvement in WM performance following stimulation, initial low performing participants were impaired in performance or were not affected by anodal tDCS over the parietal cortex. Conversely, Hsu et al. (2016) demonstrated an impairment for low performers while high performers were not affected by stimulation over the parietal cortex.

Although the reported results paint an ambiguous picture, a growing consensus suggests that individual performance level modulates the effects of stimulation. A possible explanation for a modulatory effect is given by the *excitation-inhibition balance model* (Krause, Márquez-Ruiz, & Cohen Kadosh, 2013; Okun & Lampl, 2008). Based on this model it can be assumed that there is an optimal level of prefrontal activation based on an excitation/inhibition (E/I) balance, measured by glutamate/GABA concentration (Clark, Coffman, Trumbo, & Gasparovic, 2011; Stagg et al., 2009). tES might be able to reinstate an optimal E/I balance. But regarding participants that are already at their optimum, the stimulation might lead to impairments. Talsma, Broekhuizen, Huisman, & Slagter (2018) tested the excitation-inhibition balance model by investigating the influence of anodal tDCS over the left DLPFC on WM performance. The focus of their study was whether tDCS interacts with the individual baseline cortical excitability level in form of glutatamte/GABA ratios. While the results confirmed inter-individual variability in tDCS effects, no dependency on baseline prefrontal cortical excitability was found. Although this study did not demonstrate a relationship, it seems reasonable to consider the individual functional performance level as a potential modulating factor of stimulation effects.

1.4.4 Functional State of the Brain

The effects of tES have further been shown to be influenced by cognitive activation during stimulation. A distinction between online and offline stimulation has been defined: online tES combines the stimulation with the performance of a concurrent task, while offline

tES describes the application of stimulation without a concurrent task. Based on this distinction, studies can focus on different aspects of online/offline stimulation.

First, it can be investigated whether the effects that occur during stimulation (online) differ from the effects that occur after stimulation (offline). Assumptions regarding short-term (resting membrane potential alteration) and long-term (modulation of synaptic plasticity) effects are derived. A study by Friehs & Frings (2019) compared stimulation effects on WM performance during tDCS and following tDCS (tDCS was applied without task performance) applied over the left DLPFC. They found improved WM performance for offline tDCS, but not for online tDCS. Here, the pronounced effects following stimulation might be based on the long-term effects of stimulation, inducing LTP-like plasticity. However, for studies that use stimulation durations of several minutes, it must be considered that a clear separation of online and offline effects is not possible. Even during stimulation, changes can be based on long-term effects if the stimulation has already been running for several minutes (Stagg & Nitsche, 2011).

Second, the effects of concurrent task performance during stimulation can be investigated regarding outcomes measured following stimulation. This type of studies investigates whether online (application with task) or offline tES (application without task) is more effective regarding long term tES effects, or how different kinds of tasks during stimulation affect the after-effects of stimulation. Antal et al. (2007) found a dependency of motor cortex excitability on activation during tDCS, using a cognitive task or motor exercise during stimulation (Antal et al., 2007). A review on DLPFC tDCS found stronger effects in neuropsychiatric patients following tDCS with task performance than following tDCS without task performance (Dedoncker et al., 2016). For tRNS stronger effects for combined task performance and stimulation were found than for isolated stimulation (Pirulli, Fertonani, & Miniussi, 2013).

The task-dependency of tES effects might be based on the neuromodulatory nature of tES, i.e. the influence of tES on endogenous neuronal activity. The *activity selectivity model*

assumes that tES affects the neurons in the target region that are active during task performance, leading to specific and potentially enhanced tES effects (Bikson & Rahman, 2013; Boroda, Sponheim, Fiecas, & Lim, 2020; Fertonani & Miniussi, 2017). In expanding the activity model, the *network activity-dependent model* assumes that due to the network-based nature of cognitive functions, tES effects do not only modulate active neurons in the target region but the whole network that is active during stimulation (Di Luft et al., 2014; Fertonani & Miniussi, 2017; Miniussi, Harris, & Ruzzoli, 2013). Supporting this model, Pisoni et al. (2018) found that anodal tDCS coupled with a verbal fluency task increased cortical excitability only in task-related brain networks. Besides, the difference in online and offline tES after-effects might also be explained by *metaplasticity,* i.e. the activity-dependent modulation of synaptic plasticity (Abraham & Bear, 1996; Müller-Dahlhaus & Ziemann, 2015). Specifically, *homeostatic metaplasticity* describes the activity-dependent downregulation, and *non-homeostatic metaplasticity* the activity-dependent upregulation of LTP and LTD (Müller-Dahlhaus & Ziemann, 2015). It is conceivable that due to homeostatic metaplasticity, increased activity by stimulation with concurrent task performance leads to attenuation of LPT and LTD like tES long-term effects (Müller-Dahlhaus & Ziemann, 2015). For motor cortex tDCS a stronger engagement during stimulation decreased after-effects of stimulation (Bortoletto, Pellicciari, Rodella, & Miniussi, 2015). On the other hand, following the idea of non-homeostatic plasticity, task performance during stimulation might serve as a priming leading to increased long term tES effects (Müller-Dahlhaus & Ziemann, 2015; Pirulli et al., 2013).

However, also a transfer of stimulation effects to novel tasks was found for tES with concurrent task performance (Gill, Shah-Basak, & Hamilton, 2015; Trumbo et al., 2016). At first glance, these findings might contradict the network activity-dependent model, which assumes a “winner-takes-all” principle (Fertonani & Miniussi, 2017; Maass, 2000), i.e. the task related network that is active during stimulation benefits the most. However, according to the idea of network hubs, based on graph theory, important brain hubs (connector hubs) exist in

anatomical neural networks, which are strongly connected within and between functional networks and thus play an important role for various functions (To, Ridder, Hart, & Vanneste, 2018; van den Heuvel & Sporns, 2013; see Figure 1-3). Increased functioning of such a brain hub through tES might be coupled with different functional networks depending on the (task) demands (Chadick & Gazzaley, 2011; Smith et al., 2012), leading to transfer effects of stimulation. Specifically, following the *flexible hub theory*, the FPN could serve as a connector hub, contributing to different functions through its role for cognitive control (Cole et al., 2013; Zanto & Gazzaley, 2013), which is why stimulation of the DLPFC might lead to transfer effects.

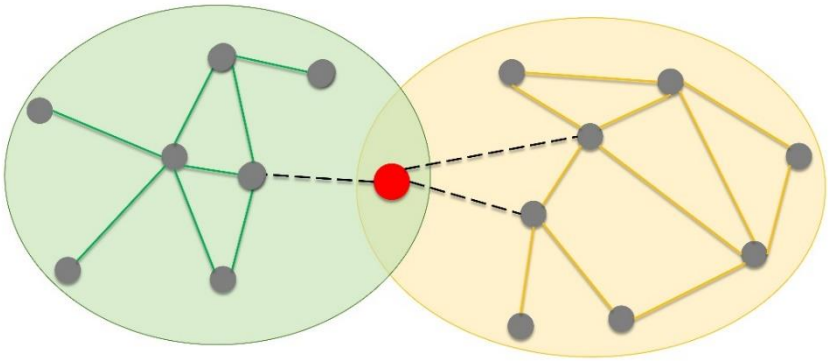

Figure 1-3. Graph representation of neural networks. Two networks (green and yellow) composed of edges (grey dots) and nodes (green and yellow lines). The connector hub (red) is connected to both networks, serving as a bridge between both networks. (Modified after Di Luft et al., 2014).

In summary, activation by a task during stimulation appears to modulate the effects of stimulation. However, the partly contradictory results show that underlying mechanisms are not sufficiently explored.

1.4.5 Physiological State of the Brain

Previous studies on tES suggest that the physiological state of the brain has an influence on stimulation effects (Krause & Cohen Kadosh, 2014; Li, Uehara, & Hanakawa, 2015). The physiological brain state is also influenced by placebo effects of stimulation, which produce changes in physiological and behavioural outcomes (Brim & Miller, 2013). To picture and control for these placebo effects in tES studies, sham stimulation protocols are used, which simulate verum stimulation (Palm et al., 2013). During sham stimulation, usually at the

beginning and end of the application period, the current is slowly ramped up and down to mimic skin sensations and give the subject the impression of verum stimulation. If effectively blinded, placebo effects should occur in the same way for verum and sham stimulation (Fonteneau et al., 2019; Turi, Mittner, Paulus, & Antal, 2017). Still, placebo effects might differ between individuals. While for some subjects changes in outcome occur after sham stimulation (responders to sham stimulation) that are due to placebo effects, other subjects remain unaffected (non-responders to sham stimulation). Kortuem et al. (2019) investigated the predictive power of this *response to sham stimulation* on effects of verum tACS and tRNS. Their investigation was based on the idea that the physiological changes produced by placebo effects might interact with the actual neurophysiological effects of verum stimulation. Based on changes in MEPs following sham stimulation, participants were classified as responder or non-responder. This susceptibility to placebo effects significantly predicted verum stimulation effects: non-responders to sham stimulation showed effects of verum stimulation, while responders did not. The authors concluded that this interaction might be due to homeostatic metaplasticity. For responders, effects of verum stimulation might be counterbalanced to avoid an overactivation by the combined effects of verum stimulation and placebo effects. Although this approach may help to elucidate the inter-individual variability in tES effects, it has not been investigated by other studies.

1.5 Safety and Tolerability of tES

As stimulation is a comparatively new neuroscientific method, questions regarding its safety and tolerability are of central importance. The available data indicate that tES can be used safely within certain limits (Antal et al., 2017). These limits relate, for example, to the duration and strength of stimulation. tES mostly leads to mild side effects, including itching or burning skin sensations and, in case of lower frequency tACS, phosphenes and tingling sensations. In order to continue to substantiate knowledge about the safety and tolerability of

stimulation, the use of standardized stimulation tolerability questionnaires is recommended for tES studies (Antal et al., 2017; Poreisz, Boros, Antal, & Paulus, 2007). This is especially important regarding new approaches of tES, such as multichannel montages, where investigations into both the safety and tolerability are necessary. Also, as only a limited number of studies investigates the effects of tES in children and adolescents, it is particularly difficult to make statements about safety and tolerability here (Davis, 2014). At the same time, it is particularly important in this vulnerable age group to ensure that subjects and patients are not exposed to unnecessary risks. Studies conducted in children and adolescents, but also in adults, should therefore always include the investigation of safety aspects.

1.6 Aim of the Thesis

tDCS, tACS and tRNS not only have the potential for investigating basic brain functioning, but also as a treatment method for neuropsychiatric disorders or neurodevelopmental diseases. However, it is also evident that the mechanisms of action of tES are complex and effects of stimulation are conditioned by a variety of influencing factors. To achieve an effective use of tES, systematic methodological studies on the influencing factors of stimulation have to be conducted. This thesis therefore aimed to investigate methodological and physiological influencing factors of tDCS, tACS and tRNS that remain insufficiently examined in the current literature. This aim is pursued by means of the following three studies.

Study I investigated the influence of different montages and individual functional performance level on anodal tDCS over the left DLPFC in healthy adults. The study focused on the comparison of an optimised multichannel montage and a classical bipolar montage. As outcomes, task performance and neurophysiological oscillatory activity, measured by EEG, were investigated. It was expected that multichannel tDCS would lead to pronounced effects on behavioural and neurophysiological outcomes due to its increased focality compared to the

bipolar montage. Further, it was predicted that stimulation effects would be modulated by the individual performance level, measured by task performance prior to stimulation.

Experimental and modelling studies show that tES works differently in children and adolescents than in adults. Yet, systematic methodological studies on tES effects in paediatric populations are lacking. Therefore, the goal of study II was to examine the effects of multichannel anodal tDCS targeting the left DLPFC in healthy children and adolescents aged 10 – 18 years, in combination with the investigation of concurrent task performance during stimulation, and individual anatomy of participants as potential influencing factors. The applied montage was identical to the multichannel montage used in study I. As outcomes, behavioural task performance and neurophysiological activity, measured by EEG correlates, were investigated. Stimulation effects were expected to be influenced by concurrent task performance during stimulation. Further, we expected higher E-field values in the target region left DLPFC to be related to stronger stimulation effects.

For tACS and tRNS methods of action are much less investigated than for tDCS. Effects of tACS and tRNS have not been directly compared in children, adolescents and adults. Study III aimed at investigating methodological factors of tACS and tRNS in healthy children and adolescents, comparing them to an adult sample. tACS with a frequency of 140 Hz and 20 Hz (Beta range) and tRNS were applied over the primary motor cortex. Stimulation effects were measured using MEPs. As previous knowledge about the effects of tACS and tRNS in children and adolescents is rare, stimulation effects were analysed in an exploratory way. Based on results by Kortuem et al. (2019), response to sham was investigated as a marker for the individual physiological brain state. It was predicted that non-responder to sham stimulation would show stronger verum tES effects than responders to sham stimulation.

In all studies, an additional focus was placed on the safety and tolerability of the stimulation, as data on these aspects remains limited for both multichannel tDCS and tES in children and adolescences.

2 Study I

Individual Baseline Performance and Electrode Montage Impact on the Effects of Anodal tDCS Over the Left Dorsolateral Prefrontal Cortex

Splittgerber, M., Salvador, R., Brauer, H., Breitling-Ziegler, C., Prehn-Kristensen, A., Krauel, K., Nowak, R., Ruffini, G., Moliadze, V., & Siniatchkin, M. (2020). Individual Baseline Performance and Electrode Montage Impact on the Effects of Anodal tDCS Over the Left Dorsolateral Prefrontal Cortex. *Frontiers in human neuroscience*, *14*, 974. https://doi.org/10.3389/fnhum.2020.00349.

2.1 Abstract

Anodal transcranial direct current stimulation (tDCS), applied over the left dorsolateral prefrontal cortex (lDLPFC), can produce significant effects on working memory (WM) performance and associated neurophysiological activity. However, results from previous studies are inconsistent and occasionally contradictory. This inconsistency may be attributed to methodological and individual differences during experiments. This study therefore investigated two hypotheses: 1) A multi-channel optimized montage was expected to be more effective than a classical bipolar montage, because of increased focality. 2) The subjects were expected to benefit differently from the stimulation depending on their initial task performance.

In a sham-controlled cross-over study, 24 healthy participants received bipolar, multi-channel and sham stimulation for 20 minutes in randomized order, targeting the lDLPFC while performing a 2-back WM task. After stimulation, EEG was recorded at rest and during 2-back and a non-target task (Continuous Performance Task; CPT) performance.

Bipolar and multi-channel stimulation were both well tolerated and effectively blinded. We found no effect of stimulation on behavioral performance or neuronal oscillations comparing the classical bipolar or the multi-channel montage with sham stimulation. We did, however, find an interaction between stimulation and initial task performance. For multi-channel stimulation initially low performing participants tended to improve their WM performance while initially high performing participants tended to worsen their performance compared to sham stimulation. Both tDCS montages induced changes in neural oscillatory power which correlated with baseline performance. The worse the participant's initial WM performance was, the more task related theta power was induced by multi-channel and bipolar stimulation. The same effect was observed for alpha power in the non-target task following multi-channel stimulation. Notably, we were not able to show a superiority of multi-channel stimulation compared to bipolar stimulation. Still, comparing both montages with sham stimulation, multi-channel stimulation led to stronger effects than bipolar stimulation.

The current study highlights the importance of investigating different parameter with potential influence on tDCS effects in combination. Our results demonstrate how individual differences in cognitive performance and electrode montages influence effects of tDCS on neuropsychological performance. These findings support the idea of an individualized and optimized stimulation setting, potentially leading to increased tDCS effects.

2.2 Introduction

Working memory (WM) is a cognitive function that underlies a multitude of our daily activities and is central to our thoughts and actions. It describes the ability to maintain information for a brief time interval in an active and easily accessible state (Baddeley, 2010, 2012; Baddeley & Della Sala, 1996; Chai, Abd Hamid, & Abdullah, 2018; Kane & Engle, 2002). A variety of mental disorders, such as schizophrenia (Galderisi et al., 2009) or Attention-Deficit/Hyperactivity Disorder (ADHD; Brennan & Arnsten, 2008) are associated with WM impairments. Improvement of WM may increase adaptability of affected individuals and their quality of life. However, most WM training have been characterized by a limited generalization and low enduring effects (Redick et al., 2013; Soveri, Antfolk, Karlsson, Salo, & Laine, 2017).

Transcranial direct current stimulation (tDCS) appears to provide a method to enhance the effectiveness of WM trainings. tDCS is a non-invasive brain stimulation technique that induces changes in cortical excitability through the modulation of the membrane potential in cortical neurons (Nitsche & Paulus, 2000) that can last beyond the duration of the stimulation (Hoy et al., 2013; Nitsche & Paulus, 2001; Paulus, Nitsche, & Antal, 2016). Studies using electroencephalography (EEG) demonstrated that tDCS can alter brain activity in different target areas and related networks (Bergmann, Karabanov, Hartwigsen, Thielscher, & Siebner, 2016; Jones, Peterson, Blacker, & Berryhill, 2017; Miller, Berger, & Sauseng, 2015; Wörsching et al., 2016; Zaehle, Sandmann, Thorne, Jäncke, & Herrmann, 2011). The left dorsolateral prefrontal cortex (lDLPFC) is a brain region strongly associated with WM processes (D'Esposito et al., 1995; Mansouri, Tanaka, & Buckley, 2009). A variety of studies have illustrated improved WM performance during or after tDCS over the lDLPFC (Fregni et al., 2005; Hoy et al., 2013; for review see Brunoni & Vanderhasselt, 2014; Dedoncker, Brunoni, Baeken, & Vanderhasselt, 2016; Hill, Fitzgerald, & Hoy, 2016). However, various studies have failed to show improved WM performance (Brunoni & Vanderhasselt, 2014; Dumont, El Mouderrib, & Théoret, 2018; Hill et al., 2016; Röhner et al., 2018) or cortical reactivity

(Boonstra, Nikolin, Meisener, Martin, & Loo, 2016; Gordon et al., 2018; Hill, Rogasch, Fitzgerald, & Hoy, 2018) caused by lDLPFC stimulation. Based on these results, it seems necessary to identify and investigate factors that have an influence on tDCS induced effects on WM.

One potential factor influencing tDCS effects is the electrode montage which affects the current flow or, equivalently, electric field (E-field) distribution. Most studies targeting the lDLPFC use a bipolar montage with the anode placed over F3 and the cathode placed over the supraorbital region, corresponding to the international 10-20 system (Herwig, Satrapi, & Schönfeldt-Lecuona, 2003). This montage leads to a rather diffuse E-field distribution and therefore poor spatial targeting, according to computation modelling studies (Laakso et al., 2016; Miranda, Mekonnen, Salvador, & Ruffini, 2013; Saturnino, Antunes, & Thielscher, 2015). One promising way of achieving more focal stimulation is through multi-channel optimized montages, using several small electrodes distributed on the head. Recently, optimized multi-channel tDCS over the motor cortex has been shown to increase motor cortex excitability, with significantly greater effects than bipolar tDCS (Fischer et al., 2017). Additionally, several studies recently investigated a ring-shaped 4×1 High-Definition tDCS (HD-tDCS) targeting the lDLPFC, showing increased effects on neurophysiological activity and WM performance (Hill et al., 2018, 2017, 2019; Nikolin, Loo, Bai, Dokos, & Martin, 2015). However, there is no study investigating multi-channel tDCS over the lDLPFC using an optimized distributed electrode montage rather than a ring-shaped HD-tDCS montage.

Another factor that can potentially explain differences in tDCS effects on WM is the inter-individual variability in participant baseline WM performance. Studies investigating the effect of tDCS on WM performance and related neural activity report inconsistent effects on initially high and low performers (Gözenman & Berryhill, 2016; Hsu, Juan, & Tseng, 2016; Jones & Berryhill, 2012; Tseng et al., 2012). Furthermore, different studies report a negative linear relationship between initial baseline performance and tDCS effects for different

modalities. The worse the subjects initially perform, the more likely they are to benefit from the stimulation (Habich et al., 2017; Rosen et al., 2016). Despite their contradictory results, these studies underline the potential predictive power of inter-individual WM capacity on tDCS outcome.

Based on these findings, in our study we combined both factors (electrode montages and individual baseline performance) as possible predictors for effects on behavioral and neurophysiological outcomes induced by tDCS over the lDLPFC. We included a 2-back WM task as target task and a Continuous Performance Task (CPT) as non-target task to test for non-specific tDCS effects and to differentiate stimulation effects more clearly. The CPT investigates response-inhibition and attention (Rosvold, Mirsky, Sarason, Bransome, & Beck, 1956), functions that are also connected to the DLPFC (Blasi et al., 2006; Oldrati, Patricelli, Colombo, & Antonietti, 2016). We expected 1) an improvement in WM performance during and after bipolar and multi-channel tDCS compared to sham, with pronounced effects for multi-channel stimulation compared to bipolar stimulation. 2) We expect that these modifications in WM performance should be reflected in changes in neural oscillatory power during task processing. Regarding the predictive role of baseline performance on tDCS outcome we expected that individual baseline performance predicts 3) changes in behavioral performance and 4) changes of oscillatory power induced by tDCS.

2.3 Methods and Materials

2.3.1 Participants

The study was approved by the Ethics Committee of the Faculty of Medicine Kiel University Kiel. All participants gave their written and informed consent prior to the start of the experiment. To calculate sample size, we used g*Power (Faul, Erdfelder, Lang, & Buchner, 2007) with the following settings: effect size f = 0.25 following Brunoni and Vanderhasselt (2014) and Dedoncker et al. (2016), α level = 0.05, power = 0.95, correlation among repeated

measures = 0.7. The minimum sample size was found to be 22. To fully counterbalance the order of stimulation conditions across participants we included 24 subjects (mean age 24.8, SD = 2.7 years; 13 females). Exclusion criteria were relevant psychological problems, assessed by the SCL-90-R (Franke, 2002), ADHD related symptoms assessed by the ADHS-E (Schmidt & Petermann, 2009), depression related symptoms assessed by the BDI-II (Hautzinger, Keller, & & Kühner, 2006), IQ score below 85, evaluated by the CFT 20-R (Weiß, 2006), history of neurological or psychiatric diseases, use of medication, pregnancy or metallic head implants (see Table 2-1). All subjects were naive to transcranial stimulation. Furthermore, besides the general information given in the consent, all subjects were naïve with regard to the aim of the study. Subjects received money or research credits for their participation.

Table 2-1

Subjects characteristics

	Mean ± Standard deviation	Exclusion criteria
Sex	13 female, 11 male	
Age	24.83 years ± 2.72	18 < age > 30
BDI II Total score	4.21 ± 3.78	BDI > 13
ADHS-E T-Value main scale	47.92 ± 8.67	T > 60
SCL-90-R T-value GSI	47.5 ± 9.23	T > 65
CFT-20-R	113.13 ± 12.72	IQ < 85

2.3.2 Experimental Design

We used a randomized, sham-controlled, single-blind, crossover design. All participants attended four sessions: one screening and baseline measurement (T1) followed by three stimulation sessions (T2-T4; Figure 2-1A). The order of stimulation sessions (sham, multi-channel and bipolar montage) was randomized and balanced across participants. The period between sessions for a single subject was minimum 7 and maximum 11 days. In 90% of the cases a time interval of 7 days was kept. Each stimulation session started with 20 minutes stimulation. After 2.5 minutes of stimulation the 2-back task started and ended 2.5 minutes before the end of stimulation to prevent distraction induced by current ramping and to allow related side effects to wear off. After the stimulation, participants filled in a questionnaire on

safety, tolerability and blinding of stimulation. Subsequently, a 64 channel EEG was then set up within 45 minutes and EEG during rest with eyes closed and opened (2x2 minutes) and during 2-back and CPT performance was recorded.

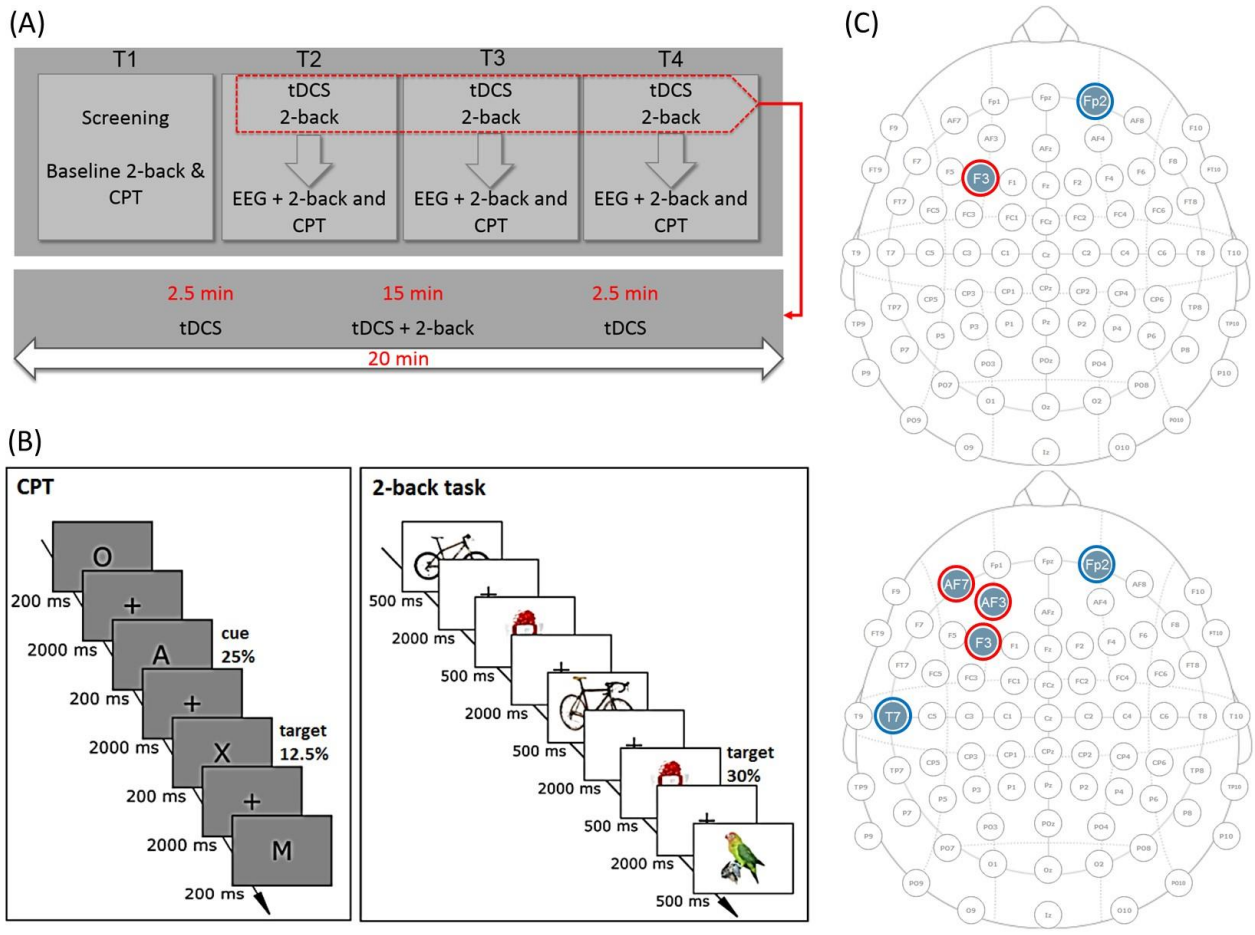

Figure 2-1. Experimental Design. (A) Time-course of the experiment. Each participant attended four sessions. At screening and baseline sessions (T1) we assessed inclusion and exclusion criteria and task baseline performance. At stimulation sessions (T2 – T4) participants were stimulated for 20 minutes with consecutive 2-back task performance. After stimulation EEG at rest and during task performance was recorded. (B) Tasks. In the 2-back task participant had to decide whether a currently presented picture was identical to the picture shown 2 steps back. In the CPT participants had to press the space bar every time the letter A was followed by the target letter "X" and withhold their response for all other letters. (C) Electrode montages for bipolar stimulation (top) and multi-channel stimulation (bottom). Red circles represent anodal, blue circles reference electrodes.

2.3.3 Tasks and Stimuli

Both tasks were programmed using the software Presentation® (Version 20.0, Neurobehavioral Systems, Inc., Berkeley, CA, www.neurobs.com). Prior to the start of each

task participants completed a training session and were instructed to react as fast and accurately as possible. The investigator made sure the tasks were fully understood.

In the 2-back task participants had to decide whether a currently presented picture was identical to the picture shown two steps back (Figure 2-1B). The 2-back task lasted approximately 15 minutes and consisted of 360 trials with 30% target trials. Participants had to press the right mouse button if the trial was a non-target or the left mouse button if the trial was a target trial. Each trial consisted of 500 ms picture presentation followed by a fixation cross presentation jittered between 1550 – 2000 ms duration, resulting in a trial duration of 2050 – 2500 ms. We used seven sets of 16 different pictures taken form the Mnemonic Similarity Task (MST), Stark lab (http://faculty.sites.uci.edu/starklab/mnemonic-similarity-task-mst), as stimuli, one for the baseline and screening session, two for each stimulation session.

In the CPT various upper-case letters were presented (Figure 2-1B). Participants had to press the space bar every time a target letter was presented (letter "X") and withhold their response for all other letters. The target was always preceded by a cue stimulus (letter "A"), whereby the cue could be followed by a target or non-target letter. The CPT lasted approximately 18 minutes and consisted of 480 trials with 25 % cue and 12.5% target letters. Each stimulus was presented for 200 ms followed by a fixation cross for 2000 ms, leading to a trial duration of 2200 ms.

