Optimising the application of transcranial direct current stimulation

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I, Evridiki Gregoriou, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Evridiki Gregoriou, July 2021

Abstract

The ability of transcranial direct current stimulation (tDCS) to modulate brain activity has vast scientific and therapeutic potential, however, its effects are often variable which limit its utility. Both current flow direction and variance in electric field intensities reaching a cortical target may be vital sources of the variable tDCS effects on neuroplastic change. Controlling for these and exploring the subsequent effects on corticospinal excitability is the aim of this thesis.

I here attempted to optimise the delivery of tDCS application by investigating the controlled application of current flow direction and whether through the use of current flow models, we can deliver comparable electric fields with reduced variability across differential montages. To assess whether current flow models are useful, I further investigated if dose-control translates to more consistent physiological outcomes.

I demonstrate, firstly, that different current flow directions did not differentially affect the two banks of the central sulcus. Secondly, with the use of dose-control, high-definition tDCS (HD-tDCS) remains focally more advantageous, even with the delivery of comparable electric field intensity and variability as posterior-anterior tDCS (PA-tDCS) to a cortical region. Thirdly, dose-controlled tDCS does not translate to reduced physiological variability.

Together, the work presented here suggests that current flow models are useful for informing dose-controlled protocols and montage comparisons for improved tDCS delivery, however, controlling for anatomical differences in the delivery of electric fields to a target is not sufficient to reduce the variability of tDCS effects in physiology. Thus, the methodology for optimised tDCS delivery remains a subject for further improvement and investigation. Advancements in this field may lead to a trusted methodology assisting stroke survivors with a more effective and efficient motor recovery journey.

Impact statement

The findings of this thesis provide further insight into the protocol specific and non-protocol specific parameters of tDCS stimulation that require controlled investigation to improve the design of tDCS application. The evidence presented in this thesis also lends further support to the hypothesis that current flow models could be leveraged to support the creation of a dose-controlled tDCS approach based on individual anatomy and exemplifies that controlling for anatomy alone does not sufficiently make tDCS more reliable. Future suggestions and directions of the work presented in this thesis may subsequently contribute to the development of improved tDCS methodologies that could benefit the scientific and clinical field. The findings of this study also point to a future role for the use of subject-specific current flow models incorporated in the product design of neuronavigation software for use in science and possibly therapeutic intervention.

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Abbreviations

ANOVA	Analysis of Variance
ANCOVA	Analysis of Covariance
AP-tDCS	Anterior-Posterior tDCS
CFM	Current Flow Models
CSE	Corticospinal Excitability
CSF	Cerebrospinal Fluid
CV	Coefficient of Variance
ECT	Electroconvulsive Therapy
EEG	Electroencephalography
EF	Electric Field
EMG	Electromyography
FDI	First Dorsal Interosseus
FEM	Finite Element Model
FWE	Family-wise Error
HCP	Human Connectome Project
HD-tDCS	High-Definition tDCS
ICN	Institute of Cognitive Neurosciences
IFCN	International Federation of Clinical Neurophysiology

IHI	Inter-Hemispheric Inhibition
LM1	Left Motor Cortex
LTP	Long-Term Potentiation
M1	Primary Motor Cortex
MH	Motor Hotspot
ML-tDCS	Medial-Lateral tDCS
MNI	Montreal Neurological Institute
MNS	Median Nerve Stimulation
MRI	Magnetic Resonance Imaging
NZ	Nasion
PA-tDCS	Posterior-Anterior tDCS
PMC	Premotor Cortex
PNS	Peripheral Nerve Stimulation
ROAST	Realistic volumetric approach to simulate transcranial electric stimulation
ROI	Region of Interest
S1	Primary Sensory Cortex
SEP	Sensory Evoked Potential
SD	Standard Deviation
SICI	Short Interval Intracortical Inhibition
tACS	Transcranial Alternating Current Stimulation
TDCS	Transcranial Direct Current Stimulation

$tDCS_{fixed}$	Fixed-dose tDCS
$tDCS_{indiv}$	Individualised-dose tDCS
TEMP	Tool for Electrode Movement Prevention
TES	Transcranial Electrical Stimulation
TMS	Transcranial Magnetic Stimulation
tRNS	Transcranial Random Noise Stimulation
VOI	Voxels of Interest
WM	Working Memory

Chapter 1 Transcranial Direct Current Stimulation: Application and Variability

1.1 Synopsis of thesis

Transcranial direct current stimulation (tDCS) is a safe form of transcranial electric stimulation (tES) that delivers a weak current to the brain and has the capacity to accelerate or decelerate neuroplastic changes (Esmaeilpour et al., 2018). Due to its ability to induce such neuromodulatory effects, it is increasingly used to promote motor plasticity in both health and disease (Das, Holland, Frens, & Donchin, 2016; Stagg & Nitsche, 2011), including stroke (Ward, 2016). However, its reliability and efficacy are questionable, since results have been extremely variable across both electrophysiological and behavioural studies (Bestmann & Ward, 2017). Both current flow direction and variance in electric field intensities reaching a cortical target may be vital sources of the variable tDCS effects on neuroplastic change. Controlling for these and exploring the subsequent effects on motor cortical excitability is the aim of this thesis.

Research utilising tDCS has only recently considered the sources of tDCS variability (Horvath et al., 2016). The orientation of the external electric field with regards to the orientation of the pyramidal neurons in the cortex plays a major role for tDCS effects on membrane polarisation and subsequently influence the direction of neuroplastic change (Dissanayaka, Zoghi, Farrell, Egan, & Jaberzadeh, 2017; Miranda, Mekonnen, Salvador, & Ruffini, 2013; Paulus, Antal, & Nitsche, 2013). However, conventional methods of tDCS do not explicitly control the direction of current flow (Bestmann & Walsh, 2017). To gain an in depth understanding of tDCS neuromodulation effects and their variability, it is important to consider the topography of the human brain and how different current flow directions alter membrane polarisation (Dmochowski, et al., 2012; Rawji et al., 2018).

Another source of the variable tDCS effects may arise from variance in individual electric field intensities reaching a cortical target site due to inter-subject anatomical differences

(Bestmann, 2015; Bestmann & Walsh, 2017; Bikson, Rahman, & Datta, 2012; Chew, Ho, & Loo, 2015; Datta et al., 2009; de Berker, Bikson, & Bestmann, 2013; Horvath, Vogrin, Carter, Cook, & Forte, 2016; Radman, Ramos, Brumberg, & Bikson, 2009). Utilising current flow models to individualise tDCS intensity has been proposed to potentially reduce the variability of the observed effects (Bestmann & Ward, 2017; Esmaeilpour et al., 2018). Thus, one needs to consider the dose-response relationship between the electric field reaching a cortical target site and subsequent changes in variance of corticospinal excitability.

In summary, this thesis explores the sources of variability in tDCS effects via neuromodulatory and current flow simulation techniques. I seek to understand the relationship of current that is delivered to the brain and neurophysiology, and whether this relationship provides a target for minimising variable tDCS outcomes. Specifically, my PhD aims to: (1) conduct validation of the importance in controlling for electric field direction in regard to the primary sensory (S1) and primary motor (M1) cortical regions, (2) assess whether individualising the effective dose for tDCS can reduce variability of electric current in the brain reaching a target area across montages and (3) examine whether this method can enhance the efficacy of tDCS physiological effects. Thereby, this thesis addresses the following questions:

1. How do different current flow directions alter membrane polarisation when considering the topography of M1 and S1? **Chapter 3**

2. While delivering spatially more constrained current, high-definition tDCS (HD-tDCS) leads to more variable electric field intensities in a cortical target across subjects. The question addressed in **Chapter 4** is whether the use of current flow models can reduce variability in intensity of electric fields whilst retaining focality advantage with HD-tDCS in comparison to conventional bipolar montages.

3. What are the electric field intensity and variability differences between two different tDCS montages when applying a fixed-dose and individualised-dose of current to a target area? What stimulator output intensity is required to deliver comparable electric field intensities with these montages? Under these

conditions, which montage produces more focal electric field in the brain? **Chapter 4**

4. Does individualised dose-control reduce variability of tDCS physiological effects? **Chapter 5**

Understanding how controlling for current flow direction, intensity and focality may translate into interindividual physiological variability may offer novel therapeutic paradigms for better control of tDCS outcomes.

1.2 Transcranial Direct Current Stimulation (tDCS)

1.2.1 Brief history of Transcranial Electrical Stimulation

Applying electrical currents to enhance human cognitive processes and study the brain has long been a focus of scientific experimentation. There is historical evidence dating as far back as 131-401 AD on using electric fish to stimulate a variety of human body parts to relieve pain and headaches (Priori, 2003). Despite such historic evidence, prior to the 17th century, little was understood about the relationship between the brain and electric currents. From the 17th century onwards, there has been vast experimentation with electrical stimulation and growth in our understanding of its potential for treating clinical conditions (Elliott, 2014).

Perhaps one of the most important techniques emerging during this time was Electroconvulsive therapy (ECT), that included applying electric currents to the brain under anaesthesia to treat psychiatric conditions, such as depression (Elliott, 2014). Nevertheless, this technique illustrated the potential of electrical stimulation and its application in clinical therapy. Since then, more recent, non-invasive brain stimulation techniques have emerged such as transcranial magnetic stimulation (TMS) and tDCS.

TDCS has gained a lot of interest over the past decade (Thair, Holloway, Newport, & Smith, 2017) and research utilizing it has skyrocketed, with promising results both in its ability to promote learning (Das et al., 2016), and in its therapeutic potential to aid

recovery for various conditions, including stroke and depression (Cappon, Jahanshahi, & Bisiacchi, 2016; Das et al., 2016; Stagg et al., 2011; Ward, 2016).

1.2.2 Basic principles

Transcranial electrical stimulation (tES) is a non-invasive brain stimulation technique used to alter brain function. TES applies an electric current to the scalp through electrodes and passes a current through the brain (Reed & Cohen Kadosh, 2018). TES can be applied with various techniques, including tDCS, transcranial alternating current stimulation (tACS) and transcranial random noise stimulation (tRNS). The differentiating factor of these techniques are the different waveforms of the electrical current. The forms of tES are typically named after the waveform they possess, which is the shape and formation of the electrical current (see Figure 1.1). This thesis focusses on tDCS, however, the points made on variability and electric field impact on the cortex, pertains to all forms of tES.

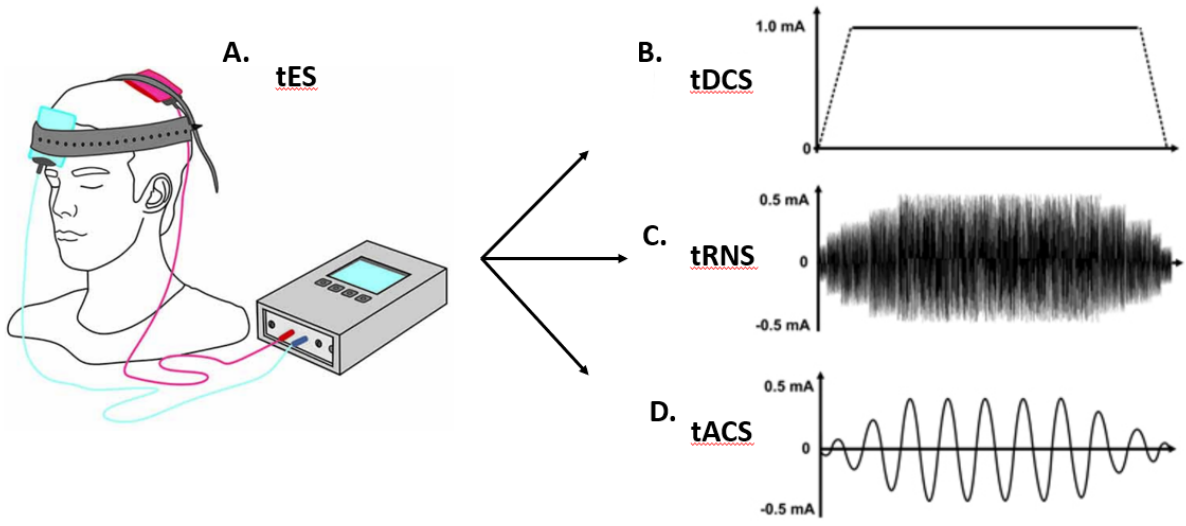

Figure 1.1. Transcranial electrical stimulation (tES) applied current with different waveforms *Schematic of TES application (tES; A). Applied tES waveforms can be direct (tDCS; B), random (tRNS; C), and alternating (TACS; D). Figure adapted from* (Yavari, Nitsche, & Ekhtiari, 2017) *and from* (Saiote, Turi, Paulus, & Antal, 2013).

TDCS is a safe form of tES that delivers a weak current to the brain via scalp electrodes and has the capacity to accelerate or decelerate neuroplastic changes (Bonaiuto & Bestmann, 2015; Esmaeilpour et al., 2018). TDCS produces a constant direct current and is the most frequently investigated method of tES, as well as the focus of this thesis. The current is thought to affect the transmembrane neuronal potential, influencing the likeliness of neurons firing (Bindman, Lippold, & Redfearn, 1964; Purpura & McMurty, 1965). The electrical stimulation applied is not strong enough to elicit an action potential (Radman et al., 2009).

TDCS current is monopolar (Bikson, Datta, Rahman, & Scaturro, 2010) and is typically ramped up for 10-30 seconds. The longer time it takes to ramp up, the less discomfort is experienced, such as a tingling sensation on the scalp under the electrodes (Salimpour, Wei, Duy, & Anderson, 2016). By applying a weak constant current to the head through the use of scalp electrodes, an electric field is created (Bonaiuto & Bestmann, 2015; Dmochowski, Datta, Bikson, Su, & Parra, 2011; Laakso, Tanaka, Koyama, De Santis, & Hirata, 2015). Traditionally, two electrodes are attached to the scalp. One electrode is the 'anode' which is positively charged, and the other is the 'cathode', which is negatively charged. During stimulation, current flows between the electrodes (from anode to cathode), passing through the head to complete the circuit.

1.2.3 Electrode montage and underlying physiology

The electrode montage refers to the placement of electrodes with the purpose of targeting a specific brain area or network. There is a multitude of ways to position electrodes in order to obtain hypothesized neuronal effects (inhibitory or excitatory) and to control for the current direction and focality. A 'bipolar montage' uses only 2 electrodes to pass a current through the brain. HD-tDCS montages refer to montages with more than two electrodes, usually designed to produce more focal electric currents (see Figure 1.2 below and section 1.6.9 TDCS electrode montage for optimised electric field targeting for details).

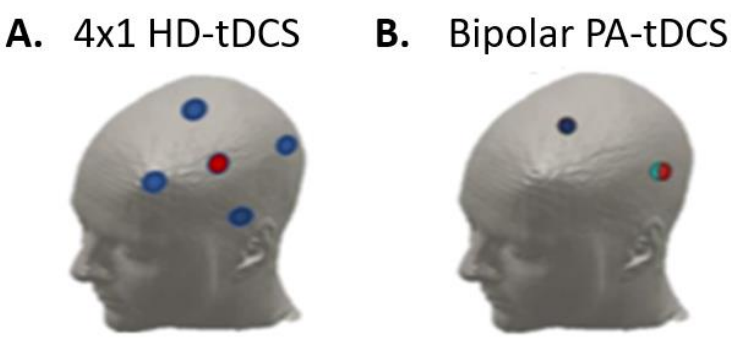

Figure 1.2. Number of electrodes used in bipolar versus more focal high-definition montages
HD-tDCS (A) montages refer to an organisation of electrode positioning with more than two electrodes and produce more focal electric currents. Bipolar montages (B) use 2 electrodes to pass current through the brain and typically produce more diffuse current flow than HD-tDCS.

It is commonly assumed that tDCS electric current facilitates cortical areas under the anode electrode and consequently that this promotes behaviour associated to those brain regions, whereas a negative 'cathodal' current, with the electrodes reversed, inhibits behaviours relative to the same cortical target (Antal, Terney, Poreisz, & Paulus, 2007; Furubayashi et al., 2008; Nitsche et al., 2008, 2005; Nitsche & Paulus, 2000; Priori, Berardelli, Rona, Accornero, & Manfredi, 1998; Uy & Ridding, 2003). In other words, depending on stimulation polarity, membrane potentials may depolarise and increase the probability of eliciting action potentials or hyperpolarise and decrease the likelihood of eliciting action potentials (see Figure 1.3; Nitsche & Paulus, 2000), which lead to the aforementioned facilitatory or inhibitory effects, respectively (Dissanayaka et al., 2017; Nitsche et al., 2008; Paulus et al., 2013; Stagg & Nitsche, 2011). Thereby, critical changes in brain excitability are thought to be a result of modulated resting membrane potentials at a cortical target (Miranda et al., 2013). This thesis challenges this simplistic view, however, whereby in humans, conventional 'anodal' stimulation with the anode electrode over the target and the cathode at a distance, simply depolarises neurons and produces excitatory effects, and 'cathodal' stimulation with electrodes reversed produces inhibitory effects.

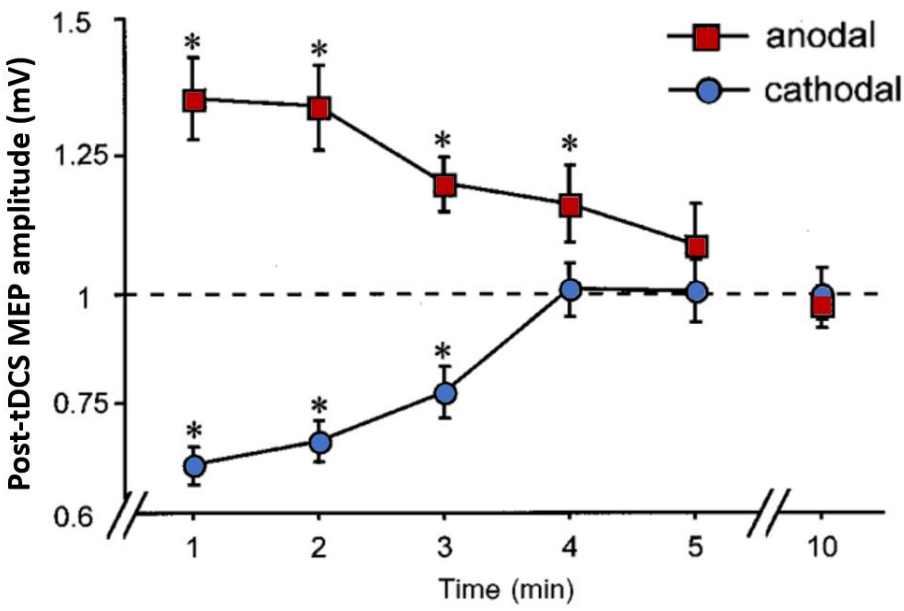

Figure 1.3. Polarity specific cortical excitability changes over a 10-minute time course
TDCS with the anode over the left primary motor cortex (LM1) increases corticospinal excitability as indexed by increased motor evoked potential (MEP) amplitudes, whereas tDCS with the cathode over the LM1 (Blue) decreases corticospinal excitability. Figure adapted from (Nitsche & Paulus, 2000) *with permission from the Journal of Physiology.*

My view is in part motivated by the observation that on a cellular level, there is no such notion as only depolarising or only hyper-polarising neurons with tDCS. Instead, the soma of pyramidal neurons (Chan, Hounsgaard, & Nicholson, 1988; Radman et al., 2009), axon terminals/synapses (Bikson et al., 2004; Chakraborty, Truong, Bikson, & Kaphzan, 2018; Rahman et al., 2013) and dendrites (Kronberg, Bridi, Abel, Bikson, & Parra, 2017) are all cellular targets of an electric current flowing through the brain. During tDCS, these sections of the neuron are depolarising while other sections are simultaneously hyperpolarising (Bikson, Rahman, Datta, Fregni, & Merabet, 2012; Chan & Nicholson, 1986). Thereby, changing the direction of current flow or 'polarity' of stimulation reverses the effects of stimulation, but the underlying mechanism is more complex than often assumed for human studies. For example, as illustrated in animal studies, stimulation with the anode over the cortical target is expected to produce an inward flow of current (relative to the cortical surface) and depolarise the soma of a pyramidal neuron whilst hyperpolarising other neuronal sections and neighbouring cells (Bikson et al., 2004; Radman et al., 2009; Rahman et al., 2013). The extent and the direction of this polarisation of different compartments of pyramidal neurons will

influence net changes in excitability (see Figure 1.4; Rahman et al., 2013). As such, at a microscopic level, it is incorrect to refer to 'anodal' stimulation as simply depolarising neurons when there are several compartments of the pyramidal neuron equally de and hyper- polarised (Bikson et al., 2004; Rahman et al., 2013). Consequently, the assumption that behavioural changes can be explained simply by uniform anode-depolarised and cathode-hyperpolarised neurons under the electrodes is flawed and ought to be questioned.