2.3.4 Transcranial Direct Current Stimulation

Participants were stimulated three times with either bipolar, multi-channel or sham stimulation over the lDLPFC for 20 minutes using the Starstim 32 stimulator (Neuroelectrics, Barcelona, Spain). Electrodes were positioned using a head cap following the 10-10 system (Figure 2-1C). For bipolar stimulation 1 mA tDCS was delivered by a pair of circular saline-soaked surface sponge electrodes (25 cm^2), with the anode positioned over F3 and cathode over Fp2. For multi-channel tDCS we used five 3.14 cm^2 circular PiStim electrodes, positioned at AF3 (897 µA), AF7 (284 µA), F3 (819 µA), Fp2 (-1000 µA) and T7 (-1000 µA), filled with

EEG electrode gel. In both conditions current was ramped up for 30 seconds at the beginning and down during 30 seconds at the end of stimulation. In the sham condition, half of the subjects received a multi-channel, the other half a bipolar montage. Current was ramped up and immediately down for 60 seconds at the beginning and end of the stimulation.

2.3.4.1 Computational Modeling of Electric Fields

The multi-channel optimized montage was obtained using the Stimweaver algorithm (Ruffini, Fox, Ripolles, Miranda, & Pascual-Leone, 2014). This realistic head modeling-based algorithm works under the assumption that the normal component of the E-field (E_n) induced by tDCS couples with long pyramidal cells in the cortex, thus leading to cortical excitability changes. The optimal multi-channel montage is determined by minimizing the least squares difference between the weighted E-field (E_n) induced by the montage and a weighted target map of E_n (E_n^{Target}). In this optimization the lDLPFC mask was defined as BA 46 (see Figure 2-2B) and the weight and E_n in this area were set to 10 and +0.25 V/m, respectively. The rest of the cortical areas were assigned to a target E_n of 0 V/m with a low weight (1). The optimization imposed constraints to the maximum current per electrode ($I_{Elec}^{Max} = 1.0$ mA) and total injected current (the sum of all the positive currents $I_{Total}^{Max} = 2.0$ mA).

The optimization was performed in a standard finite element head model of the Colin27 template (http://www.bic.mni.mcgill.ca/ServicesAtlases/Colin27) following Miranda et al., (2013) and Ruffini et al. (2014) (see Figure 2-2A-B). The head model, shown in Figure 2-2A-B, contains representations of the scalp, skull, cerebrospinal fluid (including the ventricles), gray-matter and white-matter. For the optimization, models of PiStim electrodes were placed in the scalp in a subset of positions of the international 10-10 EEG system with a radius of 1 cm and a height of 3 mm (Figure 2-2B), representing the conductive gel beneath them (conductivity of 4.0 S/m). All tissues were represented as homogeneous and isotropic materials with electrical conductivities appropriate to the DC-low frequency range: 0.33 S/m, 0.008 S/m,

1.79 S/m, 0.4 S/m and 0.15 S/m for the tissues mentioned before, respectively (Miranda et al., 2013).

The E-field distribution induced by the bipolar distribution was calculated using the same head model. The electrodes were modeled according to Neuroelectrics' SpongeStim model: conductive rubber on top of saline soaked sponge. All E-field calculations were performed in Comsol (v5.3a, www.comsol.com) using its AC/DC package.

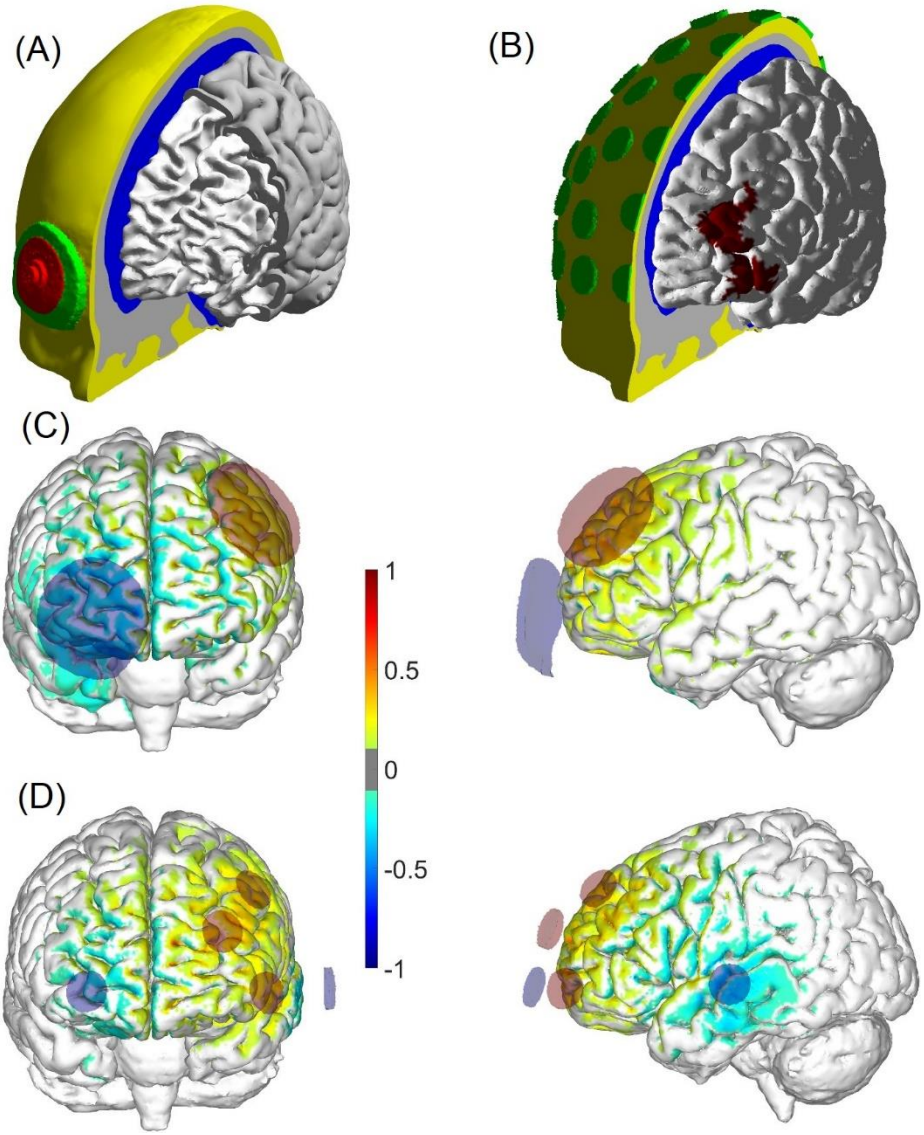

Figure 2-2. Geometry of the head model used in the optimization and E-field modeling pipeline. (A) Global view of the different tissues in the head model, as well as the SpongeStim electrode model (saline soaked sponge in green and rubber connector in red). The thickness and radius of the different components of the electrode were based on measurements of the actual electrode. (B) Global view of the head model used in the optimization pipeline, including representations of the conductive gel underneath the PiStim electrodes. The mask of the lDLPFC used in the optimization (BA 46) is also shown in the cortical surface of the head model. (C/D) Distribution of the normal component of the E-field in the bipolar/multi-channel montage (positive/negative values indicate that the E_n-field is directed into/out of the cortical surface). The field was divided by its maximum value in each montage (0.52 V/m in the bipolar montage and 0.70 V/m in the multi-channel).

The distribution of E_n in the cortical surface in the bipolar and multi-channel optimized montages are shown in Figure 2-2C and D. The multi-channel montage distributes the return electrode between the right frontal areas and left temporal cortex, leading to stronger negative

E_n values in these regions. In the bipolar montage this happens in the right frontal area, under the cathode located at Fp2. In terms of fit to the target map, the optimized montage achieved better results, expressed in the cross correlation between the weighted E_n distribution and weighted E_n^{Target} map: 0.5 and 0.3 for the multi-channel and bipolar montages, respectively. The least squares error (ERNI, in units of mV^2/mm^2, Ruffini et al., 2014) of the optimized montage is also higher (in absolute value; -6189 mV^2/mm^2) than that of the bipolar montage (-1285 mV^2/mm^2). In terms of average E_n over the lDLPFC surface area, the multi-channel montage achieved a higher value (0.07 V/m) than the bipolar montage (0.03 V/m).

2.3.5 Questionnaire on Tolerability and Participant Blinding of tDCS

Side effects and blinding effectiveness were assessed using a standardized safety questionnaire (Antal et al., 2017; Poreisz, Boros, Antal, & Paulus, 2007). Participants rated the six most common tDCS side effects on a 4-point scale, from 0 = not experienced to 4 = strongly experienced. After each stimulation, the participants gave their opinion as to whether they had received verum or sham stimulation.

2.3.6 EEG Recording and Preprocessing

We used a 64-channel electrode cap placed over the scalp according to the locations of the international 10-10 standard system with the reference electrode positioned at FCz and at the ground electrode at AFz (EasyCap, Herrsching, Germany). Electrode impedances were always kept below 10 kΩ. EEG was recorded using the BrainVision Recorder Software (Brain Products GmbH, Gilching, Germany). The EEG signal was recorded with a rate of 1000 S/s and low-pass filtered at 250 Hz.

For EEG data preprocessing we used BrainVisionAnalyzer 2 (Brain Products GmbH, Gilching, Germany). The data was down sampled at 500 S/s, re-referenced to the common average and filtered (30 Hz low-pass, 0.05 Hz high-pass filter). Semiautomatic raw data inspection and an independent components analysis were applied to remove artefacts. Task

related EEG data was segmented from -1000 to 1500 ms post stimulus onset, continuous resting state EEG data was divided in 2000 ms segments. Segmented EEG data was then exported and further analyzed using the Fieldtrip toolbox (http://fieldtrip.fcdonders.nl/). We performed a time-frequency analysis with a Hanning taper for frequencies from 1 to 30 Hz in steps of 2 Hz in a time window from -500 to 1000 ms relative to stimulus onset for both tasks, with baseline correction from -500 to 0 ms. Resting state EEG was fast Fourier transformed with a moving Hanning window in a frequency range from 1 to 30 Hz in steps of 2 Hz and averaged in every subject.

2.3.7 Statistical Analyses

2.3.7.1 Behavioral Data

Statistical analyses on task accuracy and reaction times (RT) were conducted using the computing environment R (version 3.5.1, R Core Team (2016) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/). For the 2-back task and CPT accuracy was defined as proportion of correct responses. Due to high ceiling effects in accuracy in the CPT (baseline accuracy: Mean = 99.62%, SD = 0.6 %) we restricted our analyses for the CPT to RT only. Behavioral measurements were analyzed using linear mixed effects models (LME). We assessed normality of behavioral data using Shapiro-Wilk test and visual inspection of the data (histograms and Q-Q plots). In all models, we included the maximum number of random effects that allowed the model to converge. For 2-back accuracy and RT our LME included the fixed factors *baseline* (performance at T1), *stimulation* (sham, bipolar and multi-channel stimulation), *time* (during and after stimulation) and all corresponding interactions. Random slopes for *stimulation* and a random intercept were entered as random effects. Because participants completed the CPT only after stimulation and not during stimulation, for CPT RT we fitted a LME including the fixed effects *stimulation* (sham, bipolar, multi-channel) and *baseline* and a random intercept. Degrees of freedom were approximated using the Kenward-

Rogers method, analogous to repeated measures ANOVAs (Kenward & Roger, 1997). In case of significant F-values post-hoc tests were performed using the Tukey method.

2.3.7.2 Neurophysiological Data

All analyses were performed using significance probabilities estimations based on a Monte-Carlo-Permutation tests with a cluster based approach using the Fieldtrip toolbox. This non-parametric approach solves the problem of multiple comparisons by cluster correction and avoids assumptions on normally distributed data.

Stimulation effects on task related and resting state neurophysiological outcomes were analyzed by one-way repeated measurement ANOVAs with the within subjects factor stimulation (sham, bipolar, multi-channel). For task-related time-frequency data and resting state averaged power spectra we computed separate ANOVAs for each frequency band (delta: 1–4, theta: 4–8 Hz, alpha: 8–12 Hz and beta: 12–30 Hz). In case of significant F-values we conducted paired t-tests. We decided to use an ANOVA approach for the analysis of neurophysiological data, because a combination of mixed model and cluster-based analysis is not possible using the Fieldtrip toolbox. Although a unified mixed model analysis would have been preferable in terms of comparability of behavioral and neurophysiological results, we wanted to exploit the advantages of a cluster-based approach for the analysis of physiological data and decided to apply this approach.

Additionally, the interaction of individual baseline-performance and tDCS effects on oscillatory power was examined. Stimulation induced changes in oscillatory power were computed by subtracting the sham condition from the multi-channel and bipolar conditions. This was done for task related time-frequency representations (TFRs) and resting state oscillatory power. Pearson correlations between these neurophysiological differences and behavioral baseline-performance (2-back accuracy, 2-back RT and CPT RT) were computed.

2.4 Results

2.4.1 Tolerability and Blinding of tDCS

Participants were unable to guess better than chance whether they had received active or sham stimulation for all stimulation conditions (sham: $\chi^2(1) = 0$, $p = 1.0$; bipolar: $\chi^2(1) = 0.75$, $p = 0.38$; multi-channel: $\chi^2(1) = 3.0$, $p = 0.08$). Neither incidence, nor intensity of all side effects differed significantly between stimulation conditions (see Supplementary Table 2-3).

2.4.2 tDCS Effects on 2-back and CPT Performance

Mean accuracy and RT scores can be seen in Table 2-2. Our analyses of 2-back accuracy showed significant main effects of *baseline* (F (1, 23.8) = 34.83, $p < 0.001$), *stimulation* (F (1, 23.8) = 6.39, $p = 0.005$) and *time* (F (1, 70.9) = 9.59, $p = 0.002$). Furthermore, we found a significant interaction of *baseline* × *stimulation* (F (1, 23.9) = 5.86, $p = 0.008$) and a significant interaction of *baseline* × *time* (F (1, 70.9) = 9.64, $p = 0.002$), but no significant interaction of *stimulation* × *time* (F (1, 70.9) = 0.86, $p = 0.423$) or *baseline* × *stimulation* × *time* (F (1, 70.9) = 0.91, $p = 0.411$).

Table 2-2

Mean (Standard deviation) for 2-back and CPT accuracy (%) and reaction times (ms) during and after stimulation

	Time point	Stimulation condition		
		Sham	multi-channel	bipolar
2-back accuracy	During	90.68 (5.66)	91.45 (3.82)	91.74 (5.15)
	After	90.67 (6.54)	91.75 (4.89)	91.58 (5.97)
2-back reaction time	During	509.42 (177.05)	540.61 (185.89)	547.93 (193.46)
	After	496.52 (174.71)	521.69 (201.97)	500.59 (164.33)
CPT accuracy	After	99.63 (0.41)	99.71 (0.36)	99.75 (0.29)
CPT reaction time	After	357.01 (61.34)	362.01 (68.11)	356.12 (67.49)

Post-hoc tests investigating the *baseline* × *time* interaction revealed a significant lower accuracy slope during stimulation compared to after stimulation (t (77.6) = -2.97, $p = 0.004$). Post-hoc tests based on the significant *stimulation* main effect revealed no significant effect for multi-channel or bipolar stimulation compared to sham stimulation (all $p > 0.05$, see Figure

2-3A). Post-hoc tests following the *baseline* × *stimulation* interaction revealed a significant higher accuracy slope for sham stimulation compared to multi-channel stimulation (t (25.9) = 3.11, p = 0.012; Figure 2-3B). We found no significant difference in accuracy slopes for sham stimulation compared to bipolar stimulation (t (26.2) = 1.95, p = 0.114). Importantly, the significant interaction effect of *baseline* × *stimulation* could not be explained by regression to the mean (RTM). To exclude RTM as possible explanation for this interaction, we tested whether the accuracy variances of the multi-channel and baseline condition were different (Guilford & Fruchter, 1973; Tu & Gilthorpe, 2007). This test follows the idea, that if initially low performing participants tend to improve under multi-channel stimulation and initially high performing participants do not improve or even decrease in performance, as indicated by Figure 2-3B, variance for accuracy during multi-channel stimulation should be smaller than variance for accuracy at baseline measurement. This was found to be true, as multi-channel accuracy had a significant lower variance than baseline accuracy (t (22) = 3.49, $p < 0.01$). Also, multi-channel accuracy variance was significantly lower than sham accuracy variance (t (22) = 2.59, $p < 0.02$; see Figure 2-3A). Still, this result could reflect a training effect, with a more homogenous accuracy through repetition of the 2-back task. In this case, variances for bipolar and sham stimulation should also be significantly decreased compared to baseline variance. This assumption was not confirmed, as accuracy variances were not decreased under bipolar (t (22) = 1.69, $p > 0.05$) or sham (t (22) = 1.34, $p > 0.05$) stimulation compared to baseline.

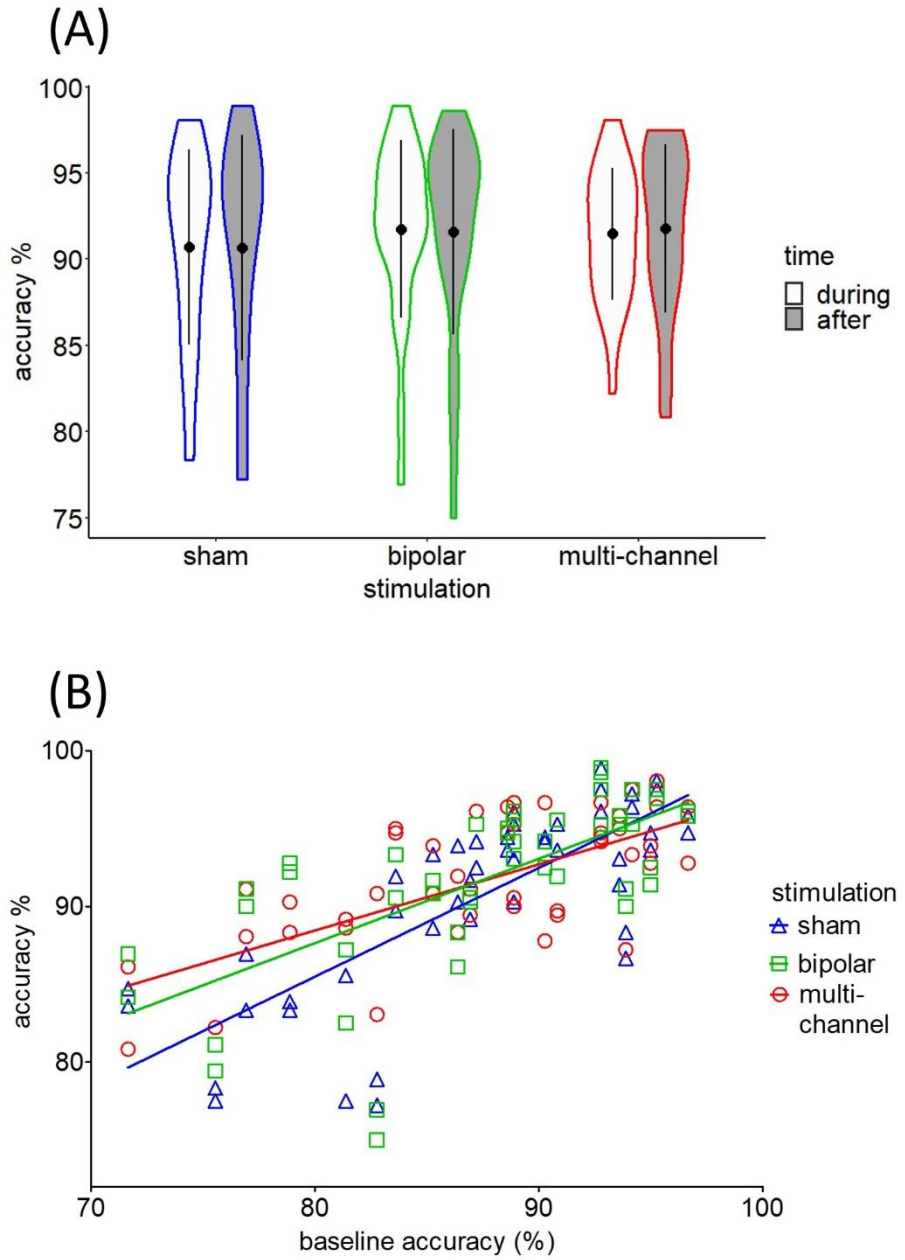

Figure 2-3. 2-back behavioral results. (A) Violin plot for mean (± SD) accuracy scores for sham, bipolar and multi-channel stimulation during and after stimulation. (B) Regression lines for multi-channel, bipolar and sham stimulation on baseline accuracy scores.

Our mixed models performed for 2-back RT and CPT RT revealed a significant main effect for *baseline* (2-back RT: F (1, 24.3) = 36.04, $p < 0.001$; CPT RT: F (1, 24) = 143.49, $p < 0.001$), all other main effects and interactions were not significant (all $p > 0.05$). Therefore, no subgroup analyses were performed.

2.4.3 tDCS Effects on Neuronal Oscillations

One-way repeated measurement ANOVAs on event-related oscillations revealed no main effect of *stimulation* for all frequency bands in the 2-back task. Correlation analyses showed no significant correlations between 2-back RT and oscillations (all $p > .05$). However, we found significant negative correlations between behavioral 2-back baseline accuracy and stimulation induced increase in power in the theta band for multi-channel ($p = 0.009$) and bipolar ($p = 0.008$) stimulation compared to sham. The worse the participant initially performed, the more theta power was detected after multi-channel and bipolar stimulation compared to sham stimulation (Figure 2-4A and B). For multi-channel stimulation this effect was seen in frontal and occipital areas from 200 to 1000 ms post stimulus (Figure 2-5A). For bipolar stimulation we observed this effect in frontal and occipital areas from 500 to 900 ms post stimulus (Figure 2-5B).

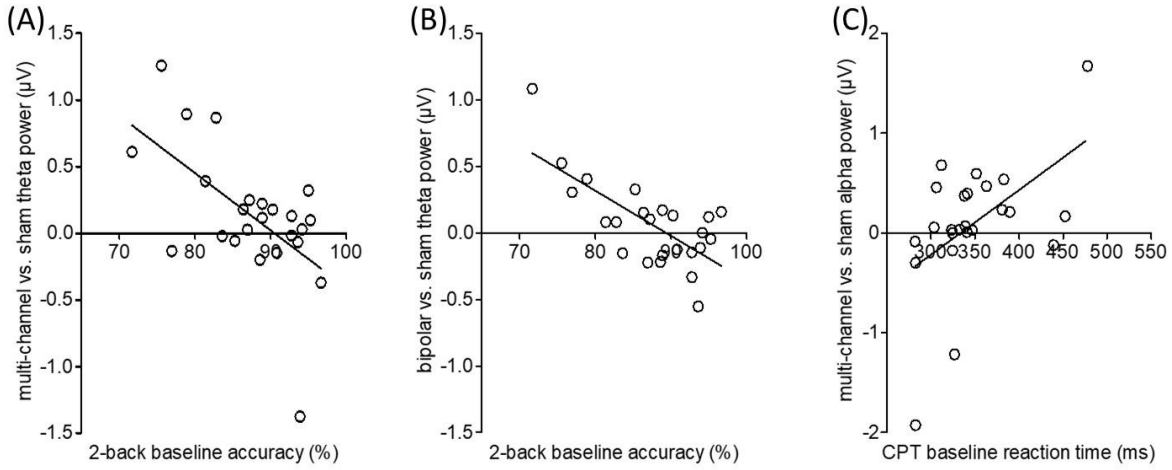

Figure 2-4. Stimulation induced changes in theta and alpha power averaged over time depending on behavioral baseline performance. (A) Correlation of multi-channel vs. sham theta power with individual 2-back baseline accuracy. The y-axis depicts the difference of mean theta power for multi-channel - sham stimulation. The regression line shows a decrease of stimulation induced theta power with increasing baseline accuracy. (B) Correlation of bipolar vs. sham theta power with individual 2-back baseline accuracy. The y-axis depicts the difference of mean theta power for bipolar - sham stimulation. The regression line shows a decrease of stimulation induced theta power with increasing baseline accuracy (C) Correlation of multi-channel vs. sham alpha power with individual CPT baseline RT. The y-axis depicts the difference of mean alpha power for multi-channel - sham stimulation. The regression line shows an increase of stimulation induced alpha power with increasing baseline accuracy.

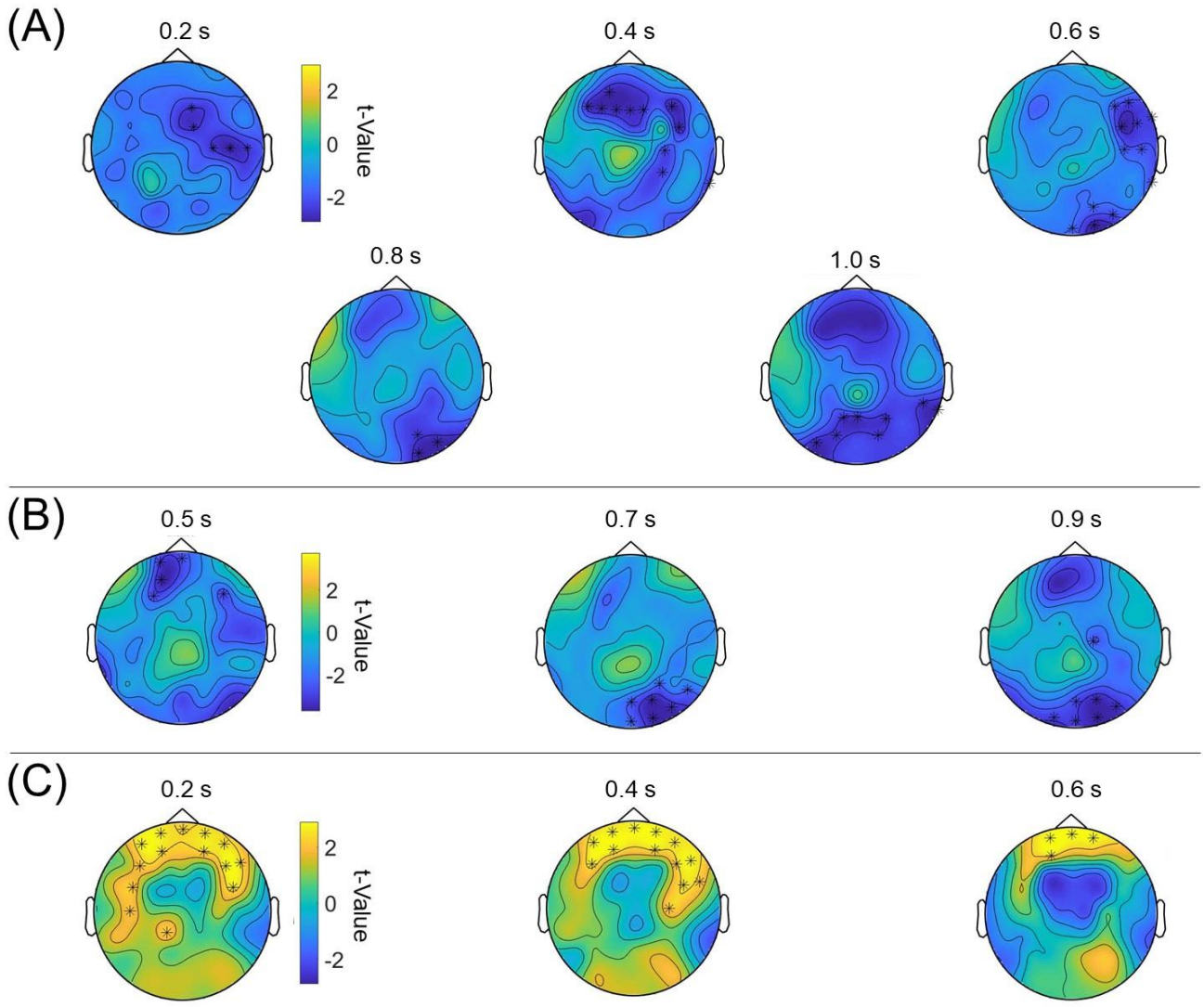

Figure 2-5. Topography of the significant correlations for theta and alpha power following multi-channel and bipolar stimulation compared to sham stimulation. (A) Correlation of multi-channel vs. sham theta power with individual 2-back baseline accuracy from 200 to 1000 ms in steps of 200 ms. Significant channels: F3, F4, C4, P4, O2, F8, T8, P8, Fz, Pz, FC2, FC6, FT10, TP10, F1, F2, P1, P2, AF3, AF4, CP4, PO3, PO4, F5, F6, C6, FT8, TP8, PO7, PO8, POz, Oz, FCz. (B) Correlation of bipolar vs. sham theta power with individual 2-back baseline accuracy from 500 to 1000 ms in steps of 200 ms. Significant channels: Fp1, F3, P4, O1, O2, T8, P7, P8, Fz, FC6, CP6, F1, C2, AF3, FC3, CP4, PO4, F6, C5, P6, PO7, PO8, Fpz, POz. (C) Correlation of multi-channel vs. sham alpha power with individual CPT baseline RT from 200 to 600 ms in steps of 200 ms. Significant channels: Fp1, Fp2, F3, C4, F8, CP1, FC5, FC6, AF3, AF4, F5, F6, C5, AF7, AF8, FT7, FT8, Fpz.