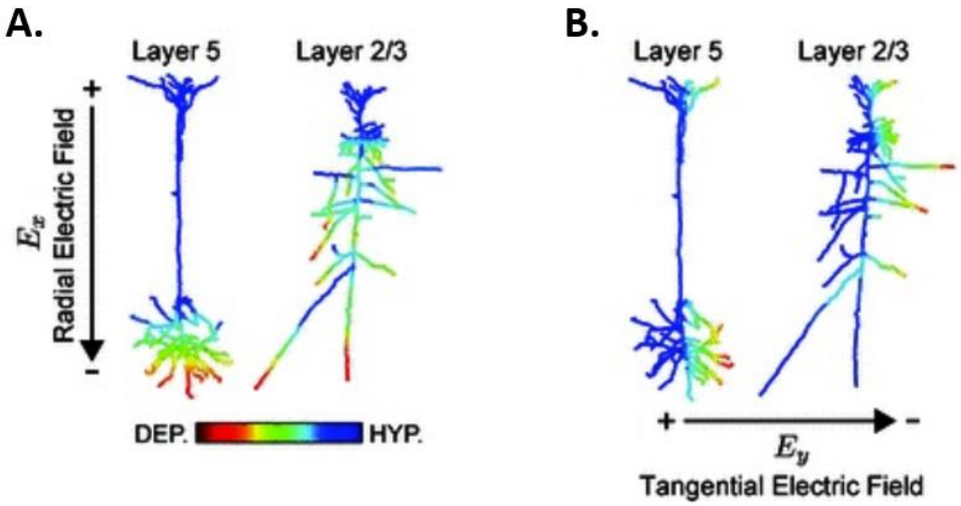

Figure 1.4. Polarisation of cellular components
Current applied superficially with a radial current flow (A; left) will depolarise (red) the cell soma whilst hyperpolarising (blue) the dendrites of a layer 5 pyramidal neuron. Layer 2-3 neurons have a more complex polarization profile independent of the neuronal body. TES applied with a tangential current flow to the somatodendritic axis of a neuron, preferentially affect processes that are oriented along the tangential field. Figure adapted from (Rahman et al., 2013).

Other than the expected polarity effects of tDCS, there are a multitude of influencing factors affecting tDCS outcomes including: the underlying brain activity (Kronberg et al., 2017), 'online' or 'offline' tDCS, current intensity, duration of stimulation, task-specific parameters and electrode parameters (see section 1.3 on 'TDCS Controlled parameter's for details). Factors such as the orientation of neurons, orientation of electric field and inter-individual anatomical and functional differences also affect stimulation outcomes (Bikson et al., 2004; Kabakov, Muller, Pascual-Leone, Jensen, & Rotenberg, 2012; Krause & Kadosh, 2014; Radman et al., 2009), see section 1.6 'Variability of tDCS effects' for

details. Nevertheless, such polarity-specific effects have been demonstrated in multiple paradigms (Antal, Kincses, Nitsche, & Paulus, 2003; Priori, 2003) both during (online) and post-stimulation (offline). Further, long-term changes such as changes in excitability indicated by synaptic plasticity (e.g., long-term potentiation; LTP (Fritsch et al., 2010; Gartside, 1968; Kronberg et al., 2017), have also been linked with the number of sessions that tDCS has been applied for, however these are not the focus of this thesis and thus are not explored in detail.

1.3 The application of tDCS

TDCS protocols and parameters of stimulation can be utilised in a myriad of combinations, with montage and stimulation largely varying across studies with different current intensities, stimulation durations and electrode montage (Boggio et al., 2006; Brunoni, Schestatsky, Lotufo, Benseñor, & Fregni, 2014; Fregni et al., 2006; Loo et al., 2012; Palm et al., 2012), all of which make it hard to compare tDCS effects across studies. To identify the fit-for-purpose montage and protocol, it is necessary to understand the impact of different tDCS parameters on physiological and behavioural outcomes.

Stimulation parameters of tDCS impact both the spatial and temporal distribution of electric field (Thair et al., 2017). These include stimulation polarity, intensity and duration of stimulation. Online vs offline stimulation and number of sessions, as well as inter-session intervals, play a vital role in the outcome of tDCS (Alonzo, Brassil, Taylor, Martin, & Loo, 2012; Gálvez, Alonzo, Martin, & Loo, 2013; Monte-Silva et al., 2013) and should also be considered when designing tDCS experiments. The following sections 1.3.1 – 1.3.4 provide oversight to these parameters.

1.3.1 Current density and electrode parameters

The combination of electrode size and current intensity produces different current densities. Current density is thought to be directly related to modulation of cortical changes (Bikson, Datta, & Elwassif, 2009) and the measurement unit of current density

is mA/cm². Current density is the amount of electric current per unit of surface area. Thereby, current density (*J*) is calculated as a function of the current intensity (*I*) and the electrode size (*A*).

Current density calculation: $J = \frac{I}{A}$

For example, if the electrical current is 2 mA and the electrode size is 5 x 7 cm, current density would be calculated as follows: $J = \frac{2}{35} = .057\ mA/cm^2$

Although current density is thought to be related to cortical excitability, results are heterogeneous. For example, tDCS with the anode over the target of three current densities (.032, .04 and .048 mA/cm², 10 min) produced no significant changes in cortical excitability (Kidgell et al., 2013), which highlights the complex non-linear effects of tDCS (Benwell, Learmonth, Miniussi, Harvey, & Thut, 2015; Esmaeilpour et al., 2018; Learmonth et al., 2017). Due to complex non-linear effects of tDCS, there is currently no consensus for optimal current densities to alter corticospinal excitability.

Most commonly, the electrodes used for bipolar tDCS are a pair of rectangular electrodes, with sizes varying typically between 3x3 (9 cm^2) and 5x7 (32 cm^2). Electrode sizes affect current intensity and distribution (Nitsche et al., 2007). Thus, changing electrode size alone can change the electric current's focality and intensity throughout the brain, and may contribute to heterogenous outcomes between studies. It has been suggested that a smaller 'anode' or target electrode can increase the focality of the electric field, whereby increasing the size of the 'cathode' or return electrode may also reduce tDCS effects due to electric field reaching undesired cortical areas (Bastani & Jaberzadeh, 2013; Nitsche et al., 2007). Comparison of 3 electrode sizes (12, 24 and 35 cm^2) showed that the smaller, 12 cm^2 electrodes produced greater effect-sizes than 24 and 35 cm^2, as current density, affected by electrode size is an important parameter affecting tDCS outcomes (Bastani & Jaberzadeh, 2013; Bikson et al., 2016). However, there were no differences between effects caused by 24 and 35cm^2 electrode sizes. This may be because the use of smaller stimulating electrodes produce more focal electric fields (Bastani & Jaberzadeh, 2013; Faria, Hallett, & Miranda, 2011; Mikkonen, Laakso, Tanaka, & Hirata, 2020; Nitsche et al., 2007; Opitz et al., 2018), and thus do not, in

comparison to larger electrodes, impact as much cortical area outside the target.. Smaller electrodes are more focal because the magnitude of current density falls faster with distance from the reference area in comparison to a larger electrode (Faria, Hallett, & Miranda, 2011). Thereby, smaller electrodes should be used to control for the focality of electric currents. Nevertheless, greater focality of distributed currents does not necessarily equate to better tDCS outcomes (see **Chapter 6** section 6.2 for an extensive discussion), and one should note that even with smaller electrodes the focality is, at best, limited compared to other stimulation techniques such as TMS.

Furthermore, there has been limited research on differential tDCS effects with various electrode shapes. It is important to note that the surface of the "electrode" thus refers to the entire conductive surface, including the surface area of the sponge and the saline solution or conductive paste and rubber electrode (Peterchev et al., 2012). A conductive paste is far easier to control in shape and form when spred on the electrode versus saline solution. Saline solution may spread further than the stimulation area and due to the shape of the cranium it is unlikely to spread homogeneously. For my experimental designs covered in this chapter, I thereby use conductive paste rather than saline solution to apply tDCS electrodes (see **Chapter 2** section 2.4 'TDCS experimental setup' for details).

1.3.2 Intensity of stimulation

The intensity and duration of stimulation are two important parameters shaping tDCS outcomes. For duration of stimulation, see next section. Regarding stimulation intensity, electrode size plays an important role in the intensity of current reaching a cortical area. An additional factor is the positioning of electrodes. Current flow simulations indicate that a greater percentage of current penetrates the cortex when electrodes are placed further apart (Miranda, Lomarev, & Hallett, 2006). When using 35 cm^2 electrodes, it is recommended that a distance of at least 8cm is accounted for (Wagner et al., 2007). However, larger distances come at a cost, as the current dissipates across the scalp, which decreases the concentration of electric field reaching a targeted brain region and thereby greater current intensities may be needed (Moliadze, Antal, & Paulus, 2010). Additionally, if inter-electrode differences are 5cm or less, the current could possibly be

shunted across the scalp and not penetrate the brain at all (Rush & Driscoll, 1968). In general terms, greater distances between electrodes are expected to increase cortical modulation, as the current will more likely penetrate the scalp and impact the targeted brain area, rather than being shunted (Bikson et al., 2010). Thereby, electrode placement should always be considered with regard to the intensity of the electric current, though currently no good models exist to inform what the ideal electrode placement might be.

Regardless of current intensities reaching the brain, most studies only considered the intensities applied by the stimulator output on the scalp and not the modelled electric field in the cortex. Studies often use between 1 and 2 mA of tDCS stimulator output (Bikson et al., 2016). Initial research assumed that with higher stimulation intensities, there would be more beneficial effects (Nitsche & Paulus, 2000), however, this notion has been questioned (Bastani & Jaberzadeh, 2013; Batsikadze, Moliadze, Paulus, Kuo, & Nitsche, 2013; Esmaeilpour et al., 2018; Kidgell et al., 2013). Further evidence portrayed the expected cortical excitability increase with 1 mA tDCS with the anode over the target and expected decrease with the cathode over the target (Batsikadze et al., 2013). However 2mA of tDCS with the cathode over the target reversed the effects and found an increase in corticospinal excitability following twenty minutes of stimulation (Batsikadze et al., 2013). Nevertheless, excitability changes relative to stimulator output intensities have been widely investigated with varying tDCS parameters. A crossover-design study investigated the effects of ten minutes 0.8, 1 and 1.2 mA of tDCS stimulation on corticospinal changes during weak voluntary contraction and found no significant differences between these stimulation intensities (Kidgell et al., 2013). On the contrary, examination of a larger range of stimulator output (i.e 0.3, 0.7, 1.4 and 2 mA) exhibited greater facilitation with 2 mA stimulation than 1.4 mA (Bastani & Jaberzadeh, 2013). However, the same study also illustrated higher motor facilitatory effects with 0.3 mA than with 0.7 mA which contradicts their initial findings that a stronger current has larger modulatory effects in corticospinal outcomes (Bastani & Jaberzadeh, 2013).

In some cases, it is still expected that 2 mA would be more likely to induce increased excitability changes compared to weaker stimulator outputs (Ammann, Lindquist, & Celnik, 2017). However, tDCS effects of current intensity are complex and non-linear

(Esmaeilpour et al., 2018). This is still highly dependent on the electric current reaching cortical target areas. Inter-individual differences in anatomy influence current intensities reaching a cortical target across subjects and some individuals may be more responsive to the same current intensities than others (Bestmann, 2015; Bestmann & Walsh, 2017; Bikson, Rahman, Datta, et al., 2012; Chew et al., 2015; Datta et al., 2009; de Berker et al., 2013; Horvath, Carter, & Forte, 2014; Laakso et al., 2015; Opitz, Paulus, Will, Antunes, & Thielscher, 2015; Radman et al., 2009). As such, tDCS protocols typically applying 1-2 mA dose across a population will lead to substantial differences in the variation of electric field intensities reaching a targeted cortical site (Evans et al., 2019; Laakso et al., 2015). Controlling for electric field intensity is therefore crucial for making tDCS effects more consistent. Such lack of dose-control challenges the comparability of tDCS effects (see sections 1.6.3 and 1.6.4 for details on interindividual dose-response differences and responders vs non-responders that contribute to variable tDCS electric field intensity and distribution).

1.3.3 Duration of stimulation

Regarding the duration of stimulation, 10-20 minutes is widely used (Bastani & Jaberzadeh, 2012b). This is based on early, 1960's animal studies by Bindman and colleagues (Bindman, Lippold, & Redfearn, 1962; Bindman et al., 1964) that showed excitability changes increasing with time and outlasting the stimulation period. Thus, there is an expected relationship between the duration of stimulation and effects of tDCS on brain excitability following tDCS. Early human studies of tDCS in the motor cortex suggest that stimulation durations of approximately 10 minutes are required to produce effects that can last up to one hour after the stimulation (Nitsche et al., 2003; Nitsche, Paulus, & Nitsche, 2001; Nitsche et al., 2005; Nitsche & Paulus, 2000). TDCS administered for 5-7 minutes with the anode over the cortical target increased changes in excitability further than the stimulation period of up to 5 minutes, whereas 9 minutes of stimulation showed increased corticospinal excitability for 30 minutes (Nitsche et al., 2001). A longer stimulation time of 13 minutes showed prolonged corticospinal excitability effects for over 90 minutes (Nitsche et al., 2001).

The notion of longer stimulation periods leading to longer lasting effects is not universally true. Longer stimulation periods have also shown to reverse expected outcomes (Fricke et al., 2011; Monte-Silva et al., 2013). TDCS application for 26 minutes with the anode over the primary motor area resulted in an inhibitory response rather than excitatory (Monte-Silva et al., 2013). These results were dependent on the rest time between two stimulation periods. Indeed, research shows that differential effects can be obtained when applying short- or long- lasting rest periods (Fricke et al., 2011). Fricke and colleagues (2011) tested multiple stimulation periods, and reported motor evoked potentials that index corticospinal excitability change, acquired for 5 minutes in 2 periods. In between these periods, different resting times were intersected (0, 3 and 30 minutes). Results indicated that with no break, the aftereffects were prolonged in comparison to having breaks. The effects with the 30-minute break were the same in the second period as in the first period, whereas a 3-minute break resulted in opposite effects to the first period (Fricke et al., 2011). The effects of resting-time on tDCS outcomes was further explored with up to three stimulation sessions, but there was no clear consensus on resting time between two stimuli (Bastani & Jaberzadeh, 2014), because the experimental paradigms differed (Besson, Perrey, Teo, & Muthalib, 2016). Thereby, resting time differences between studies may be a factor adding to the inter-study variability of tDCS effects, and thus, when deciding on stimulation period with resting times, one should consider the possible mediated outcomes.

Additionally, effects of duration of stimulation are not necessarily linear, nor consistent, as there is a multitude of studies applying tDCS to other cortical areas with stimulation times of 30-40 minutes and have found no reversal of effects (Bolognini et al., 2011; Brunoni et al., 2012; Ho et al., 2015; Tortella, 2015).

1.3.4 'Online' and 'offline' tDCS

Another factor for tDCS application is whether tDCS is applied during a task or at rest. 'Online' brain stimulation refers to tDCS stimulation paradigms that involve a subject undertaking a behavioural task whilst receiving stimulation. On the other hand, offline stimulation paradigms involve tDCS application at rest (Thair et al., 2017). It has been highlighted that a person's brain state during stimulation can interfere with stimulation

effects (Horvath et al., 2014). This suggests that the decision to use online or offline protocols may play a role in modulating tDCS effects. Classically, tDCS is applied before a behavioural task. Yet, little is known about the effectiveness between online (tDCS and task are concurrent) and offline (tDCS is adjacent to task) effects from tDCS protocols. Some studies promote online tDCS with a task due to their promising results (Galea & Celnik, 2009; Nitsche et al., 2003; Stagg et al., 2011). Performing a task involves multiple brain regions and thereby targeting these neurons is expected to strengthen such networks (Miniussi, Harris, & Ruzzoli, 2013). This is referred to as 'functional targeting' (Bikson & Rahman, 2013). As tDCS may apply very weak currents to the brain, a task may activate relevant neuronal networks to the task at hand and thus these networks are more active than other irrelevant neuronal networks and may become more susceptible to tDCS (Jackson et al., 2016). For example, if tDCS is to be used during motor rehabilitation training, the neuronal network that is already active during training and undergoing plasticity would be targeted by tDCS, while other networks that are inert, would not be modulated (Kronberg et al., 2017). This is reflective of the dependency of tDCS effects on brain state (Bikson & Rahman, 2013; Li et al., 2019; Luft, Zioga, & Bhattacharya, 2018; Petti et al., 2017; Wang et al., 2018), which could be a factor needing control in physiological studies or leverage in motor training circumstances (Bergmann, 2018). Concurrent stimulation protocols are thereby a promising approach to increase the effects of tDCS.

Research shows contradictory results of polarity specific effects when the subject undertaking tDCS is occupied with an irrelevant task (Nozari, Woodard, & Thompson-Schill, 2014). An important example is a research study that applied tDCS with the cathode over the cortical target, during an unrelated task, but found facilitatory effects on a relevant task (the Flanker task). However, when the same relevant task (Flanker task) was undertaken during stimulation, the expected inhibitory effect was observed (Nozari et al., 2014).

Furthermore, when participants imagine tasks before or after the stimulation, this interferes with the effects of stimulation (Antal et al., 2007; Gladwin, den Uyl, Fregni, & Wiers, 2012; Quartarone et al., 2004). For example, a differential effect of the polarity of stimulation was demonstrated when participants were asked to imagine motor tasks

before or after stimulation (Quartarone et al., 2004). Furthermore, a large decrease in motor evoked potential amplitude was apparent when inhibitory stimulation was applied following motor imagery than at rest, whereas effects of tDCS of the opposite polarity were not interfered with by motor imagery (Quartarone et al., 2004). Thereby, when applying tDCS, it should be considered whether the subject is undertaking a task during or before stimulation and whether there are any breaks in between. Even if considering such parameters, there are other participant behaviours that may be hard to control for in such experiments, such as finger tapping and minor movements that affect tDCS effects (Horvath et al., 2014).

When tDCS experiments are undertaken with no relevant tasks, it is worth considering the excitability of the target cortical area prior to stimulation as this can alter tDCS effects (Filmer, Dux, & Mattingley, 2014). It has been shown that neuronal populations that are less active are more susceptible to brain stimulation such as single pulse TMS (Silvanto, Muggleton, & Walsh, 2008). This could very well mean the same for other types of brain stimulation, including tDCS, and should be considered. Whilst online tDCS paradigms show promising effects relative to a task, when looking at physiology, a task may affect the outcomes and thus it is important to control for.

Overall, it is essential to understand the external and internal factors that influence tDCS polarity effects, to aid the improvement of future tDCS application protocols and impact. Nevertheless, it is difficult to decide and control for all the aforementioned factors as there is currently no consensus which of these may contribute most to the effects of stimulation. Experimental design in this thesis therefore considered the aforementioned factors that may produce variability arising from intensity and montage differences, but note, that this is not to say other factors are not relevant (see **Chapter 2** for tDCS experimental design and parameters used in this thesis).

1.4 Measuring corticospinal excitability

TDCS's ability to modulate cortical excitability and promote neuroplastic changes is one of the reasons behind its vast use in both health and disease (Das et al., 2016; Stagg et al., 2011), including stroke (Ward, 2016). The most widely used read-out of tDCS induced excitability change are motor evoked potentials (MEPs) elicited in peripheral muscles when applying TMS over the human motor cortex (Bestmann & Krakauer, 2015; Horvath et al., 2016). MEPs provide an index of corticospinal excitability (CSE) (Di Lazzaro et al., 1999, 1998; Di Lazzaro & Rothwell, 2014). Changes in the amplitude of MEPs are thereby thought to reflect changes in CSE. For this reason, tDCS effects on CSE are thought as a main potential biomarker for assessing the effects of tDCS in health and disease.

1.4.1 Transcranial magnetic stimulation induced corticospinal excitability

TMS is an established neurostimulation device utilised for its ability to stimulate corticomotor pathways and examine these in health and disease. TMS elicits electromagnetic pulses, through a coil placed tangentially on the head over a cortical target to activate cortical neurons (Barker, Jalinous, & Freeston, 1985). With the coil placed tangentially on the head, a magnetic pulse penetrates the scalp and induces a secondary electric current in the conductive brain tissue. The orientation of the electric field created is perpendicular to that of the magnetic field and is in opposite direction to the electric current in the TMS coil. As human brain anatomy is not homogeneous, and there are differences in regional conductive tissues, the current distribution is distorted and difficult to predict interindividually (Yang et al., 2006). To understand the current distribution of TMS electric fields, current distribution based on different tissue types and conductivities have been modelled (Yang et al., 2006). Stimulation strengths of the magnetic field that TMS produces peaks between 1–2.5 Tesla and is very short lasting (<1 ms). The term 'Magnetic' stimulation only refers to the pulse administered and not the 'stimulation' of the brain, as the magnetic field itself does not stimulate cortical neurons and is only used to induce an electric current in the brain.

TMS was introduced as a non-invasive brain stimulation device to directly stimulate the brain, safely and painlessly (Barker et al., 1985). It was demonstrated that a single TMS

pulse over the primary motor cortex could activate corticomotor neurons associated with the brain region and elicit responses to the corresponding muscles in the contralateral part of the body (Barker et al., 1985). These motor evoked potentials, are easily detected through surface electromyography (EMG) used to record peripheral muscle activity, and are considered an index of corticospinal excitability (see Figure 1.5). This has made TMS an attractive and vastly adopted technique for routine method to measure CSE changes both in health and disease (Bestmann, 2012; Chen et al., 2008; Day et al., 1987; Di Lazzaro et al., 2004; Mills, Murray, & Hess, 1987; Rothwell, 1997; Rothwell, Day, Thompson, Dick, & Marsden, 1987; Rothwell et al., 1999; Rothwell, Thompson, et al., 1987; Terao et al., 1995).

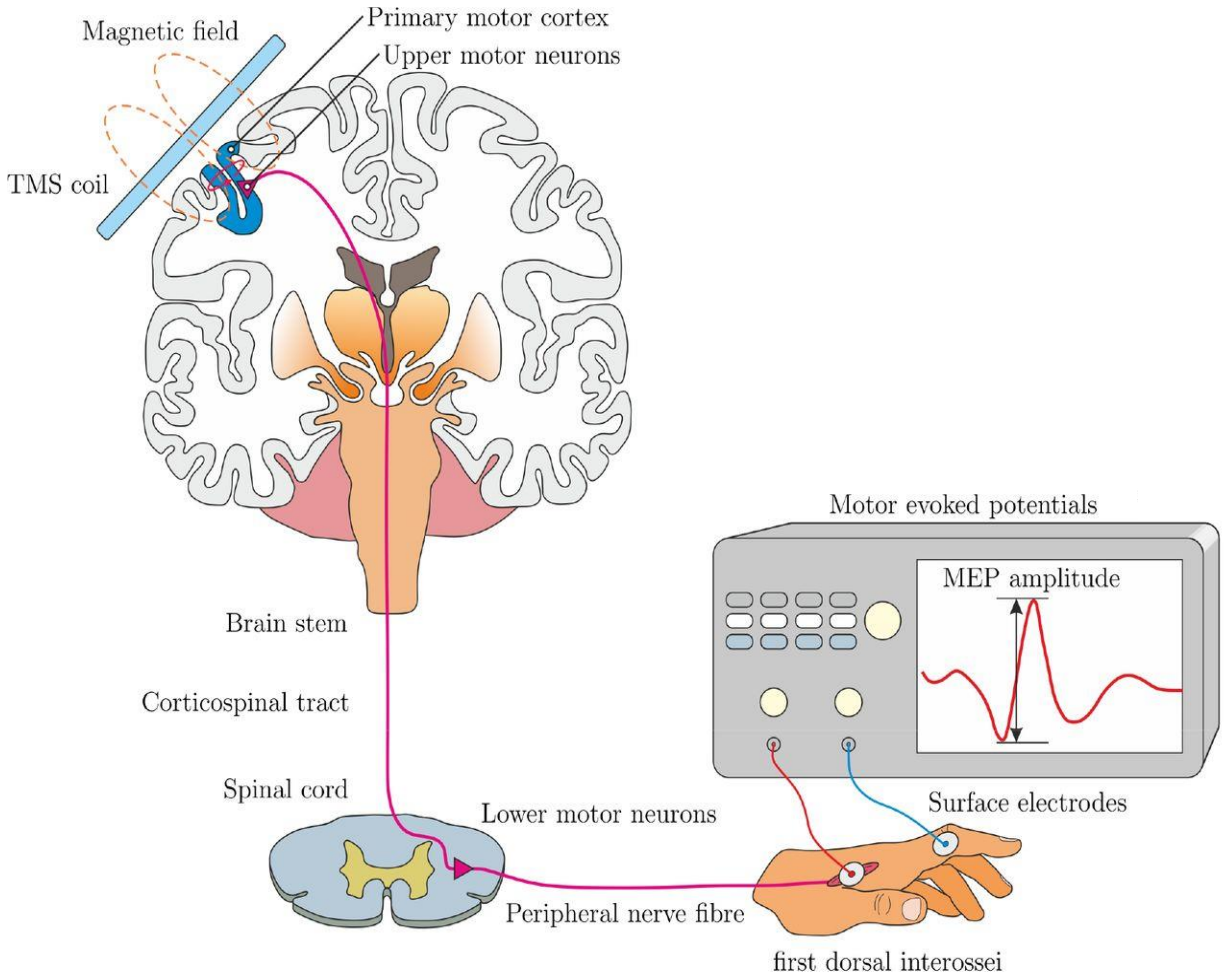

Figure 1.5 Schematic representation of the TMS-induced MEP measurement protocol
The TMS coil is oriented tangentially over the primary motor cortex (top left). The TMS magnetic pulse generates an electric field in the brain that depolarizes upper motor neurons with corticospinal efferent neurons. Upper motor neuron action potentials excite lower motor neurons

(bottom). Action potentials travel through the peripheral nerves to the first dorsal interosseous (FDI) of the hand (bottom right). MEPs are recorded using electromyographic electrodes, using a classical belly tendon montage, i.e, active electrode over the 'belly' of the FDI (red electrode; bottom right) and reference electrode (blue electrode; bottom right) over the proximal interphalangeal joint of the index finger. Adapted from Weise et al., (2019)

More specifically, TMS applies electromagnetic pulses through the use of a coil placed tangentially on the head over a cortical target, and induces short-lived electrical current in the underlying cortical tissue (~200 µs; Barker et al., 1985). Depending on the resting state of the neurons, and the stimulation intensity, these currents can evoke a contralateral muscle response, MEP, consisting of both cortical and spinal neuronal contributions. Corticospinal neurons are activated both directly and trans-synaptically (Abbruzzese & Trompetto, 2002). The electric field generated by TMS over the primary motor cortex depolarises upper motor neurons. Upper motor neuron action potentials excite lower motor neurons and travel through the peripheral nerves to hand muscles including the first dorsal interosseous muscle. Electromyographic activity of the FDI as a response to TMS (MEP) is then recorded with electrodes over the FDI (see Figure 1.5 for a schematic representation of this process).

The magnitude of MEPs are thereby considered a response consisting of a combination of both cortical and spinal contributions which can be difficult to dissociate as it depends on a variety of processes and presented characteristics such as its temporal dispersion and mechanisms of the brain and spine (Abbruzzese & Trompetto, 2002). As such, MEP magnitude can be utilised as gross measure of corticospinal excitability but cannot be used to distinguish between subcortical and cortical changes in excitability when using single pulse TMS (Abbruzzese & Trompetto, 2002; Chen, 2000). On the contrary, paired-pulse TMS protocols for short interval intracortical inhibition (SICI) and interhemispheric inhibition (IHI) can provide greater insight in to the nature of direct and indirect mechanisms of cortico-cortical and corticospinal circuitry that is activated by TMS (Perez & Cohen, 2009). However, this thesis utilised single-pulse TMS and thereby does not go into detail with other TMS protocols.

Furthermore, the latency of the MEP reflects a simple measure, which is the conduction velocity for neural impulses to reach peripheral muscles from the cortex, which includes

the speed of the fastest cortico-spinal projections, the amount of descending volleys at the spine and the conduction velocity along the peripheral motoneurons (Bestmann & Krakauer, 2015). At each of these three levels, any state changes can significantly impact the latency of the MEP (Bestmann & Krakauer, 2015). Additionally, the magnitude and latency of the MEP can be a critical surrogate marker for motor impairment (Freund, Rothwell, Craggs, Thompson, & Bestmann, 2011; Jayaram et al., 2012; Reis et al., 2008; Stinear et al., 2007; Ward et al., 2006).

MEPs in response to the same stimulation intensity have shown to be highly variable. MEP variability is a result of various influencing factors, including intrinsic and extrinsic factors. Intrinsic factors include fluctuations in corticomotor neuronal excitability (Kiers, Cros, Chiappa, & Fang, 1993; Thickbroom, Byrnes, & Mastaglia, 1999), physiological noise (Kischka, Fajfr, Fellenberg, & Hess, 1993), the level of muscle activation (Darling, Wolf, & Butler, 2006), attention (Bell, Lauer, Lench, & Hanlon, 2018), time of day (Tamm, Lagerquist, Ley, & Collins, 2009), age and neuronal motor threshold (Pitcher, Ogston, & Miles, 2003). Further extrinsic factors include TMS coil location and placement (Kiers et al., 1993), stochastic noise, environmental conditions, skill levels of the experimental researcher, and measurement bias (Goetz, Luber, Lisanby, & Peterchev, 2014). Although it is difficult to control for all these factors, and to further understand the exact mechanisms of effect of each of these factors on MEP variability, some techniques are suggested, including the use of magnetic resonance imaging-guided TMS neuronavigation that may increase the consistency of MEPs (Bashir, Edwards, & Pascual-Leone, 2011; Gugino et al., 2001). Additionally, obtaining a larger number of MEPs is more reliable. Although there is no consensus regarding ideal intensity and MEP count, a minimum of five MEPs to an ideal number of 30 has been suggested (Bashir et al., 2017; Bastani & Jaberzadeh, 2012a; Cavaleri, Schabrun, & Chipchase, 2017; Christie, Fling, Crews, Mulwitz, & Kamen, 2007; Lewis, Signal, & Taylor, 2014). For further details on Neuronavigation techniques see **Chapter 2** section 2.2.10 and 2.2.11.

In summary, TMS applied over the motor cortex provides an objective measure of corticospinal excitability through MEPs, and thus, the effects of tDCS on excitability have been widely investigated for this region. This makes TMS-induced MEPs, although variable, the primary source to visualise CSE changes induced by TDCS. Using techniques

such as neuronavigation and increased MEP measurements have been suggested to control for MEP variability.

1.4.2 TDCS effects on corticospinal excitability

TDCS has a number of advantages over TMS, such as the fact that the device is small, portable, easy to use and cheaper, making it more appealing for home and clinical use (Elder & Taylor, 2014). The earliest study examining tDCS effects on motor cortical excitability was conducted by Priori and colleagues in 1998 (Priori et al., 1998). This work was then followed by a classic demonstration by Nitsche and Paulus (Nitsche & Paulus, 2000; Nitsche et al., 2001) on the effects of tDCS on CSE. The early studies of Nitsche and Paulus provided the classical tDCS protocol to induce CSE changes (Nitsche & Paulus, 2000). The conventional tDCS montage for modulating CSE (see Figure 1.9; A) places one electrode over the hand location of the central sulcus, and another electrode over the contralateral orbit (Nitsche & Paulus, 2000). They demonstrated that 1 mA (using 35 cm^2 electrodes) tDCS with anode over the cortical target and cathode over the contralateral orbit, applied for 13 minutes, increased TMS-induced MEPs. On the contrary, tDCS applied with the anode/cathode electrodes positions reversed decreased MEPs with effects outlasting the stimulation period for up to 5 minutes (see Figure 1.3; Nitsche & Paulus, 2000). Due to these findings, tDCS became desirable for use in rehabilitation of clinical motor disorders.

1.5 TDCS clinical potential for motor recovery after stroke

Given that tDCS can increase or decrease cortical excitability, its application in neurorehabilitation is thereby not surprising, given it is also safe and well-tolerated (see **Chapter 2** section 2.4.5 for safety considerations of tDCS). The underlying principle is that the same physiological mechanisms that allow for the reorganisation of the nervous system when CSE is promoted, are the same mechanisms that are affected after abrupt trauma or deterioration in healthy motor function caused by injury or disease. However, an injured brain undergoes different brain processes, both functionally and structurally,

and there should not be an expectation that tDCS effects in healthy individuals will translate in injury or disease.

The advancements in our understanding of the brain in health and disease provides a basis for developing neurorehabilitation protocols with the use of technologies such as tDCS (Di Pino et al., 2014; Khan, Amatya, Galea, Gonzenbach, & Kesselring, 2017; Pellicciari et al., 2018). There is a plethora of research highlighting tDCS effects on CSE, also evident by multiple in vivo and in vitro research in animals (Guan, Yi, Xie, & Huang, 1989; Jackson et al., 2016; Kim et al., 2010; Notturno et al., 2014; Pikhovych et al., 2016; Yoon, Oh, & Kim, 2012). For instance, in vivo stroke animal models have shown that reduced infarct volume correlated with the application of fourty-five minutes of tDCS (Notturno et al., 2014) and behavioural improvement with tDCS application was evident within one week of an ischemic event (Yoon et al., 2012). In the latter study, tDCS was applied for five days at either one week or five days after the ischemic event, and although both groups had improved effects with tDCS than the sham group, the group receiving stimulation one week post-stroke onset had the most beneficial outcomes on behavioural tests (Yoon et al., 2012). While research on the impact of tDCS for improving stroke outcomes is just in its infancy, such studies promote its use for modulation of motor plasticity and learning in both health and disease, especially stroke (Das et al., 2016; Stagg et al., 2011; Ward, 2016).

Motor rehabilitation following stroke is one of the most widely investigated rehabilitation applications of tDCS. Beneficial effects following tDCS application have been demonstrated both for upper- (Butler et al., 2013; Chhatbar et al., 2016; Elsner, Kugler, Pohl, & Mehrholz, 2016; Ward, 2016) and lower- limb (Kang, Summers, & Cauraugh, 2016b) functions and mobility in general (Kang, Summers, & Cauraugh, 2016a; Kang et al., 2016b). However, as the mechanisms underlying tDCS are still unclear and results are variable and inconsistent across studies, the high number of studies reporting results from tDCS demands a close examination of stroke rehabilitation. A systematic review highlights the inconsistency of results across studies (Lüdemann-Podubecká, Bösl, Rothhardt, Verheyden, & Nowak, 2014) for improved post-stroke motor performance and long-term sustained improvements (Lüdemann-Podubecká et al., 2014). Two meta-analyses examined long-term motor learning following tDCS and

were unable to resolve the controversy due to the excessive heterogeneity in the few studies analysed and the confound of comparing studies with differential tDCS protocols such as applying tDCS with online or offline paradigms (Elsner, Kugler, Pohl, & Mehrholz, 2013; Marquez, van Vliet, Mcelduff, Lagopoulos, & Parsons, 2015).

1.5.1 Montage selection for stroke populations

There is currently no consensus for selecting tDCS protocol and montage for stroke patients, as tDCS effects across clinical studies remain largely variable. Various tDCS montages are suggested for stroke patients based on the principle of post-stroke abnormal interhemispheric inhibition (Lindenberg, Renga, Zhu, Nair, & Schlaug, 2010; Schlaug, Renga, & Nair, 2008). The idea is that modulation of cortical excitability may interfere with post-stroke maladaptive brain processes which imbalance interhemispheric inhibition (Hummel & Cohen, 2006; Murase, Duque, Mazzocchio, & Cohen, 2004; Nowak, Grefkes, Ameli, & Fink, 2009). Stroke may affect the imbalance of neural networks between the two hemispheres as neural activity following a stroke is enhanced in the motor areas of the unaffected hemisphere (Hummel & Cohen, 2006; Murase et al., 2004). By reducing the influence of brain regions that are disturbing the physiological network in the affected hemisphere, this may show improved performance in the stroke-affected hand. Based on the post-stroke abnormal interhemispheric inhibition model, several montages have been investigated (Nowak et al., 2009; Takeuchi, Oouchida, & Izumi, 2012) to normalise the imbalance of transcallosal inhibition between the hemispheres to improve function (Gomez Palacio Schjetnan, Faraji, Metz, Tatsuno, & Luczak, 2013).

A series of proof-of-concept studies investigated the neuromodulatory benefits of tDCS on motor rehabilitation training following stroke (Hummel et al., 2005). One of the initial studies (Lindenberg, Renga, Zhu, Betzler, et al., 2010) demonstrated that tDCS with the anode located over ipsilesional and cathode over contralesional M1 combined with an occupational and physical therapy protocol over a period of five days, significantly improved motor function relative to sham tDCS in the upper-limb of stroke patients. This was indicated through improvements on the Fugl-Meyer Assessment and Wolff Motor Function test. In a similar study, Kim et al. (2010) administered ten days of tDCS with the

cathode located over contralesional M1 and anode over ipsilesional supraorbital region combined with occupational therapy. This improved upper limb impairment and activities of daily living when compared to sham tDCS (Kim et al., 2010). These effects persisted up to six months post-tDCS. However, the latter 'dual montage' produced more beneficial effects in stroke impairment (Bolognini et al., 2011; Lindenberg, Renga, Zhu, Nair, et al., 2010).

Apart from M1, other brain regions have also been targeted. Areas such as the sensorimotor association cortex, primary somatosensory cortex (S1) (Allman et al., 2016), premotor cortex (PMC) (Rocha et al., 2016) and cerebellum (Wessel & Hummel, 2018) have all shown beneficial motor rehabilitation effects following tDCS. These beneficial effects were illustrated in different phases of stroke impairment such as acute (Barros Galvão, Borba Costa Dos Santos, Borba Dos Santos, Cabral, & Monte-Silva, 2014; Picelli et al., 2018), subacute (Marangolo, Fiori, Caltagirone, Pisano, & Priori, 2018) and chronic phases (Chang, Kim, & Park, 2015; Viana et al., 2014).

Nevertheless, there is no current tDCS protocol for stroke producing consistent and reliable outcomes and the myriad of combinations of target area, stroke phase, type of stimulation and task make it difficult to compare across studies. Additionally, the heterogeneity of stroke type, lesion location and size, chronicity and cortical tract integrity influence the effects of tDCS (Bestmann & Ward, 2017; Feng et al., 2018) and perhaps individualising stimulation to the patient is one way to reduce such variability. Currently, there is lack of control and individualisation of tDCS effects and developing personalised tDCS protocols and identifying ways to reduce the largely inter-subject and inter-study variability of tDCS outcomes in motor physiology is still a subject of research.

1.6 Variability of tDCS effects limit its clinical application

Several studies (Bastani & Jaberzadeh, 2013; Batsikadze et al., 2013; Kidgell et al., 2013; Monte-Silva et al., 2013; Wiethoff, Hamada, & Rothwell, 2014) have not found the expected tDCS outcomes observed initially by Nitsche and Paulus (Nitsche & Paulus, 2000; Nitsche et al., 2001) which make tDCS effects variable in healthy populations. This

variability is exacerbated in clinical populations due to the added complexity of pathological brain function. More patient-specific and sophisticated approaches are required to effectively apply tDCS clinically. TDCS has not yet been clinically approved as a commercial rehabilitation device since results have been extremely variable across both electrophysiological and behavioural studies.