For CPT event-related oscillations we found no main effect of *stimulation* but a significant positive correlation between behavioral baseline CPT RT and stimulation induced changes in alpha power for multi-channel compared to sham stimulation (p = 0.01). Subjects

with higher baseline RT showed higher alpha power after multi-channel compared to sham stimulation (Figure 2-4C). This effect occurred in a frontal area from 200 to 600 ms post stimulus onset (Figure 2-5C).

Analyses on resting state oscillations revealed no significant effects for *stimulation* or significant correlations for behavioral and oscillatory activity.

2.5 Discussion

Here we compared the effects of two different tDCS montages targeting the lDLPFC taking into account the influence of individual baseline performance. Bipolar and multi-channel stimulation were both well tolerated and effectively blinded. We found no effect of stimulation on behavioral performance or neuronal oscillations comparing the classical bipolar or the multi-channel montage with sham stimulation. However, we observed an interaction of stimulation and baseline performance for behavioral and neurophysiological outcomes. Multi-channel stimulation influenced WM performance depending on the baseline performance level, leading to decreased variability in accuracy between subjects. Initially low performing participants tended to improve their WM performance while initially high performing participants tended to worsen their performance compared to sham stimulation. Furthermore, changes in neuronal oscillations following tDCS correlated with behavioral baseline performance. The worse the participant initially performed, the more the WM task related theta power was increased following multi-channel and bipolar stimulation compared to sham stimulation. Interestingly, alpha power in the non-target task was also influenced by multi-channel stimulation depending on initial baseline performance.

In line with previous studies, we partly show that multi-channel stimulation might lead to pronounced tDCS effects when compared to sham stimulation. However, we were not able to show a superiority of multi-channel stimulation compared to bipolar stimulation as it was demonstrated in resting-state motor network (Fischer et al., 2017). Using the sham stimulation

as a reference, multi-channel montage produced stronger effects on behavioral and neurophysiological outcomes than the bipolar montage. Comparing multi-channel and bipolar effects directly, no superiority can be seen for neither of the two montages. Possible explanation could be that enhanced effects of tDCS on the motor network cannot generalize to other brain areas. Additionally, it could be argued that resting state and task specific active networks are affected differently by tDCS. According to the network model, the interaction of different factors has an influence on the stimulation effects. With online brain activity, unlike offline brain activity in resting state networks, different factors influence the effects of stimulation such as state of activation, task difficulty, level of performance and cognitive functions or strategies involved (Fertonani & Miniussi, 2017; Li et al., 2015). Also, it is important to note that working memory is a complex, high-level cognitive function, composed of different subprocesses (Baddeley, 2003).

The lack of significant effects comparing multi-channel and bipolar stimulation with sham stimulation for behavioral and neurophysiological outcomes could be due to inter-individual factors which vary the responses to tDCS. Recent meta-analyses report minor or even negative effects of tDCS on WM performance (Brunoni & Vanderhasselt, 2014; Dedoncker et al., 2016; Hill et al., 2016; Mancuso, Ilieva, Hamilton, & Farah, 2016), stressing the need to investigate factors potentially influencing tDCS outcome, such as anatomical features, baseline neurophysiological state or development and aging (Filmer, Ehrhardt, Bollmann, Mattingley, & Dux, 2019; Horvath, Carter, & Forte, 2014; Krause & Cohen Kadosh, 2014; Moliadze et al., 2015; Moliadze et al., 2018; for review see Li, Uehara, & Hanakawa, 2015). Accordingly, when including participant's individual baseline performance in our analyses, a significant interaction between stimulation and baseline performance can be observed. Importantly, this effect was not due to RTM as tests on variances show significant decreased accuracy variances following multi-channel stimulation, but not following bipolar or sham stimulation, compared to baseline accuracy variance. Following Krause, Márquez-Ruiz

and Cohen (2013) it can be assumed that there is an optimal level of prefrontal activation based on an excitation/inhibition (E/I) balance, measured by glutamate/GABA concentration (Clark, Coffman, Trumbo, & Gasparovic, 2011; Stagg et al., 2009). Based on this theory, tDCS can lead to reinstate an optimal E/I balance but can also lead to an over activation and worsening of performance. This might be the reason for improved WM performance in initially low performers and worse performance in initially high performers. This interaction is in line with previous studies reporting an increase for low performing and a decrease for high performing participants for different WM parameters (Gözenman & Berryhill, 2016; London & Slagter, 2015).

Our results for task related oscillations correspond to previous studies reporting increased theta and alpha oscillations following lDLPFC stimulation (Boonstra et al., 2016; Jones et al., 2017; Zaehle et al., 2011). Theta activity has been shown to be crucial for WM processes (Gevins, Smith, McEvoy, & Yu, 1997; Klimesch, Schack, & Sauseng, 2005; Lisman, 2010; Pesonen, Hämäläinen, & Krause, 2007), memory maintenance (Jensen & Tesche, 2002) and retrieval (Klimesch et al., 2001). The stimulation induced change in theta power we have observed, depending on initially baseline performance, may therefore indicate increased cognitive processing for initially low performing participants. Our non-target task, the CPT, investigated response inhibition and attention (Rosvold et al., 1956). Alpha oscillations following stimulus onset have been increased after multi-channel stimulation depending on initially baseline performance. Stimulation induced changes of alpha oscillations during this non-target task suggest a transfer effect of the stimulation to functions that have not been entrained during stimulation (Allenby et al., 2018). The increase in alpha following stimulus onset could reflect increased response inhibition through the inhibition of related cortical areas (Klimesch, 1996; Schmiedt-Fehr, Mathes, & Basar-Eroglu, 2009).

In contrast to Zaehle et al. (2011) changes in oscillatory power were not associated with alterations in WM performance after stimulation. A reason for missing performance changes

might be that effects of stimulation on WM performance tend to be relatively small (Hill et al., 2016; Mancuso et al., 2016). Neurophysiological activity is potentially more sensitive to stimulation than behavioral performance. The neurophysiological effects therefore suggest that tDCS together with the 2-back WM task has activated the underlying neurophysiological network beyond the duration of stimulation but not to a sufficient extent to lead to effects at the behavioral level. Following this idea, both stimulation and task engagement led to neurophysiological changes. This could also explain the missing stimulation effects on resting state oscillations. In line with previous studies, we did not observe effects on resting state oscillatory power following stimulation (Gordon et al., 2018; Hill et al., 2019; Horvath, Forte, & Carter, 2015). Our results suggest, that effects on neurophysiological outcomes are only detectable during network activation through task performance, representing a state-dependency of stimulation effects (Learmonth, Thut, Benwell, & Harvey, 2015; Silvanto, Muggleton, Cowey, & Walsh, 2007; for review see Hsu et al., 2016).

Multi-channel stimulation, in combination with the initial behavioral baseline performance, led to effects on both behavioral and neurophysiological outcomes, while bipolar stimulation only affected oscillations in the target WM task. While the maximum injected current was the same across active montages, the observed differences may have arisen due to the higher total current used in the multi-channel compared to the bipolar montage, thus leading to a higher average En-field in the lDLPFC. Increasing the current in the bipolar montage would increase the average En-field in the same proportion. A previous study seems to point to a relationship between current density in the lDLPFC and improvement in WM performance (Kim et al., 2014). This study, however, has some technical limitations, especially in the electrical conductivities assigned to the tissues represented in the models. Another potential factor that can affect the results is the focality of the En-field distribution, which is much higher in the optimized montage than in the bipolar montage, especially in the area outside the target. Lack of focality of the bipolar montage introduces confounding factors when analyzing the

data, as stimulation of other cortical areas might affect performance of the subjects in these tasks. It should be noted here that although it is possible to use multi-channel montages that achieve greater focality of stimulation (like the 4x1 montage with one central anode), this comes at the cost of a lower average En-field on the target area, which may reduce the effects of stimulation. The used multi-channel approach strikes a balance between focality and average En on target.

Additionally, the multi-channel and bipolar montage used different electrodes, which may have provided sensory cues to the subjects as to the method of stimulation. Thus, no effective blinding of the applied montage at each visit could take place. Therefore, we cannot exclude that the subjects had implicit assumptions about the effectiveness of the different montages, which in turn may have had an impact on the measured outcomes. However, for both montages we achieved effective blinding in terms of verum or sham stimulation and all subjects were naive to stimulation and the study aim of comparing the effectiveness of montages.

Another limitation of this study is related to the lack of subject-specific personalized head models. These are important, as inter-individual anatomical features such as skull thickness and cortex folding, have been shown to have large influence on tDCS current flow (Laakso et al., 2016; Opitz, Paulus, Will, Antunes, & Thielscher, 2015). These personalized models would provide means to calculate the average En in the lDLPFC for each subject, which could then be used as an additional term in the statistical analysis of the data, as performed by Laakso, Mikkonen, Koyama, Hirata & Tanaka (2019). Also, the influence of baseline performance could have been investigated more detailed if a larger sample size would have been collected. This would have allowed us to study the effects of stimulation in different subgroups.

In summary, and considering the limitations we have highlighted, our results demonstrate the importance of taking into account inter-individual baseline performance and montage when stimulating the lDLPFC. Several studies have shown a limited effectiveness of

tDCS on WM, often expressed in a low response rate. Therefore, our study helps to identify the factors that determine whether a subject benefits from stimulation. Moreover, sharing partly "null results" will have (1) positive impact on future research questions and (2) will improve knowledge acquisition of non-invasive transcranial brain stimulation techniques.

2.6 References

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2.7 Supplementary

Table 2-3

Incidence (in %) and intensity (scale 1–5, Ø) of side effects for multi-channel, bipolar and sham stimulation.

		multi vs. bipolar	multi vs. sham	bipolar vs. sham
Itching sensation	Incidence	79.2/75	79.2/65.2	75/65.2
	Intensity	1.57/1.42	1.57/1.33	1.42/1.33
Pain	Incidence	16.7/16.7	16.7/21.7	16.7/21.7
	Intensity	1.25/1.25	1.25/1	1.25/1
Burning sensation	Incidence	58.3/45.8	58.3/52.2	45.8/52.2
	Intensity	1.36/1.5	1.36/1.11	1.5/1.11
Warmth/Heat	Incidence	20.8/29.2	20.8/30.4	33.3/30.4
	Intensity	1/1.5	1/1	1.5/1
Metallic/Iron taste	Incidence	0/0	0/0	0/0
	Intensity	n.a.	n.a.	n.a.
Fatigue/Decreased alertness	Incidence	33.3/25	33.3/30.4	27.8/30.4
	Intensity	1.5/1.2	1.5/1.67	1.2/1.67

Note. n.a. = not applicable.

3 Study II

Multichannel Anodal tDCS Over the Left Dorsolateral Prefrontal Cortex in a Paediatric Population

Splittgerber, M., Borzikowsky, C., Salvador, R., Puonti, O., Papadimitriou, K., Merschformann, C., Biagi, M. C., Stenner, T., Brauer, H., Breitling-Ziegler, C., Prehn-Kristensen, A., Krauel, K., Ruffini, G., Pedersen, A., Nees, F., Thielscher, A., Dempfle, A., Siniatchkin, M., & Moliadze, V. (2021). *Multichannel Anodal tDCS Over the Left Dorsolateral Prefrontal Cortex in a Paediatric Population*. Manuscript submitted for publication.

3.1 Abstract

Background: Methodological studies investigating transcranial direct current stimulation (tDCS) over the left dorsolateral prefrontal cortex (DLPFC) in paediatric populations are limited. Especially the use of multichannel montages and the influence of offline/online tDCS has not been investigated in this age group.

Objective: We investigated in a paediatric population whether stimulation success of multichannel tDCS over the left DLPFC depends on concurrent task performance during stimulation and individual head anatomy.

Methods: In a randomised, sham-controlled, double-blind crossover study 22 healthy participants (10 – 18 years) received 2 mA anodal or sham multichannel tDCS over the left DLPFC with and without a target 2-back working memory task. After stimulation, the 2-back task and a Flanker task (non-target task) were performed. Resting state and task related EEG were recorded. In 16 participants we calculated the individual electric field (E-field) distribution.

Results: Performance and neurophysiological activity in the 2-back task during and after stimulation and resting state activity were not affected by tDCS. TDCS led to reduced reaction time in the Flanker task, independent of whether tDCS had been combined with the 2-back task. Increased Flanker task related beta oscillation was observed only following stimulation without concurrent 2-back task performance. TDCS effects were not correlated with the individual E-field.

Conclusion: Our results show transfer effects of multichannel tDCS in children/adolescents. While on the behavioural level this transfer was independent of concurrent task performance, neurophysiological activity might be more sensitive to cognitive activation during stimulation. Our study demonstrates the importance to include control tasks, since stimulation effects might otherwise remain undetected.

3.2 Introduction

Transcranial direct current stimulation (tDCS) is a promising neuromodulatory technique in research and clinical application (Ciullo et al., 2020; Kekic, Boysen, Campbell, & Schmidt, 2016; for review see Woods et al., 2016). Although the use of tDCS in children and adolescents is increasingly being investigated, important insights into how tDCS affects and interacts with the developing brain are missing. Therefore, the clinical application of tDCS in paediatric populations is still limited (Lee, Kenney-Jung, Blacker, Doruk Camsari, & Lewis, 2019; Palm et al., 2016). Previous studies show that findings on tDCS effects obtained in adults cannot simply be assumed to be valid for tDCS application in children and adolescents. Compared to adults, children show different conductivity of the skull tissue, different white and gray matter content and cerebrospinal fluid (CSF) volume as well as a smaller brain-scalp distance, all of which influence the electric-field (E-field) distribution (Beauchamp et al., 2011; Kessler et al., 2013). As demonstrated previously in motor cortex, age of participants may affect efficacy of tDCS and even inverse stimulation effects (Moliadze et al., 2015; Moliadze et al., 2018). However, due to differences in cortical architecture, receptor distribution and anatomical factors, it is not clear if findings from motor cortex are transferable to other cortical areas (Knotkova, Nitsche, & Polania, 2019).

The left dorsolateral prefrontal cortex (DLPFC) is an area often used as target region for electrical stimulation, due to its role for various cognitive and executive functions such as working memory (WM) or decision making (D'Esposito et al., 1995; Heekeren, Marrett, Bandettini, & Ungerleider, 2004). Previous studies have shown that tDCS has the potential to modulate neuronal activity in the left DLPFC and associated cognitive functions in adults (Dedoncker, Brunoni, Baeken, & Vanderhasselt, 2016; Wörsching et al., 2016) and in clinical samples in children and adolescents (Lee et al., 2019; Soff, Sotnikova, Christiansen, Becker, & Siniatchkin, 2017; Sotnikova, Soff, Tagliazucchi, Becker, & Siniatchkin, 2017). However, to

date, no methodological studies have been conducted on the effects of tDCS over the left DLPFC in normally developed children and adolescents.

In adults, it has been shown that tDCS effects are dependent on the current intensity in the target area, with higher intensities leading to stronger stimulation effects (Albizu et al., 2020; Antonenko et al., 2019; Kim et al., 2014). Previous studies that stimulated the left DLPFC using tDCS have mostly used classical bipolar montages, which produce a relative diffuse electric field (E-field) and poor targeting (Laakso et al., 2016; Miranda, Mekonnen, Salvador, & Ruffini, 2013; Saturnino, Antunes, & Thielscher, 2015). An alternative could be optimised multichannel montages, for which modelling studies predict a comparatively strong but also focal stimulation of the target area (Salvador et al., 2021; Saturnino, Siebner, Thielscher, & Madsen, 2019; Wagner, Burger, & Wolters, 2016). For adults, an increased effectiveness of an optimised multichannel montage has already been shown for motor cortex stimulation (Fischer et al., 2017) as well as left DLPFC stimulation (Splittgerber et al., 2020).

A relevant methodological factor in tDCS studies is the pairing of stimulation with a concurrent task, i.e. whether tDCS is applied without (offline) or with concurrent task performance (online). Studies in adults suggest that cognitive engagement during stimulation has an impact on the type and strength of tDCS after-effects (Gill, Shah-Basak, & Hamilton, 2015; Trumbo et al., 2016). Regarding left DLPFC stimulation, both a superiority of online (Gill et al., 2015; Martin, Liu, Alonzo, Green, & Loo, 2014) and of offline stimulation have been proposed (Friehs & Frings, 2019; Hill, Fitzgerald, & Hoy, 2016). Especially in the context of a potential therapeutic use of tDCS, it is relevant to generate broad, transferring effects. According to the activity selectivity model, the activity in neuronal populations that are involved into task execution during the stimulation is primarily strengthened, which might lead to task specific after-effects (Bikson & Rahman, 2013; Boroda, Sponheim, Fiecas, & Lim, 2020; Fertonani & Miniussi, 2017). Besides, cognitive tasks almost always involve effort for patients. If it were shown that concurrent task processing during stimulation is not necessary to

evoke stimulation effects, the clinical applicability of tDCS would be substantially enhanced. Unfortunately, online and offline stimulation is rarely compared within the same study.

An important tool to illustrate stimulation effects is provided by neurophysiological correlates such as event-related potentials (ERPs) and event-related and resting state oscillatory power recorded by EEG. Studies in adults demonstrate effects of anodal tDCS over the left DLPFC on neurophysiological activity. TDCS led to increased task related N2 and P3 amplitudes (Dubreuil-Vall, Chau, Ruffini, Widge, & Camprodon, 2019; Keeser et al., 2011). Additionally, theta and alpha oscillatory power have been shown to be influenced by tDCS (Hoy et al., 2013; Splittgerber et al., 2020; Zaehle, Sandmann, Thorne, Jäncke, & Herrmann, 2011). Also, changes in resting state oscillatory power have been demonstrated following anodal tDCS (Boonstra, Nikolin, Meisener, Martin, & Loo, 2016; Keeser et al., 2011). However, several studies did not prove an effect of tDCS over the left DLPFC on resting state oscillatory power (Gordon et al., 2018; Hill, Rogasch, Fitzgerald, & Hoy, 2019; Horvath, Forte, & Carter, 2015).

Up to now, the previously mentioned factors have not been targeted in a methodological study on tDCS effects in children and adolescents. Therefore, the current study aimed at investigating multichannel anodal tDCS over the left DLPFC in children and adolescents aged 10 – 18 years. We explored whether tDCS effects are influenced by concurrent target task performance during stimulation as well as individual anatomy. As outcomes we used behavioural and neurophysiological variables. We expected anodal tDCS to lead to improved performance in a 2-back WM task, which we used as target task. We assumed that task-related N2 and P3 amplitudes increase following anodal compared to sham stimulation. We also expected tDCS to influence theta and alpha task related oscillations. In addition, we investigated changes in beta oscillation, as it has been shown to be relevant for WM processes (Schmidt et al., 2019). For resting state oscillatory power, we investigated the theta, alpha and beta band but without a certain expectation, as previous tDCS studies in adults are contradictory. Further,

we expected stimulation effects to be influenced by concurrent task performance during stimulation. Since studies in adults do not show a clear superiority of online or offline stimulation, this is treated as an exploratory hypothesis. To investigate whether tDCS effects are task specific, we used a Flanker task as non-target task that investigates interference control (Eriksen & Eriksen, 1974). Following the activity selectivity model, we did not expect tDCS to influence Flanker task performance and related neurophysiological activity. Furthermore, we assumed that the effects of tDCS are modulated by individual anatomy, with higher E-field distributions in the target area leading to stronger tDCS effects. Because respective research in typically developing children and adolescents is lacking, we also investigated aspects of tolerability for multichannel anodal tDCS.

3.3 Materials and Methods

3.3.1 Participants

The study was approved by the local ethics committee of the Medical Faculty at Kiel University, Kiel, Germany and was carried out in accordance with the latest revision of the Declaration of Helsinki (Trial DRKS00008207). This study is part of the EU research project STIPED (Stimulation in pediatrics; grant agreement No 731827, www.stiped.eu), which investigates tDCS as potential treatment approach for neurodevelopmental disorders in children and adolescents. All participants and their parents were instructed about the study and written informed consent prior to the experiment was obtained. In total, 34 participants were recruited. After a screening procedure we included 29 healthy children and adolescents between 10 and 18 years. Due to study dropout and technical problems during data recording, a total of 22 participants were included in the final data analyses (14 females, mean age: 15.18 years, SD: 1.9). Except for one case, the reason given for study termination was that the study participation was too time-consuming. One 13-year old girl developed an epileptic disease during her study participation and had to be excluded from the study (Splittgerber et al., 2019). Exclusion criteria

were an IQ score < 80 (CFT-20-R, Grundintelligenztest Skala 2 – Revision; Weiß, 2006), a birth weight < 2500 gr. or a birth before the 37th week of pregnancy, past or present chronical internistic disorders or neurological diseases/brain surgery or psychiatric disorders, epilepsy/epileptic seizure(s) in the past or in the family, substance consumption or regular medication, any body electronic devices or implants and pregnancy. Health and social impairments were further assessed using the CBCL (Child Behavior Checklist; Döpfner, Plück, & Kinnen, 2014), FBB-ADHS (Fremdbeurteilungsbogen für Aufmerksamkeitsdefizit-/Hyperaktivitätsstörungen; Döpfner, Görtz-Dorten, & Lehmkuhl, 2008) and SRS (Social Responsiveness Scale; Constantino & Gruber, 2007). All participants' characteristics can be found in Table 3-1.

Table 3-1

Participants characteristics.

	Mean ± Standard deviation	Exclusion criteria
Sex	14 female, 8 male	
Age	15.12 years ± 1.9	10 < age > 18
CFT-20-R	106.68 ± 8.91	IQ < 80
CBCL Competence	59.86 ± 7.66	T < 37
CBCL Problems	48 ± 8.14	T > 69
FBB-ADHS	0.19 ± 0.18	KW > 0.5
SRS	45 ± 9.39	T > 60

Note. CFT-20-R = Grundintelligenztest Skala 2 – Revision; CBCL = Child Behavior Checklist; FBB-ADHS = Fremdbeurteilungsbogen für Aufmerksamkeitsdefizit-/Hyperaktivitätsstörungen; SRS = Social Responsiveness Scale.

3.3.2 Experimental Design

We used a randomised, sham-controlled, double-blind, crossover study design (see Figure 3-1). Participants underwent six experimental sessions: one screening and baseline measurement (T1) followed by four stimulation sessions (T2 – T4) and one optional MRI session (T6). In the stimulation sessions each participant received four different stimulation conditions in randomised order: anodal tDCS with or without concurrent 2-back task performance and sham tDCS with or without concurrent 2-back task performance. The

minimum period between stimulation sessions for a single participant was seven days. At the start of each stimulation session, participants filled in a diary, asking for any adverse events since the last session and their current mood and motivation. After preparation of the stimulation head cap, we recorded a resting state EEG (2 min. eyes open, 2 min. eyes closed), followed by 20 minutes stimulation. In case of concurrent task performance during stimulation, the 2-back task started after 2.5 minutes of tDCS and ended 2.5 minutes before the end of stimulation. During non-concurrent stimulation, participants were instructed to sit relaxed with opened eyes. After stimulation, we recorded a second resting state EEG (2 min. eyes open, 2 min. eyes closed). Afterwards participants performed the 2-back task, a Flanker task and a Continuous Performance Task (CPT) during EEG recording. Eventually, participants filled in a questionnaire on safety, tolerability and blinding of stimulation. Since this study is part of a larger project, several questions were investigated simultaneously. The present study is limited to the analysis of the stimulation sessions (T2 – T4). Of the tasks performed, the 2-back and Flanker task were evaluated, while the CPT was performed for later comparisons with patient groups.

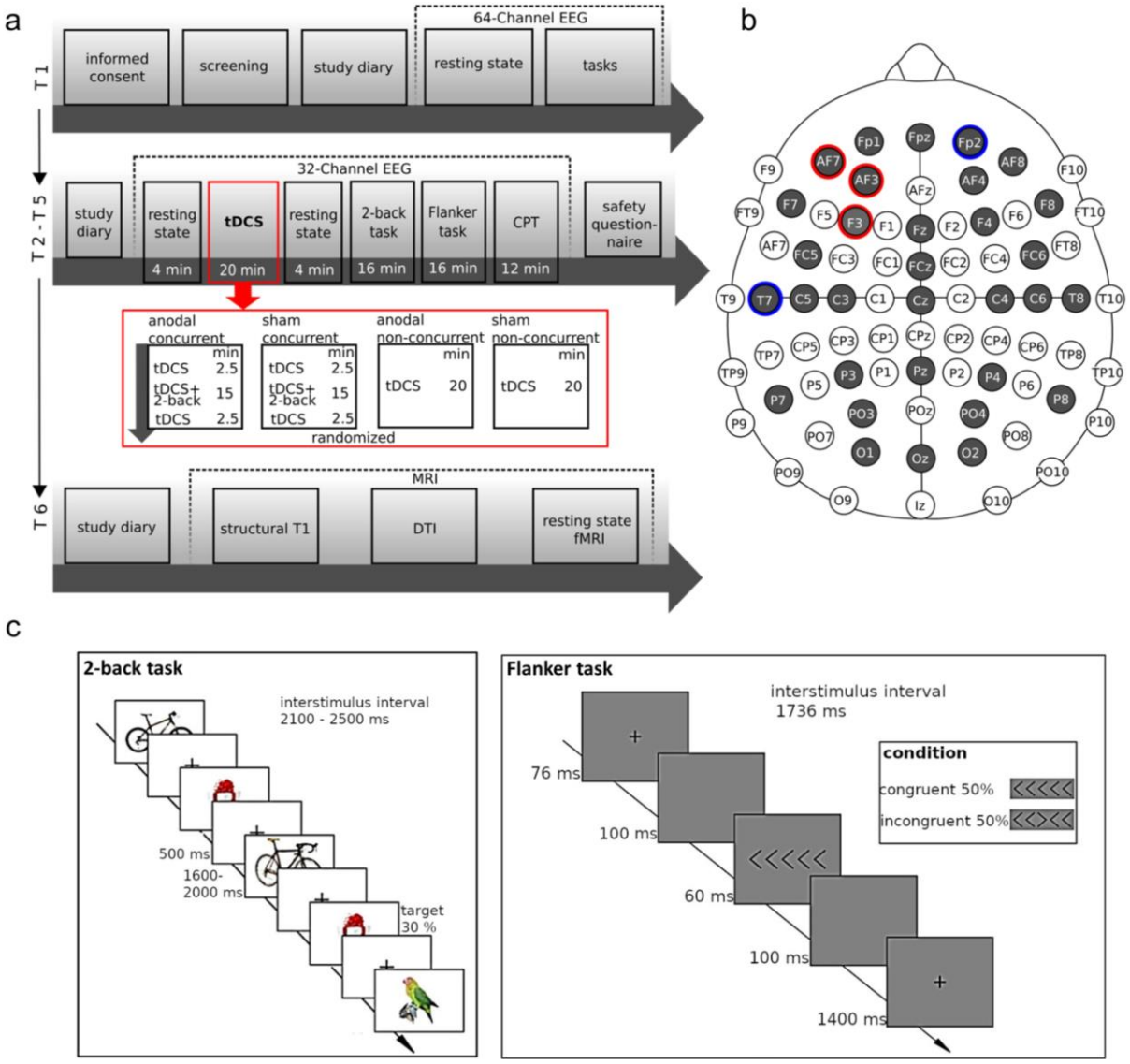

Figure 3-1. Experimental Design. (a) Time-course of the experiment. Each participant was stimulated four times (T2–T5) with the following condition: anodal tDCS with 2-back performance, sham tDCS with 2-back performance, anodal tDCS without task performance, sham tDCS without task performance. After stimulation, the participants always performed the 2-back task, Flanker task and Continuous Performance Task (CPT). EEG was recorded at rest (pre and post tDCS), during stimulation and after stimulation during task performance. At an optional session (T6) MRI data for individual modelling was obtained. (b) Electrode montage for EEG and multichannel stimulation. Red circles represent anodal, blue circles reference electrodes. Grey colour indicates EEG electrodes. (c) Tasks. In the 2-back task participants had to decide whether a currently presented picture was identical to the picture shown 2 steps back. In the Flanker task participants had to indicate via button press if the middle target arrow pointed to the right or to the left.

3.3.3 Tasks and Stimuli

All tasks were programmed using the software Presentation® (Version 20.0, Neurobehavioral Systems, Inc., Berkeley, CA, www.neurobs.com). For the 2-back task pictures were taken from the Stark Lab Mnemonic Similarity Task (MST; Stark, Kirwan, & Stark, 2019). To make the task more demanding, we included lure pictures that were very similar but not identical. Participants had to decide whether a currently presented picture was identical to the picture shown two steps back (Figure 3-1c). Participants had to press the right mouse button if the trial was a non-target or the left mouse button if the trial was a target trial. The 2-back task lasted approximately 16 minutes and contained 366 trials with 30% target trials. Each trial consisted of 500 ms picture presentation followed by fixation cross presentation jittered between 1600 – 2000 ms duration, resulting in a trial duration of 2100 – 2500 ms.

In the Flanker task (Figure 3-1c), stimuli consisted of five arrows. Participants had to indicate via button press if the middle target arrow pointed to the right or to the left. The outer arrows served as distractors. The task had about 16 minutes duration and 528 trials in total with 50% of the trials being congruent and incongruent, divided into 3 blocks with short breaks in between. Stimuli were presented for 60 ms with an inter stimulus interval of 1676 ms and a trial duration of 1736 ms.

Accuracy and RT for both tasks were analysed using the computing environment R (version 3.6.1, R Core Team (2019) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/). As measurement of accuracy in the 2-back task we computed d' scores. This score is calculated by subtracting the z-transformed false alarm rate from the z-transformed hit rate: $d' = Z_{HIT} - Z_{FA}$ (Macmillan & Creelman, 1990). RT were analysed for target trial hits. For the Flanker task accuracy was defined as proportion of correct responses for congruent and incongruent trials separately. RT as well were analysed for correct responses for congruent and incongruent trials.