The majority of clinical and healthy population studies applying tDCS, use a simplistic approach, whereby excitability is increased or decreased under the anode and cathode, respectively when applying a fixed dose. However, tDCS effects are much more complex than simple 'anodal' excitation/'cathodal' inhibition and there is a complex relationship between stimulation parameters (as aforementioned in section 1.2) and outcomes. Subject-specific parameters play a vital role in tDCS effects and include anatomy, behaviour and brain state at the time of stimulation. The effects of tDCS stimulation are thereby a result of a combination of subject specific differences and parameter selection. Any modification even of a single factor will vary the effects of tDCS. Further factors affecting inter-individual variability include genetics, gender, age, hormone level, time of day, parallel motor activity, attention level and habituation to the presented task (Chew et al., 2015; Huang, Datta, Bikson, & Parra, 2017; Wiethoff et al., 2014) however these are not discussed in detail in this thesis. Studies are advised to experimentally consider and control for factors that influence tDCS effects as much as possible, especially when comparing effects across studies that apply tDCS in different populations (Krause & Kadosh, 2014; Li, Uehara, & Hanakawa, 2015; Terranova et al., 2019).

Given the above, it is not easy to decide upon the most effective design for a given experiment. Firstly, there is great variability in protocol and set-up across published studies, of which many are often under-powered due to small sample sizes (Li et al., 2015). Thereby, it is difficult to acquire consistent results across studies as there are various ways of applying tDCS and there is not yet an optimum protocol to induce specific outcome reliably. Secondly, there is variance in the effects of tDCS when the same protocol is used, and this is exacerbated due to inter-individual differences. Variance in the effects of tDCS is portrayed by a great cohort of studies that often fail to replicate tDCS effects, thus lowering its reliability and efficacy (Galli, Vadillo, Sirota,

Feurra, & Medvedeva, 2019; Hordacre et al., 2017; Horvath, Forte, & Carter, 2015b; Horvath et al., 2016; Tremblay et al., 2016). This threatens the uptake of tDCS in a therapeutic setting (Bestmann & Walsh, 2017; Terranova et al., 2019).

Investigating the sources of variability of tDCS effects is therefore of paramount importance to ensure reliable delivery and improve its application for clinical use. Both current flow direction and variance in electric field intensities reaching a cortical target may be vital sources of the variable tDCS effects on neuroplastic change. Controlling for these and exploring the subsequent effects on CSE is the aim of this thesis.

1.6.1 Variability in tDCS effects due to lack of control for current flow direction

Research utilising tDCS has only recently considered the sources of tDCS variability (Horvath et al., 2016). The orientation of the external electric field with regards to the orientation of the pyramidal neurons in the cortex plays a major role for tDCS effects on membrane polarisation and subsequently influence the direction of neuroplastic change (Dissanayaka et al., 2017; Miranda et al., 2013; Paulus et al., 2013). However, conventional methods of tDCS do not explicitly control the direction of current flow (Bestmann & Walsh, 2017). To gain an in depth understanding of tDCS neuromodulation effects and their variability, it is important to consider the topography of the human brain and how different current flow directions alter membrane polarisation. The next section (section 1.6.2) explores this in detail.

1.6.2 Current flow direction effects on membrane polarisation

Intracranial recordings (Huang, Liu, et al., 2017) and current flow modelling studies (Datta et al., 2009; Dmochowski et al., 2011) show that bipolar montages produce current flow in brain areas between electrodes. However, as conventionally practiced, applying one electrode over the target and the other at a distance creates a sparse distribution of current flow in the brain (Bestmann & Walsh, 2017; Bikson, Rahman, Datta, et al., 2012), such that tDCS induced changes in measured outcomes may be reflective of excitability changes occurring as a result of stimulated brain regions outside

the targeted area (Alam, Truong, Khadka, & Bikson, 2016; Edwards et al., 2013; Karvigh, Motamedi, Arzani, & Roshan, 2017). Thus, it is important to control for current flow direction in a target and understand its effects on physiology.

Conventionally, it is expected that there is an increase in cortical excitability under the anode and a decrease under the cathode. However, this is more complex. Cell membrane polarisation depends on the orientation of external field with regards to the orientation of pyramidal neurons in cortex (Bestmann & Walsh, 2017; Esmaeilpour et al., 2018). This was initially demonstrated by Nitsche and Paulus (2000), who found that the effects of tDCS on the primary motor cortex were abolished when changing the position of the cathode (Nitsche & Paulus, 2000). One parameter that could explain this is the change in the direction of current flow. Thus, controlling for the direction of current flow within a target is important (Bikson et al., 2004; Radman et al., 2009; Rawji et al., 2018). The complicating factor is that different types of neurons are oriented differentially in the cortex. For example, pyramidal neurons are aligned perpendicular to the cortical surface, whereas interneurons are aligned parallel to the cortical surface (Dmochowski, Bikson, & Parra, 2012). This is further complicated as the same pyramidal neurons are oriented differentially in the cortex due its curvature and the gyri and location of the sulci within it. *In vitro* rat studies show that neurons oriented parallel to the electric field respond differently to those aligned perpendicularly (Bikson et al., 2004; Kabakov et al., 2012). Thus, changing the direction of current flow will polarise neurons differentially.

Due to inter-individual differences in the anatomy, organisation of brain tissue types, such as tissue geometry and gyri structure, each individual will have a unique pattern of current flow direction based on the location of electrodes (Datta et al., 2009; Miranda et al., 2013). Current flow direction is further affected by the conductivity of cerebrospinal fluid, which is more conductive than white or grey matter (Bikson, Rahman, Datta, et al., 2012) and impacts brain tissue that is directly adjacent to the cerebrospinal fluid at a greater level (Neuling, Rach, Wagner, Wolters, & Herrmann, 2012).

Previous research (Rawji et al., 2018) indicates that tDCS induced CSE changes depend on the current flow direction with respect to the principal axis of the central sulcus.

Rawji and colleagues (2018) investigated the effects of different orientations of current flow on corticospinal excitability, as indicated by MEPs. Current flowing along the precentral gyrus (i.e. in medio-lateral orientation) did not affect CSE. On the contrary, current flowing perpendicularly across the wall of the central sulcus (i.e. in anterior-posterior direction) reliably modulated CSE (Rawji et al., 2018). This may be because the primary motor cortex is located in the anterior wall of the central sulcus, and optimal polarisation of pyramidal neurons in this region should occur when current flows along the principal dendritic axis of these neurons i.e, perpendicularly with regards to the cortical surface (Bikson & Rahman, 2013; de Berker et al., 2013; Esmaeilpour et al., 2018; Rawji et al., 2018).

Since the primary sensory cortex (S1) is positioned on the posterior wall of the central sulcus, and the orientation of pyramidal neurons is opposite in the two banks of a sulcus (Rahman et al., 2013), current directed perpendicular through the cortical surface of pre- and post-central sulcus should lead to opposite polarisation effects in M1 and S1, respectively (see Figure 1.6). However, this has yet to be explored. One of the key questions addressed in my first experimental chapter (**Chapter 3**) is: How do different current flow directions alter membrane polarisation when considering the topography of M1 and S1? More specifically, does controlling for the direction of current flow lead to opposite polarisation effects in M1 and S1? To investigate this, I applied current flow direction across and along the central sulcus, and concurrently measured the impact of stimulation in these two regions (M1/S1).

Despite compelling evidence from animal studies that the effects of externally applied current depend on cell morphology and orientation of pyramidal neurons (Rahman et al., 2013), the majority of tDCS studies in humans give no consideration to the effects of current flow direction in a target region. Such demonstration would be important to provide a rationale for optimizing the application of tDCS.

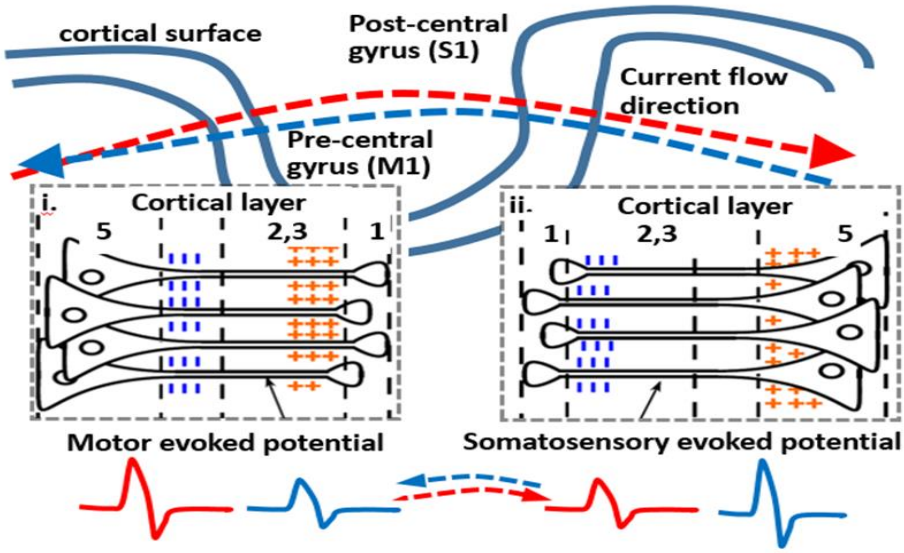

Figure 1.6. Orientation of pyramidal neurons on the opposite banks of the central sulcus and DCS effect

This figure illustrates the orientation of pyramidal neurons in the banks of the pre- and post-central sulcus. The red arrow illustrates the anterior to posterior tDCS current (AP-tDCS) whereas the blue arrow illustrates the tDCS current flowing from posterior to anterior (PA-tDCS). As described in the hypothesis, I expect that PA-tDCS and AP-tDCS will have opposite polarisation effects.

1.6.3 Interindividual dose-response differences contribute to variable tDCS intensity and distribution

Another source of the variable tDCS effects may arise from variance in individual electric field intensities reaching a cortical target site due to inter-subject anatomical differences (Bestmann, 2015; Bestmann & Walsh, 2017; Bikson, Rahman, Datta, et al., 2012; Chew et al., 2015; Datta et al., 2009; de Berker et al., 2013; Evans et al., 2019; Horvath et al., 2016; Radman et al., 2009). The amount of current 'dose' and the site of current 'target' entering the brain are of major consequence, though surprisingly, neither are assessed in tDCS studies (Bestmann & Ward, 2017). Exploration of this source of variance is vital for the improvement of tDCS delivery and its progression in clinical rehabilitation.

Inter-individual differences in anatomical structures, such as skull thickness and cerebrospinal fluid (CSF) volume, have different resistive properties which vary both the amount and spread of the electric field in the brain across a population (Bestmann, 2015; Bestmann & Walsh, 2017; Bikson, Rahman, Datta, et al., 2012; Chew et al., 2015;

Datta et al., 2009; de Berker et al., 2013; Evans et al., 2019; Horvath et al., 2014; Laakso et al., 2015; Opitz et al., 2015; Radman et al., 2009). As such, one specific tDCS protocol may lead to substantially different effects than another. Conventional tDCS protocols typically apply 1-2mA dose across a population, leading to substantial inter-individual differences in the variation of electric field intensities reaching a targeted cortical site (e.g left M1; Laakso et al., 2015). Current flow induced by conventional bipolar montages is not limited to the target site, but diffuse and heterogeneous, with current reaching large parts of the cortex (Datta et al., 2012). Thereby, current flow does not necessarily peak at the cortical target site and its location is dependent on individual anatomy (Laakso et al., 2015). Such distinctions may vary by a factor of two or more across studies for the same sample sizes (Evans et al., 2019). This lack of dose-control undermines the comparability of observed tDCS effects. Further exploration is crucial for reducing the variability of tDCS effects and improving its potential to be applied in therapeutic settings.

The dose-response relationship of tDCS remains largely undetermined, partly because its investigation requires a level of dose-control that current protocols have not yet achieved and it is still unclear if the effects increase linearly with applied current intensity (Bestmann & Walsh, 2017; Esmaeilpour et al., 2018). Increasing the stimulation dose does not necessarily result in greater brain excitability changes (Bastani & Jaberzadeh, 2013; Kidgell et al., 2013). In some cases, this has shown to reverse effects (Batsikadze et al., 2013; Mosayebi Samani, Agboada, Jamil, Kuo, & Nitsche, 2019).

1.6.4 Variability of electric field leads to variability of tDCS effects

If we assume there is a direct relationship between the electric field intensity reaching a cortical target site and the magnitude of tDCS effects, then reasonably, variability of electric fields will inevitably lead to variable tDCS responses. Evidence for this is highlighted by a study that demonstrated inter-individual differences in electric field at M1 were directly related to differences in CSE changes following tDCS (Laakso, Mikkonen, Koyama, Hirata, & Tanaka, 2019). Subjects that received the highest and lowest electric fields responded with opposite effects to the same tDCS output intensity (Laakso et al., 2019). Further validation comes from a more recent study (Mosayebi-

Samani et al., 2021), demonstrating that inter-individual variability of physical factors such as cerebrospinal fluid thickness, and regional electrode-to-cortex distance were directly related to inter-individual variability of neurophysiological changes in MEPs and cerebral blood flow changes induced by tDCS (Mosayebi-Samani et al., 2021). Furthermore, on a behavioural level, working memory (WM) improvement outcomes, following tDCS during a WM task, was correlated with the amount of current reaching the dorsolateral prefrontal cortex (Kim et al., 2014). These observations validate that electric field intensity is an important source of inter-individual variability of tDCS effects and may account for the low efficacy of tDCS observed in some studies.

1.6.5 Responders versus non-responders

Differences in tDCS effects are also due to substantial inter-individual differences in the response to tDCS. Evidence suggests that post-tDCS CSE changes showing weak or non-significant effects at group-level may be due to people classified as 'Responders' and 'Non-responders', with responders including 20 to 61 % of subjects (Horvath et al., 2016). Responders are classed as participants who show effects in an expected direction, whereas non-responders are participants who show minor effects, no effects, or even effects opposite to the expected direction (Chew et al., 2015; López-Alonso, Cheeran, Río-Rodríguez, & Fernández-Del-Olmo, 2014; Strube et al., 2016; Wiethoff et al., 2014). For example, one study highlights that although three-quarters of the participants responded with facilitatory effects to 2mA tDCS, only 38 % responded with the expected facilitatory effects with the anode over the target and inhibitory effects with the cathode over the target (see Figure 1.7; Wiethoff et al., 2014). Non-responders may not receive adequate current intensities at the cortical target site to cause the predicted change of excitability, or they simply may receive too much current (Strube et al., 2016). Note the terms 'responders' and non-responders are referring to classifications of people with expected effects versus individuals who responded to tDCS with non-expected effects, respectively. Surprisingly, the vast majority of tDCS studies do not account for interindividual differences in response to tDCS. Instead, they apply a fixed tDCS stimulation intensity for each subject and do not consider that differences in anatomy may play a role in varying tDCS effects due to electric fields varying across their sample

(Bestmann & Ward, 2017). Controlling for electric field intensity is therefore important for making tDCS effects more consistent.

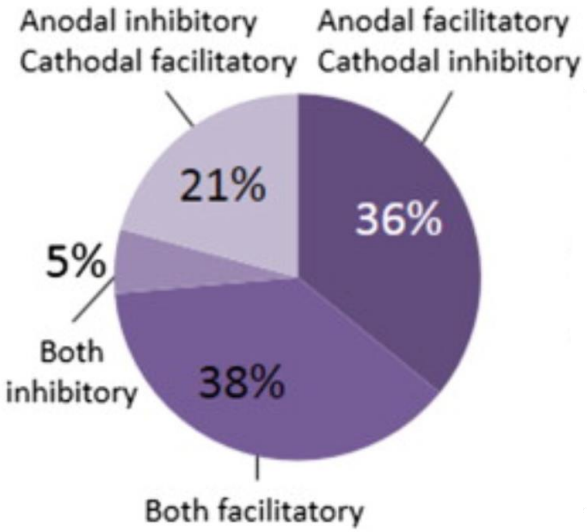

Figure 1.7 Response profile following tDCS
This figure provides a responders' profile to tDCS and highlights the high inter-individual variability of response to tDCS effects. Responders and non-responders were classified based on whether the mean post-TDCS effects on corticospinal excitability were greater or less than 1, respectively. Just over one third of participants had the expected tDCS response, i.e increased CSE excitability following TDCS with the anode over the target and decreased CSE with the cathode over the target (36 %). However, a similar proportion had increased CSE effects following both stimulation types (38 %). A lower proportion had the opposite effects than expected (21 %). Figure taken from (Wiethoff et al., 2014).

1.6.6 Current flow models

Utilising current flow models (CFMs) to individualise tDCS intensity has been proposed to potentially reduce the variability of the observed effects (Bestmann & Ward, 2017; Esmaeilpour et al., 2018). Current flow models are a promising tool that provides individualised computational estimates of current flow for a given tDCS montage based on Magnetic Resonance Imaging (MRI) scans (Bikson et al., 2015; Bonaiuto & Bestmann, 2015; Datta, Baker, Bikson, & Fridriksson, 2011; Huang, Datta, et al., 2017).

CFMs have recently been validated in primate and human studies using intracranial recordings (Huang, Liu, et al., 2017; Opitz et al., 2016). Opitz and colleagues in 2016,

used implanted electrodes in cebus monkeys and pre-surgery epilepsy patients to measure the distribution of an oscillating electric current in cortical space and time that was induced by three different tACS protocols. As expected, the electric field intensity measured by the electrodes exhibited large variation across subjects, however, these intensities were in line with those predicted by previous current flow modelling studies (Opitz et al., 2016). Subsequent work measuring current electric field intensity and distribution intracranially with epilepsy patients, compared current flow modelling estimates using their MRI scans. The electric field estimates produced by the models highly correlated with the actual electric field recorded in patients' intracranially (Huang, Liu, et al., 2017). Both studies report electric fields in the range of 0.2-0.5 V/m, produced by transcranial electrical stimulation output range of 1-2 mA. Such electric field strengths were present throughout the brain including deep brain structures and not only at the surface, under the stimulation electrode. The above studies validated that current flow models present reliable estimates of tES current flow and promoted its potential use for further research investigating their use in individualising tDCS protocols based on individual anatomical factors.

1.6.7 Individualising current dose with the use of current flow models

Utilising current flow modelling to individualise tDCS intensity has been proposed to potentially reduce the variability of the observed effects (Bestmann & Ward, 2017; Esmaeilpour et al., 2018). Such models can be used, in principle, to provide an estimate of individualised dose and deliver comparable electric fields in a target across a population (Bestmann & Ward, 2017; Evans et al., 2019). Based on individual estimates of electric fields in a cortical target, it is possible to estimate the tDCS dose required for each individual so that a specific amount of electric field reaches a selected target across a population (Figure 1.8 illustrates this concept). A recent study that modelled current flow in a large cohort proved the feasibility of this approach (Evans et al., 2019)[1]. Evans and colleagues (2019) modelled current flow in 50 MRI scans and demonstrated that by

[1] Personal contributions to this research paper include overview on the methodology and publication as well as contributions to discussions around the design and implementation of the research.

selecting a target region and extracting the electric field intensities reaching this target, one can reverse calculate the necessary stimulator output intensity so that all subjects can receive the same electric field strengths in that region (Figure 1.8 illustrates this concept). Thereby, with the use of current flow models, one can substantially reduce variability of electric fields reaching a cortical target across a population (Evans et al., 2019). The tDCS output intensities remained below 4 mA when individualising for a 2mA montage (Evans et al., 2019), which is considered a safe dose (Bikson et al., 2016).

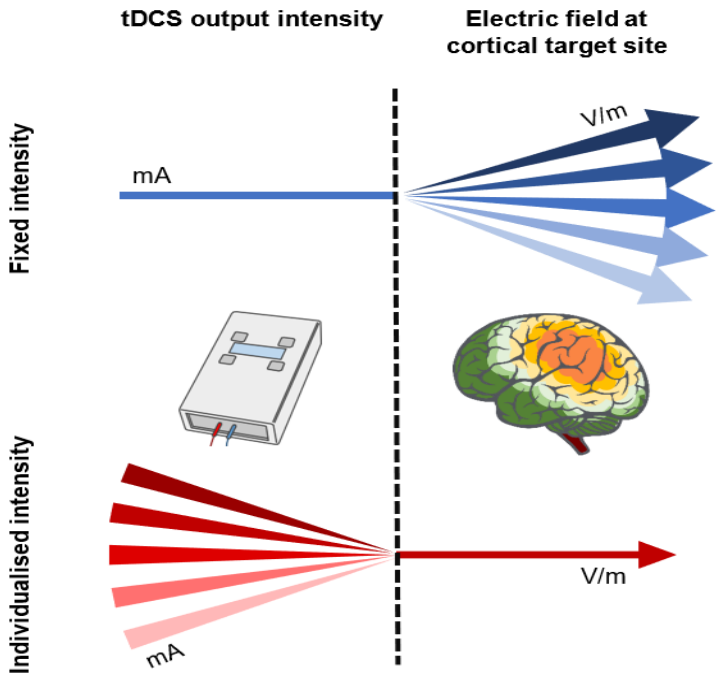

Figure 1.8 Electric field variability with individualised vs fixed applied doses
A fixed tDCS output intensity results in variable electric field intensities at the cortical target site across participants (Blue). Individualised tDCS output intensities for each participant results in constant electric field intensities at the cortical target site (Red). Figure adapted from (Evans et al., 2019).

1.6.8 Current flow is diffuse

Current flow induced by conventional tDCS montages is diffuse and has brain-wide distribution (Datta et al., 2012). Inter-subject anatomical variation also influences electric field distribution across the brain, such that weaker or stronger electric fields

are distributed differentially across individuals (Laakso et al., 2015). This is problematic since peak electric fields do not necessarily concentrate in the target area, and tDCS induced change in outcome measures (e.g MEP amplitude) may reflect excitability effects from electric fields spreading toward untargeted cortical areas. Thereby, it is as important to control for the focality of current as it is to control for the intensity.

1.6.9 TDCS electrode montage for optimised electric field targeting

To improve stimulation control, studies have examined the focality of current delivered by tDCS (Bortoletto, Rodella, Salvador, Miranda, & Miniussi, 2016; Faria et al., 2011; Mikkonen et al., 2020; Moliadze et al., 2010). Despite wide-spread electric fields delivered by tDCS, questions remain unanswered about the design of electrode montages, including electrode size and location and their subsequent effects on physiology. Changing the size and location of electrodes will affect the focality of electric fields and subsequently change the physiological outcomes (Mikkonen et al., 2020; Moliadze et al., 2010). As such, montage selection should always be considered.

To achieve more focal electric fields, previous studies have investigated various approaches, including: the use of smaller stimulating electrodes which have shown to produce more focal electric fields the smaller they are (Bastani & Jaberzadeh, 2013; Faria et al., 2011; Mikkonen et al., 2020; Nitsche et al., 2007; Opitz et al., 2018) and various high-definition (Bortoletto et al., 2016; Datta et al., 2009) and multifocal (Fischer et al., 2017) montages. These tDCS applications have shown to deliver more focal electric fields (Mikkonen et al., 2020).

Specifically, HD-tDCS has been developed for its focality advantage over conventional montages, which utilises more than two, and smaller, electrodes (Datta et al., 2009; Dmochowski et al., 2011; Shekhawat et al., 2016). Based on current flow models, the 4 x 1 deployment of HD-tDCS, where four return electrodes are arranged around a target with a fixed ring-radius distance, delivers peak electric fields under the central electrode and constrains current flow within the ring's radius (Datta et al., 2011; Edwards et al., 2013). Thus, the focality of 4 x 1 HD-tDCS minimises impact on untargeted areas that may alter physiological outcome measures (Alam et al., 2016; Edwards et al., 2013;

Karvigh et al., 2017) compared to conventional tDCS that apply more diffuse electric field spread in the brain (see Figure 1.9).

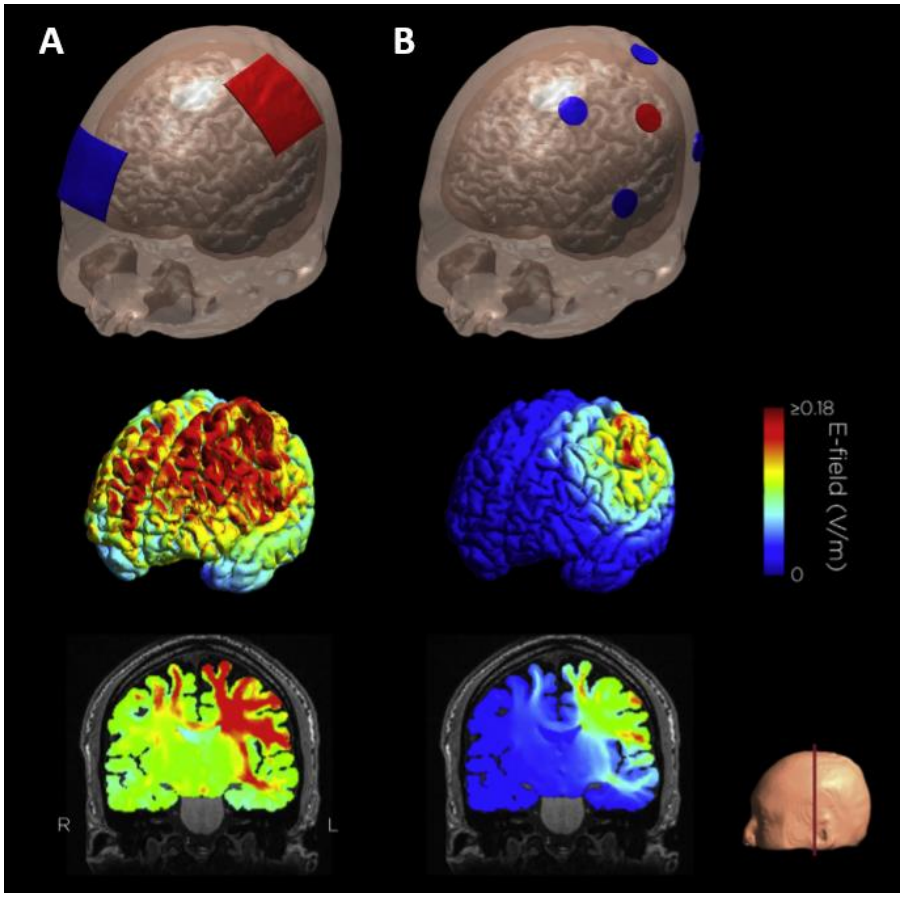

Figure 1.9 Electric field distribution and focality for conventional bipolar tDCS and 4x1 HD-tDCS montages

A. The conventional tDCS montage (A; top left) with the anode electrode (red) over the left primary motor cortex and the cathode (blue) over the supraorbital ridge ("M1-SO" montage) and its corresponding electric field distribution (A; middle and bottom). B. 4x1 high-definition tDCS (4x1 HD-tDCS) montage (B; top right) with the anode (red) over the left primary motor cortex, surrounded by four cathode electrodes (blue) its corresponding electric field distribution (B; middle and bottom). The electric field was simulated using an amplitude of 1 mA. Figure adapted from (Bikson et al., 2018)

Even though HD-tDCS may indeed deliver more focal electric fields, it comes at the cost of increased inter-individual variability of electric fields reaching a target (Mikkonen et al., 2020). One study explored the differential electric field distribution, magnitude and variability of thirteen montages with different electrode sizes and shapes targeting the motor cortex and illustrated that with decreasing electrode size, there is an increase in

focality, intensity and variability of electric fields at the cortical target (Mikkonen et al., 2020). Indeed, this is in line with previous research reporting higher variability in the after-effects of TDCS with 12 cm^2 electrodes versus 24 cm^2 electrodes (Bastani & Jaberzadeh, 2013). As previously suggested (Borckardt et al., 2012), 4x1 HD-tDCS exhibited the most focal electric fields, however, the magnitude and focality of the electric field was inversely proportional such that higher electric field magnitudes led to lower focality (Mikkonen et al., 2020). Furthermore, bipolar montages that control for the direction of current within a target (i.e posterior-anterior tDCS; PA-tDCS, and mediolateral tDCS; ML-tDCS) achieved more focal electric field than conventional tDCS (i.e anode over cortical target and cathode at supraorbital region), with equally as strong electric field strengths as 4x1 HD-tDCS and with lower inter-individual variability than 4x1 HD-tDCS and conventional tDCS (Mikkonen et al., 2020).

Overall, HD-tDCS provides more focal stimulation than other montages, but given the same stimulator output, the electric field reaching the brain varies in magnitude and in spread across montages. While delivering spatially more constrained current, HD-tDCS does not help the problem of variable intensity (Mikkonen et al., 2020). The question that I address in **Chapter 4** is whether, through the use of current flow models, reduced variability in intensity can be achieved whilst retaining focality advantage with high-definition tDCS than PA-tDCS, when applying comparable electric field intensities to a target. To the best of my knowledge this has yet to be investigated.

1.6.10 Translation to physiology

Current flow models are valuable for predicting electric current flow and magnitude through the brain and individualising tDCS dose, however, are they useful?

In order to demonstrate the importance of current flow models, we need to show that dose-control (Evans et al., 2019; Mikkonen et al., 2020) can lead to a reduction in variance of tDCS effects. Current flow modelling helps guide tDCS protocols to deliver less variable tDCS current to a cortical target, interindividually. However, it is still unknown whether delivering less variable tDCS current leads to improved effect sizes and reduced inter-subject variability in physiological outcomes. Making that connection

is an important step forward to improve tDCS application and increase its adaptability in a therapeutic setting.

It seems logical that the stimulation of a system as complex as the brain does not lead to simple results at the physiological level. One study investigated whether the electric field was related to the after-effects of tDCS (Laakso, Takenobu, Hirata, & Ugawa, 2018). By first calculating the electric fields and then applying 1 mA conventional tDCS with the anode over the target for 20 min on the right M1, they demonstrated that a large part of inter-individual variability in tDCS-induced MEPs are due to differences in the electric fields and suggested that electric field dosimetry could be useful for controlling the neuroplastic effects of tDCS. However, corticospinal excitability modulation differs between different montage types (Kuo et al., 2013). Possible differences in interindividual variability of stimulation outcomes, in corticospinal excitability, between the different montage types when individualising dose, have not yet been fully addressed. A systematic review revealed higher standard deviation of motor evoked potential amplitudes for high current density montages than for low current density montages (Horvath, Forte, & Carter, 2015a). Thus, one needs to consider the dose-response relationship between the electric field reaching a cortical target site and the subsequent variability effects on corticospinal excitability for different montages.

Electric field magnitude and focality has been linked to evoked muscle responses (Edwards et al., 2013). So far, however, no studies have validated whether controlling electric field variability translates to reduced inter-individual variability of corticospinal excitability responses (Bestmann & Walsh, 2017). This is the focus of experimental **Chapter 5**. Following my current modelling study's results (**Chapter 4**), I investigated whether individualised current flow models of dose control can reduce mean variability of tDCS CSE effects for posterior-anterior tDCS (**Experiment 5.1**) and for HD-tDCS (**Experiment 5.2**)[2].

[2] Data collection for Chapter 5 (Experiment 5.2) was kindly performed by Susanne Munz, an MSc student at the Department of Clinical and Movement Neurosciences, Institute of Neurology, supervised by myself.

1.7 Chapter overview

Chapter 1: Provided an overview of tDCS and neurophysiological parameters that play a vital role in its effects, and how, with the use of neuromodulatory technologies and simulation methods we can address variability of its application.

Chapter 2: The methodological techniques and parameters for consideration when designing an experiment to induce and measure current flow and brain excitability changes are discussed. Furthermore, the methodologies used in experimental **Chapters 3, 4** and **5** are outlined.

Chapter 3: Experimental chapter which is focused on the direction of current flow within the central sulcus and how different electric field directions impact the S1 and M1 cortical locations.

Chapter 4: Experimental chapter whereby current flow modelling techniques are used to identify electric field differences between a directional tDCS montage and high definition tDCS. CFMs are used to individualise electric field for both montages targeting the motor cortex. How this impacts stimulator output and focality for each montage is further investigated.

Chapter 5: Experimental chapter that challenges the utility of current flow models by realising the techniques used in **Chapter 4** and, in healthy individuals, illustrates whether dose-control translates into reduced inter-individual variability in physiological outcomes of tDCS for both a directional montage (**Experiment 5.1**) and an HD-tDCS montage (**Experiment 5.2**).

Chapter 6: Draws together the findings of research presented in this thesis and discusses the implications of the work for use in basic and clinical research. Limitations and future recommendations for further investigation in the field are outlined.

Chapter 2 Methodologies

Numerous non-invasive brain stimulation methods are used to induce and measure corticomotor and sensory excitability including tDCS, TMS and Peripheral Nerve stimulation (PNS). Although there is a multitude of research on tDCS, selecting a specific protocol for inducing specific outcomes in a reliable manner is still challenging. Current flow models are increasingly being used for targeting, post-hoc evaluation of tDCS protocols, and induced electric fields in the brain, however we are yet to understand their utility and translation in physiology.

In this chapter, I introduce the experimental designs and methodological techniques used in experimental **Chapters 3**, **4** and **5**. I thereby outline the procedures and methodologies for 1) measuring excitability of the primary sensory and motor cortices, 2) using neuronavigated TMS for coil monitoring and placement of tDCS electrodes based on MRI scans, and 3) applying current flow modelling to individualise tDCS intensities. I further overview the data analyses techniques used in these chapters.

2.1 Experimental designs and protocols

To identify any montage that may reduce variability of tDCS effects in corticospinal excitability, I first established whether directing the current across or along the primary motor area indeed produced differential effects on sensory and motor outcomes **(Chapter 3).** This study sought to validate the importance of controlled electric field direction when targeting the primary motor cortex in humans (Dmochowski, Bikson, Datta, et al., 2012; Hannah, Iacovou, & Rothwell, 2019; Rawji et al., 2018), as previously seen in animal studies (Bikson et al., 2004; Radman et al., 2009; Rahman et al., 2013). For this purpose, I used three groups of different tDCS montages that direct the tDCS current in different directions relative to the central sulcus; posterior-anterior tDCS (PA-tDCS), medio-lateral tDCS (ML-tDCS), and anterior-posterior tDCS (AP-tDCS). Excitability changes in S1 and M1 were assessed before, during, and after tDCS. Motor and sensory measurements were separated in alternating blocks (see Figure 2.1). To quantify motor

cortical excitability, I used TMS-induced MEPs and to quantify sensory excitability I used sensory evoked potentials (SEPs) induced by Median Nerve Stimulation (MNS). Each TMS-MEP block consisted of thirty-seven trials, and each MNS-SEP block consisted of six-hundred trials (see sections 2.2.6 and 2.2.7 for procedure details on MNS-induced SEPs and TMS-induced MEPs, respectively). The equivalent number of trials for each measure was selected based on previous research highlighting the sufficient number of trials necessary for a reliable measure of each outcome (Goldsworthy, Hordacre, & Ridding, 2016).

The protocol included one block of MEPs and one block of SEPs collected before tDCS (see Figure 5; Phase 1) and lasted approximately five minutes (2.1 minutes for each SEP Block; 2.6 minutes for each MEP block). 1mA tDCS was then applied for 20 minutes; throughout this phase, 4 blocks of SEPs and 4 blocks of MEPs were measured, in alternating fashion (see Figure 5; Phase 2). TDCS intensity ramped up/down for thirty seconds at the beginning/end of stimulation. Five minutes following tDCS stimulation, I further recorded two blocks of MEPs and two blocks of SEPs (see Figure 5; Phase 3).

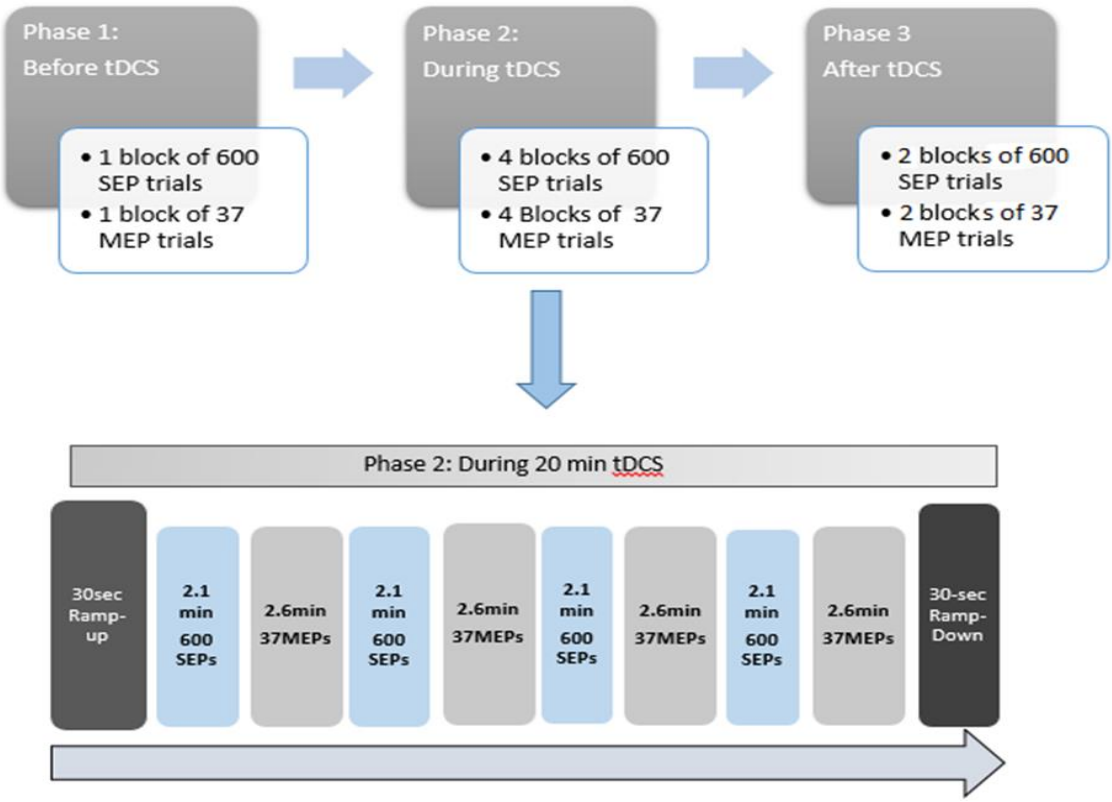

Figure 2.1 The experimental protocol for Chapter 3.
The effects of tDCS were assessed before- (Phase 1), during- (Phase 2), and after- (Phase 3) tDCS. MEP and SEP measurements were separated in time and applied in alternating blocks. Each TMS-MEP block consisted of 37 trials and each MNS-SEP block consisted of 600 trials. 1 block of MEPs and 1 block of SEPs were collected before tDCS (Phase 1) that lasts approximately 5 minutes (2.1 minutes for each SEP Block; 2.6 minutes for each MEP block). TDCS was then applied for 20 minutes; throughout this phase, 4 blocks of SEPs and 4 blocks of MEPs were measured, in alternating fashion (Phase 2). TDCS intensity ramped up/down for 30seconds at the beginning/end of stimulation. 5 minutes following tDCS stimulation we recorded another block of MEPs and SEPs. 2 blocks of MEPs and 2 blocks of SEPs will be collected post-tDCS (Phase 3).

In **Chapter 4,** I investigate through dose-control, whether we can improve the delivery of tDCS electric field intensities to a target using a more focal montage, HD-tDCS, compared to PA-tDCS montage. To do this, I simulated the tDCS induced electric field through the cortex using current flow modelling and compared electric field intensity and distribution between PA-tDCS and HD-tDCS, when using a fixed-dose or individualised-dose of current, respectively (see section 2.3 for details on current flow modelling procedures).