3.3.4 Transcranial Direct Current Stimulation

We applied 2 mA tDCS over the left DLPFC using a Starstim 32 stimulator (Neuroelectrics, Barcelona, Spain). Five 3.14 cm² circular PiStim electrodes were positioned at AF3 (897 µA), AF7 (284 µA), F3 (819 µA), Fp2 (-1000 µA) and T7 (-1000 µA), filled with EEG electrode gel. Electrodes were positioned using a head cap following the 10-10 system (Figure 3-1b). For anodal stimulation current was ramped up for 30 seconds at the beginning and down during 30 seconds at the end of stimulation, for sham stimulation current was ramped up and immediately down for 60 seconds at the beginning and end of the stimulation.

3.3.4.1 Montage Optimisation

The multichannel montage used in this study was derived from an optimisation algorithm applied to a template head model (Colin head model; Miranda et al., 2013). The optimisation was conducted using the Stimweaver algorithm (Ruffini, Fox, Ripolles, Miranda, & Pascual-Leone, 2014). This method determines the montage that minimise the least squares difference between a weighted target electric field (E-field) map and the weighted E-field induced by the montage. In this study, we focused on the component of the E-field normal to the cortical surface (E_n) of the head model, since this component is thought to induce the strongest polarisations in the pyramidal neurons, aligned along this direction (lambda-E model; Ruffini et al., 2014). For the target map we created a maximum excitation region (maximum weight, 10), and a positive target E_n field of +0.50 V/m on the left hemisphere based on Brodmann area 46/left DLPFC. The target *En*-field on the remaining areas was set to 0 V/m with a lower weight (2). The electrode positions were selected from those available in Neuroelectrics PRO cap (64 positions of the 10-10 EEG system). The maximum current in each electrode (in absolute value) was constrained to 1.0 mA and the total injected current was limited to a maximum of 2.0 mA. The distribution of E_n in the cortical surface of the template head model is shown in Figure 3-2a.

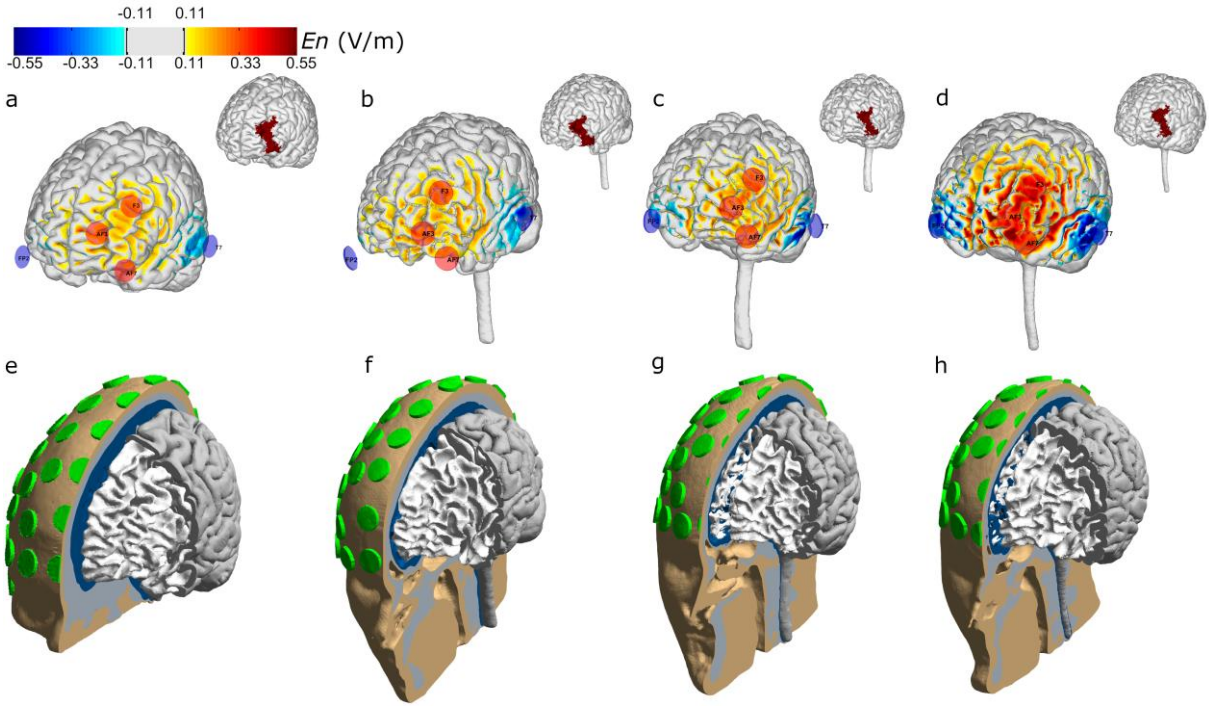

Figure 3-2. Distribution of the normal component of the E-field in the cortical surface of 4 of the head models used in this study. The distribution induced by the optimized montage on the cortical surface are shown in the top row for: the template head model (a) and the participants with the lowest, median and highest average E_n in the left DLPFC (b-d, respectively). The head models for each participant are shown in the bottom row (e-h), including the 61 electrodes present in the headcap used in this study.

3.3.5 EEG Recording and Preprocessing

EEG was recorded from 32 channels during the stimulation sessions using the Starstim 32 stimulator and NG PiStim electrodes (Neuroelectrics, Barcelona, Spain) and NIC software v2.0.10 and v2.0.11 (Neuroelectrics, Barcelona, Spain). The EEG signal was recorded with a rate of 500 S/s and with a bandwidth of 0 to 125 Hz (DC coupled). The electrode positions corresponded to the positions of the international 10-10 standard system, with the reference and ground electrodes located on the right mastoid. Electrode impedances were kept below 10 kΩ.

We preprocessed the EEG data using BrainVisionAnalyzer 2 (Brain Products GmbH, Gilching, Germany). The data was re-referenced to the common average and filtered (120 Hz low-pass, 0.53 Hz high-pass filter, 50 Hz notch filter). We applied a semiautomatic raw data inspection to remove artefacts. Further, ocular and ECG artefacts were removed using an independent component analysis. Resting state EEG data was then segmented in 2-seconds

intervals. Task related EEG data was segmented relative to the stimulus onset (2-back: -500 to 1250 ms; Flanker: -500 to 1000 ms). Finally, we rejected any remaining artefacts from the segmented data using a semiautomatic artefact rejection. For the 2-back task a mean number of 191 trials (SD: 35) and for the Flanker task a mean number of 210 trials (SD: 37) was included in the following analyses.

EEG data was further analysed using the Fieldtrip toolbox (Oostenveld, Fries, Maris, & Schoffelen, 2011). Due to a limited number of target hits in the 2-back task for several participants we restricted our analysis of event-related potentials (ERPs) and time-frequency representation to correct rejections trials in the 2-back task. For the 2-back task we analysed the N2 (200 – 270 ms) in the FCz electrode as region of interest (ROI) and the P3 (250 – 500 ms post stimulus) component in the Pz electrode as ROI. For the Flanker task ERPs and time-frequency representations were analysed for correctly answered incongruent trials. Here, we analysed the N2 component (250 – 350 ms) in the FCz electrode and P3 (350 – 600 ms) component in the Pz electrode. ROIs were based on previous investigations of task related ERPs (Danielmeier, Wessel, Steinhauser, & Ullsperger, 2009; Folstein & van Petten, 2008; Pfueller et al., 2011). The time windows of all component were defined based on visual inspection of the grand average ERP from all participants.

For task related oscillatory activity we performed a time-frequency analysis with a Hanning taper in steps of 2 Hz for the theta (4 – 6 Hz), alpha (8 – 12 Hz) and beta frequency band (14 – 30 Hz). For all frequency bands event-related synchronization/desynchronization (ERS/ERD; Pfurtscheller & Lopes da Silva, 1999) was computed with respect to a pre stimulus baseline (-250 – 0 ms), with positive values reflecting an increase in power (ERS) and negative values a decrease in power (ERD) from the baseline interval to the post stimulus interval. In the 2-back task we analysed the post stimulus interval from 0 – 1000 ms, in the Flanker task we analysed the post stimulus interval from 0 – 700 ms.

Resting state EEG was Fourier-transformed with a moving Hanning window in steps of 0.5 Hz for the theta (4–7.5 Hz), alpha (8–12 Hz) and beta (12.5–30 Hz) frequency band and averaged in every participant. Next, we computed pre to post stimulation changes in frequency power for all frequency bands.

3.3.6 Individual Calculation of E-field Distribution

From the included 22 participants 16 individuals underwent structural head scanning on a 3 T Philips Achieva scanner, during which the following sequences were acquired: a T1-weighted scan (1 mm3, TR =2530 ms, TE = 3.5 ms, TI = 1100 ms, FA = 7º, fast water excitation), a T2-weighted scan (1 mm3, TR =3200 ms, TE = 300 ms, no fat suppression), and a diffusion MRI (dMRI) scan (2 mm3, TR = 6300 ms, TE = 51 ms, 67 directions, b = 1000). For these 16 participants, personalised head models were built. Each participants MRI was segmented using an in-house implementation combining extra-cerebral tissue segmentations from a new segmentation approach, which will be included in a future version of the open-source simulation toolbox SimNIBS (Puonti et al., 2020), with brain tissue segmentations and cortical gray matter surface reconstructions from FreeSurfer (Fischl et al., 2002). Finite element head models were then generated, including representations of the scalp, skull, CSF, gray matter and white matter. The head models also contained representations of Pistim electrodes (1 cm radius, cylindrical Ag/AgCl electrodes) placed in 61 positions of the 10-10 EEG system. The head models of 3 of the individuals participating in this study are shown in Figure 3-2f-h. For the electrodes, only the conductive gel underneath the metal connector was represented in the head model. Unless otherwise stated, the scalp, skull, and CSF were modelled as isotropic with conductivities of 0.33 S/m, 0.008 S/m, and 1.79 S/m, respectively, which are appropriate values for the DC-low frequency values used in transcranial current stimulation (Miranda et al., 2013). The gray and white matter were modelled as anisotropic (volume normalization (Opitz, Windhoff, Heidemann, Turner, & Thielscher, 2011), with isotropic conductivity values used for diffusion tensor scaling of 0.40 S/m–0.15 S/m, for the gray matter – white matter (Miranda

et al., 2013)). The E-field distribution induced by the common optimised montage used in this study was calculated for each participant (see Figure 3-2b-d). All E-field calculations were performed in COMSOL, using second- order tetrahedral mesh elements to solve Laplace's equation. For each participant the surface-average value of the normal component of the E-field was calculated for the left DLPFC (Brodmann area 46), as region targeted by the optimisation.

3.3.7 Questionnaire on Tolerability and Participant Blinding of tDCS

For assessment of side effects and blinding effectiveness we used a standardised safety questionnaire (Antal et al., 2017; Poreisz, Boros, Antal, & Paulus, 2007). Participants rated the incidence and intensity (0 = not experienced to 3 = strongly experienced) of the six most common tDCS side effects on a 4-point scale. Additionally, following each stimulation application, the participants gave their opinion as to whether they had received anodal or sham stimulation.

3.3.8 Statistical Analyses

3.3.8.1 Behavioural Data

Throughout all analyses, results were regarded as statistically significant with a two-tailed p value of less than 0.05. Differences in accuracy and RT were analysed using linear mixed effects models (LME) with type III sum of squares. For the 2-back task we computed four models investigating performance: One model for each outcome (accuracy or RT) for each time point (during or after stimulation). The models of 2-back task performance during stimulation included the fixed factors *stimulation* (sham, anodal) and *age* (centred at the mean) and their interaction as well as *visit* (tDCS session 1 to 4). The models of 2-back task performance after stimulation included the fixed factors *stimulation*, *tDCS+target task* (tDCS application with or without concurrent 2-back task performance), *age* and all corresponding interactions as well as *visit*. For the Flanker task we computed two models investigating accuracy and RT with the fixed factors *stimulation*, *trial* (congruent or incongruent),

tDCS+target task, *age* and all corresponding interactions, except for the four-way interaction, as well as *visit*. In all models we included a random intercept. The assumptions of normality and homogeneity were assessed using Shapiro-Wilk-tests and Q-Q as well as scatter plots. Degrees of freedom were approximated using the Satterthwaite method. In case of violations of assumptions, we computed robust linear mixed models using the robustlmm package (Koller, 2016).

3.3.8.2 Neurophysiological Data

Analyses of neurophysiological data were performed using the Fieldtrip toolbox (Oostenveld et al., 2011). Differences in ERPs of correct rejection 2-back trials and of incongruent Flanker trials were investigated using two-way repeated measurement ANOVAs with the factors *stimulation* (sham, anodal) and *tDCS+target task* (tDCS application with or without concurrent 2-back task performance) based on the respective ROIs and time intervals. Regarding task related ERD/ERS and resting state post-pre frequency changes, we used two-way repeated measurement ANOVAs with the factors *stimulation* and *tDCS+target task* with a cluster based approach. This non-parametric approach solves the problem of multiple comparisons by cluster correction and avoids assumptions on normally distributed data. In all ANOVAs we restricted our analysis to the main effect of *stimulation* and the interaction of *stimulation* and *tDCS+target task*. Due to the number of ANOVAs computed for EEG analysis we used a Bonferroni-Holm correction to adjust the alpha level. Following the ANOVAs, in case of a significant main or interaction effect paired t-tests for the specific significant time window and cluster electrodes or ROI were conducted.

3.3.8.3 Correlation of E-field and Stimulation Effects

We computed correlations between stimulation effects on behavioural and neurophysiological data and the individual normal E-field values in the stimulation target area. On the behavioural level, these correlations were defined for 2-back task d’ scores, and RT during and after stimulation and for Flanker task accuracy and RT after stimulation. For

neurophysiological data correlations between the E-field and 2-back and Flanker task ERPs and ERD/ERS were computed. Stimulation effects were defined as difference between the sham and anodal condition. Due to the high number of correlations a Bonferroni-Holm Alpha correction was performed.

3.3.8.4 Tolerability and Blinding of tDCS

Differences in experience of side effects between sham and anodal stimulation were investigated using Wilcoxon signed-rank tests. Blinding effectiveness was assessed using a Pearson's chi-squared test.

3.4 Results

3.4.1 Behavioural Data

Mean accuracy and RT values for both tasks are displayed in Table 3-2. Additionally, Figure 3-3 displays 2-back accuracy during and after stimulation. Results of the LMM of 2-back d' scores and RT are summarized in Table 3-3. Neither for 2-back performance during nor after stimulation we found a significant main effect of or interaction with *stimulation* on d' scores or RT. There was a significant effect of *age* for d' scores during stimulation, showing an increase in d' scores with increasing age ($\beta = 0.162$, $t\ (19.8) = 2.22$, $p = 0.038$). For 2-back RT after stimulation we found a reduction of RT at the third stimulation visit compared to the overall RT mean ($\beta = -24.386$, $t\ (57) = -2.55$, $p = 0.013$).

Table 3-2

Mean (Standard deviation) for 2-back d' score, accuracy (%) and reaction times (ms) and Flanker accuracy (%) and reaction times (ms).

Task	Time point	Outcome	Stimulation condition	
			sham	anodal
2-back	during	d'	2.38 (0.78)	2.35 (0.71)
		accuracy	67.02 (18.01)	65.75 (22.64)
		RT	580.21 (155.71)	577.43 (173.37)
	after	d'	2.23 (0.78)	2.21 (0.81)
		accuracy	64.18 (22.94)	64.37 (23.57)
		RT	594.12 (197.29)	566.71 (189.41)
Flanker	after	accuracy	92.83 (11.35)	94.07 (6.66)
		RT	521.43 (129.44)	512.08 (126.82)

Note. RT = reaction time.

Table 3-3

Coefficients and corresponding t values and p values for 2-back accuracy (d') and reaction time during and after stimulation.

during stimulation						
predictors	accuracy			reaction time		
	β	*t* value	*p* value	β	*t* value	*p* value
stimulation (sham vs. anodal)	-0.016	-0.258	0.799	-2.068	-0.299	0.769
age	**0.162**	**2.223**	**0.038**	22.041	1.256	0.224
visit 1	-0.172	-1.234	0.231	-3.995	-0.244	0.810
visit 2	0.025	0.202	0.842	-16.325	-1.140	0.269
visit 3	0.019	0.152	0.880	26.069	1.775	0.093
stimulation×age	-0.001	-0.023	0.982	-0.144	-0.038	0.970
after stimulation						
predictor	accuracy			reaction time		
	β	*t* value	*p* value	β	*t* value	*p* value
stimulation (sham vs. anodal)	-0.016	-0.485	0.634	-8.478	-1.545	0.128
task (con vs. non-con)	0.049	1.453	0.162	11.058	2.005	0.050
age	0.033	0.720	0.479	-4.857	-0.632	0.529
visit 1	0.000	0.003	0.997	19.157	1.914	0.061
visit 2	0.088	1.492	0.151	0.693	0.073	0.942
visit 3	0.039	0.673	0.510	**-24.386**	**-2.553**	**0.013**
stimulation×task	0.007	0.215	0.832	-4.193	-0.760	0.450
stimulation×age	-0.034	-1.825	0.086	-2.307	-0.757	0.453
concurrent×age	0.009	0.448	0.659	-0.505	-0.166	0.869
stimulation×task×age	0.004	0.233	0.818	-1.580	-0.510	0.612

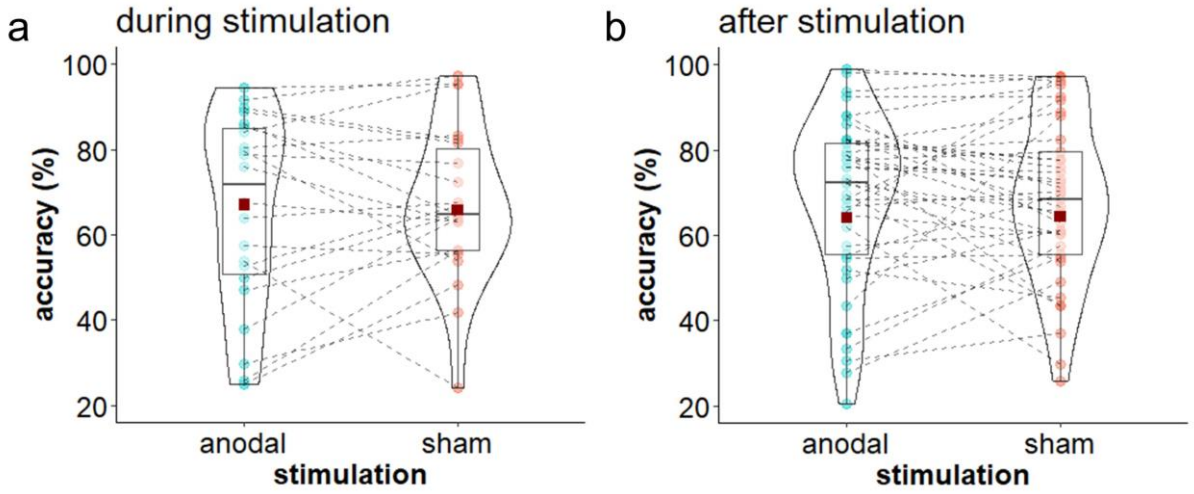

Figure 3-3. Stimulation effects on 2-back performance. (a) Violin plot for mean (± SD) and individual 2-back accuracy for sham and multichannel stimulation during stimulation. (b) Violin plot for mean (± SD) and individual 2-back accuracy for sham and multichannel stimulation after stimulation, averaged over concurrent and non-concurrent task condition. Red squares indicate mean accuracy, coloured dots indicate individual accuracy.

Analysis of the Flanker task showed no *stimulation* effect on accuracy but an effect of *stimulation* on RT (β = -6.438, t (57) = -2.47, p = 0.015; see Table 3-4). Following anodal stimulation RT were generally reduced compared to sham stimulation (see Figure 3-4a). Importantly, there was no trade-off between RT and accuracy, meaning while RT were reduced following anodal compared to sham stimulation, accuracy did not decrease. Furthermore, we found an interference effect reflected in a significant effect of *trial* for both accuracy (β = -2.145, t (138) = -10.42, p < 0.001) and RT (β = 35.701, t (138) = 13.81, p < 0.001): Compared to congruent trials accuracy scores were lower and RT were higher for incongruent trials. Besides, we found a significant interaction of *trial* and *age* for RT (β = 3.298, t (138) = 2.36, p = 0.019). While RT decreased with increasing age for both trial types, this decrease was steeper for congruent than for incongruent trials.

Table 3-4

Coefficients and corresponding t values and p values for Flanker accuracy and reaction time.

predictors	accuracy			reaction time		
	β	*t* value	*p* value	β	*t* value	*p* value
stimulation (sham vs. anodal)	0.103	0.498	0.619	**-6.438**	**-2.472**	**0.015**
task (con vs. non-con)	-0.129	-0.622	0.535	-0.428	-0.163	0.870
trial (congr. vs. incongr.)	**-2.145**	**-10.420**	**<0.001**	**35.700**	**13.804**	**<0.001**
age	0.168	0.720	0.473	3.601	0.987	0.325
visit 1	-0.546	-1.460	0.147	8.616	1.813	0.072
visit 2	-0.275	-0.766	0.445	7.161	1.583	0.116
visit 3	0.354	0.982	0.328	-6.078	-1.340	0.182
stimulation×task	-0.004	-0.021	0.983	0.738	0.282	0.778
stimulation×trial	-0.025	-0.123	0.902	0.185	0.072	0.943
concurrent×trial	-0.298	-1.447	0.150	0.110	0.043	0.966
stimulation×age	-0.225	-1.961	0.052	-0.328	-0.226	0.821
task×age	0.185	1.607	0.110	1.864	1.287	0.200
trial×age	0.057	0.511	0.610	**3.298**	**2.364**	**0.019**
stimulation×task×trial	-0.295	-1.432	0.154	1.479	0.571	0.569
stimulation×task×age	-0.014	-0.119	0.905	-2.092	-1.421	0.157
stimulation×trial×age	0.004	0.032	0.975	0.085	0.061	0.951
task×trial×age	0.114	1.023	0.308	-1.175	-0.842	0.401

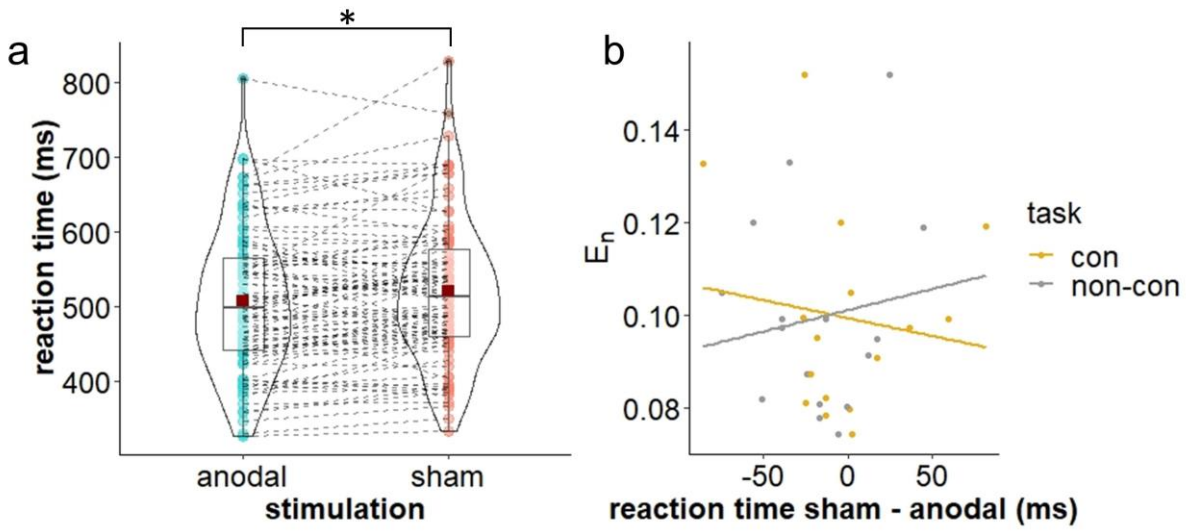

Figure 3-4. Stimulation effects on Flanker performance. (a) Violin plot for mean (± SD) and individual Flanker RT for sham and multichannel stimulation, averaged over concurrent and non-concurrent task condition. Red squares indicate mean RT, coloured dots indicate individual RT (*$p < 0.05$). (b) Scatter plots with regression lines of stimulation effect (sham-anodal) on Flanker RT and individual normal E-field component (E_n) for concurrent (con) and non-concurrent (non-con) task condition.

3.4.2 Neurophysiological Data

The ANOVAs of 2-back ERPs for non-target trials did not show a significant effect of stimulation or a significant stimulation*tDCS+target task interaction. Our ANOVAs of Flanker task ERPs for incongruent trials as well showed no significant effects.

Similarly, our analyses of 2-back task related alpha, theta and beta ERD/ERS revealed no significant *stimulation* effect or *stimulation*tDCS+target task* interaction. For the Flanker task ERD/ERS our analyses did not show a main effect of *stimulation* but a significant interaction effect of *stimulation*tDCS+target task* for beta ERD/ERS in an area covering the stimulation target area (AF7, Fp1, AF3, C5, Fpz, F3, F7, FC5, T7; 430 – 700 ms; see Figure 3-5). Pairwise comparisons showed increased oscillatory beta power following anodal compared to sham stimulation in the non-concurrent *tDCS+target task* condition (t = -2.744, p = 0.014; see Figure 3-5a). Additionally, following anodal stimulation beta power was increased for non-concurrent compared to the concurrent *tDCS+target task* condition (t = -4.16, p = 0.002).

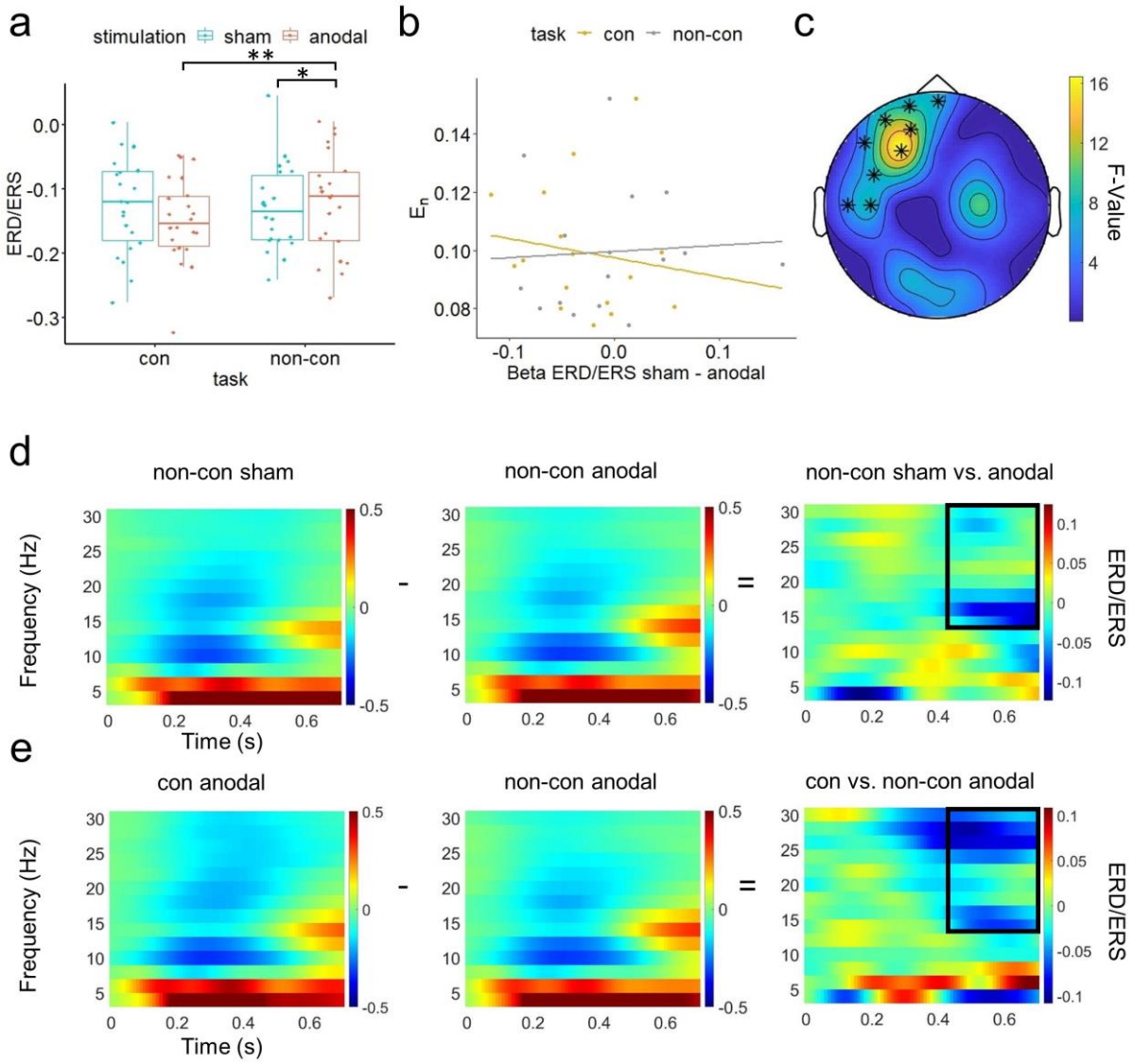

Figure 3-5. Stimulation*tDCS+target task interaction effect on Flanker task related beta ERD/ERS. (a) Boxplots of individual beta ERD/ERS averaged over channels and time in significant *stimulation*tDCS+target task* interaction cluster (AF7, Fp1, AF3, C5, Fpz, F3, F7, FC5, T7; 430 – 700 ms) for sham and anodal stimulation, separated for concurrent (con) and non-concurrent (non-con) task condition (*$p < 0.05$, **$p < 0.01$). (b) Scatter plot with regression lines of stimulation effect (sham-anodal) on Beta ERD/ERS and individual normal E-field component (E_n) for concurrent (con) and non-concurrent (non-con) task condition. (c) Topography of significant *stimulation*tDCS+target task* interaction effect of Flanker related beta ERD/ERS marked by asterisks. (d) Flanker task related time-frequency representation (TFR), averaged across electrodes forming significant *stimulation*tDCS+target task* interaction cluster. From left to right: Flanker task TFR following non-concurrent sham stimulation, TFR following non-concurrent anodal stimulation, difference in TFR for non-concurrent condition between sham and anodal stimulation. The black box indicates the significant difference in beta oscillatory power between both conditions. (e) Flanker TFR, averaged across electrodes forming significant *stimulation*tDCS+target task* interaction cluster. From left to right:

Flanker task TFR following concurrent anodal stimulation, TFR following non-concurrent anodal stimulation, difference in TFR for anodal stimulation condition between concurrent and non-concurrent task condition. The black box indicates significant difference in beta oscillatory power between both conditions.

Two-way repeated measures ANOVAs of resting state frequency data did not show a significant *stimulation* effect for any frequency band. Also, the interaction of *stimulation*tDCS+target task* was not significant in all frequency band.

3.4.3 Correlation of E-field and Stimulation Effects

We did not find a significant correlation between the individual normal component of the E-field and stimulation effects on behavioural or neurophysiological outcomes. Especially the significant stimulation effect on Flanker RT was not correlated with the individual E-field component (see Figure 3-4b), neither for the concurrent stimulation condition (r = -0.13, p = 0.612) nor for the non-concurrent *tDCS+target task* condition (r = 0.13, p = 0.619). The same was true for the stimulation effect on Flanker task related beta oscillatory power and the individual E-field component for the concurrent (r = -0.15, p = 0.578) and non-concurrent *tDCS+target task* condition (r = 0.06, p = 0.816; see Figure 3-5b).

3.4.4 Tolerability and Blinding of tDCS

For the conditions sham tDCS without concurrent 2-back task ($\chi 2$ (1, N=22) = 0.18, p = 0.669), sham tDCS with concurrent 2-back task ($\chi 2$ (1, N=22) = 1.81, p = 0.179) and anodal tDCS with concurrent 2-back task ($\chi 2$ (1, N=20) = 2.91, p = 0.088) participants were unable to guess better than chance whether they had received anodal or sham stimulation, while for the anodal tDCS without concurrent 2-back task condition the rate of correct assumptions was significantly higher than guess probability ($\chi 2$ (1, N=22) = 4.54, p = 0.033). In general, participants had a higher hit rate for anodal (70.5 % correct guess vs. 29.5 % incorrect guess) than for sham stimulation (54.7 % correct guess vs. 45.3 % incorrect guess). The intensity of perceived side effects was generally low (see Supplementary Table 3-5). Only the intensity of

perceived itching during stimulation was significantly higher under anodal compared to sham stimulation ($z = 2.52$, $p = 0.012$). All other side effects did not differ significantly between both stimulation conditions.

3.5 Discussion

The goal of the present study was to examine the effects of multichannel anodal tDCS targeting the left DLPFC in children and adolescents aged 10 – 18 years in combination with the investigation of concurrent task performance during stimulation and individual anatomy as potential influencing factors. We could show that 1) anodal multichannel stimulation resulted in only limited effects compared to sham stimulation. We could not demonstrate a stimulation effect on WM performance in a 2-back task (target task) neither during nor following tDCS. The 2-back task related as well as resting state neurophysiological activity was also not affected by the stimulation. 2) In a Flanker task (non-target task), which was performed after tDCS application and following the performance of the 2-back task, we found a reduction of RT following anodal tDCS. 3) This tDCS effect was independent of whether tDCS had been combined with a cognitive task (2-back task). However, increased Flanker task related beta oscillation was observed only following stimulation without concurrent 2-back task performance. 4) The individual normal E-field component was not correlated with stimulation effects. 5) The stimulation led to minor side effects. However, one participant experienced a serious adverse event during her study participation.

3.5.1 tDCS Effects on Target (2-back Task) Behavioural and Neurophysiological Outcomes

Unlike our study with adults (Splittgerber et al., 2020), the current study showed no effect on WM performance and task-related neurophysiological activity, using the same multichannel montage targeting the left DLPFC. Based on results in tDCS over the motor cortex, anodal stimulation with the same intensity should have an excitatory effect in both

children and adults (Moliadze et al., 2015). However, tDCS results cannot easily be transferred from one brain area to another.

Our partly null finding could be due to a suboptimal combination of stimulation parameters. Based on computational modelling, a multichannel montage leads to a more focused stimulation while a classical bipolar montage leads to a comparatively diffuse current flow in the brain (Laakso et al., 2016; Salvador et al., 2021). However, it is not clear whether a more focal stimulation also causes stronger tDCS effects. WM is a cognitive process based on a widely distributed neural network (Eriksson, Vogel, Lansner, Bergström, & Nyberg, 2015; Rottschy et al., 2012). A multichannel montage may not be able to sufficiently activate this underlying network due to its focality, which may lead to reduced effects on performance, while larger electrodes allow simultaneous activation of different relevant brain regions. This assumption is supported by the partly null results of Hill et al. (2019) and Savic, Müri and Meier (2019), who investigated the effects of HD tDCS over the DLPFC on WM and implicit task sequence learning and consolidation. Furthermore, Sotnikova et al. (2017) showed that classical bipolar tDCS over the left DLPFC compared to sham stimulation caused greater activation in several, distributed areas, including the left DLPFC, left premotor cortex, left supplementary motor area and precuneus.

Furthermore, the 2 mA total injected current used in this study may not have been sufficient in every participant in terms of induced E-field strength in the target area to lead to detectable tDCS effects (Vöröslakos et al., 2018). More important than simply increasing the current intensity seems to be the individualisation of the stimulation in order to ensure that a sufficient and at the same time safe current intensity is applied to each participant (Evans et al., 2020; Salvador et al., 2021). The multi-channel montage we used was optimised for the target region left DLPFC, but only based on a standard brain. A next step would be to individualise the optimisation with respect to the participant specific neuroanatomy.

Another explanation for the missing stimulation effects on WM could be the lack of fit between our WM task and the healthy sample we examined. Healthy participants often seem to be close to or at the optimum of their performance and leave little room for a stimulation effect (Hill et al., 2019). In adults it has been shown for various outcome measures that low performers are more likely to benefit from stimulation than high performers (Gözenman & Berryhill, 2016; Ruf, Fallgatter, & Plewnia, 2017; Tseng et al., 2012). Regarding WM performance, imaging studies also prove that the activation of the DLPFC is dependent on cognitive load (Manoach et al., 1997). A higher cognitive load therefore leads to a stronger neural activation and thus potentially enables stronger tDCS effects. At the same time the DLPFC activation is related to the individual WM capacity (van Snellenberg et al., 2015) and shows a nonmonotonic, inverted-U response to WM load (*inverted-U-hypothesis*; Callicott et al., 1999). Therefore, an excessive cognitive load could also be an inhibitor for a possible tDCS effect. Hoy et al. (2013) demonstrated a tDCS effect on 2-back but not 3-back task performance and suspected a "cognitive resources ceiling effect" during the more effortful 3-back task. Compared to a standard 2-back task, the 2-back task we used was complicated by the requirement to respond to each stimulus, not only target stimuli, and the use of images that were very similar but not identical (lures). The 2-back accuracy during stimulation was shown to be dependent on the age of the participants, which reflects differences in performance levels between the participants and may indicate a possible overload in younger children. Thus, it can be hypothesised that some participants were overchallenged by the task and the optimum of their DLPFC activation had already been exceeded. Hence, the WM task used may have been too simple for some participants while it was too difficult for others to allow tDCS effects to occur. An alternative could be an adaptive n-back task, which ensures maximum cognitive load for each participant individually, as it has been shown for adults (Ruf et al., 2017).

3.5.2 tDCS Effects on Resting State Neurophysiological Activity

Results of the resting-state EEG recordings showed that theta, alpha and beta power did not differ significantly between active and sham anodal tDCS conditions. These results correspond to previous studies in adults demonstrating no tDCS effect on resting state neurophysiological activity (Gordon et al., 2018; Hill et al., 2019; Horvath et al., 2015). The missing tDCS influence on resting state EEG might be due to a state dependency of stimulation effects (Hsu, Juan, & Tseng, 2016; Silvanto, Muggleton, Cowey, & Walsh, 2007).

3.5.3 tDCS Effects on Non-Target (Flanker Task) Behavioural and Neurophysiological Outcomes

In contrast to the 2-back task, we found a tDCS effect on behavioural and neurophysiological outcomes in the non-target Flanker task. Our results are consistent with previous studies in adults that also found reduced RT in a Flanker task after tDCS over the left DLPFC (Dubreuil-Vall et al., 2019; Karuza et al., 2016). Still, it should be emphasised that the stimulation effects in the Flanker task were small and limited to RT and specific neuronal oscillations. As for WM functioning, the functions relevant for the Flanker task, such as interference control, sustained attention and response inhibition, are based on a network of different brain areas. This network includes the DLPFC and the anterior cingulate cortex as critical hubs (Fan, Flombaum, McCandliss, Thomas, & Posner, 2003). We cannot exclude the possibility that our multichannel montage activated the circuits involved in the Flanker task more strongly than the 2-back related WM network due to the distribution of the electrodes on the skull.

3.5.4 Influence of Concurrent Task Performance During Stimulation

Interestingly, the tDCS effect on Flanker task performance occurred regardless of whether tDCS had been combined with a cognitive task or not. The fact that improvements in Flanker task performance were also found after anodal tDCS with concurrent 2-back task performance argues against task-specific effects of stimulation, as assumed by the activity

selectivity model (Bikson & Rahman, 2013; Fertonani & Miniussi, 2017). The Flanker task is mainly applied to investigate interference control, but also relies on WM processes. Working memory, in turn, is composed of different executive functions (Baddeley, 2012; Diamond, 2013). While the 2-back task particularly requires an updating component (Collette & van der Linden, 2002), the Flanker task especially requires an inhibition component (Sanders & Lamers, 2002). Both WM components seem to be closely connected functionally (Scharinger, Soutschek, Schubert, & Gerjets, 2015; Szmalec, Verbruggen, Vandierendonck, & Kemps, 2011) and structurally (Nee et al., 2013), which is why a transfer of stimulation effects is conceivable. Additionally, following the flexible hub theory, a general enhancement of the left DLPFC activity could contribute to the improvement in different tasks (Cole et al., 2013; Zanto & Gazzaley, 2013). At the same time, the changes in Flanker task performance cannot be clearly attributed to stimulation in isolation since this task was performed as second task after the application of stimulation and 2-back task performance. This increased strain on the target area left DLPFC through the stimulation and the following execution of the 2-back task after stimulation might have had effects on the neuroplasticity and thus on the performance in the Flanker task (Vosskuhl, Strüber, & Herrmann, 2018).

Besides the behavioural changes, we found increased Flanker task related beta activity over frontal channels after anodal tDCS applied without concurrent 2-back task performance. Beta oscillation is often associated with the sensorimotor system (Schmidt et al., 2019). In the prefrontal cortex a connection of beta oscillations with WM, executive control of action and distraction prevention was observed (Miller, Lundqvist, & Bastos, 2018; Swann et al., 2009; Zavala, Jang, & Zaghloul, 2017). Tafuro et al. (2019) have investigated the role of beta oscillations for cognitive control. They found an engagement of beta frequencies in frontal regions in a stroop task, which seemed to be especially involved in interference control processes. These results correspond to a study by Ambrosini and Vallesi (2017) who also found an association between left lateralized prefrontal beta activity and interference control.

Studies in adults suggest an activity selectivity of neurophysiological changes following tDCS combined with task performance (Hill et al., 2019; Pisoni et al., 2018). In our study, the 2-back task during stimulation could also have led to more selective activation, which may have counteracted a transfer effect. However, in this case, neurophysiological activity seems to be more sensitive to this influence than behavioural activity. Therefore, regarding our hypothesis that the effects of stimulation depend on the performance of a task during stimulation, we cannot draw a definite conclusion.

3.5.5 Influence of Anatomy

The individual (brain) development status influences the current flow, as modelling studies suggest (Kessler et al., 2013; Opitz, Paulus, Will, Antunes, & Thielscher, 2015), which subsequently might affect stimulation effects (Albizu et al., 2020; Antonenko et al., 2019). Further, studies in adults confirm stronger tDCS effects with higher current intensities in the stimulation target area (Albizu et al., 2020; Antonenko et al., 2019; Kim et al., 2014). However, we did not find a connection between tDCS effects and calculations of the individual E-field. This could be because we computed the individual E-field only for a subsample, which may have masked possible correlations. Besides, we determined the participant-specific stimulation effect only in relation to the sham condition. Other studies that have investigated the relationship between individual E-field and tDCS effects have mostly determined the stimulation effect in relation to a pre-stimulation baseline (Kim et al., 2014; Laakso, Mikkonen, Koyama, Hirata, & Tanaka, 2019). In this way, tDCS effects can be examined more precisely, for example by considering the participants day-specific individual performance level.

3.5.6 Aspects of Tolerability for Multichannel Anodal tDCS

For adults we have shown previously that multichannel tDCS targeting the left DLPFC is well tolerated (Splittgerber et al., 2020). In children and adolescents, a 2 mA multichannel montage has not been investigated so far. In the current study, all participants reported only

mild side effects directly following stimulation. Nevertheless, our study demonstrates the importance to adapt tDCS for applications in a paediatric group, also regarding safety aspects. The case of a female participant, who developed an epileptic disease during her participation in this study, shows that the screening procedure needs to ask more specifically for neurological abnormalities (Sierawska et al., 2020; Splittgerber et al., 2019). Additionally, our study demonstrates the importance to proactively ask for adverse events following stimulation sessions, as it is procedure in clinical trials.

3.5.7 Limitations

There are some limitations in our study. One focus of our study was the correlation between the individual anatomy and the effects of the stimulation. Due to a relatively large drop-out, we were only able to include a comparatively small sample (n = 22) in the evaluation. Furthermore, the E-field distribution was only computed for a subsample. The small sample size is a methodological limitation that cannot be denied. At the same time, experiments on children should start with small samples to avoid exposing a large population to potential risks.

Furthermore, the acquisition of a task baseline at each visit would have been an additional control for intraindividual differences. Especially the investigation of the influence of individual E-field on tDCS effects would have benefited from such a baseline. Still, we were able to determine stimulation effects with regards to sham stimulation.

Another limitation is that participants were not effectively blinded whether they had received anodal or sham stimulation in the non-concurrent anodal tDCS condition. However, the tDCS effect on Flanker task performance was independent of whether tDCS was applied with concurrent or non-concurrent task performance. Besides, no stimulation effect in the target 2-back task was observed.

3.5.8 Conclusion

Our study demonstrates the importance of methodologically well-defined study designs, including the use of control tasks, since stimulation effects might otherwise remain undetected. While no tDCS related changes in a target WM task were observed, tDCS improved performance in a non-target task investigating interference control, independent of concurrent task performance during stimulation. While this behavioural data argues against an activity selectivity effect of stimulation, neurophysiological activity was only affected following offline stimulation. Therefore, neurophysiological activity might be stronger affected by concurrent task performance than behavioural outcomes. Further, tDCS studies in children and adolescents should use screening procedures adapted to this age group and inquire about adverse events after each stimulation session, to prevent dangers from the stimulation.

3.6 References

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3.7 Supplementary

Table 3-5

Mean intensity (scale 0–3, Ø) and comparison of side effects for anodal and sham stimulation.

	sham	anodal	z	p
Itching sensation	**0.38**	**0.81**	**- 2.51**	**0.012**
Pain	0.23	0.35	- 1.01	0.314
Burning sensation	0.23	0.42	- 1.41	0.161
Warmth/Heat	0.04	0.19	- 1.62	0.105
Metallic/Iron taste	0.02	0.00	0	1
Fatigue/Decreased alertness	0.97	0.92	0.09	0.923

4 Study III

The Effects of 1 mA tACS and tRNS on Children/Adolescents and Adults: Investigating Age and Sensitivity to Sham Stimulation.

Splittgerber, M., Suwelack, J. H., Kadish, N. E., & Moliadze, V. (2020). The Effects of 1 mA tACS and tRNS on Children/Adolescents and Adults: Investigating Age and Sensitivity to Sham Stimulation. *Neural Plasticity, 2020*, 8896423. https://doi.org/10.1155/2020/8896423

4.1 Abstract

The aim of this study was to investigate the effect of transcranial random noise (tRNS) and transcranial alternating current (tACS) stimulation on motor cortex excitability in healthy children and adolescents. Additionally, based on our recent results on the individual response to sham in adults, we explored this effect in the pediatric population.

We included 15 children and adolescents (10–16 years) and 28 adults (20–30 years). Participants were stimulated four times with 20 Hz and 140 Hz tACS, tRNS and sham stimulation (1 mA) for 10 minutes over the left $M1_{HAND}$. Single pulse MEPs (motor evoked potential), short interval intracortical inhibition and facilitation were measured by TMS before and after stimulation (baseline, 0, 30, 60 minutes). We also investigated aspects of tolerability. According to the individual MEPs response immediately after sham stimulation compared to baseline (Wilcoxon signed rank test), subjects were regarded as responder or non-responder to sham.

We did not find a significant age effect. Regardless of age, 140 Hz tACS led to increased excitability. Incidence and intensity of side effects did not differ between age groups or type of stimulation. Analyses on *responders* and *non-responders* to sham stimulation showed effects of 140 Hz, 20 Hz tACS and tRNS on single pulse MEPs only for *non-responders*.

In this study, children and adolescents responded to 1mA tRNS and tACS comparably to adults regarding the modulation of motor cortex excitability. This study contributes to the findings that noninvasive brain stimulation is well tolerated in children and adolescents including tACS, which has not been studied before. Finally, our study supports a modulating role of sensitivity to sham stimulation on responsiveness to a broader stimulation and age range.

4.2 Introduction

Non-invasive transcranial brain stimulation (NTBS) may modulate cortical excitability, outlasting the period of NTBS itself from several minutes to more than one hour (Huang et al., 2017). Transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) are the most commonly used methods of NTBS (Huang et al., 2017). Transcranial alternating current stimulation (tACS) is an increasingly popular NTBS technique (Herrmann, Rach, Neuling, & Strüber, 2013), with the advantage of enabling manipulation and entrainment of intrinsic oscillations through the injection of sinusoidal currents (Antal & Paulus, 2013; Paulus, 2011; Thut, Schyns, & Gross, 2011). The transcranial random noise stimulation (tRNS) paradigm was developed with a potential to desynchronize normal and pathological cortical rhythms. The frequency band of tRNS can encompass a full range (typically from 0.1 to 640 Hz) or can be delivered at low (0.1–100 Hz)- or high-frequency (101–640 Hz). The concept of tRNS is to enhance the stochastic dynamics of neurons and thus facilitate the neural processing and the related behavior (Pavan et al., 2019; Terney, Chaieb, Moliadze, Antal, & Paulus, 2008) for review see (Antal & Herrmann, 2016).

Until recently, NTBS has been mainly investigated in adults, while studies in children and adolescents are still limited, focusing on TMS and tDCS (Finisguerra, Borgatti, & Urgesi, 2019). Yet, effects in this group are of interest, as they might have accelerated neural plasticity compared to adults after brain stimulation (Brunoni et al., 2012). Therefore, NTBS is expected to have even greater potential to regulate and enhance plasticity in the pediatric population. Indeed, rather than considering it as a small adult brain, a child's brain should be considered as a unique physiological entity (Davis, 2014; Maslen, Earp, Cohen Kadosh, & Savulescu, 2014). At the same time, extreme caution is needed while dealing with a developing brain, mainly because of a lack of translational studies from adults to children (Cohen Kadosh, Levy, O'Shea, Shea, & Savulescu, 2012).

In the pediatric population, tRNS has rarely been applied (Looi et al., 2017) and tACS has not yet been studied. In fact, investigating oscillation-specific effects in children is of special interest. In fact, investigating oscillation-specific effects in children is of special interest e.g. developing the basis for potential treatment options (tRNS/tACS) with a promising safety profile in a vulnerable young population. Therefore, we investigated tRNS and tACS with frequencies both within and outside the conventional electroencephalography (EEG) frequency range (20 Hz and 140 Hz) in children and adolescents. In adults, stimulation in the beta frequency range (~13 – 30 Hz) have been studied extensively, using EEG, they are linked to a variety of cognitive and sensorimotor processes (Baker, 2007). For example, Pogosyan and colleagues used a stimulation frequency of 20 Hz, a prominent beta band oscillatory frequency found in the motor system, to study the effect of tACS on movement speed (Pogosyan, Gaynor, Eusebio, & Brown, 2009). The results show that, whilst reaction times were not affected, the subject's voluntary movements were decreased in velocity. Additionally, more recent studies show that tACS in the ripple range (especially 140 Hz) can modulate cortical excitability (Ambrus et al., 2015; Moliadze, Antal, & Paulus, 2010a). tRNS in healthy adults can modulate cortical excitability and improve high-level cognitive functions (Chaieb, Paulus, & Antal, 2011; Fertonani, Pirulli, & Miniussi, 2011; Popescu et al., 2016; Terney et al., 2008).

In this study, we aim to understand factors determining efficacy of NTBS and individual differences in response in relation to age. A classical experimental design was chosen in order to compare results of the current study with previous results obtained in healthy adults (Kortuem, Kadish, Siniatchkin, & Moliadze, 2019; Moliadze et al., 2010a; Moliadze, Atalay, Antal, & Paulus, 2012; Terney et al., 2008). Specifically, the aim of the current study was:

1) To provide an exploratory investigation of tRNS and 20 Hz as well as 140 Hz tACS in children and adolescents comparing them to adults. The exploratory nature of our analysis is based on the following assumptions: On the one hand children generally show increased plasticity relative to adults and are thus expected to respond more favorably to non-invasive

brain stimulation. However, on the other hand 1mA anodal tDCS shows the same excitatory effect both in children and adults (Moliadze, Schmanke, et al., 2015). Based on the excitatory nature of 1mA 140 Hz tACS and tRNS (Moliadze et al., 2010a; Moliadze et al., 2012; Terney et al., 2008), one could therefore expect excitatory effects for these stimulation types for all ages. Additionally, in the case of tRNS and tACS not only intensity but also frequency plays a role in how it affects the brain. Therefore, we cannot predict the influence of frequency in the developing brain.

Regarding 20 Hz, previous results are heterogenous with some studies showing an inhibitory effect in adults (for review see (Wischnewski, Schutter, & Nitsche, 2019)); therefore, this too is treated as an exploratory hypothesis.

2) In the light of the lack of respective research in pediatrics, we also investigated aspects of tolerability for tACS and tRNS.

3) In our recent study (Kortuem et al., 2019) we explored whether neurophysiological response to sham over the motor cortex could influence response to active stimulation. Response to sham was evaluated based on changes in MEPs immediately after sham stimulation compared to baseline MEPs with a Wilcoxson signed rank test. We found that subjects who responded to sham stimulation turned out to be non-responders to verum stimulation when applying tRNS and 140 Hz tACS, while non-responders to sham showed the expected effects to verum stimulation. Based on this role of the individual response to sham in adults, we explored this effect in the pediatric population. We were therefore interested to see whether sensitivity to sham affects response to verum stimulation and whether a possible effect might be more predictive of response than age.

4.3 Materials and Methods

The study was part of a project investigating different factors which influence variability of tACS and tRNS (Kortuem et al., 2019). Experimental procedures were approved by the local

ethics committee of the Kiel University, Kiel, Germany. All participants and their parents were instructed about the study and written informed consent according to the Declaration of Helsinki on biomedical research involving human subjects was obtained.

4.3.1 Subjects

We included 15 healthy children and adolescents (8 males) aged 10–16 years (M 13.3; SD 2.1) and 28 healthy young adults (19 males) aged 20–30 years (M 24.4; SD 2.5; for details see Table 4-1). The adult sample in this study has been included in our previous study (Kortuem et al., 2019). All participants were right-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971). Exclusion criteria were pregnancy, history of migraine, unexplained loss of consciousness, or brain related injury, IQ < 90, history or family history of epileptic seizures, history of other neurological, psychiatric or chronical internistic disorders, intake of central nervous system-effective medication, brain- or cardiac- pacemakers, or not removable metal head implants.

Table 4-1

Subject characteristics and thresholds before stimulation for age groups (children/adolescents, adults) and response to sham groups (responder to sham, non-responder to sham)

Age groups	*n*	Sex	Age ± SD	SI_{1mV} (%) ± SD	RMT (%) ± SD	AMT (%) ± SD	Baseline MEP (mV) ± SD
Children/ Adolescents	15	8M/ 7F	13.3 ± 2.1				
Sham				61.7 ± 10.5	52.8 ± 4.9	44.8 ± 8.7	0.98 ± 0.18
tRNS				64.1 ± 10.6	54.7 ± 6.5	46.2 ± 8.4	0.94 ± 0.19
140 Hz tACS				62.9 ± 10.6	52.8 ± 6.6	45.1 ± 8.2	0.93 ± 0.16
20 Hz tACS				63.2 ± 10.8	54.5 ± 6.1	46.6 ± 8.9	0.99 ± 0.18
Adults	28	19M/ 9F	24.4 ± 2.5				
Sham				56.2 ± 10.1	48.5 ± 8.2	40.8 ± 8.1	0.96 ± 0.08
tRNS				56.7 ± 10.8	48.8 ± 9.1	40.9 ± 8.7	0.96 ± 0.09
140 Hz tACS				55.5 ± 10.3	47.4 ± 8.6	39.1 ± 8.1	0.98 ± 0.09
20 Hz tACS				55.5 ± 10.8	47.9 ± 8.9	40.2 ± 7.6	0.98 ± 0.09
Response to sham groups	*n*	Sex	Age ± SD (adults/ children)	SI_{1mV} (%) ± SD	RMT (%) ± SD	AMT (%) ± SD	Baseline MEP (mV) ± SD
Responder to sham	21	13M/ 8F	20.9 ± 4.7 (16/5)				
Sham				58.8 ± 8.5	50.4 ± 6.8	43.1 ± 7.4	0.97 ± 0.11
tRNS				58.3 ± 10.5	49.3 ± 8.1	42.8 ± 8.5	0.94 ± 0.14
140 Hz tACS				57.3 ± 8.7	48.1 ± 6.3	41.1 ± 6.7	0.98 ± 0.09
20 Hz tACS				57.9 ± 7.9	49.1 ± 6.5	42.3 ± 6.6	0.94 ± 0.11
Non-responder to sham	22	14M/ 8F	20.1 ± 6.9 (12/10)				
Sham				57.5 ± 12.2	48.4 ± 8.7	41.3 ± 9.4	0.96 ± 0.14
tRNS				60.2 ± 12.1	51.3 ± 9.7	42.7 ± 9.5	0.97 ± 0.11
140 Hz tACS				58.9 ± 12.8	49.3 ± 10.6	41.3 ± 10.2	0.96 ± 0.15
20 Hz tACS				58.4 ± 14.1	49.8 ± 10.9	42.5 ± 10.3	1.03 ± 0.14

Note. Data presented in means ± SD; SD = Standard deviation; F = female; M = male; SI = stimulus intensity; RMT = resting motor threshold; AMT = active motor threshold; MEP = motor evoked potential.

4.3.2 Stimulation Techniques

tACS/tRNS was delivered by a DC stimulator (NeuroConn GmbH, Ilmenau, Germany) through a pair of saline-soaked rectangular sponge electrodes (5 × 7 cm). The motor cortex electrode was fixed over the area representing the right first dorsal interosseus (FDI) muscle as

identified by TMS. The other electrode was fixed over the contralateral supraorbital area. This electrode set-up has been shown to be the optimal combination to enhance excitability of the M1 (Moliadze, Antal, & Paulus, 2010b). The electrodes were held in place by rubber bands. Stimulation was applied at 20, 140 Hz, tRNS and sham for 10 minutes. Peak-to-peak current intensity was 1mA (between -0.5mA and 0.5mA). Ramping at the beginning and the end of the stimulation was 5 s in all stimulation conditions. In the sham condition, 30 s of tACS was applied.

The waveform of the 20 Hz and 140 Hz stimulation was sinusoidal (no DC offset, no phase shift). For whole spectrum tRNS in the stimulation mode "noise" there was a random level of current generated for every sample (sampling rate 1280 sps). The random numbers were normally distributed; the probability density function followed a bell-shaped curve. In the frequency spectrum, all coefficients had a similar size ("white noise"). The noise signal contained all frequencies up to half of the sampling rate, i.e. a maximum of 640 Hz. Due to the statistical characteristics, the signal had no DC offset.