In **Chapter 5,** I investigated whether dose-control translates into reduced physiological variability. In this chapter, I present two experiments, one for HD-tDCS and one for PA-tDCS. For **Experiment 5.1**, each participant took part in two, two-hour sessions. In each

session, they received either fixed- or individualised- tDCS intensity. For **Experiment 5.2**, each participant took part in three two-hour sessions. In each session, they received either fixed-, individualised- or sham- tDCS. Data collection for **Experiment 5.2** was assisted by an MSc Clinical Neurosciences student, Susanne Munz. My contribution to this project involved supervision, coding contributions of the MatLab analyses scripts, construction of the tDCS protocols, data analysis, invigilation of the experimental testing and contributions to discussions around the design and implementation of **Experiment 5.2**. For both experiments, the protocol consisted of a baseline block of TMS (BL) to record pre-tDCS MEPs, followed by 20 minutes of tDCS during which five more blocks of TMS where administered (during-tDCS). Immediately and five minutes after the end of tDCS, two more blocks of TMS were applied (post-tDCS, P1). This amounted to a 2 x 8 (tDCS-type x TMS-block) within-subject design, with the dependent variable being MEP amplitude. Sessions were separated by at least 48 hours in order to avoid carry-over effects of brain stimulation (Dissanayaka et al., 2017). In **Experiment 5.2**, the order in which participants went through tDCS conditions was fully counterbalanced and double-blinded. After the last session, blinding effectiveness was confirmed with a questionnaire (Appendix; A).

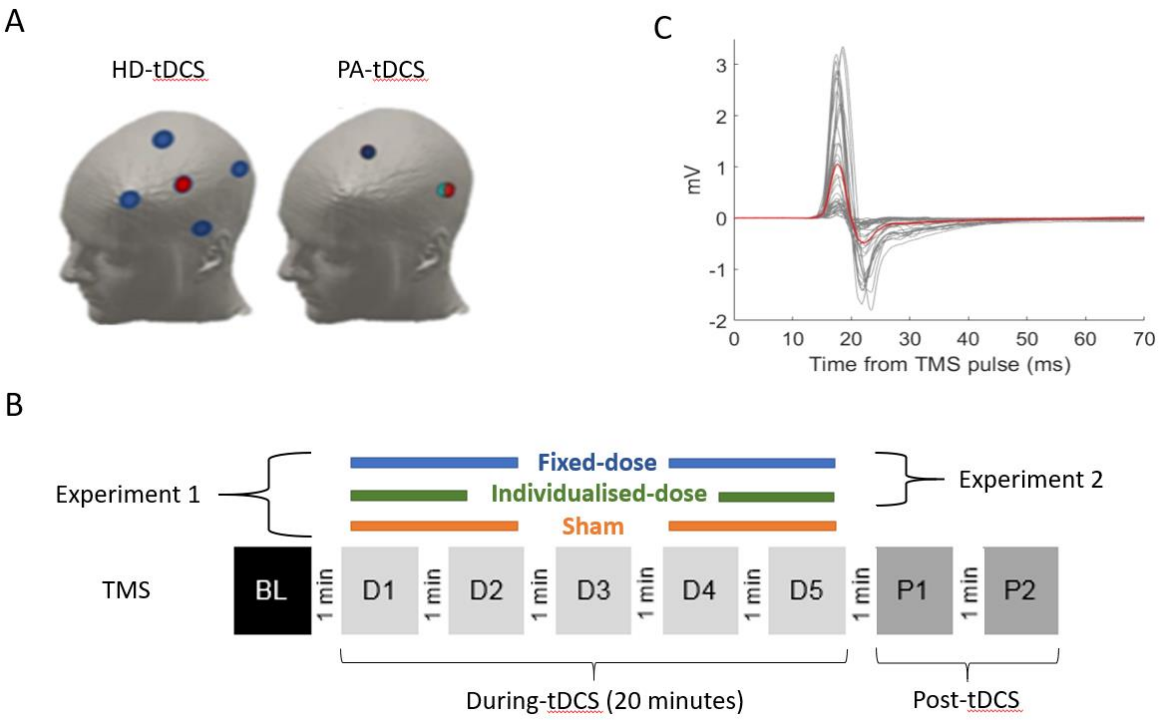

Figure 2.2 Details of the stimulation protocol for Chapter 5
A. *4x1 HD-tDCS (left;* ***Experiment 5.1****) montage with anode over C3, and cathodes over F3, Cz, P3 and T7 and PA-tDCS (right;* ***Experiment 5.2****) montage with anode over CP5 and cathode over FC1.*

B. *Stimulation protocol consisting of a block of baseline MEPs (BL) followed by 20 min of tDCS in 2 sessions of different tDCS conditions for* ***Experiment 5.1*** *(fixed-dose and individualised-dose), and 3 sessions for* ***Experiment 5.2*** *(fixed-dose, individualised-dose and sham). During tDCS, five blocks of MEPs (D1-5) were recorded, followed by two blocks of MEPs at 0 and 5 min post-tDCS (P1-2).*

C. *Block of 37 MEPs from an exemplary participant (grey: individual MEPs; red: block average).*

2.2 Measuring M1 and S1 excitability changes

As discussed in **Chapter 1**, factors that may influence the variability of tDCS effects include: (1) current flow direction (Bikson et al., 2004; Dmochowski, Bikson, Datta, et al., 2012; Kabakov et al., 2012; Rawji et al., 2018) and (2) focality of current (Laakso et al., 2015; Laakso, Tanaka, Mikkonen, Koyama, & Hirata, 2017; Mikkonen et al., 2020; Moliadze et al., 2010). Controlling for the direction of current across or along the central sulcus has differential effects for CSE (Rawji et al., 2018). As the orientation of pyramidal neurons is opposite in the two banks of a sulcus (Rahman et al., 2013), directing the current across the central sulcus should affect S1 and M1 excitability differentially. I here examined this by controlling for the direction of current flow across or along the central

sulcus and asked if different current flow directions impact MEPs and SEPs differentially. To assess this, I measured changes in SEPs and MEPs by applying different directions of tDCS current flow. The sections below outline the underlying neurophysiology of these cortical areas and the techniques for acquiring biomarkers from S1 and M1.

2.2.1 The somatosensory system

The somatosensory system is made up of elements of the peripheral nervous system and the central nervous system with regard to touch, vibration, temperature, pain and kinaesthesia (Arezzo, Schaumburg, & Spencer, 1982). The somatosensory pathway of communication between the peripheral nervous system and central nervous system is neurologically known as the dorsal column-medial lemniscal pathway (see Figure 2.3). The trajectory of a peripheral signal to the brain for processing sensation starts from peripheral receptors and afferent neurons entering the dorsal root ganglion and ascend the spinal cord to the medulla, then cross to the contralateral hemisphere. The pathway here continues to the thalamus (ventral posterior lateral nucleus) and finally arrives and is processed at the primary somatosensory cortex (Leeman, 2007). The compound action potential of somatosensory stimulation transmitted within the pathway, evoked by tactile, vibrational, painful or electrical stimuli, can be recorded using electrodes to study the post-stimulus characteristics (Bartley & Heinbecker, 1937; Heinbecker, Bishop, & O'Leary, 1934).

SEPs are brain and spinal cord electrical responses measured at the skin's surface following sensory stimulation (Passmore, Murphy, & Lee, 2014; Yamada, 2014). Although more natural stimuli such as pain or touch can yield SEPs, technical complexities and the relatively small responses hinder their clinical value and therefore, most clinical and scientific research typical measure SEPs induced via electrical stimulation of peripheral nerves (Dawson, 1947; Yamada, 2014). SEPs are thereby neurophysiological measurements of somatosensory processing and have been established as meaningful assays of physiological changes in both clinical and research domains (Passmore et al., 2014).

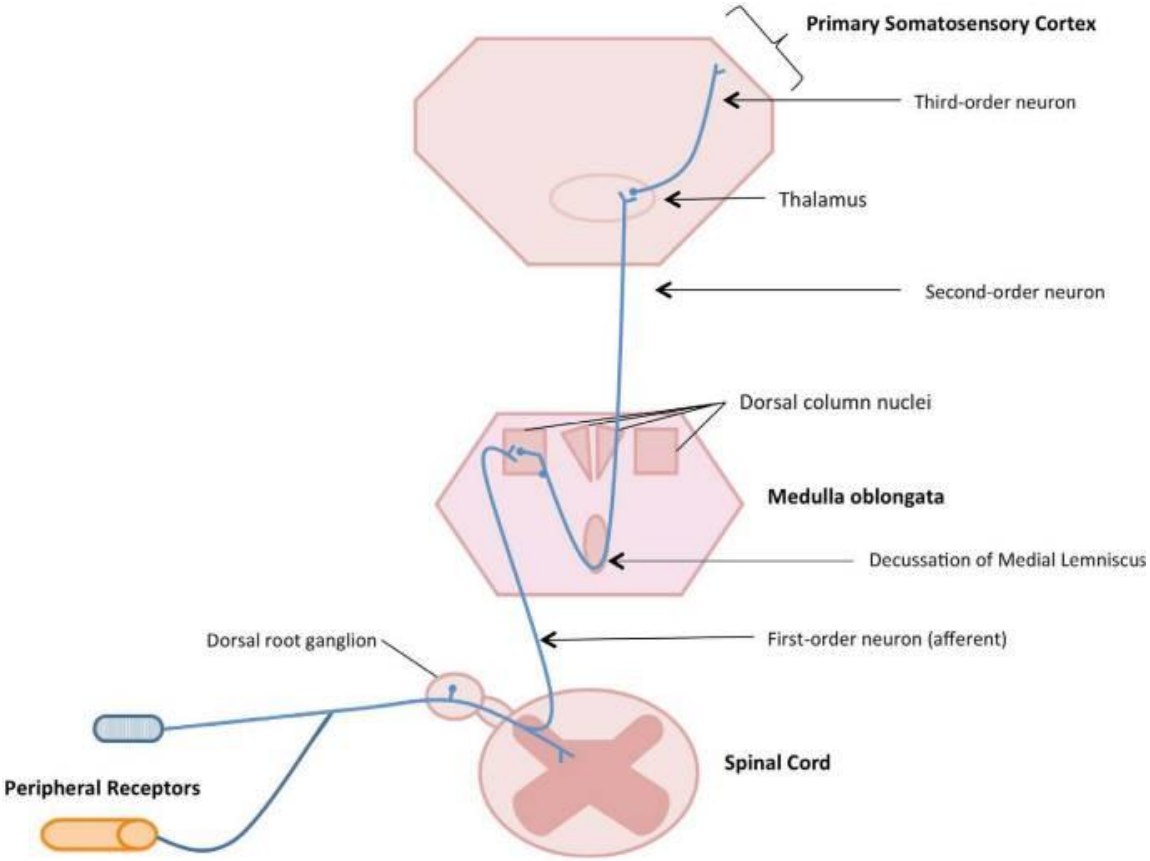

Figure 2.3 Peripheral stimulation trajectory to S1 from Dorsal column-medial lemniscal pathway.
The trajectory of a peripheral signal to the brain for processing sensation starts from peripheral receptors and afferent neurons entering the dorsal root ganglion and ascending the spinal cord to the medulla and crossing to the contralateral hemisphere. The pathway here continues to the thalamus (ventral posterior lateral nucleus) and finally arrives and is processed at the primary somatosensory cortex. Taken from (Leeman, 2007).

2.2.2 Peripheral nerve stimulation-induced somatosensory evoked potentials

Peripheral nerve stimulation is commonly used to induce SEPs due the flexibility provided to control and manipulate stimulation parameters (Aminoff & Eisen, 1998; Yamada, 2014). Electrical stimulation is delivered with a square wave pulse and parameters that can be manipulated include the duration and frequency of the electrical stimulus. In light of the International Federation of Clinical Neurophysiology (IFCN) guidelines, the recommended stimulus duration is 0.1–0.2 ms (Cruccu et al., 2008) and stimulation pulse frequency should be between 3 and 5 Hz. These pulses are applied transcutaneously over the targeted nerve. Muscle afferents that most likely dominate

cortical potentials produced by peripheral stimulation are of the median nerve at the wrist (Gandevia & Burke, 1988; Gandevia, Burke, & Mc Keon, 1984). Thus, the median nerve is a commonly targeted peripheral nerve of the upper limb in regard to somatosensory evoked potentials measured from the brain (Leeman, 2007; Passmore et al., 2014). Peripheral stimulation of the median nerve is by the primary sensory cortex through the dorsal column-medial lemniscal pathway and is measured through Electroencephalography (EEG) with electrodes placed on the scalp, above the S1.

2.2.3 Measuring SEPs with EEG

EEG is an electrophysiological-based human brain imaging technique that uses electrodes placed on the scalp and records the summed, synchronous post-synaptic activity of neurons (Passmore et al., 2014). SEPs are time-locked to a stimulus and are not recorded following spontaneous stimuli. In order to record SEPs most effectively, several methods can be used to localize the electrode placement. The most common method is the use of the 10-20 EEG system (Klem, Lüders, Jasper, & Elger, 1999) to identify the location of the primary sensory cortex (Mauguière, 2012). The 10:20 EEG system involves measuring the participants head based on locations of anatomical landmarks to accurately identify the location of a targeted region (Klem et al., 1999). For the primary somatosensory cortex, this is CP3, which refers to the location central and parietal to the centre of the head (Cz). Placing EEG electrodes through functional targeting is also common. For example, by identifying the motor cortex with TMS-induced MEPs, one can count ~2-3 cm posterior to the hand knob representation of the primary motor cortex in order to identify the primary somatosensory cortex (Woods et al., 2016). This technique was used in **Chapter 3** to identify the somatosensory area.

Disc-shaped EEG electrodes require the application of conductive gel between the electrode and skin for measuring cortical activity. It is common practice to scrub the skin with an alcohol agent prior to the placement of the electrode and add conductive gel in order to reduce electrode impedance levels. It is recommended that EEG readings should have less than 5 kΩ impedance (Cruccu et al., 2008). Applying between 500-2000 stimuli is recommended for averaging signal responses for SEPs to clearly differentiate the signal from noise. To isolate reproducible waves from background noise, a filtering

bandwidth with a high pass of 3 Hz and low pass of over 2000 Hz is commonly used (Cruccu et al., 2008). SEP peak amplitudes are under 10 μV range (Mauguière, 2012). See section 2.2.6 for procedures undertaken in **Chapter 3**.

2.2.4 SEP peak nomenclature and interpretation

SEP waveform peaks are assigned a letter (P or N) depending on their polarity (positive peaks or negative peaks) and an integer follows depending on the peak latency (Cruccu et al., 2008). Conventionally, negative polarity is shown as an upward deflection of the waveform and on the contrary, a downward deflection of the waveform reflects positive polarity. For example, a waveform with an upward inclination at 20 ms post-stimulus would be assigned as N20 (Passmore et al., 2014; Yamada, 2014).

The amplitude and latencies of the SEP are thought to reflect a combination of peripheral and central nervous system processing of an external stimulus. SEP amplitude reflects the magnitude of the incoming afferent volley, whereas the latency represents the relay of information along the somatosensory pathway (Mauguière, 2012). SEP peaks are divided in 'short' and 'long' latency peaks. Short latency peaks are considered the most reliable across healthy individuals and are present within the first 40ms post-stimulus for the upper-limb (Allison, Mccarthy, Wood, & Jones, 1991). Long latency peaks, further than 45 ms are susceptible to cognitive influences, making them more variable (Cruccu et al., 2008). Thereby, changes in peak latencies and amplitudes allow visualization of neural changes associated with perceptual, cognitive, and motor conditions.

2.2.5 The N20 peak

The primary sensory cortex contralateral to the peripheral nerve stimulation is the main generator of both the cortical early (20–40 ms) and late (60–120 ms) responses to stimulation (Baumgärtner, Vogel, Ohara, Treede, & Lenz, 2010). This brain region lies in the posterior bank of the Rolandic fissure and is approximately underneath the CP3 electrode position when using the 10:20 EEG system (Rehmann et al., 2016). This is the location of the N20 peak generation (Desmedt & Cheron, 1980), which is known to

respond consistently to contralateral external stimuli (Hlushchuk & Hari, 2006) and represents the earliest cortical processing of the primary somatosensory cortex (Desmedt & Ozaki, 1991). The 20 ms time delay reflects the travel time for the afferent signal from the median nerve to reach to arrive at S1. Thereby the latency of the signal may slightly vary depending on the height of an individual.

2.2.6 Median nerve stimulation and somatosensory evoked potentials

Somatosensory evoked potentials were induced and monitored only in the experiments of **Chapter 3**. For this, electrical stimulation was applied to the right median nerve at the wrist, with a pulse width of 0.2 micro-seconds at 5 kHz (Digitimer DS7-A), which is within the recommended IFS guideline (Cruccu et al., 2008). The anode was placed just proximal to the palmar crease, and the cathode was placed between the tendons of the palmaris longus muscle, 3cm proximal to the anode (Legatt, 2014). See Figure 2.4 section A for an illustration of the electrode placement. The intensity of stimulation was fixed just below the threshold for evoking muscle movement in the hand. To do this, I first ramped up the intensity until an observable muscle movement was induced in the hand and then lowered the intensity to an amplitude where there was no longer a muscle twitch. This was checked throughout the course of the experiment, by monitoring the evoked EMG response of the FDI muscle. At this stimulus intensity, SEPs are usually submaximal in amplitude. There have been a number of studies reporting the effect of stimulus intensity on SEP and it is generally agreed that the amplitude of scalp recorded SEPs reach plateau with the intensity of stimulus at 1.5 times the motor threshold or 2-3 times the sensory threshold (Huttunen, 1995; Shiga et al., 2001). However, to quantify the impact of tDCS on MEPs, uncontaminated by overt muscle movement during MNS stimulation, subthreshold MNS stimulation intensities were applied.

SEPs were recorded from two scalp electrodes (3mm Ag/AgCl Disposable Cup Electrodes) positions. One electrode was placed at 3 cm posterior from the motor hotspot in the orientation of the TMS coil. Based on pilot measurements, this electrode location corresponds to approximately CP4-5 standard EEG electrode position (depending on the individual). The other electrode was positioned 3cm posterior to the motor hotspot (not in the orientation of the TMS coil) at the 10-20 system location of

CP3 approximately above the primary sensory area whereby the SEP N20 component is greatest in size (see Figure 2.4; B). Both scalp electrodes were referenced to Fz (Kirimoto et al., 2011; Legatt, 2014). A bandpass filter of 3Hz to 3 kHz was used with a Digitimer D360 amplifier (Gain: 100.000x).