4.3.3 Monitoring of Motor Cortical Excitability

Stimulus intensities of TMS were measured as percentage of maximal stimulator output (MSO %) and determined at the beginning of each experiment. To detect changes in cortical excitability, MEPs of the right FDI were recorded following a single-pulse TMS of its representation area on M1. A Magstim 200 magnetic stimulator (Magstim Company, Whiteland, Wales, UK) with a figure-of-eight standard double magnetic coil (diameter of one winding 70 mm; peak magnetic field 2.2 T; average inductance 16.35μH) was used. A surface electromyogram (EMG) was recorded from the right FDI through a pair of Ag-AgCl surface electrodes in a belly tendon montage (Nihon Kohden Europe, Rosbach, Germany). The amplified raw-data was band-pass filtered (2Hz–2kHz; sampling rate, 5kHz) and digitized with a micro 1401 AD converter (Cambridge Electronic Design, Cambridge, UK) controlled by

Signal Software (Cambridge electronic Design, version2.13). For offline analysis, data was stored on a computer. Complete relaxation was controlled through visual feedback of EMG activity; in case of tension the subject was reminded to relax. The eight-curved coil was held tangentially to the skull at 45∘ from the sagittal-line, which results in a posterior to anterior direction of current flow in the brain. The optimum position was defined as the site where TMS resulted consistently in the largest and most stable MEP in the resting muscle. This spot was marked with a skin marker pencil to ensure that the coil was held in the correct position throughout the experiment.

4.3.3.1 Motor Threshold Determination

The resting motor threshold (RMT) was determined as the minimum stimulator output needed to produce a response of at least 50 μV in the relaxed FDI in at least 3 of 6 consecutive trials. The active motor threshold (AMT) was defined as the lowest stimulus intensity at which 5 out of 10 consecutive stimuli elicited reliable MEPs (above 200 μV in amplitude) during isometric contraction of the contralateral FDI muscle in at least 3 of 6 recordings (Awiszus, 2003; Rothwell et al., 1999).

4.3.3.2 Single-Pulse MEPs (SI 1mV)

The intensity required to evoke a MEP of ~ 1mV peak-to-peak amplitude (SI 1mV) and a baseline of TMS-evoked MEPs (20 stimuli) were recorded at 0.25 Hz.

4.3.3.3 Intracortical Inhibition and Facilitation

Changes in intracortical excitability were monitored using short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF). A conditioning stimulus (CS, first pulse) was set to 80 % of the AMT, while the test pulse (TS, second stimulus) was set to the SI 1mV threshold. The conditioning stimulus inhibits the MEP amplitude elicited by the test stimulus at short interval (1 - 5 ms) whereas it facilitates it at longer interval (6 – 20 ms; Kujirai et al., 1993; Rossini et al., 2015). In the present study, we measured SICI at 2 ms ISI because inhibition was reported to be maximal and expressed without contamination by short-interval

intracortical facilitation (SICF), ICF or any refractoriness of neural elements at this interval (Di Lazzaro et al., 2000; Wagle-Shukla, Ni, Gunraj, Bahl, & Chen, 2009; ULF Ziemann, Rothwell, & Ridding, 1996). For ICF, we chose an ISI of 12 ms because we expected a maximal increase in MEP amplitude at the median ISI (6–20 ms) known to induce MEP facilitation (Fresnoza et al., 2018). We recorded 15 MEPs evoked by the TS and 15 MEPs evoked by the paired pulses (CS + TS) for SICI and ICF, separately.

4.3.4 Experimental Design and Procedure

For study design see Figure 4-1. A randomized sham-controlled study with a double-blind, within-subject design was implemented conducting all stimulation conditions in each participant. The order of the stimulation conditions (sham, tRNS, 140 Hz tACS, 20 Hz tACS) was counterbalanced across subjects. Sessions were separated by at least 7 days to avoid carry over effects. In each subject, the experimental sessions were performed at the same time during the day.

The subjects were seated in a comfortable chair with head and arm rests. First, the hotspot (the coil position that produced the largest MEPs of the right FDI) was identified by TMS. Then the stimulation intensity was adjusted to elicit single-pulse MEPs with peak-to-peak amplitudes of an average of 1mV and 20 MEPs were recorded prior to stimulation. After determination of SI1mv, RMT and AMT were obtained. After measuring AMT, a 15 minutes break followed to avoid an effect of muscle contraction on the next measurements. After this break SICI/ICF were measured.

Afterwards, 1 mA stimulation (sham, tRNS, 140 Hz tACS, 20 Hz tACS) was administered over 10 minutes. Following stimulation, 20 single test-pulse MEPs, followed by SICI and ICF in counterbalanced order were recorded at intervals of directly after (T0), 30 min (T30) and 60 min (T60) post stimulation. For SI 1mV TMS intensity was kept constant for the post-stimulation assessment; for the SICI/ICF, TMS intensity was readjusted to obtain single test pulse amplitudes of 1 mV, if needed.

After finishing each experimental session, the participant was asked to complete a stimulation side effects questionnaire adapted from (Poreisz, Boros, Antal, & Paulus, 2007). The questionnaire contains items pertaining to the presence and severity of headaches, change or difficulties in concentration, mood, visual perception, presence of fatigue, and discomforting sensations like pain, tingling, itching or burning.

Subjects as well as the investigator, who made the MEP measurements, were blinded for stimulation conditions in all studies. The stimulations were done by another investigator.

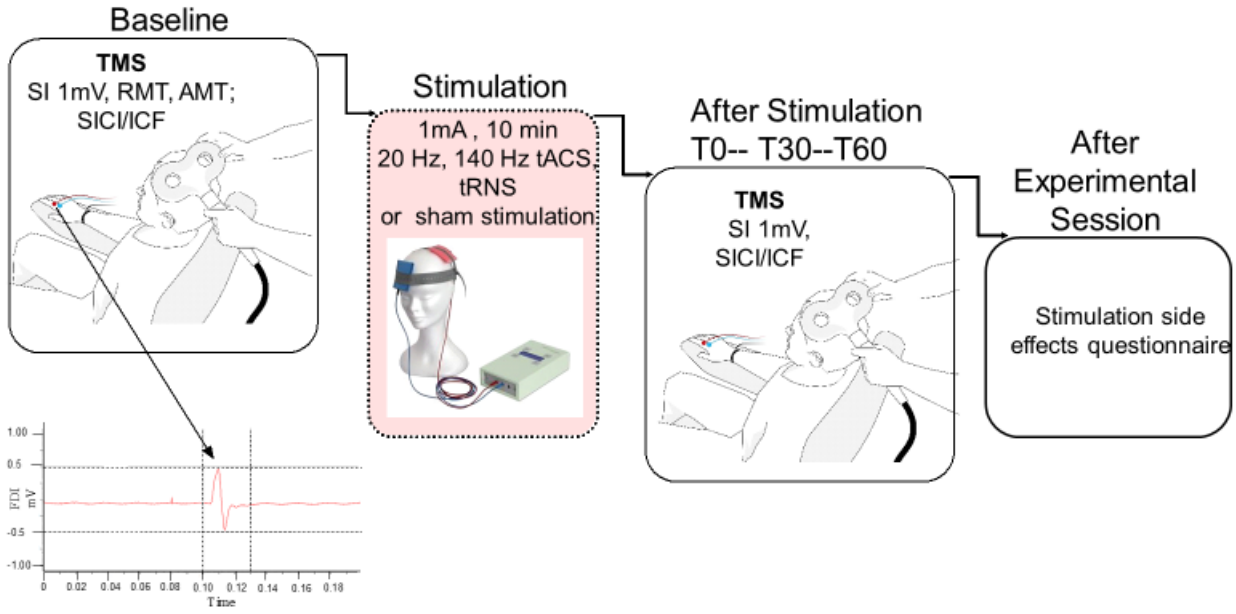

Figure 4-1. Design of the study. The figure illustrates the procedure for each experimental session. In the beginning of each session, 20 baseline single-pulse MEPs of SI 1mV amplitude, RMT, AMT, SICI/ICF were recorded. Afterwards 1mA tRNS, 20 Hz tACS, 140 Hz tACS or sham stimulation was applied over the left primary motor cortex for 10 minutes and then the SI 1mV, SICI/ICF were recorded again directly as well as 30 and 60 minutes after stimulation (T0-T60). After finishing each experimental session, the participant was asked to complete a stimulation side effects questionnaire.

4.3.5 Data Analysis and Statistics

4.3.5.1 MEP Analysis

Data analysis was completed manually by visual inspection of offline EMG data. Traces showing any muscle activity prior to the stimulus were removed from the analysis as well as MEPs with a distance of two standard deviation or more to the individual mean.

The MEP means of the participants for the SI 1mV and the means for each interstimulus interval in SICI (2ms) and ICF (12ms) were calculated for the adults and children group before and after stimulation. Post stimulation means of the SI 1mV threshold were standardized to the pre-stimulation mean, whereas the mean of the paired stimulation protocols (SICI and ICF) were normalized to the respective single pulse test condition.

4.3.5.2 Statistical Comparisons

All statistical analyses were conducted using the computing environment R (version 3.6.1, R Core Team (2016) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/). Throughout all analyses, results were regarded as statistically significant with a two-tailed p value of less than 0.05.

Stimulus intensities, baseline MEPs, RMT and AMT were compared between age groups (children/adolescents, adults) using Wilcoxon signed rank tests for matched samples, because of failed normal distribution. Furthermore, these baseline values were compared within the children/adolescents and the adults group using Friedman rank sum test, to exclude baseline differences between the different stimulation conditions.

MEPs for single-pulse (SI 1 mV) and paired-pulse TMS (SICI and ICF) were analyzed separately using linear mixed-effects models. Homogeneity of variances was inspected using Levene's test. SI 1 mv, SICI and ICF MEPs were log transformed to achieve normal distribution. In all models, we included the maximum number of random effects that allowed the model to converge. Each model included the fixed factors stimulation (sham, tRNS, 140 Hz tACS, 20 Hz tACS), time (T0, T30, T60), age group (children/adolescents, adults) and all corresponding interactions, as well as a random intercept for each participant as random factor.

Differences between baseline and post stimulation (T0, T30, T60) SI 1 mv in each age group were investigated using paired-samples t-tests or, in case of failed normal distribution, Wilcoxon signed rank tests for matched samples with Bonferroni-Holm correction.

In addition, according to our analyses described in Kortuem et al. (2019), the effect of corticospinal activity during sham stimulation on the individual response to tRNS and tACS was investigated. Therefore, response to sham was taken into consideration according to the previously published procedure. Response to sham was evaluated for each individual based on change in MEP amplitudes directly after stimulation (T0) compared to baseline MEP with a Wilcoxon signed rank test for matched samples. Based on the result of this test, subjects were categorized as either "responder" or "non-responder" to sham stimulation. Age and baseline parameters of TMS were compared between and within these groups using Wilcoxon signed rank tests and Friedman tests as described above. To assess whether responders and non-responders to sham were affected differently by tACS or tRNS, linear mixed models on log transformed MEPs for single-pulse (SI 1 mV) and paired-pulse TMS (SICI and ICF) were computed for both subgroups. The models contained the fixed factors stimulation (sham, tRNS, 140 Hz tACS, 20 Hz tACS) and time (T0, T30, T60) as well as a random intercept for each participant as random factor. Because of our restricted sample size (see Table 4-1) we did not include age as an additional factor. Also, differences between baseline and post stimulation (T0, T30, T60) SI 1 mv were investigated for both subgroups using paired-samples t-tests or, in case of failed normal distribution, Wilcoxon signed rank tests for matched samples with Bonferroni-Holm correction.

For all models degrees of freedom were approximated using the Kenward-Rogers method, analogous to repeated measures ANOVAs (Kenward & Roger, 1997). In case of significant F-values post-hoc tests comparing verum and sham stimulation were performed using the Tukey method.

4.3.5.3 Adverse Events Questionnaire

The incidence of each side effect was coded in a binary system (yes = 1, no = 0). The severity of each side effect was rated on a numerical analogue scale (NAS) from one to five, one being very mild and five being an extremely high intensity of any given side-effect.

Incidence and intensity of adverse effects were compared between age groups using Mann–Whitney U test. Furthermore, the number of adverse effects were compared between stimulation conditions using Cochran's Q test and McNemar's test, intensity was compared using Friedman test and Wilcoxon signed-rank test.

4.4 Results

4.4.1 Analyses on Age Groups

There was a significant difference in the MSO % needed to elicit a 1 mV peak-to-peak MEP amplitude for children/adolescents compared to adults, with higher intensities for children/adolescents than for adults (z = 3.98, p < 0.001). Furthermore, children/adolescents had significantly higher RMT and AMT compared to adults (RMT: z = 5.85, p < 0.001; AMT: z = 4.29, p < 0.001). Baseline MEPs did not differ between both groups (z = - 0.31, p = 0.763). Within the groups, these baseline TMS values (stimulus intensities, baseline MEPs, RMT and AMT) did not differ between the different stimulation conditions. For details see Table 4-1.

4.4.1.1 Single-Pulse MEPs (SI 1mV)

The linear mixed model for the SI 1mV MEP amplitudes revealed a significant main effect of *stimulation* (p < 0.001) and a trend for *time* (p = 0.073; see Table 4-2). The main effect of *age group* as well as all interactions were non-significant (all p > 0.05). Our post hoc tests for the factor *stimulation* showed significant higher SI 1 mV MEP amplitudes following 140 Hz stimulation compared to sham stimulation (t (451) = -2.64, p = 0.025, see Figure 4-2a). The SI 1mV MEP amplitudes did not differ between age groups following stimulation for any of the stimulation conditions (all p > 0.05; see Figure 4-2b).

Our investigations on differences between baseline and post stimulation SI 1 mV amplitudes showed significant results only for the adult group. Here, amplitudes were significantly increased 0 minutes (t (27) = 2.47, p = 0.039) and 60 minutes (t (27) = 2.68, p = 0.037) compared to baseline values following 140 Hz tACS.

Table 4-2

The results of linear mixed models (LMM) performed on SI 1mV, SICI and ICF for all participants, non-responder and responder to sham.

	Numerator *df*	Denominator *df*	*F* value	*p* value
SI 1 mv				
Stimulation	3	451	6.69	< **0.001**
Time	2	451	2.62	0.073
age group	1	41	0.57	0.451
stimulation × age group	3	451	0.31	0.816
time × age group	2	451	0.34	0.705
stimulation × time	6	451	1.01	0.423
stimulation × time × age group	6	451	0.59	0.736
SICI				
Stimulation	3	448	0.31	0.816
Time	2	448	0.11	0.897
age group	1	41	1.84	0.182
stimulation × age group	3	448	0.82	0.486
time × age group	2	448	1.19	0.304
stimulation × time	6	448	0.36	0.901
stimulation × time × age group	6	448	1.63	0.135
ICF				
Stimulation	3	448	0.19	0.901
Time	2	448	0.85	0.425
age group	1	41	0.64	0.426
stimulation × age group	3	448	0.28	0.839
time × age group	2	448	0.67	0.509
stimulation × time	6	448	0.28	0.839
stimulation × time × age group	6	448	1.15	0.328
Non-Responder to sham	Numerator *df*	Denominator *df*	*F* value	*p* value
SI 1 mv				
Stimulation	3	231	13.14	< **0.001**
Time	2	231	3.16	**0.044**
stimulation × time	6	231	0.51	0.803
SICI				
Stimulation	3	228	0.71	0.546
Time	2	228	1.91	0.149
stimulation × time	6	228	0.67	0.672
ICF				
Stimulation	3	228	1.29	0.277
Time	2	228	0.31	0.729
stimulation × time	6	228	0.85	0.531

(continued on next page)

Table 4-2 *(continued)*

Responder to sham	Numerator *df*	Denominator *df*	*F* value	*p* value
SI 1 mv				
Stimulation	3	231	0.76	0.513
Time	2	231	0.27	0.764
stimulation × time	6	231	1.02	0.411
SICI				
Stimulation	3	220	0.95	0.414
Time	2	220	1.48	0.229
stimulation × time	6	220	0.09	0.997
ICF				
Stimulation	3	220	0.12	0.942
Time	2	220	0.51	0.601
stimulation × time	6	220	1.17	0.319

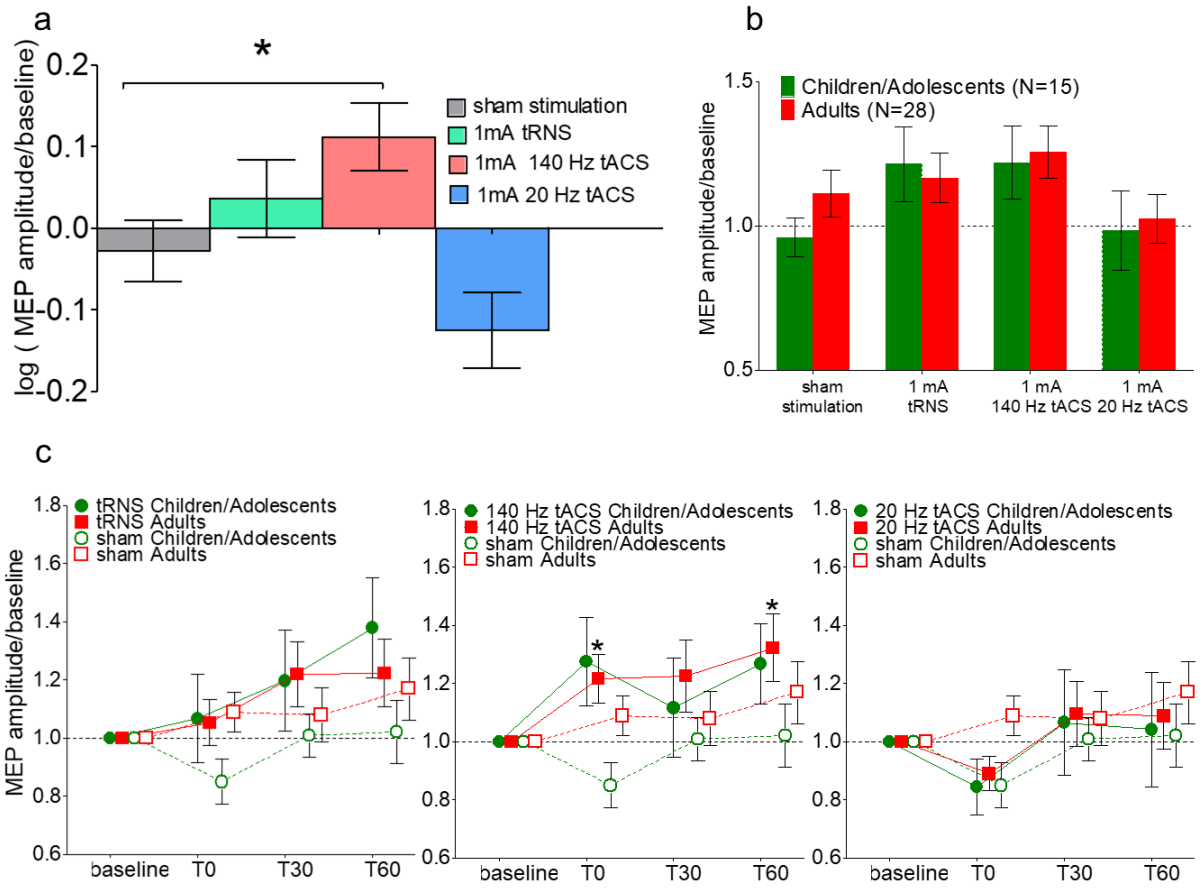

Figure 4-2. Analyses on age groups. The main effect of *age group* as well as all interactions were non-significant (all $p > .05$)**.** An asterisk indicates $p < 0.05$. **(A)** Mean SI 1mV MEP amplitudes (± SEM) of stimulation conditions. The y-axis depicts normalized and log transformed MEP amplitudes. The linear mixed model for the SI 1mV MEP amplitudes revealed a significant main effect of *stimulation* ($p < 0.001$). The post hoc tests for all 43 participants showed significant higher amplitudes following 140 Hz stimulation compared to sham stimulation **(B)** Mean SI 1mV MEP amplitudes (± SEM) of stimulation conditions in the children/adolescents and adults group. The y-axis depicts normalized MEP amplitudes. The SI 1mV MEP amplitudes did not differ between age groups for any of the stimulation conditions (all $p > 0.05$). **(C)** Time course of mean SI 1mV MEP amplitudes (± SEM) for each age group and each stimulation. The x-axis depicts the timepoints before and after stimulation. The y-axis displays normalized MEP amplitudes.

4.4.1.2 Intracortical Inhibition and Facilitation

The mixed models for SICI and ICF MEP amplitudes showed no significant effect of *stimulation*, *time*, *age group* or any interaction (all $p > 0.05$).

4.4.2 Side-Effects and Sensations

Table 4-3 displays the frequency and intensity of side effects for children/adolescents and adults. Incidence and intensity did not differ between age groups for any side effect. Comparisons between stimulation conditions were significant only for the adverse effects flickering, tingling and unpleasantness. Pairwise comparisons to sham stimulation showed increased incidence ($p < 0.001$) and intensity ($z = -3.71$, $p < 0.001$) of flickering for 20 Hz tACS and decreased incidence of flickering for tRNS ($p = 0.016$). Incidence but not intensity of unpleasantness was increased for 20 Hz tACS compared sham stimulation ($p = 0.031$).

Table 4-3

Incidence (sum) and intensity (scale 1-4, Ø) of side effects for the different stimulation conditions in the children/adolescents and adult subgroups

Children/ Adolescents	Incidence				Intensity			
	Sham	tRNS	140 Hz	20 Hz	Sham	tRNS	140 Hz	20 Hz
Burning	1	0	0	0	2	-	-	-
Difficulties in concentration	1	1	1	1	3	-	3	1
Fatigue	2	0	4	2	2.5	1	2.5	1
Flickering	2	2	0	6	1.5	-	-	2.5
Headache	0	1	0	0	-	1	-	-
Itching	1	0	1	2	1	1	1	1.5
Nervousness	0	0	0	1	-	-	-	1
Pain	1	2	0	1	2	-	-	2
Tingling	1	0	1	3	1	1	1	1.7
Unpleasantness	1	2	0	3	2	-	-	1
Adults	Incidence				Intensity			
	Sham	tRNS	140 Hz	20 Hz	Sham	tRNS	140 Hz	20 Hz
Burning	2	1	2	2	1.5	1	1	1.5
Difficulties in concentration	0	2	0	1	-	1.5	-	2
Fatigue	7	10	7	6	2.4	1.5	1.6	1.8
Flickering	6	2	1	16	1.5	2	2	2.1
Headache	1	1	0	0	1	1	-	-
Itching	1	0	3	4	2	-	1.7	1.3
Nervousness	1	2	1	3	2	1.5	1	1
Pain	2	3	0	3	1.5	1	-	1.7
Tingling	5	1	2	8	1.2	2	2	1.5
Unpleasantness	1	2	1	5	1	1.5	1	1.2

4.4.3 Analyses on Response to Sham Groups

Figure 4-3a shows the individual response to sham for all 43 participants and mean SI 1mV MEPs for the children/adolescents and adults group. Our analyses classified 22 participants as *non-responders* to sham and 21 participants as *responders* to sham. Age and baseline parameters of TMS did not differ between *non-responders* and *responders* to sham

and within each group for the different stimulation conditions (all $p > 0.05$). For details see Table 4-1.

4.4.3.1 Single-Pulse MEPs (SI 1mV) for Non-Responders to Sham Stimulation

For *non-responders* to sham, our linear mixed model for the SI 1 mV MEP amplitudes revealed a significant effect of *stimulation* ($p < 0.001$) and *time* ($p = 0.044$; see Table 4-2). Post hoc tests investigating the *stimulation* factor showed significant higher SI 1 mv amplitudes following 140 Hz tACS compared to sham stimulation (t (231) = -2.91, $p = 0.012$). Also, we found higher amplitudes following tRNS compared to sham stimulation (t (231) = -2.447, $p = 0.016$). Additionally, amplitudes were significantly lower following 20 Hz tACS compared to sham stimulation (t (231) = 2.66, $p = 0.016$, see Figure 4-3b).

We also conducted post hoc tests investigating the main effect of the factor *time.* These tests revealed that SI 1 mv amplitudes were generally lower at 0 minutes post stimulation compared to 60 minutes post stimulation (t (231) = -2.51, $p = 0.034$).

Our pairwise comparisons on baseline to post stimulation changes in SI 1 mV amplitudes showed increased amplitudes 0 ($z = 2.46$, $p = 0.018$), 30 ($z = 2.59$, $p = 0.018$) and 60 ($z = 2.98$, $p = 0.008$) minutes following 140 Hz tACS. Amplitudes were also increased 30 ($z = 2.66$, $p = 0.015$) and 60 ($z = 3.27$, $p = 0.003$) minutes following tRNS (see Figure 4-3c).

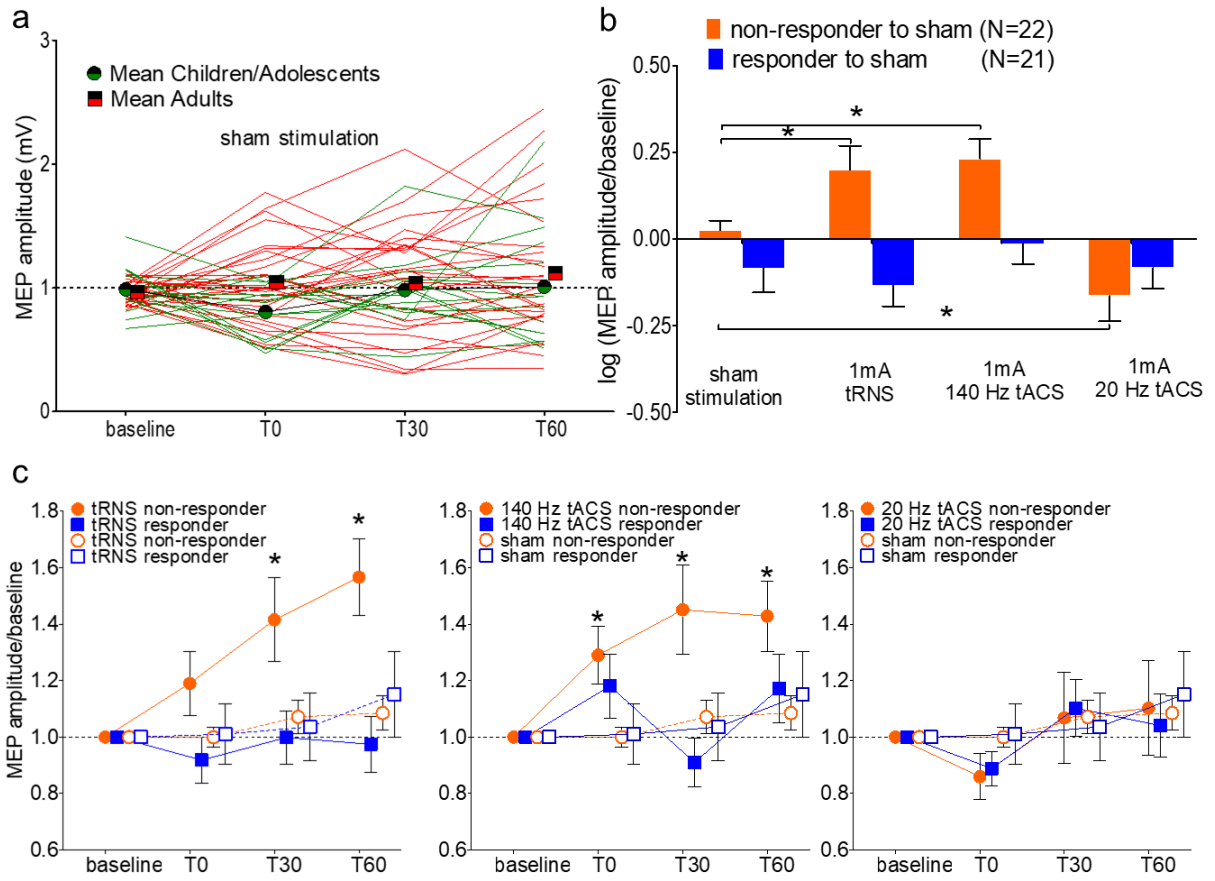

Figure 4-3. Analyses on response to sham groups. For *non-responders* to sham, the linear mixed model for the SI 1 mV MEP amplitudes revealed a significant effect of *stimulation* and *time*. An asterisk indicates $p < 0.05$. (A) The individual response to sham for all 43 participants and mean SI 1mV MEPs for the children/adolescents and adults group. (B) Mean SI 1mV MEP amplitudes (± SEM) of stimulation conditions. The y-axis depicts normalized and log transformed MEP amplitudes. For non-responders, post hoc tests showed significant higher SI 1 mv amplitudes following 140 Hz tACS compared to sham stimulation. Also, we found significant lower amplitudes following 20 Hz tACS compared to sham stimulation. (C) Time course of mean SI 1mV MEP amplitudes (± SEM) for each age group and each stimulation. The x-axis depicts the timepoints before and after stimulation. The y-axis displays normalized MEP amplitudes. We found increase amplitudes 0, 30 and 60 minutes following 140 Hz tACS, as well as 30 and 60 minutes following tRNS. The inhibitory effect of 20 Hz tACS has only been demonstrated in comparison to sham, but not for the baseline -post stimulation comparison.

4.4.3.2 Intracortical Inhibition and Facilitation for Non-Responders to Sham Stimulation

Our investigations on SICI and ICF did not show any significant effect for the *stimulation* or *time* factor or for the *stimulation* × *time* interaction for *non-responders* to sham (all $p > 0.05$).

4.4.3.3 Single-pulse MEPs (SI 1mV) for Responders to Sham stimulation

Our mixed model for *responders* to sham investigating the SI 1 mv amplitude did not reveal a significant main effect for *stimulation*, *time* or for the *stimulation* × *time* interaction (all $p > 0.05$; see Table 4-2). The pairwise comparisons on baseline to post stimulation changes in SI 1 mv amplitudes revealed no significant results.

4.4.3.4 Intracortical Inhibition and Facilitation for Responders to Sham

Our mixed models for SICI and ICF MEP amplitudes did not show any significant effects or interactions for *responders* to sham (all $p > 0.05$).

4.5 Discussion

The current study aimed at investigating age as well as response to sham stimulation as factors potentially determining efficacy of tACS and tRNS on motor cortex excitability. We found an effect of verum stimulation that was not influenced by age but by response to sham. Specifically, we observed an excitatory effect of 140 Hz tACS for all participants compared to sham, while tRNS and 20 Hz tACS did not influence corticospinal excitability; yet, these effects did not differ between children/adolescents and adults. Importantly, all types of stimulation were well tolerated by children/adolescents and adults with only minor side effects.

The exploratory analysis of response to sham as a predictor of response to verum stimulation showed that only in non-responders to sham, 140 Hz tACS and tRNS increased and 20 Hz tACS decreased excitability, while responders to sham showed no effect to verum stimulation. For both factors the effects were limited to single pulse TMS. For SICI and ICF no effects were observed.

4.5.1 Effect of Age

To the best of our knowledge, no previous study has explored tACS and tRNS effects on motor cortex excitability in a pediatric population. Existing knowledge from adult populations cannot simply be transferred to children, as the child's brain is still developing. For

tDCS it has been demonstrated that the age of subjects and developmental stage of the brain may affect the efficacy of stimulation and even inverse stimulation effects (Moliadze, Schmanke, et al., 2015). In our study, we did not observe differences in stimulation effects between children/adolescents and adults for any of the stimulation conditions. This may have several reasons discussed below.

As expected, 140 Hz tACS led to increased motor cortex excitability with no difference between age groups. This effect is due to the excitatory nature of 1mA 140 Hz tACS. For anodal tDCS it has already been shown that excitatory effects occur in the same way in children and adults (Moliadze, Schmanke, et al., 2015). The current results are also in line with previous studies showing excitatory effects on motor cortex excitability for frequencies ≥ 140 Hz (Dissanayaka, Zoghi, Farrell, Egan, & Jaberzadeh, 2017). This effect might be based on short-term synaptic plasticity induced by stimulation (Chaieb et al., 2011; Citri & Malenka, 2008). Moliadze et al. (2010) reported increased MEP up to one hour following 1 mA 140 Hz tACS and decreased (SICI), an electrophysiological marker of GABA receptor mediated inhibition (Moliadze et al., 2010a). Beneficial effects of 140 Hz tACS have also been demonstrated for memory consolidation (Ambrus et al., 2015).

Similarly, our hypothesis that children/adolescents react to the full spectrum tRNS due to increased neuronal plasticity has been confirmed by our results. Previous studies in adults report increased excitability following high-frequency tRNS (Dissanayaka et al., 2017; Terney et al., 2008), showing that the excitatory effect of tRNS derives from higher frequencies (< 100 Hz). Different mechanisms have been discussion as explanation for this effect, including stochastic resonance (Wiesenfeld & Moss, 1995) or repetitive opening of Na+ channels (Schoen & Fromherz, 2008; Terney et al., 2008). Our results suggest that in both adults and children/adolescents the full spectrum tRNS is not able to influence excitability. However, it is possible that the sample size is simply too small to detect a small-moderate effect of tRNS. The lack of an effect of tRNA in the children/adolescents groups could be due to the comparatively

high age of the participants (10-16 years), as an improvement in performance and learning by full-spectrum tRNS was demonstrated for younger children (8.5-10.9 years; Looi et al., 2017). At the same time, no effect of high frequency tRNS on phoneme processing was observed in children aged 10-16 years (Rufener, Krauel, Meyer, Heinze, & Zaehle, 2019). Therefore, based on our current results and those in adults, future studies could investigate whether high-frequency tRNS can lead to excitatory effects in children.

Since beta activity in motor cortical areas is associated with suppression of prepared movements in go-nogo tasks (N. Swann et al., 2009; N. C. Swann et al., 2012), it can be assumed that tACS at 20 Hz would enhance automatic inhibition and therefore decrease motor cortex excitability. However, previous studies report heterogeneous results for 20 Hz tACS applied over the motor cortex area. Our results are in line with several other studies that did not find stimulation effects on motor cortex excitability (Rjosk et al., 2016; Wach et al., 2013), while Cappon et al. (2016) demonstrated inhibitory effects following 20 Hz stimulation (Cappon, D'Ostilio, Garraux, Rothwell, & Bisiacchi, 2016). Unlike Cappon (2016), we did not use a task during stimulation; this activation during stimulation might influence the effects of tACS, since previous studies showed state dependent effects of tACS (Feurra et al., 2013; Fusca, Ruhnau, Neuling, & Weisz, 2018). Importantly, no difference between children/adolescents and adults was observed in our study. Additional factors may influence the effect of 20 Hz tACS, such as intensity, phase and duration of stimulation, electrode montage and activation during stimulation. For example, a recent meta-analysis on effects of beta tACS showed that only intensities above 1 mA are able to introduce excitatory effects on MCE (Wischnewski et al., 2019).

Our study is in accordance with earlier studies which show that motor thresholds are higher in children and adolescents compared to adults (Frye, Rotenberg, Ousley, & Pascual-Leone, 2008; Garvey & Mall, 2008; Moliadze, Schmanke, et al., 2015). Corticospinal tract maturation is a complex process affected by dynamic factors such as synaptic pruning and

development, myelination, changes in axonal diameter and length and organization of pyramidal neuron firing patterns (Chiappa et al., 1991; J. Eyre, Miller, & Ramesh, 1991; J. A. Eyre, Taylor, Villagra, Smith, & Miller, 2001; Papadelis et al., 2019; Paus et al., 2001). All these factors may also play a role in MEP threshold development. Maturation of the representation of the FDI in the dominant motor cortex is not complete at puberty (Garvey et al., 2003).

4.5.2 Safety and Tolerability

Extreme diligence is required while carrying out NTBS studies in children, as there are few publications, and in case of tACS even no prior experience, concerning the (side) effects of stimulation in this age group. Therefore, the present study is not only relevant with regard to the effects of stimulation on MCE, but also concerning the safety and tolerability of stimulation. For tDCS it has already been shown that this technique is well tolerated by children (Krishnan, Santos, Peterson, & Ehinger, 2015). Studies conducted in minors did not report considerable side effects, except itching and tingling skin sensations and transient redness of the skin under the electrodes (Andrade et al., 2014; Antal et al., 2017; Mattai et al., 2011; Moliadze, Andreas, et al., 2015). Nevertheless, it should be noted that the use of NTBS in children is a relatively young field of research. One of our previous studies has shown that especially in children and adolescents a more thorough screening for a possible epileptic pathology is necessary (Splittgerber et al., 2019).

The present study indicates that tACS and tRNS are well tolerated in children and adolescents. The reported side effects were of low to moderate intensity. In addition, there were no differences in the frequency and intensity of reported adverse events between children and adults.

4.5.3 Effect of Response to Sham Stimulation

Our results show different effects depending on the individual response to sham stimulation. Non-responders to sham showed excitatory effects after 140 Hz and tRNS, as well as inhibitory effects after 20 Hz stimulation. Still, it should be emphasized that the inhibitory effect of 20 Hz tACS has only been demonstrated in comparison to sham, but not for the baseline to post stimulation comparison. The effect has thus not been fully confirmed for all frequencies of stimulation. In contrast, responders to sham showed no stimulation effects. Recently we demonstrated the influence of response to sham in an adult subgroup for tRNS and 140 Hz stimulation and discussed several possible explanations (Kortuem et al., 2019). Though we were not able to investigate the influence of response to sham in adults versus children/adolescents due to our small sample size for children/adolescents, the effects are robust in the extended sample of adults and children/adolescents combined.

The dependency of stimulation effects on the response to sham might be based on metaplasticity. Neurons are known to be able to change their activity via synaptic plasticity in the form of long-term potentiation (LTP) or depression (LTD; Abbott & Nelson, 2000). The basic idea of metaplasticity is that the threshold for activity-dependent synaptic plasticity is not static but dynamic and it is also a function of the integrated prior activity of the postsynaptic neuron. It refers to synaptic or cellular activity that primes the ability to induce subsequent synaptic plasticity, such as LTP or LTD (for review see (Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015).

However, in our study we did not measure task-evoked state dependency but rather physiological state dependency. Some current papers and reviews refer to this as “baseline activity”, interchangeably it is also called “individual physiological brain state” (for a review see (Krause & Cohen Kadosh, 2014). Therefore, individual physiological state dependency might have contributed to the different outcomes in responders and non-responders to sham (Krause & Cohen Kadosh, 2014; Silvanto, Muggleton, & Walsh, 2008). Following this idea,

the interindividual state of neural activity influences the outcome of stimulation (Ammann, Lindquist, & Celnik, 2017).

As we discussed previously (Kortuem et al., 2019) our results could add further support the Bienenstock–Cooper–Munro (BCM) theory (Bienenstock, Cooper, & Munro, 1982), which claims that high levels of prior activity favor the induction of LTD, while low levels of prior activity favor LTP (Karabanov et al., 2015; Lang, Nitsche, Paulus, Rothwell, & Lemon, 2004; Siebner et al., 2004; U. Ziemann et al., 2008). However, it should be noted that based on the design of our study the stability of a physiological state as assumed for the BCM theory may not be applicable to our results, given that sham stimulation was only performed once with a gap of least a 7-days to verum stimulation. The BCM theory also does not explain why SICI and ICF were unaffected in our study. If prior neural activity affected LTP/LTD, it would stand to reason that either SICI or ICF measures would be similarly altered (e.g. Golaszewski et al., 2010).

The relationship between SICI and CS intensity is non-linear and varies between individuals (Chen et al., 1998; Rossini et al., 2015), therefore using a single CS intensity may contribute to the variability of the outcome. Later studies with threshold-tracking, parameters obtained from SICI recruitment curve showed better reproducibility (Samusyte, Bostock, Rothwell, & Koltzenburg, 2018).

In our study TMS was delivered at 0,25 Hz. It could be suggested that the TMS with low frequencies may be needed to prevent neuromodulatory effects (Chen et al., 1997). For example, Manganotti et al. (2012) shown that, single, paired, and transcallosal TMS applied on the sensorimotorareas induced rapid desynchronization over the frontal and central-parietal electrodes mainly in the alpha and beta bands (Manganotti et al., 2012). However, given the low number of stimuli used in the present study, this is unlikely to alter cortical excitability and result in inhibitory effects.

4.6 Limitations

This study is characterized by some limitations. Due to the small sample size, results for tRNS in our study may be vulnerable to overlooking effects (type II statistical error). Therefore, specifically for these null-results, additional studies with larger sample sizes are needed.

Another limiting factor of our study is that we were not able to investigate the effect of response to sham in adults versus children/adolescents. This was due to the comparatively small sample of children and adolescents, which did not allow us to perform a comparison within that group. Therefore, it remains unclear whether there is an interaction between age and response to sham.

In addition, our study investigated only one stimulation intensity. It is conceivable that the stimulation effects vary depending on the intensity and that these intensity effects may also interact with the age of the subjects, as it has been shown for tDCS (Moliadze et al., 2018; Moliadze, Schmanke, et al., 2015). However, the aim of the present study was rather to investigate different stimulation conditions and frequencies to lay the foundation for future tACS and tRNS studies in children, especially with regard to safety and tolerability.

Furthermore, the use of neuro-navigation would have been beneficial in order to objectively monitor the coil position and reduce possible distortions caused by the examiner.

4.7 Conclusion

The present study is intended to serve as a basis for further studies investigating tACS and tRNS in the pediatric population as there are currently only few studies. Our results suggest that tACS and tRNS are well tolerated children and adolescents and no serious adverse events occurred; the evaluation of safety will require longer-lasting investigations. While 140 Hz tACS facilitated excitability, full-spectrum tRNS and 20 Hz tACS did not influence MCE. Interestingly, the effects of stimulation were not modulated by age. At the same time, our study

suggests, that the net corticospinal excitability modulation induced during tRNS and 140 Hz and 20 Hz tACS critically depends on the individual sensitivity to sham stimulation. Therefore, it is worth considering the individual response to sham stimulation as a marker for the physiological brain state and an opportunity to understand the variability in the interindividual response to verum stimulation.

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5 General Discussion and Conclusions

tES is able to influence neuronal activity in the human cortex (Paulus et al., 2016; Reed & Cohen Kadosh, 2018). However, a huge inter-individual variability exists in reported tES effects, limiting the overall effectiveness of stimulation. Repeatedly it has been shown that only approximately 50% of subjects respond to tES as expected (López-Alonso, Cheeran, Río-Rodríguez, & Fernández-Del-Olmo, 2014; Wiethoff, Hamada, & Rothwell, 2014; Ziemann & Siebner, 2015). There is now evidence that various methodological and physiological factors modulate the effects of stimulation (Li et al., 2015; Polanía et al., 2018). To effectively use tES as a research or potential treatment method, these influencing factors need to be further examined. The aim of this thesis therefore was to investigate methodological and physiological factors influencing tES effects.

Study I investigated the influence of montage and individual functional performance level on the effects of anodal tDCS over the left DLPFC. The study compared a classical bipolar montage with a multichannel montage. Directly compared to each other no montage was superior regarding behavioural and neurophysiological activity. Comparing both montages with sham stimulation, multichannel stimulation led to stronger effects than bipolar stimulation. However, for both montages the effects of stimulation were dependent on the functional performance level of participants, with decreasing stimulation effects with increasing performance level.

It has been shown that age-related anatomical and functional features of the head and brain may affect the outcome of tES (Beauchamp et al., 2011; Kessler et al., 2013), which is why results of tES studies in adults cannot simply be transferred to children and adolescents. Study II therefore explored the effects of multichannel tDCS over the left DLPFC in children and adolescents. The montage corresponded to the multichannel montage developed for study I. The study explored whether tDCS effects are influenced by concurrent target task

performance during stimulation as well as individual anatomy of participants. Though accounting for these factors, the stimulation did not affect the target behavioural and related neurophysiological outcomes. And, unexpectedly, stimulation influenced non-target behavioural and neurophysiological outcomes. An influence of concurrent target task performance during stimulation was only seen for oscillatory neuronal activity. In general, the individual head anatomy had no influence on stimulation effects.

Study III focused on the systematic investigation of tACS and tRNS effects in children and adolescents in comparison to adults. Additionally, the individual response to sham stimulation was investigated as a marker for the individual physiological brain state. Different to studies I and II, stimulation was applied over the primary motor cortex. The results showed an effect of stimulation that was not influenced by age. Specifically, an excitatory effect of 140 Hz tACS compared to sham stimulation was shown, while tRNS and 20 Hz tACS did not influence corticospinal excitability. Further, stimulation effects depended on the individual response to sham stimulation. Only in non-responders to sham stimulation, 140 Hz tACS and tRNS increased and 20 Hz tACS decreased excitability, while responders showed no effect to verum stimulation.

Additionally, all studies reported minor side effects of stimulation. However, in study II one female participant developed an epileptic disease during her study participation.

A differentiated discussion of the individual study results can be found in the original studies (chapters 2 to 4). In the following, the study results are discussed in an integrative manner and framed in a common context.

5.1 Montage and Age Specific Effects

Study I confirmed a montage specific effect for tDCS targeting the left DLPFC, indicating that an optimised multichannel montage might be more effective than a bipolar montage in terms of WM and associated neurophysiological activity. Modelling computations

for both montages showed that the multichannel montage led to a higher average E-field in the target area than the bipolar montage, which may explain the observed increased efficacy of multichannel stimulation. This assumption is in line with a study by Kim et al. (2014) demonstrating a relationship between the individual current density in the left DLPFC and WM performance. A recent tDCS study targeting the left DLPFC and WM predicted tDCS treatment response based on individual current models (Albizu et al., 2020). However, the multichannel montage was not effective in a paediatric population in study II regarding effects on the target WM performance and related neurophysiological activity. Importantly, no relationship between the individual normal E-field value in the left DLPFC and stimulation effects were found.

This result could be indicative of an age effect, interacting with the multichannel montage. However, since there was no direct comparison between the age groups, and the studies used different designs, albeit with an identical WM task and the multichannel montage, no strong conclusions regarding an age effect are possible. The differences in effects could be based on differences in anatomy between children and adults, which in turn affect the E-field (Beauchamp et al., 2011; Kessler et al., 2013; Opitz et al., 2015). Therefore, a comparison at the anatomic level with the adult sample would have been desirable. Within a subsample of the child and adolescent age group, the association between anatomy and tDCS effects was examined; however, this too was unable to confirm a relationship. Still, an explanation for a possible age effect could also be found in other age-specific factors. Besides anatomical maturation processes, children and adolescents show developmental changes in GABA-ergic and glutamatergic systems (Arain et al., 2013; Li & Xu, 2008), which are known to be influenced by tDCS. Additional, cortical networks are subject to age-specific changes. The development of the prefrontal cortex is not complete until early adulthood (Casey et al., 2008) and is associated with an increase of myelin in the frontal lobes throughout adolescence (Arain et al., 2013; Giedd et al., 1999). This increase in turn leads to improved connectivity between brain regions (Giedd, 2004). TDCS alters neuronal activity not only in the target area, but also

in functionally and structurally linked regions (Jones, Peterson, Blacker, & Berryhill, 2017; Park et al., 2013; To et al., 2018). This effect was also demonstrated by changes in oscillatory activity in the theta, alpha and beta band in frontal and occipital regions in studies I and II. Multichannel montages use a wide distribution of anodes and cathodes to optimally stimulate the target region. At the same time, these distributed electrodes might affect excitability in other regions than the target region. In combination with the above-mentioned changes in the course of brain development, multichannel montages could lead to deviating tDCS effects in children and adolescents compared to adults.

It is likely that maturation factors also influence tACS and tRNS effects, since all tES techniques share the same basic principles. Still, in study III, no age effect was present following investigation into 140 Hz, 20 Hz and full spectrum tRNS effects on motor cortex excitability. So far, age effects of tACS and tRNS have marginally been investigated. Studies comparing effects of motor cortex tACS at alpha frequency in younger and older adults found both, a difference between age group (Fresnoza et al., 2020) and no difference at all (Fresnoza et al., 2018). In study III, 140 Hz tACS led to increased excitability regardless of age. However, it remains unclear to what extent these results can be transferred to other cortex areas. In adults, a strong frequency dependence of the tACS effects has been shown (Klink, Paßmann, Kasten, & Peter, 2020). At the same time, this review found that this frequency effect is dependent on the target cognitive function, and related target regions, that were stimulated.

Taken together, the studies suggest that age effects cannot easily be generalised. Even though general mechanisms of action are assumed for tES, these are obviously influenced by the interplay of age-specific factors and other variables (e.g. frequency, target region or montage), to an extent that makes tES effects difficult to predict. Therefore, when applying these methodologies in real life situations, e.g. for therapeutic usage, the stimulation effects should be investigated directly in the age group for which statements are to be derived.

5.2 Brain State Dependent Effects

This thesis investigated different factors that picture or influence the individual brain states of participants: individual functional performance level, concurrent task performance during stimulation, and the response to sham stimulation.

For WM performance following tDCS over the left DLPFC, study I found an increase in stimulation effect with decreasing individual baseline performance. The influence of individual functional performance level on stimulation effects has been investigated in numerous studies (Gözenman & Berryhill, 2016; Hsu et al., 2016; Jones & Berryhill, 2012; Li et al., 2015). However, due to contradictory results and inconsistent study designs, a comparative analysis between the studies is difficult. An explanation for the different effect of high and low performers is often seen in E/I balance, which can differ depending on the individual performance level and could be normalised through tDCS in case of an imbalance (Clark et al., 2011; Krause et al., 2013). In general, differences in performance levels imply that subjects are differently stressed by the task, with low performers having a greater control demand than high performers. Different studies suggest that tDCS long-term effects are only detectable in a challenging task (Jones & Berryhill, 2012; Pope, Brenton, & Miall, 2015; Wu et al., 2014). However, results in study II imply that for some participants the task may have been too challenging. Therefore, one way to reduce variability in tDCS effects could be through the use of an adaptive task, thus employing an optimal stress level for each subject.

Regarding concurrent task performance, both studies I and II showed stimulation effects only after and not during tDCS. This finding indicates that stimulation effects developed over time and can be traced back to long-term effects of tDCS, which appear to be based on changes in GABAergic and glutamatergic activity (Liebetanz et al., 2002; Stagg & Johansen-Berg, 2013). In addition, it was examined whether additional task engagement during stimulation modulates tDCS effects. Study I did not compare the effects of concurrent and non-concurrent task performance during stimulation; Stimulation was always combined with the target 2-back

task performance. However, the behavioural effects found on 2-back task and not CPT, both performed after stimulation, could argue for the network activity-dependent model. This model implies that activity in neuronal networks, that are active during the stimulation, is primarily strengthened by the stimulation (Di Luft et al., 2014; Fertonani & Miniussi, 2017). However, oscillatory alpha power during CPT performance was affected by the stimulation, which shows a transfer of stimulation effects. Additionally, since it is unclear whether stimulation without task performance would have produced the same effects and the CPT showed strong ceiling effects, conclusions regarding a network activity-dependent effect of stimulation are speculative. Interestingly, in study II tDCS only affected non-target outcomes. For these outcomes, on the behavioural level there was no influence of concurrent task performance during stimulation. In regard to the neurophysiological outcome, an increase in beta activity was only seen for non-concurrent task performance. These transfer effects support the flexible hub theory, assuming that the FPN strengthened by stimulation can be coupled with different networks at different times (Zanto & Gazzaley, 2013).

Unlike the other influencing factors of tES investigated in this thesis, the response to sham has not yet been examined in other studies. A subsample of the sample examined in study III was previously analysed by Kortuem et al. (2019). The previous analysis also confirmed an influence of response to sham stimulation on the response to verum stimulation. Various approaches, such as metaplasticity (Müller-Dahlhaus & Ziemann, 2015) or the Bienestock-Cooper-Munro theory (Bienenstock, Cooper, & Munro, 1982), are conceivable as explanations for the observed effects. The division into responders and non-responders based on MEP changes following sham stimulation aimed to capture individual placebo effects. Still, it is unclear whether the individual responsiveness to placebo effects is stable between stimulation sessions. The changes in motor cortex excitability following sham stimulation could also reflect spontaneous intra-individual variations in MEP outcomes (Horvath, Vogrin, Carter, Cook, & Forte, 2016). For responders to sham stimulation, these intra-individual variations might be

generally stronger, and allowed verum tES effects to be superimposed. In any case, follow-up studies are needed to investigate whether the response to sham actually has predictive power for stimulation effects and, if so, on what mechanisms this relationship is based.

5.3 Findings on Safety and Tolerability

All studies included in this thesis indicate, that tES was well tolerated and led to only mild side effects in children, adolescents and adults. The investigation of side effects and safety is of central importance, as the mechanisms of tES and thus its possible (side) effects still leave many questions unanswered. This applies especially to novel tES approaches, such as multichannel stimulation, or to vulnerable and less studied populations, such as children and adolescents. Although our results are in line with previous findings on safety of tES (Antal et al., 2017), the case of a female participant in study II, who developed an epileptic disease during her study participations, demonstrates the necessity to adopt screening procedures to new findings (Sierawska et al., 2020; Splittgerber et al., 2019). This participant had shown signs of juvenile myoclonic epilepsy prior to study participation; however, they were not detected in the screening process. Hence, tES routines should be reviewed and, if necessary, adapted for the use in children and adolescents. There is an obvious need for screening procedures to be validated as effective for children and adolescents, thus ensuring the safe application of tES in this age group. Although no causal relationship between the occurrence of the epileptic seizure and tDCS has been proven, pre-diseased individuals should under no circumstances be included in studies without clarification to exclude possible negative effects of tES.

5.4 Limitations

Several limitations are evident in the studies conducted for this thesis. All three studies examined comparatively small samples, resulting in low statistical power (Button et al., 2013). This poses a problem especially in the context of examining the influence of various factors as

continuous or categorical variables. Small sample sizes are a general problem of tES studies and, combined with publication bias, lead to overestimation of effect sizes (Medina & Cason, 2017; Minarik et al., 2016). Therefore, the sample size calculated for study I might be underestimated. However, the risk of an underpowered study also exists for studies II and III, for which such a priori calculations were not performed. At the same time, children and adolescents, which were investigated in studies II and III, are a particularly vulnerable population group for tES studies. Since tES techniques have not been well studied within this age group, it is difficult to exclude adverse effects with certainty. Accordingly, paediatric samples should be kept as small as possible.

Since it is known that tES is influence by inter and intra-individual variability (Chew, Ho, & Loo, 2015; Ziemann & Siebner, 2015), changes during or following stimulation should be relativized to the individual baseline at each session. While for study III a pre-post stimulation comparison within individual sessions was performed, for studies I and II only between-session comparisons (verum vs. sham) were made. A pre-post comparison would have also been useful in these studies to better compensate, at least in part, for the influence of intra-individual variation between measurements.

Another limitation lies in the exploratory nature of the studies conducted within the framework of this thesis. For many of the influencing factors investigated in this thesis, conflicting results were found in previous studies, while other factors have rarely been investigated before. Based on this, broad hypotheses were chosen, both in terms of behavioural and neurophysiological outcomes. Although such "trial and error" approaches are common in tES research, they only allow general statements as to whether a factor has an influence on tES outcome. However, it is difficult to derive specific conclusions, for example, about the strength of the influence or in regard to the basis of the relationship. In this respect, a more model-guided approach (Bestmann et al., 2015), would have been advantageous. Furthermore, a stronger methodological effort would have lead to increasingly conclusive and mechanistically informed

evidence (Polanía et al., 2018). However, it must be noted, that the studies conducted for this thesis employed several of these aspects, such as, the investigation of different tACS frequencies, the investigation of neurophysiological outcomes or the use of control tasks.

Another limitation is evident in the statistical analyses. In study III, responders and non-responders to sham stimulation were divided based on their MEP amplitude changes after sham stimulation. The effect of verum stimulation (140 Hz, 20 Hz tACS and tRNS) was determined as a pre-post comparison within each stimulation session. Additionally, a comparison was made between the sham session and the verum stimulation sessions. A comparison of sham stimulation to examine the effectiveness of verum stimulation while also using this sham measure to classify responder/non-responder, is problematic. The variance in MEP amplitudes is by definition larger in the group of responders than in the group of non-responders to sham stimulation, at least for the test time T0 used to divide the groups. Therefore, significant differences in non-responders to sham stimulation were also more likely to be found between sham and verum stimulation. In any case, an independent determination of the response to sham would have been advantageous. Still, effects of verum stimulation in the non-response to sham group were also found in pre-post comparisons within the verum stimulation condition.

A similar problem applies to the statistical analyses conducted for study II. The influence of the individual functional performance level was defined here as baseline performance in the 2-back task. At the same time, however, 2-back task performance was the primary outcome for determining stimulation effects at each stimulation visit. As in the previous case, an independent measure to determine the performance level would have been advantageous. In this way, it could have been more reliably ruled out that the effects found were due to a *possible regression to the mean* phenomenon (Blomqvist, 1987).

5.5 Future Directions

Clearly, there is a need for further investigation of the factors that influence tES, to be able to reduce inter and intra-subject variability in tES effects. To obtain reliable and valid results, several methodological aspects should be considered and implemented in future studies on factors influencing tES.

For TMS an individualisation of stimulation is standard practice (Rossini et al., 2015). Similarly, the approach of optimised and individualised stimulation should be further pursued in tES research. A first step in this direction is the optimised multichannel montage, based on a standard head model, that was used in studies I and II. The next step will be to individualise these optimised multichannel montages for every participant or patient, based on individual head modelling. A modelling study by Salvador et al. (2021) using the MRI measurements obtained in study II, investigated the use of an individualised montage. The aforementioned study confirmed a reduced inter-individual variation in the E-field, as well as stronger current densities in the target region, compared to a standard montage. However, the effect of this individualisation has not yet been investigated in experimental studies. To ensure the correct placement of electrodes and induction of an E-field in the brain as predicted, the application of individualised multichannel montages should be combined with the use of neuronavigation (Opitz et al., 2018; Witte et al., 2018). An additional open question is in regard to the priority for which aspects of a montage should be optimised. For example, it is still unclear whether it is better to perform stimulation on one target region or different parts of a neural network. While the multichannel montage that was applied in studies I and II used one target region, montages that target different hubs of one network might lead to increased effects (Chen et al., 2019; Di Luft et al., 2014; Fischer et al., 2017). However, the definition of optimisation might differ between cognitive functions or behaviours that are targeted by stimulation and even between individuals (Lynch et al., 2019). The modelling of individualised, optimised multichannel montages should therefore also consider theoretical frameworks of cognitive functions or

behaviours that are to be influenced by tES (Polanía, Paulus, Antal, & Nitsche, 2011; To et al., 2018).