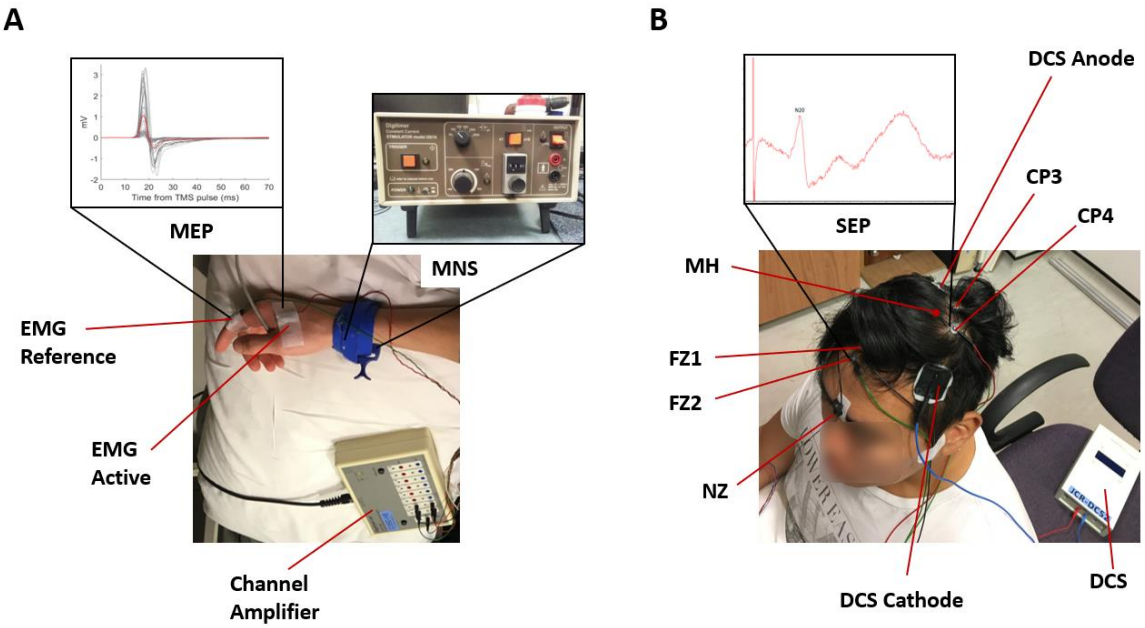

Figure 2.4 Experimental setup to for concurrent tDCS and MEP/SEP recording
A*) Motor evoked potentials (MEPs; top left) were recorded via two electromyographic electrodes; an active electrode placed on the FDI muscle (EMG Active) of the contralateral hand (to the TMS stimulated region, LM1) and a reference electrode placed proximal to the interphalangeal joint (EMG Reference). Median nerve stimulation was applied with the anode of the peripheral nerve stimulator placed just proximal to the palmar crease, and the cathode placed between the tendons of the palmaris longus muscle, 3cm proximal to the anode (MNS; top right).*
B*) Sensory evoked potentials (SEPs; top left) were recorded with from two scalp electrodes positions. One electrode was placed at 3 cm posterior from the motor hotspot (MH) in the orientation of the TMS coil which corresponds to approximately CP4 standard EEG electrode position (depending on the individual). The other electrode was positioned 3cm posterior to the motor hotspot (MH), not in the orientation of the TMS coil, at the 10-20 system location of CP3 approximately above the primary sensory area. Both scalp electrodes were referenced to FZ1 and FZ2, respectively. The ground electrode was placed at the nasion (NZ).*

2.2.7 TMS-MEP measures of corticospinal excitability

In **Chapters 3** and **5,** tDCS induced changes in corticospinal excitability, as reflected by TMS-induced MEPs, were assessed. Details on MEP recordings are summarized in **Chapter 1** section 1.4.1.

Single-pulse TMS was applied over the M1 to elicit MEPs in the contralateral FDI muscle during and after tDCS stimulation. TMS was administered using a MagStim BiStim2 (The Magstim Co. Ltd). A standard figure of eight (Magstim, 70mm) alpha coil was placed over the motor area in approximately 45-degree angle perpendicular to the central sulcus, with the coil handle pointing backwards from the midline for posterior to anterior stimulation. The location (motor hotspot) for activation of the FDI muscle was determined by moving the coil in 1 cm steps around the presumed M1. TMS was delivered at 0.2 Hz and signals were digitized at 5 kHz (CED Power 1401; Cambridge Electronic Design, Cambridge, UK).

To avoid the coil pressing on the EEG scalp electrodes in **Chapter 3**, which may move the electrodes or disperse the electrode paste, I designed a TMS Tool for Electrode Movement Prevention (TEMP) made out of foam core (AIREX T10) 5 x 1 x 1.5 cm (Length x width x breadth), that was fixed at the center of the TMS coil where the current is strongest (see Figure 2.5). TEMP causes the TMS coil to be positioned marginally higher from the scalp, thus the TMS stimulator output intensity was on average 12 % higher in **Chapter 3** than **Chapter 5**. TEMP was not needed for **Chapter 5** as I did not measure sensory excitability, and thereby did not use EEG electrodes in this study that could be affected from the TMS coil.

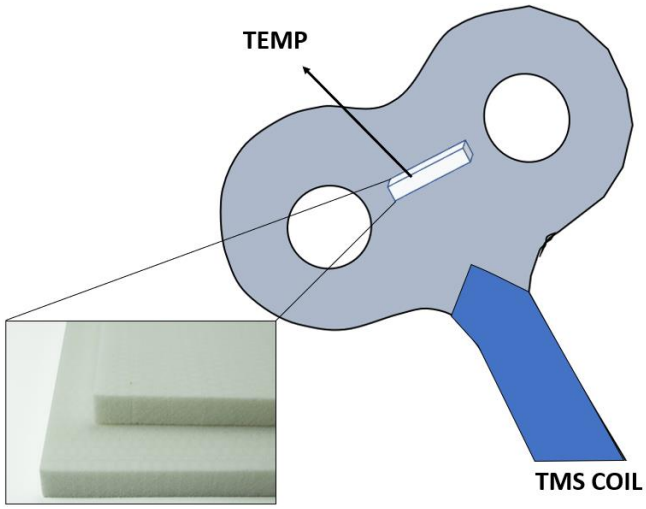

Figure 2.5 Custom-made Tool for Electrode Movement Prevention (TEMP)

TEMP was added at the center of a figure 8 TMS coil to avoid the coil pressing on the EEG scalp electrodes. TEMP is made of foam core material (bottom left) with 5 x 1 x 1.5 (Length x width x breadth) dimensions.

2.2.8 Safety and contraindications

TMS is generally considered a safe method of non-invasive brain stimulation (Rossi et al., 2021, 2009). Contraindications to TMS include: a presence of metal implants in the brain, a history of seizures and medications that alter the nervous system excitability, a history of skull fracture and/or concussion, and brainstem/cerebellar lesions or the presence of cognitive impairments (Rossi et al., 2021, 2009). All participants were checked for any of these contraindications through a questionnaire administered prior to each session (Appendix; B). Any of the above criteria would deem them ineligible for TMS.

2.2.9 MEP recordings

TMS-evoked MEPs are thought to index changes in corticospinal excitability and thus provide a direct read-out of the impact of tDCS at the time of TMS application (Bestmann, 2012; Bestmann & Krakauer, 2015), see **Chapter 1** section 1.4.1 for details. TMS-evoked MEPs were measured via electromyographic (EMG) Ag/AgCl electrodes (19x38 mm Ambu WhiteSensor) with an active electrode placed on the FDI muscle of the contralateral hand, and the reference proximal to the interphalangeal joint (Figure 2.4; A). For **Chapter 5**, the ground electrode was placed on the head of the ulnar bone. For optimal readout in **Chapter 3**, the ground electrode was placed on the nasion, since one ground was needed for both EMG and EEG electrodes (Figure 2.4; B). In **Chapter 5**, signals were amplified (gain; 1000) and band-pass filtered (5-2500) (Digitimer D360). MEP data was stored on a computer for offline analysis (Signal Version 6.0, Cambridge Electronic Design, UK).

2.2.10 Neuronavigated TMS and tDCS

One factor affecting the variability of MEPs across studies is intra-subject variability in TMS coil location and placement (Kiers et al., 1993). The precise and consistent

positioning of the TMS coil over the targeted cortical site for the entirety of the TMS protocol is one of the most challenging experimental procedures (Sparing, Buelte, Meister, Pauš, & Fink, 2008) and movement in the placement of TMS position may increase the inter-trial variability of TMS-evoked MEPs, and thereby, for the correct interpretation of stimulation effects, the TMS coil should be placed as consistently as possible throughout the entirety of the experimental session. Since TMS experiments are usually conducted in blocks lasting from minutes to hours, the coil needs to be either locked in place or repositioned between blocks. One technique suggested to control for coil location and placement is neuronavigation. Neuronavigated TMS may increase the intra- and inter-subject and between-group consistency of MEPs (Bashir et al., 2011; Brett, Johnsrude, & Owen, 2002; Gugino et al., 2001). Therefore, this technique was used to control and monitor coil position errors in **Chapter 5**. Additionally, I further evaluated whether TMS coil errors explained part of the variability in MEPs (see section 2.6.4 for details).

Instead of using anatomical targeting such as the 10–20 EEG system or individual MRI scans, the TMS coil was positioned using a function-guided approach: by localising M1 based on the optimal location and orientation of the TMS coil that elicits 1mV peak-to-peak MEPs. With the use of Brainsight® neuronavigation system, I registered this position and orientation to monitor coil placement and movement throughout the entirety of the stimulation protocol (20 minutes) to control for lower intra- and inter-subject variability caused by TMS coil movement (see next section for details). Even minimal movements away from the optimal stimulation region may lead to attenuation of the MEP amplitude, thus this technique is optimal in comparison to manual stimulation methods. Neuronavigation uses infrared sensors attached to the TMS coil and tracked by a camera to monitor its position relative to the subject's head.

2.2.11 Neuronavigation procedure for tDCS electrode positioning and coil monitoring

Correct positioning of tDCS electrodes in **Chapter 5** was ensured with the neuronavigation software Brainsight® TMS Navigation (Brainbox Ltd.) to replicate the

montage used in the individuals' current flow models as precisely as possible (see Figure 2.6).

To achieve this, before the experimental session, I imported the ROAST segmented images of the subject's skin and the positions and dimensions of electrodes in the neuronavigation system and coregistered them. Next, six landmarks were selected (nasion, inion, left and right pre-auricular, tip of the nose and philtrum (above lip)) for registration of the scan to the subject's head. A tracker was then attached and calibrated on the TMS coil to monitor its exact position, using the calibration block in the view of the camera (Polaris Tracker). The Polaris optical position sensor used with Brainsight™ requires that there is no obstruction between its line of sight and the trackers on the coil, pointer and subject. To achieve this while allowing free movement around the subject requires planning, and thus, I set-up the TMS device, chair and trackers prior to the subject's arrival. The Polaris tracker was always positioned above the subject's head in a diagonal view looking down at the entire area surrounding the subject's head. This minimizes the chances of standing between the camera and a tracker.

During the experiment, subjects wore tracking glasses and sat comfortably at a lower diagonal view of the Polaris Tracker. The subject tracker (tracking glasses) was fixed onto the subject's head with tape so it would not move during the study, nor interfere with the TMS coil or any other objects on the subject's head. I then registered the virtual pre-assigned landmarks to the subject's head, using the pointer tracker. To do this, the pointer is used to identify and sample the location of the landmarks on the subject. Following registration of the scan to the subject, the pointer was then used to trace the subject's head to assess whether registration was optimal as indicated by the distance of the pointer to the registered head on the neuronavigation system. Registration was assessed when there were less than 2mm gaps between the individual's head and the pointer position. Less distance reflected more accurate scan registration. I then traced the location of the tDCS electrodes on the subject head and added a marker to indicate the centre position of that electrode. This was done for all electrode positions in **Chapter 5**.

Following TMS coil positioning (as described in section 2.2.7), the targeted coil position was saved on the Brainsight system as a reference position for the entirety of the

experiment. For each TMS pulse, Brainsight recorded the displacement of the TMS coil from the targeted stimulation site, i.e. the motor hotspot, and saved four types of coil errors. Firstly, the 'Target error', is the shortest distance between the hotspot and the vector projecting into the head from the centre of the coil. Secondly the 'Angular error', reflects the tilt of the coil with respect to the target trajectory. Thirdly, the 'Twist' error, indicates the coil's rotation within the plane perpendicular to its target trajectory. Finally, the 'Distance to Target error' is the Euclidean distance between the coil's centre and the hotspot.

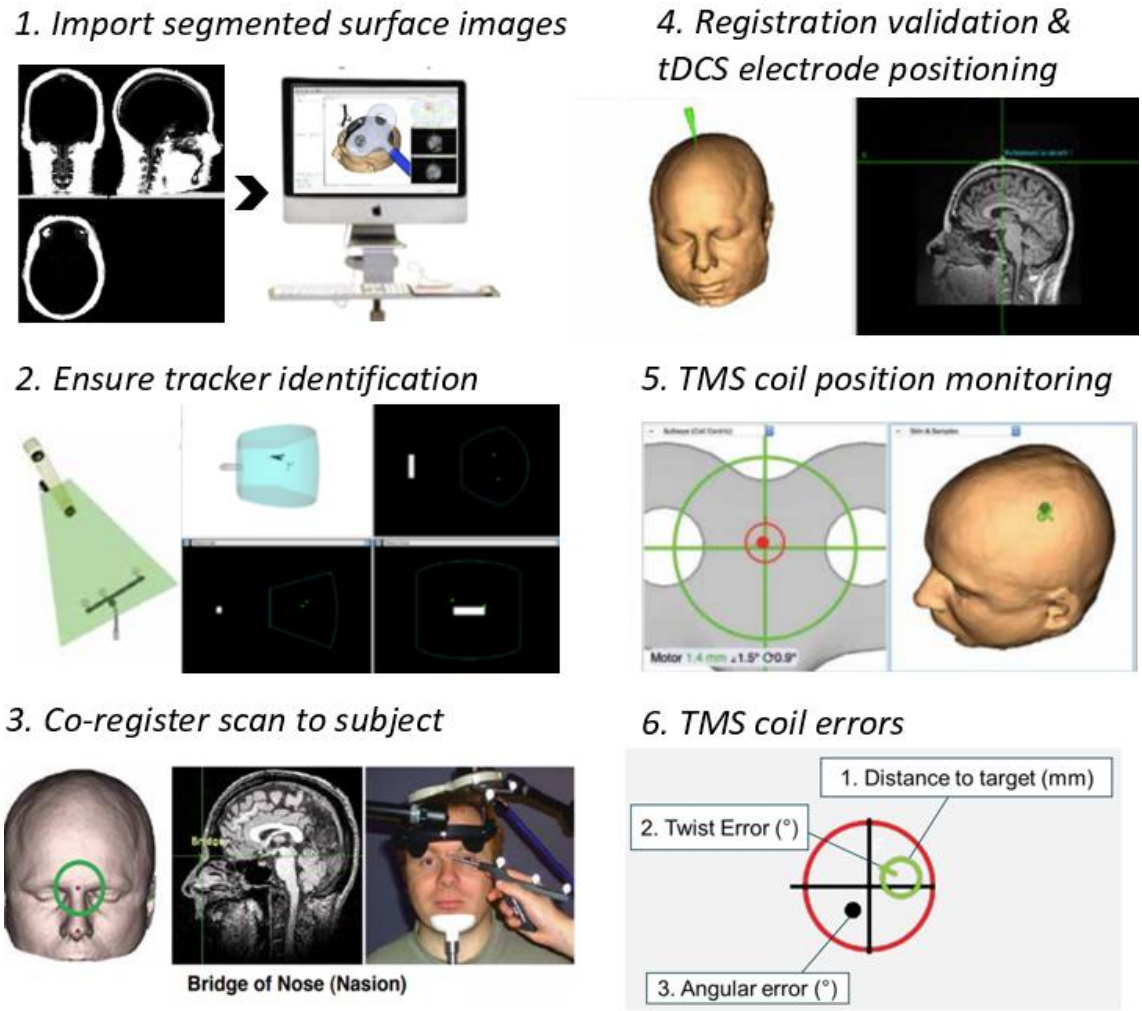

Figure 2.6 Neuronavigation procedure for placement of tDCS electrodes and tMS coil monitoring.

1. Imported and coregistered segmented MRI surface images (skin and electrode images) of subject's scan to the brainsight software. 2. The subject was then seated with the tracking glasses in a diagonal point of view under the Polaris tracker. No obstruction between the polaris line of sight and the trackers on the coil, pointer and subject was ensured in a 3-dimensional view from the tracker. 3. Imported MRI surface images were co-registered to subject's head using six pre-assigned landmarks. The image here shows an example of registering 1 landmark; the Nasion.

4. The pointer was then used to trace the subject's head to a) assess whether registration was accurate enough as indicated by less than 2mm distance between the pointer to the registered scan and b) identify the location of the tDCS electrodes on the subject head as indicated by the registered electrode scan on the neuronavigation system. A marker was added on the subject's head to indicate the centre position of each electrode. 5. The TMS hotspot was identified functionally, and the position was saved on the neuronavigation system for monitoring and consistent coil positioning. 6. Four TMS coil errors were monitored, 'Distance to Target', 'Twist', 'Angular' and 'Target' errors.

2.3 Current flow models

Current flow modelling was used on MRI scans to provide numerical estimates of the electric field induced by PA-tDCS and HD-tDCS. See **Chapter 1** section 1.6.6 for details on current flow modelling. In **Chapter 5**, prior to the first testing session, current flow modelling was performed on each participant's structural MRI scan. This section details the procedures of using the Realistic Volumetric Approach to Simulate Transcranial Electric Stimulation (ROAST) toolbox (Huang, Datta, Bikson, & Parra, 2019) to estimate electric fields in MRI scans and how these can then be used to individualise tDCS intensity. Current flow modelling and statistical analyses were conducted using ROAST v2.7.1, and SPM12 (Wellcome Trust Centre for Neuroimaging, www.fil.ion.ucl.ac.uk/spm) on MATLAB version 2016b (The MathWorks, Inc., Natick, MA, USA).

2.3.1 Estimating electric field intensity at the left M1 with current flow modelling

For **Chapters 4** and **5** I simulated current flow in the LM1, using two different montages. ROAST processes individual MRIs and creates a 3D model of electric field distribution, based on user-defined tDCS protocols. ROAST segments 1x1x1 mm isotropic voxel size resolution scans using SPM12 into grey matter, white matter, cerebrospinal fluid (CSF), bone, skin, and air cavities. The segmentations are corrected for any incongruities that may interfere with the model using morphological operations and simple heuristics (detailed in Huang et al., 2019). The model then places electrodes at assigned 10-10 EEG coordinates and an Matlab-based mesh generation and processing toolbox (Iso2Mesh; Fang & Boas, 2009) is used for the computation of the Finite Element Model (FEM). The FEM model is further solved for voltage and electric field distribution using a finite element solver (getDP; Dular, Geuzaine, Henrotte, & Legros, 1998). The assigned

conductivity values for each tissue type are (in S/m): grey matter: 0.276; white matter: 0.126; CSF: 1.65; bone: 0.01; skin: 0.465; air: 2.5×10^{-14}; gel: 0.3; electrode: 5.9×10^{7} (Datta et al., 2009; Wagner et al., 2007).

Following current flow modelling, electric field intensities and distribution were extracted following pre-processing of the electric field images produced by ROAST on SPM12. I then spatially normalised structural and electric field images (resampled to 2 x 2 x 2mm voxels) into Montreal Neurological Institute (MNI) space and smoothed the normalised images using a Gaussian filter with a 4 x 4 x 4mm smoothing kernel. I applied an explicit binary mask computed of grey and white matter using the normalised structural images to confine analysis to voxels within the mask. See Figure 2.6 for the current flow modelling pipeline & procedure (adapted from Evans et al., 2019).

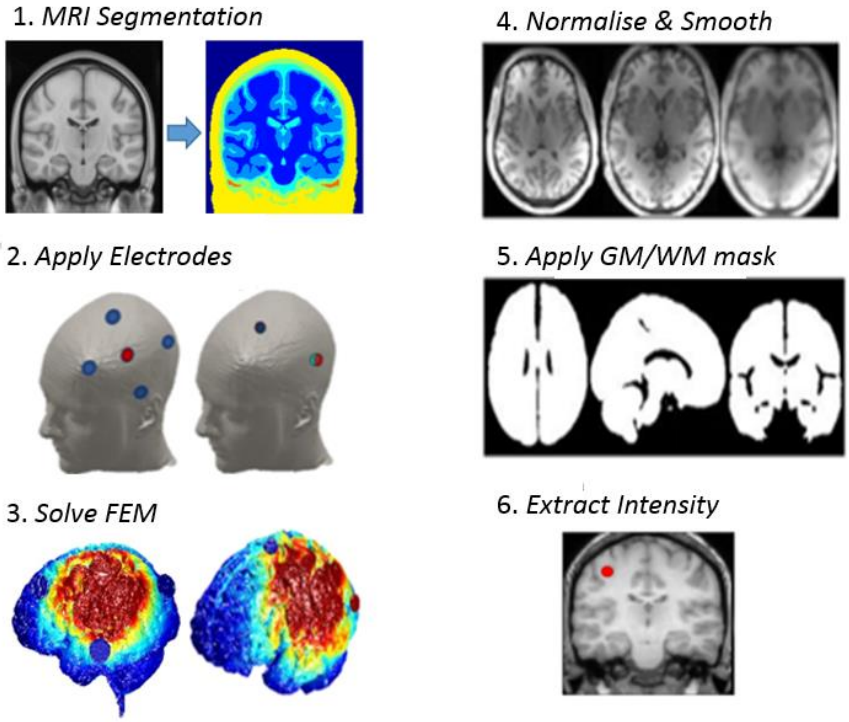

Figure 2.7 Current flow modelling pipeline.
Steps 1-3 are generated by ROAST v2.7.1. Steps 4-6 are generated using SPM12. T1 weighted structural MRI is used and segmented in different tissue types (1), the pre-defined electrode montage is applied (2) and the finite element model is solved (3). SPM 12 is used to normalise and smooth the structural MRI and EF images (4), create a grey and white matter explicit mask (5) and then applied to the EF to limit analyses within the cortex (6). Step 6 shows an example of the region of interest used in this study; the LM1 (red). Adapted from (Evans et al., 2019).