Further, future studies must build on each other more strongly, which can be facilitated by methods-reporting checklists (Polanía et al., 2018). In study I the individual baseline performance predicted tDCS effects, which is in line with previous studies on the influence of the individual functional performance level (Gözenman & Berryhill, 2016; Hsu et al., 2016; Jones & Berryhill, 2012).But the direction of prediction differs between these studies. These differences in results are probably partly due to differences in study designs, including target area, investigated cognitive function and tES montage. Also, especially for new approaches like the investigation of response to sham stimulation as predictor of verum stimulation, follow-up studies are necessary. To be able to compare results and derive general conclusions, studies should examine only a clearly defined area and specific brain-behaviour relationship. Pooling these studies together, meta-analyses or studies using artificial neuronal network models might enable deeper insights into the nature of modulating factors and predictions about individual tES responses (Li et al., 2015; Polanía et al., 2018).

Based on the current results, it also seems useful to consider different influencing factors in combination within a study. In study I, an interaction of individual functional performance level and tDCS montage was observed. In study III tACS frequencies interacted with the individual response to sham stimulation. Recent studies that investigated modulation of tES effects also demonstrate that tES modulatory factors do not influence tES in isolation, but in combination (Evans, Banissy, & Charlton, 2018; Heise, Monteiro, Leunissen, Mantini, & Swinnen, 2019; Li et al., 2019). In this context, larger samples than those collected in the studies conducted here would be advantageous. However, this is a general aspect relatable to a large number of other stimulation studies (Héroux et al., 2017; Minarik et al., 2016), to help avoid type I and type II errors (Button et al., 2013).

In addition, future studies should further investigate the safety and tolerability of stimulation, for example by means of safety questionnaires. This is particularly important for vulnerable groups such as children and adolescents (Davis, 2014). Only in this way can risks be minimised for new tES approaches, such as multichannel montages or increased current strengths of up to 4 mA (Khadka et al., 2020).

5.6 Conclusions

This thesis confirms that tES effects are not homogeneous but influenced by different methodological and physiological factors. For tDCS over the left DLPFC an influence of montage and individual functional performance level was shown in adults in study I. In Study II, investigating children and adolescents, concurrent task performance during stimulation had a minor influence on effects of tDCS over the left DLPFC, while individual head anatomy did not predict tDCS effects. Moreover, studies I and II demonstrated transfer effects of stimulation. In study III, tACS and tRNS over the motor cortex was not modulated by age but by individual response to sham stimulation as a marker for the physiological brain state.

Additionally, all studies have shown that tES resulted in few side effects. However, one serious adverse event occurred in Study II. The investigation of safety and tolerability aspects is especially important and necessary for previously unexplored approaches, such as multichannel montages or tACS in children and adolescents. Guidelines should be continuously updated and disseminated not only regarding tES parameters, but also for accompanying procedures, such as the screening process, to minimise potential risks of stimulation.

In all studies, several null findings were observed, indicating that parameters of stimulation were not optimal in each participant regarding the investigated processes and populations. The results of this thesis give further indications how tES parameters can be adjusted to achieve a more effective stimulation. Optimal stimulation can only be achieved if all relevant influencing factors are considered and the parameters of stimulation (e.g. current

intensity, distribution of electrodes or combined cognitive activation), are determined in each individuum separately. This means, an optimisation of simulation through an individualisation of stimulation. That can only be achieved by further advancing the understanding of tES influencing factors.

6 Deutsche Zusammenfassung (Summary in German)

6.1 Einleitung

Die nichtinvasive transkranielle elektrische Hirnstimulation (tES) ist eine vielversprechende neuromodulatorische Technik in Hinblick auf ihre Anwendung in der Forschung (Ziemann et al., 2008) und als potentielle Behandlungsmethode für verschiedene neuropsychiatrische Störungen (Ciullo et al., 2020; Kekic, Boysen, Campbell, & Schmidt, 2016). Das Ziel der tES ist es, neuronale Aktivität in kortikalen Arealen und damit verbundene Gehirnfunktionen zu verändern. Dazu wird Strom mit geringer Intensität (≤ 4mA) über einen kurzen (einige Sekunden) oder längeren (typischerweise 10-40 Minuten) Zeitraum über am Kopf platzierte Elektroden appliziert (Paulus et al., 2016). Der Strom induziert ein elektrisches Feld (E-Feld) im Gehirn, das zu einer Polarisation der neuronalen Membranen führt und damit die endogene neuronale Aktivität auch über die Dauer der Stimulation hinaus beeinflussen kann (Paulus et al., 2016).

Zu den tES Techniken gehören die transkranielle Gleichstromstimulation (transcranial direct current stimulation, tDCS), die transkranielle Wechselstromstimulation (transcranial alternating current stimulation, tACS) und die transkranielle Rauschstromstimulation (transcranial random noise stimulation, tRNS), welche eine Sonderform der tACS ist. Bei der tDCS wird ein konstanter Strom zwischen mindestens einer Anode und einer Kathode angelegt (Gebodh et al., 2019). Es wird vereinfacht angenommen, dass eine anodale Stimulation zu einer somatischen Depolarisation und damit zu einer erhöhten neuronalen Erregbarkeit führt, während eine kathodale Stimulation zu einer Hyperpolarisation und reduzierter Erregbarkeit führt (Ziemann et al., 2008). Bei der tACS wird ein sinusförmiger Strom mit einer bestimmten Frequenz an das Gehirn angelegt (Antal & Herrmann, 2016). tACS beeinflusst nachweislich endogene neuronale Oszillationen, indem es die Amplitude erhöht oder die Phase der Oszillationen verschiebt (Herrmann, Rach, Neuling, & Strüber, 2013). Auch die tRNS

verwendet einen Wechselstrom (Antal & Herrmann, 2016). Allerdings variieren die Frequenz und die Intensität des Stroms zufällig innerhalb eines bestimmten Bereichs. Es wurde gezeigt, dass tRNS, ähnlich wie anodale tDCS, die kortikale Erregbarkeit erhöhen kann (Inukai et al., 2016; Terney et al., 2008).

tES-Elektroden werden üblicherweise über bestimmten Hirnregionen platziert mit dem Ziel, die neuronale Aktivität in diesen Bereichen zu verändern. Ein häufig als Zielregion für tES verwendetes Hirnareal ist der motorische Cortex (Dissanayaka et al., 2017), wo mittels transkranieller Magnetstimulation (TMS) motorisch evozierte Potenziale (MEP) induziert werden können (Legatt, 2014), die quantitative Aussagen über tES-Effekte erlauben. Außerdem wird der dorsolaterale präfrontale Cortex (DLPFC) häufig als Zielregion für die elektrische Stimulation verwendet, da er eine wichtige Rolle für verschiedene kognitive Funktionen, wie das Arbeitsgedächtnis (AG) oder die kognitive Kontrolle spielt (D'Esposito et al., 1995; Fan, Flombaum, McCandliss, Thomas, & Posner, 2003). tES kann nachweislich die DLPFC-Aktivität beeinflussen und diesem Areal zugeordnete kognitive Funktionen verbessern (Dedoncker et al., 2016; Meiron & Lavidor, 2014).

Allerdings berichten viele Studien, unabhängig von ihrer Zielregion, eine hohe interindividuelle Variabilität der tES-Effekte, sowie fehlende oder geringe Effekte (Héroux et al., 2017; Horvath et al., 2015). In diesem Zusammenhang ist es notwendig und sinnvoll, methodische Studien durchzuführen, um Faktoren zu untersuchen, die einen Einfluss auf die Effekte der Stimulation haben.

Bislang konnte gezeigt werden, dass insbesondere die tES-Montage in Verbindung mit der individuellen Anatomie die Dichte und Verteilung der elektrischen Ströme und damit die Effekte der tES beeinflussen (Albizu et al., 2020; Kasten et al., 2019; Opitz et al., 2015). In tES-Studien werden meist klassische bipolare Montagen verwendet, die ein relativ diffuses E-Feld erzeugen und eine schlechte räumliche Zielerfassung bewirken (Laakso et al., 2016; Miranda, Mekonnen, Salvador, & Ruffini, 2013; Saturnino, Antunes, & Thielscher, 2015). Eine

Alternative könnten optimierte multichannel Montagen sein, für die eine vergleichsweise starke, aber auch fokale Stimulation des Zielbereichs vorhergesagt wird (Salvador et al., 2021; Saturnino et al., 2019).

Zudem zeigen Studien eine Abhängigkeit der tES-Effekte vom individuellen Alter der Probanden. Im Vergleich zu Erwachsenen weisen Kinder eine andere Leitfähigkeit des Schädelgewebes, ein anderes Verhältnis an weißer und grauer Substanz, ein anderes Liquorvolumen und einen geringeren Abstand zwischen Gehirn und Schädel auf, wodurch die Verteilung des E-Feldes beeinflusst wird (Beauchamp et al., 2011; Kessler et al., 2013). In experimentellen Studien hat sich gezeigt, dass das Alter der Teilnehmer die Wirksamkeit der tES beeinflussen und sogar inverse Stimulationseffekte hervorrufen kann (Moliadze et al., 2015; Moliadze et al., 2018).

Auch das individuelle funktionelle Leistungsniveau scheint tES Effekte zu beeinflussen. In mehreren tDCS-Studien konnte ein negativer Zusammenhang zwischen individueller kognitiver Ausgangsleistung und tDCS-Effekten nachgewiesen werden: Je schlechter die Leistung der Teilnehmer vor der Stimulation war, desto mehr profitierten sie von einer Stimulation (Gözenman & Berryhill, 2016; Habich et al., 2017; Rosen et al., 2016). In anderen Studien zeigten sich allerdings auch abweichende Effekte. Jones & Berryhill (2012) fanden beispielsweise, dass anfänglich leistungsstarke Teilnehmer nach der Stimulation eine Verbesserung der AG-Leistung zeigten, während anfänglich leistungsschwache Teilnehmer in ihrer Leistung beeinträchtigt oder nicht beeinflusst wurden.

Es wurde außerdem gezeigt, dass tES Effekte durch kognitive Beanspruchung während der Stimulation beeinflusst werden. Dabei wird online tES (Stimulation mit gleichzeitiger Durchführung einer Aufgabe) und offline tES (Stimulation ohne/vor Durchführung einer Aufgabe) unterschieden. Studien zeigen Unterschiede in den Effekten, die während (online) und nach der Stimulation (offline) auftreten, was vermutlich unterschiedliche Kurz- und Langzeitwirkungen der tES auf die kortikale Aktivität abbildet (Friehs & Frings, 2019; Hill et

al., 2016; Stagg & Nitsche, 2011). Zudem unterscheiden sich auch die Art und Stärke der Effekte nach der Stimulation in Abhängigkeit davon, ob und welche Art von Aufgabe während der Stimulation durchgeführt wurde (Bortoletto et al., 2015; Dedoncker et al., 2016; Gill et al., 2015; Hill et al., 2019). Das *network activity-dependent model* geht davon aus, dass tES das neuronale Netzwerk beeinflusst, das primär während der simultanen Aufgabenausführung während der Stimulation aktiv ist (Di Luft et al., 2014; Fertonani & Miniussi, 2017). Allerdings wurde auch ein Transfer von Stimulationseffekten auf neuartige Aufgaben nach tES mit gleichzeitiger Aufgabendurchführung gefunden (Gill et al., 2015). Der *flexible hub theory* folgend, kann eine neuronale Region oder ein Netzwerk zu unterschiedlichen Zeiten mit verschiedenen Netzwerken gekoppelt sein (Zanto & Gazzaley, 2013), was zu Transfereffekten führen könnte.

Zudem scheint der physiologische Zustand des Gehirns einen Einfluss auf die Stimulationseffekte zu haben (Krause & Cohen Kadosh, 2014; Li, Uehara, & Hanakawa, 2015). Dieser hängt auch von Placebo-Effekten der Stimulation ab (Brim & Miller, 2013). Um diese Placeboeffekte in tES-Studien abzubilden und zu kontrollieren, werden sham Stimulationsprotokolle verwendet, die eine verum Stimulation simulieren (Palm et al., 2013). Placeboeffekte könnten sich allerdings zwischen Individuen unterscheiden und Effekte der verum Stimulation modulieren. Kortuem et al. (2019) untersuchten die Vorhersagekraft dieser Placebo Reaktion, bezeichnet als *response to sham stimulation*, auf die Effekte von verum tACS und tRNS. Die individuelle Anfälligkeit für Placebo-Effekte sagte die Effekte der verum Stimulation signifikant voraus.

Um tES effektiv und sicher einsetzen zu können, ist ein ausreichendes Verständnis dieser modulierenden Faktoren unumgänglich. Ziel dieser Arbeit war es daher, Einflussfaktoren auf die Wirkung von tDCS, tACS und tRNS zu untersuchen. Dabei wurde ein Schwerpunkt auf die Untersuchung von Effekten und Verträglichkeit der Stimulation in pädiatrischen

Populationen gelegt, da systematische methodische Studien zu tES-Effekten bei Kindern und Jugendlichen fehlen.

6.2 Studie I

Studie I untersuchte den Einfluss verschiedener Montagen und des individuellen funktionellen Leistungsniveaus auf die Effekte der anodalen tDCS über dem linken DLPFC bei gesunden Erwachsenen. Die Studie verglich die Effekte einer optimierten multichannel Montage und einer klassischen bipolaren Montage. Zur Messung der tDCS Effekte wurde die Leistung in neuropsychologischen Aufgaben, sowie die neurophysiologische oszillatorische Aktivität, gemessen mittels EEG, untersucht. Es wurde erwartet, dass eine optimierte multichannel Montage aufgrund erhöhter Fokalität zu stärkeren tDCS Effekten als eine klassische bipolare Montage führt. Außerdem wurde erwartet, dass die Probanden in Abhängigkeit von ihrer kognitiven Ausgangsleistung unterschiedlich von der Stimulation profitieren würden.

In einem sham-kontrollierten, cross-over Design erhielten 24 gesunde Probanden jeweils 20 Minuten lang in randomisierter Reihenfolge bipolare, multichannel und sham tDCS über dem linken DLPFC, während sie eine 2-back Aufgabe (target Aufgabe) durchführten, welche die AG-Leistung prüft (Jonides et al., 1997; Kirchner, 1958). Nach der Stimulation wurde ein Ruhe-EEG aufgenommen. Anschließend führten die Probanden erneut die 2-back Aufgabe und eine non-target Aufgabe, die Continuous Performance Task (CPT), durch, während ein EEG aufgezeichnet wurde.

Im direkten Vergleich mit der sham Stimulation zeigten weder die bipolare noch die multichannel Stimulation einen Effekt auf die Verhaltensmaße oder auf neuronale Oszillationen. Es zeigte sich jedoch eine Interaktion zwischen der Stimulation und der kognitiven Ausgangsleistung. Nach multichannel Stimulation verbesserten anfänglich leistungsschwache Teilnehmer tendenziell ihre AG-Leistung, während anfänglich

leistungsstarke Teilnehmer ihre Leistung im Vergleich zur sham Stimulation tendenziell verschlechterten. In Anlehnung an Krause et al. (2013) kann davon ausgegangen werden, dass es ein optimales Niveau der präfrontalen Aktivierung gibt, das auf einem Erregungs-/Hemmungs-Gleichgewicht (excitation/inhibition, E/I) basiert, das durch die Glutamat/GABA-Konzentration gemessen werden kann (Clark et al., 2011; Stagg et al., 2009). Basierend auf dieser Theorie kann tDCS zur Wiederherstellung einer optimalen E/I-Balance führen, aber auch zu einer Überaktivierung und Leistungsverschlechterung. Dies könnte der Grund für eine verbesserte AG-Leistung bei niedriger Ausgangsleistung und eine schlechtere Leistung bei hoher Ausgangsleistung sein.

Beide tDCS-Montagen induzierten Veränderungen in der neuronalen oszillatorischen Aktivität, die mit der kognitiven Ausgangsleistung korrelierten. Je schlechter die anfängliche individuelle AG-Leistung, desto mehr Theta-Aktivität wurde während der 2-back Aufgabe durch multichannel und bipolare Stimulation induziert. Theta-Aktivität ist nachweislich entscheidend für AG-Prozesse (Gevins, Smith, McEvoy, & Yu, 1997; Klimesch, Schack, & Sauseng, 2005; Pesonen et al., 2007). Die von uns beobachtete stimulationsinduzierte Veränderung der Theta-Leistung, abhängig von der anfänglichen Ausgangsleistung, könnte daher auf eine erhöhte kognitive Verarbeitung bei anfänglich leistungsschwachen Teilnehmern hinweisen. Je schlechter die anfängliche AG-Leistung war, desto höher war zudem die Alpha-Aktivität in der non-target Aufgabe nach multichannel Stimulation, was einen Transfereffekt der tDCS Wirkung nahelegt. Es zeigte sich ein Anstieg von Alpha nach Stimulus Erscheinen, was eine erhöhte Reaktionshemmung durch die Inhibition verbundener kortikaler Areale widerspiegeln könnte (Klimesch, 1996; Schmiedt-Fehr et al., 2009).

Hervorzuheben ist, dass keine Überlegenheit der multichannel Stimulation im direkten Vergleich zur bipolaren Stimulation gezeigt werden konnte. Dennoch führte die multichannel Stimulation zu stärkeren Effekten als die bipolare Stimulation, beim Vergleich beider Stimulationsarten mit sham Stimulation. Dies könnte auf die Fokalität der E-Feld-Verteilung

zurückgehen, die basierend auf Modellberechnungen bei der multichannel Montage als deutlich höher anzunehmen ist als bei der bipolaren Montage.

Die Ergebnisse demonstrieren, dass Unterschiede in der individuellen kognitiven Leistung und der Elektrodenmontage die Effekte der tDCS auf die neuropsychologische Leistung beeinflussen.

6.3 Studie II

In Studie II wurden die Effekte von multichannel tDCS über dem linken DLPFC bei gesunden Kindern und Jugendlichen im Alter von 10 – 18 Jahren untersucht, wobei der Einfluss der Durchführung einer kognitiven Aufgabe während der Stimulation, sowie der individuellen Anatomie berücksichtigt wurde. Die angewandte Montage war identisch zu der in Studie I verwendeten multichannel Montage. Zur Untersuchung der tDCS Effekte wurden aufgabenbezogene Verhaltens- und neurophysiologische Variablen erhoben.

Es wurde erwartet, dass anodale multichannel tDCS zu einer verbesserten Leistung in einer 2-back AG-Aufgabe, die als target Aufgabe verwendet wurde, sowohl während als auch nach der Stimulation führen würde. Zudem wurde angenommen, dass die Stimulation ereigniskorrelierte Potentiale (N2 und P3 Komponente) und aufgabenbezogene und Ruhezustands-Oszillationen (Theta-, Alpha und Beta-Band) beeinflusst. Es wurde erwartet, dass die Effekte der Stimulation davon beeinflusst werden, ob während der Stimulation die 2-back Aufgabe durchgeführt worden war oder nicht. Außerdem wurde eine Flanker Aufgabe als non-target Aufgabe nach der Stimulation durchgeführt, um mögliche Transfereffekte der Stimulation zu untersuchen (Eriksen & Eriksen, 1974). Angesichts eines Mangels an entsprechender Forschung bei Kindern und Jugendlichen untersuchten wir auch Aspekte der Verträglichkeit für multichannel tDCS.

In einem randomisierten, scheinkontrollierten, doppelblinden Crossover-Design erhielten 22 gesunde Kinder und Jugendliche an vier separaten Sitzungen anodale oder sham

multichannel tDCS über dem linken DLPFC mit und ohne gleichzeitige Durchführung der 2-back Aufgabe. Nach der Stimulation führten die Teilnehmer die 2-back Aufgabe und die Flanker-Aufgabe durch. EEG wurde im Ruhezustand, sowie während der Aufgabendurchführung aufgezeichnet. Bei einer Teilstichprobe von 16 Teilnehmern wurde auf Basis von anatomischen MRT Aufnahmen die individuelle Normalkomponente des E-Feldes im linken DLPFC berechnet.

Weder die Leistung in der 2-back Aufgabe, noch die neurophysiologische Aktivität während der 2-back Aufgabe sowie im Ruhezustand wurden durch die Stimulation beeinflusst. TDCS führte zu einer reduzierten Reaktionszeit in der Flanker-Aufgabe, unabhängig davon, ob die tDCS-Applikation mit der 2-back Aufgabe kombiniert worden war oder nicht. Im Vergleich zur sham Stimulation wurde während der Flanker Aufgabe eine erhöhte Beta-Oszillation gefunden, wenn tDCS ohne die 2-back Aufgabe appliziert worden war. Insgesamt waren die tDCS Effekte nicht mit der individuellen Normalkomponente des E-Feldes in der Zielregion korreliert. Die Stimulation führte zu geringen Nebenwirkungen. Eine Teilnehmerin erlebte jedoch während ihrer Studienteilnahme ein schwerwiegendes unerwünschtes Ereignis, in Form des Ausbruchs einer epileptischen Erkrankung.

Es kann nicht ausgeschlossen werden, dass die multichannel Montage aufgrund der Verteilung der Elektroden auf dem Schädel die an der Flanker-Aufgabe beteiligten Netzwerke stärker aktivierte als die für die 2-back Aufgabe relevanten Netzwerke. Die Tatsache, dass Verbesserungen in der Flanker Aufgabe auch nach anodaler tDCS bei gleichzeitiger 2-back Aufgabendurchführung gefunden wurden, spricht gegen aufgabenspezifische Effekte der Stimulation, wie sie im network activity-dependent model angenommen werden (Bikson & Rahman, 2013; Fertonani & Miniussi, 2017). Da beide Aufgaben aber auf Netzwerke zurückgreifen, die den DLPFC umfassen, könnte der flexible hub theory folgend eine generelle Verstärkung der Aktivität des linken DLPFC zur Verbesserung in verschiedenen Aufgaben beitragen (Cole et al., 2013; Zanto & Gazzaley, 2013). Während auf der Verhaltensebene dieser

Transfer unabhängig von der gleichzeitigen Aufgabenbearbeitung war, könnte die neurophysiologische Aktivität empfindlicher auf die kognitive Aktivierung während der Stimulation reagieren. Studien bei Erwachsenen deuten auf eine Aktivitätsselektivität der neurophysiologischen Veränderungen nach tDCS in Kombination mit der Aufgabenausführung hin (Hill et al., 2019; Pisoni et al., 2018). In unserer Studie könnte die 2-back Aufgabe während der Stimulation ebenfalls zu einer selektiveren Aktivierung geführt haben, was einem Transfereffekt auf neurophysiologischer Ebene entgegengewirkt haben könnte.

6.4 Studie III

Ziel der Studie III war es, die Wirkung der tACS und tRNS auf die Erregbarkeit des motorischen Cortex bei gesunden Kindern und Jugendlichen zu untersuchen, da Erkenntnisse zu beiden Techniken in dieser Altersgruppe bisher unzureichend sind. Hierzu wurde eine explorative Untersuchung der Effekte von 140 Hz und 20 Hz tACS, sowie tRNS über dem Motorcortex durchgeführt. Zusätzlich wurde der Einfluss der individuellen response to sham stimulation untersucht, basierend auf der zuvor erwähnten Studie von Kortuem et al. (2019).

Insgesamt wurden 15 Kinder und Jugendliche (10-16 Jahre), sowie 28 Erwachsene (20-30 Jahre) in die Studie eingeschlossen. Jeder Teilnehmer wurden in randomisierter Reihenfolge an vier Terminen mit 140 Hz, 20 Hz tACS oder tRNS und sham Stimulation (1 mA) für 10 Minuten über dem linken Motorcortex ($M1_{HAND}$) stimuliert. Einzelpuls-MEPs, kurzzeitige intrakortikale Inhibition und Fazilitation wurden mittels TMS vor und nach der Stimulation (Baseline, 0, 30, 60 Minuten) induziert. Zudem wurde die Verträglichkeit der Stimulation erfasst. Entsprechend der individuellen MEP Amplitude unmittelbar nach der sham stimulation im Vergleich zur Baseline wurden die Probanden mittels Wilcoxon signed rank Tests als responder oder non-responder to sham stimulation eingestuft.

Es zeigte sich kein signifikanter Alterseffekt. Unabhängig vom Alter führte 140 Hz tACS zu einer erhöhten Erregbarkeit des Motorcortex im Vergleich zu sham Stimulation. tRNS

und 20 Hz tACS beeinflussten die Motorcortex Erregbarkeit nicht. Die Analyse der response to sham stimulation als Prädiktor für die Reaktion auf die verum-Stimulation zeigte, dass nur bei non-respondern to sham stimulation, 140 Hz tACS und tRNS die Motorcortex Erregbarkeit erhöhten und 20 Hz tACS die Erregbarkeit senkten, während responder to sham stimulation keinen Effekt auf die verum Stimulation zeigten. Für beide Faktoren waren die Effekte auf Einzelpuls-TMS beschränkt. Für kurzzeitige intrakortikale Inhibition und Fazilitation wurden keine Effekte beobachtet. Die Stimulation verursachte wenige und geringe Nebenwirkungen. Inzidenz und Intensität der Nebenwirkungen unterschieden sich nicht zwischen den Altersgruppen oder der Art der Stimulation.

Dass 140 Hz tACS unabhängig vom Alter der Probanden zu einer erhöhten Erregbarkeit des Motorcortex führte, könnte auf die exzitatorische Natur dieser Frequenz zurückgehen. So konnten auch für anodale tDCS, für die ebenfalls exzitatorische Wirkung angenommen wird, vergleichbare Effekte bei Kindern, Jugendlichen und Erwachsenen gezeigt werden (Moliadze et al., 2015). Die fehlenden Effekte der tRNS könnten darauf zurückzuführen sein, dass ein breiter Frequenzbereich verwendet wurde. Bisherige Studien bei Erwachsenen zeigten verstärkt Effekte für tRNS mit einem höheren Frequenzspektrum (Dissanayaka et al., 2017; Terney et al., 2008). Da Beta-Aktivität in Bereichen des Motorcortex mit der Unterdrückung vorbereiteter Bewegungen in Go-Nogo-Aufgaben assoziiert ist (Swann et al., 2009), kann angenommen werden, dass 20 Hz tACS die Erregbarkeit des motorischen Cortex verringern würde. Allerdings berichten frühere Studien von heterogenen Ergebnissen. Die hier gefundenen Ergebnisse stehen im Einklang mit mehreren anderen Studien, die für 20 Hz tACS keine Stimulationseffekte auf die Erregbarkeit des motorischen Cortex fanden (Rjosk et al., 2016; Wach et al., 2013).

Die Abhängigkeit der Stimulationseffekte von der individuellen response to sham stimulation könnten auf Effekte der *homöostatischen Metaplastizität* (Müller-Dahlhaus & Ziemann, 2015) zurückgehen, sowie durch die *Bienenstock-Cooper-Munro theory* erklärt

werden (Bienenstock et al., 1982). So könnte angenommen werden, dass durch die Stimulation hervorgerufene Effekte in respondern to sham stimulation ausgeglichen wurden. Die Ergebnisse könnten aber auch spontane intra-individuelle Fluktuationen in den MEPs widerspiegeln (Horvath et al., 2016). Bei respondern to sham stimulation könnten diese intra-individuellen Fluktuationen generell stärker sein, weshalb verum tES-Effekte überlagert werden könnten.

6.5 Diskussion

Die im Rahmen dieser Arbeit durchgeführten Studien bestätigen, dass tES-Effekte nicht homogen sind, sondern von verschiedenen methodischen und physiologischen Faktoren beeinflusst werden. Für tDCS über dem linken DLPFC zeigte sich bei Erwachsenen in Studie I ein Einfluss der Montage und des individuellen funktionellen Leistungsniveaus. In Studie II, in der Kinder und Jugendliche untersucht wurden, hatte die gleichzeitige Aufgabendurchführung während der Stimulation einen geringen Einfluss auf die Effekte der tDCS über dem linken DLPFC, während die individuelle Anatomie keine Vorhersagekraft bezüglich der tDCS-Effekte hatte. Zudem zeigten sich in Studie I und II Transfereffekte der Stimulation. In Studie III wurde tACS und tRNS über dem motorischen Cortex nicht durch das Alter moduliert, sondern durch die individuelle response to sham stimulation als Marker für den physiologischen Gehirnzustand.

Darüber hinaus haben alle Studien gezeigt, dass die tES mit Ausnahme eines schwerwiegenden unerwünschten Ereignisses nur wenige Nebenwirkungen hatte. Die Untersuchung von Sicherheitsaspekten ist besonders wichtig und notwendig für bisher unerforschte Ansätze, wie z. B. multichannel Montagen oder tACS bei Kindern und Jugendlichen. Richtlinien sollten nicht nur hinsichtlich der tES-Parameter, sondern auch für begleitende Verfahren, wie z. B. den Screening-Prozess, kontinuierlich aktualisiert und verbreitet werden, um mögliche Risiken der Stimulation zu minimieren.

In allen Studien wurden unerwartete Effekte und teilweise geringe Effekte beobachtet, was darauf hindeutet, dass die Parameter der Stimulation bei den einzelnen Teilnehmern in Bezug auf die untersuchten Prozesse und Populationen nicht optimal waren. Die Ergebnisse dieser Arbeit geben weitere Hinweise, wie die tES-Parameter angepasst werden können, um eine effektivere Stimulation zu ermöglichen. Eine optimale Stimulation kann nur erreicht werden, wenn alle relevanten Einflussfaktoren berücksichtigt werden und die Parameter der Stimulation, wie z. B. Frequenz, Verteilung der Elektroden oder kombinierte kognitive Aktivierung, bei jedem Individuum separat bestimmt werden. Das heißt, eine Optimierung der Stimulation impliziert eine Individualisierung der Stimulation. Dies kann nur erreicht werden, wenn das Verständnis der tES-Einflussfaktoren weiter vorangetrieben wird.

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