2.3.2 Fixed and individualised tDCS output intensity

The fixed tDCS stimulation dose for **Chapters 4 and 5** was 1mA, however, the individualised dose in these chapters was dependent on anatomical differences between the subjects.

In **Chapter 4**, the current flow induced by 1 mA PA-tDCS and HD-tDCS was modelled in 50 subjects from the Human Connectome Project (https://ida.loni.usc.edu/login.jsp). In this population, the average electric field was 0.185 V/m for PA-tDCS and 0.128 V/m for HD-tDCS at the LM1 (See section 2.6.5 Electric field analysis for details on how these values were extracted). Next, I determined the individual tDCS output intensity required to induce an electric field equivalent to the average electric field at the left M1 for each montage. This was based the following formula (Evans et al., 2019):

$$\text{Individualised-dose } (tDCS_{indiv}) = (M1_{TI}/M1_{AI}) \times \text{Fixed-dose } (tDCS_{fixed})$$

Individualised doses ranged between 0.8 mA - 1.9 mA, remaining under the safe current density measures when applied on healthy participants in **Chapter 5.** This is in line with previous evidence of substantial variability of electric field reaching a target across a population (Evans et al., 2019; Laakso et al., 2019).

2.4 TDCS experimental setup

2.4.1 Procedure

To apply tDCS in an experimental setup, the locations for electrode placement were first identified. The location of the electrodes on the scalp were marked with a wax-based pencil (Chinagraph Pencil). Conductive paste (Ten20; Weaver & Co., Aurora, USA) was applied at these locations and spread evenly across the electrode to affix it to the scalp (see section 2.4.3 below for details on electrode conductive agents and impedance). To add the electrodes to the scalp of the participant, alcohol wipes were first used to lightly scrub the scalp where the electrode would be placed and allowed to dry for minimising impedence. The participant's hair was then parted to ensure good contact between the

scalp and electrode. Electrode wires were then attached to the stimulator's positive or negative ports depending on the polarity of stimulation. Next, the dose and duration parameters were inputted in the tDCS system to start the stimulation session. The participant was informed about the procedure taking place during the application of the tDCS electrodes.

2.4.2 TDCS intensity, duration, and online application

A NeuroConn DC-STIMULATOR MC (Brainbox Ltd., Cardiff, UK) was used to administer 20 minutes of tDCS, plus ramp-up and ramp-down periods of 30 seconds, at 1 mA or the subject's individual intensity. This is in line with most studies that use current intensities of up to 2 mA for 10-40 minutes (Brunoni et al., 2012; Nitsche et al., 2008; Shiozawa et al., 2014; Tortella, 2015). This duration is sufficient to produce long lasting effects without reversing tDCS effects.

In **Chapter 5, Experiment 5.2**, I also applied sham stimulation. To apply sham stimulation, the electric current was ramped up and down for 30 seconds at the beginning and end of the 20-minute stimulation period, with no stimulation in between and the electrodes were kept on for the duration of the experimental sham condition. The participant may only feel a slight tingling sensation during the ramp-up and down period and the subject thinks to be stimulated while no current is being applied. Oftentimes, participant questionnaires are used to monitor their perception of the stimulation session (Kessler, Turkeltaub, Benson, & Hamilton, 2012). Furthermore, tDCS was applied during acquisition of physiological measurements, and not before or after, as research highlights that tDCS has immediate cortical effects (Liu et al., 2018). During stimulation, participants did not undergo any task. This design was selected as there may be task-specific effects of tDCS (Bikson & Rahman, 2013; Miniussi et al., 2013), however, I was only interested in the immediate physiological effects and thus, subjects were not required to perform any tasks during the course of the experiment.

2.4.3 Electrode conductive agent and impedance

Saline is the most common conductive agent for ensuring current conductivity between spongy electrodes and the skin. If impedance levels are high, there needs to be reapplication of the saline solution (Loo et al., 2011), however, over-soaking the electrodes can saturate hair and lead to changes in the spread and direction of current flow achieved (Fertonani, Ferrari, & Miniussi, 2015; Horvath et al., 2014) and may add to inter-individual heterogeinity of cortical excitability effects. Reliable and consistent application of tDCS requires good contact with the scalp to maintain conductivity through the circuit. High impedance levels are an indicator of poor conductivity. This could be a result of poor electrode set-up such as inadequate parting of the hair to allow good contact with the scalp, or insufficient conductive agent between the scalp and the electrode. I used electro-conductive paste (Ten20; Weaver & Co., Aurora, USA), which creates better conductivity than saline solution for participants with thick hair and may control the distribution of the current more effectively and evenly than saline solution of spongy electrodes (DaSilva, Volz, Bikson, & Fregni, 2011). Impedance levels were monitored and kept under 5 kΩs, as generally recommended (DaSilva et al., 2011).

2.4.4 TDCS montages

The electrode montage refers to the number, type, size and placement of electrodes attached to the scalp. First, depending on the electrode montage, the desired locations of electrode placement were ascertained. In **Chapter 3**, I used a function-guided approach (see Neuronavigated TMS and tDCS electrode positioning sections 2.2.10 and 2.2.11). I administered tDCS so that current flows in different directions across and along the primary sensory and motor cortices. I administered three montages using 9cm^2 (3 x 3 cm) electrodes, with posterior-anterior, anterior-posterior and mediolateral current flow directions relative to the M1 and S1 cortical areas. Participants were divided into three groups of tDCS current direction; Group 1 received PA-tDCS, where the anode was positioned 7cm posterior- and the cathode 7cm anterior- to the hand representation of central sulcus (5.5cm from the hotspot to the electrode edge), in a right angle to the presumed orientation of precentral sulcus at 45 degrees with respect to the central fissure. Group 2 received AP-tDCS, with the anode and cathode in reversed positions to Group 1. Finally, I administered ML-tDCS for Group 3 with the anode positioned 7cm

medial and the cathode 7cm lateral (5.5cm from the hotspot to the electrode edge) to the hand representation of the cortical surface of the central sulcus (Rawji et al., 2018), in parallel to the presumed orientation of the central sulcus at 45 degrees with respect to the central fissure (see Figure 2.8).

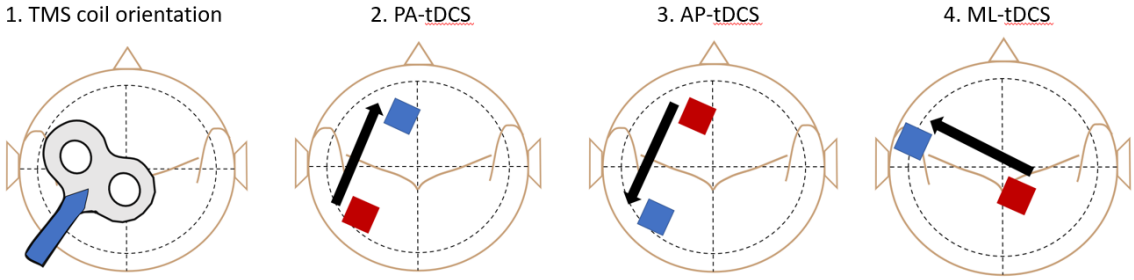

Figure 2.8 Transcranial Direct Current Stimulation electrode montage
TDCS electrodes on the head as applied across (2; PA-tDCS) the central sulcus, with anode 7 cm posterior (red) and cathode (blue) 7cm anterior to the motor hotspot (5.5cm from electrode edge) in the orientation of the TMS coil (1; left). Electrodes were reversed for the AP-tDCS (3; AP-tDCS) current flow montage. ML-tDCS (4; ML-tDCS) directed the current along the central sulcus with the tDCS anode (red) 7cm medial and the cathode (blue) 7cm lateral.

In **Chapter 4,** two tDCS montages were simulated (HD-tDCS and PA-tDCS), using disc electrodes (6mm radius, 2mm height) on ROAST which are the default electrode sizes used by roast. I used these electrode sizes to correspond to montages simulated in a previous study (Evans et al., 2019). These montages were used to simulate electric fields to assess whether we can improve the electric field intensity and focality delivered to the brain and identify which montage may deliver more focal electric fields with comparable intensities. Electrode positions for both montages were selected to target the LM1 hand region. For the bipolar montage, I used a posterior-anterior bipolar tDCS montage that controls for the direction of current flow across primary motor cortex (i.e., traversing central sulcus in posterior-anterior direction), which recently has been shown to produce consistent corticospinal excitability changes (Hannah et al., 2019; Rawji et al., 2018). For PA-tDCS, the electrode positions were CP5 (Anode) and FC1 (Cathode). For comparable results, PA-tDCS electrode dimensions and locations were replicated from our previous study (Evans et al., 2019). This montage directs current in a posterior-

anterior direction across the central sulcus and at either side of LM1 (Rawji et al., 2018). For HD-tDCS, the electrode positions were C3 (Anode), F3, P3, T7 & Cz (Cathodes), respectively, with approximately 7.5 cm ring-radius distance as typically applied (DaSilva et al., 2015). The 4x1 montage can also be applied with less distance between the electrodes. The electrode setup for tDCS stimulation, such as the distance between the electrodes, influences the amount of current that is shunted across the scalp. While the electric current is sufficient to penetrate through skin and scalp to reach the brain, a substantial amount of the current is shunted across the scalp (Suh, Kim, Lee, & Kim, 2009). There is less shunting when the electrodes are positioned further apart (Moliadze et al., 2010; T. Wagner et al., 2007). I thereby used the largest distance reported for the 4x1 HD-tDCS montage.

For **Chapter 5,** I used the same electrode montages simulated in **Chapter 4** (PA-TDCS and HD-tDCS) however with larger, round rubber electrodes of 2 cm diameter and 1 mm height (Brainbox Ltd). Correct positioning of the electrodes was ensured with the neuronavigation software Brainsight® TMS Navigation (Brainbox Ltd.) to replicate the montage used in the individuals' current flow models as precise as possible.

2.4.5 Safety of tES

TES is generally considered a safe method when all safety considerations are implemented following guidelines (Loo et al., 2011; Nitsche et al., 2008, 2003). Any serious side effects are rare.

The most important safety consideration is current density. As aforementioned in **Chapter 1**, this is subject to the combination of size of the electrodes and the stimulation intensity. Most studies use tDCS stimulation of up to 2 mA with 35 cm^2 sized electrodes, typically producing average current densities of 0.057 mA/cm^2. It is recommended that current densities do not exceed 0.1 mA/cm^2 (The Federal Institute for Drugs and Medical Devices, Germany). With studies that use smaller electrodes, such as with the use of HD-tDCS, stimulation has been safely applied with 2 mA stimulation using 6mm radius electrodes with current density of 1.77 mA/cm^2 (Borckardt et al., 2012). None of our experimental designs exceeded this amount of current densities. Indeed, Liebetanz and

colleagues (2009) illustrated that it requires current applied for 10 minutes with densities reaching 14.29 mA/cm^2 to produce brain lesions in rats (Liebetanz et al., 2009). That is more than 100 times the recommended dose (Liebetanz et al., 2009; Pikhovych et al., 2016).

Although theoretically possible, seizures are not a main risk for healthy subjects. In animals studies, tDCS does not cause epileptic seizures nor lower the seizure threshold (San-Juan et al., 2015). Note, tDCS should never be painful, although, cutaneous sensations have been reported. The most commonly reported side effects are scalp sensation such as tingling, or itching or heating sensation under the target electrode position and only during stimulation electrodes (Frank et al., 2010) and in rare cases, slight pain is reported (Fertonani et al., 2015). Redness of the skin under the electrodes is also common. Further reports such as fatigue and headaches or dizziness have been occasionally reported (Fertonani et al., 2015). A meta-analysis on data from 48 studies indicates side effects are generally mild and transient, including: tingling (11.5%), itching (5.8%), redness (4.7%), scalp discomfort (3.1%), (Krishnan, Santos, Peterson, & Ehinger, 2015). In my experiments, slight tingling and heating sensations were reported during tDCS but this was well tolerated.

The parameters that I used have been applied safely in many studies and are consistent with the established safety guidelines. Furthermore, none of the material presented or tDCS procedures offended or distressed volunteers. This risk was obviated by pre-screening subjects with a safety questionnaire (Appendix; B) and informing them of all procedures and methodologies taking place during the experimental session (Appendix; C).

2.5 Participants

Subjects were independently recruited for each experimental study. In addition to recruiting participants for the studies, pilot experiments were undertaken prior to each main experiment to assess feasibility and successful implementation of protocols and techniques used in each study. In total, 124 participants were recruited with a total of

199 testing sessions conducted (**Chapter 3**; 70 subjects, 85 sessions and **Chapter 4**, 54 subjects, 114 sessions). 70 participants were recruited for **Chapter 3**, and data used for the study was collected from 54 participants. Thereby, 16 additional participants were used for the purpose of pilot testing. For **Chapter 5**, 54 participants were recruited and data from 41 participants in total was used (25 for **Experiment 5.1** and 16 for **Experiment 5.2**). For **Chapter 5**, I selected participants who were in possession of a T1 structural MRI scan from the Wellcome Trust Centre for Neuroimaging at UCL. Participants were recruited from the UCL Psychology and Institute of Cognitive Neuroscience (ICN) subject pools.

Inclusion criteria for **Chapters 3 and 5** consisted of right-handed volunteers between the ages of 18-40 with no current or previous history of neurological conditions. Right-handed participants were used as there have been observable differences with modulating tDCS effects in cortical excitability between left- and right-handed individuals (Guadalupe et al., 2014; Schade, Moliadze, Paulus, & Antal, 2012). There are known changes in cortical excitability and motor learning in healthy ageing, and our age range of 18-40 minimizes variance arising from these changes (Seidler et al., 2010). All participants gave their written informed consent and were screened for tES eligibility (Appendix; B). An information sheet was provided explaining all the procedures of the study and all procedures were explained prior to the beginning of the experimental session (Appendix; C).

In **Chapters 4 and 5** individual MRI scans were used to simulate electric field in the brain. For **Chapter 4**, 50 structural MRI scans of healthy adults (aged 22-35, 23 males, 27 females) from the Human Connectome Project (HCP) database (https://ida.loni.usc.edu/login.jsp) were used; courtesy of the Laboratory of Neuro Imaging and Martinos Center for Biomedical Imaging, Consortium of the Human Connectome Project (Van Essen et al., 2012). The database's images were obtained using a 3.0 T Siemens Connectome Skyra scanner with a standard 32 channel Siemens receiver head coil. The selected T1-weighted scans are identical to ones used in our previous study (Evans et al., 2019). In **Chapter 5** the participants' own structural MRI scans were used.

2.5.1 Sample size

Sample size calculations were conducted to determine the minimum number of subjects to recruit for adequate study power (80%). For this, I used the formulae by Rosner's (2011) (see Figure 2.9) and based our calculations on the means and variance reported in Rawji and colleagues' study (Rawji et al., 2018; Rosner, 2011).

$$k = \frac{n_2}{n_1} = 1$$

$$n_1 = \frac{(\sigma_1^2 + \sigma_2^2/K)(z_{1-\alpha/2} + z_{1-\beta})^2}{\Delta^2}$$

$$n_2 = K * n_1$$

Figure 2.9 Sample size calculation
*This figure shows the calculations and formulae used for assessing number of subjects that need to be enrolled in **Chapter 3** in order to have sufficient statistical power to detect tDCS effects (18 in each group). Here, Δ = |μ2-μ1, corresponding to the absolute difference between two means. σ1 and σ2 are the expected variance of the mean (expected means and variance based on a previous study by Rawji et al., 2018). n1 and n2 is the sample size recommended for each group. 'A' corresponds to the probability of type I error (usually 0.05) and β corresponds to the probability of type II error (usually 0.2). z = critical Z value for a given α or β and k stands for the ratio of sample size for group 2 to group 1 (Rosner, 2011).*

2.6 Data analyses

Statistical analysis of SEPs and MEPs was performed using my own custom-written routines in MATLAB and group differences were analysed on SPSS (IBM SPSS Statistics Version 26).

Conventional statistical methods were further used including, Analysis of Variance (ANOVA), t-tests, multiple regression and grand average analyses to analyse physiological data between subjects in **Chapters 3** and **5**. Significance level was set at the alpha p-value level of 0.05. Details of these analyses are described in the relevant chapters. In the next section I briefly outline physiological data pre-processing, ANOVA/ANCOVA approaches.

2.6.1 SEP and MEP data pre-processing

Electromyographic data was collected on Signal (Signal Version 6.0; Cambridge electronic design, UK) and converted from Signal (.csf) to MatLab (.mat) format. Peak-to-peak amplitudes of MEPs were measured after excluding trials with artefacts. The first 5 MEP trials of each block were discarded (Julkunen, Säisänen, Hukkanen, Danner, & Könönen, 2012). This ascertains that the TMS coil is positioned in the same location throughout the experiment. Furthermore, a window of observation (100ms) before the TMS trigger was analysed to identify pre-contraction in the FDI muscle. Pre-contraction in the FDI muscle is non-TMS induced EMG activity. Thereby, trials contaminated with pre-stimulus contraction of the FDI muscle were rejected (Farzan et al., 2013). To do this, any signal exceeding two standard deviations from the pre-TMS (100ms) EMG signal was ruled out. Trials with TMS-evoked MEP amplitudes <0.1 mV were discarded (Mars et al., 2009). Finally, MEPs that were two standard deviations (SDs) larger than the block's mean (MEP + 2 SD) were discarded, as these were considered outliers. Following pre-processing, the average MEP amplitude was calculated per block.

In **Chapter 3** I also analysed SEPs by computing the average in each block on a separate computer using Matlab similar to previous studies (Kirimoto et al., 2011; Matsunaga, Nitsche, Tsuji, & Rothwell, 2004). Averaging SEPs across multiple trials increases the signal-to-noise ratio that is substantially low for SEPs. With more trials, spontaneous waves in EEG disappear and the evoked potential wave becomes more prominent (Dawson, 1954; Hu, Zhang, Liu, Luk, & Hu, 2015). In **Chapter 3**, I used an adequate amount of trials to reduce SEP noise through averaging (600 trials per block). For S1 excitability, I measured the mean amplitude of the N20 component of SEPs, identified as the first peak indicated by 2 SDs larger than the baseline value of the SEP signal between 17 – 24 ms following the stimulus artefact.

2.6.2 Repeated measures ANOVA

In **Chapters 3** and **5** I investigate pre- and post- tDCS effects of different group conditions on a repeated measure of SEPs and MEPs. In **Chapter 3**, I administered a two-way repeated measures analysis of variance (ANOVA) to analyse the mean differences of our dependent variables' 'biomarker' (SEP/MEP) peak to peak amplitudes between two factors 'current direction', 'time' and their interaction. One factor is a between-subjects factor; 'current direction' (AP-tDCS, PA-tDCS and ML-tDCS) and the other, are two within-subjects factors; 'biomarker' (MEP/SEP) x 'time' (before- and post- tDCS). Post-tDCS MEP/SEP block means were presented as a fraction % of their baseline across groups and conditions. I did this to allow for comparison of 2 factors that are on different metrics and scale differentially (MEP: mV and SEP: μV). Variability of MEPs were calculated as the coefficient of variance (CV).

In **Chapter 5**, to assess effects of the different types of tDCS on the variability of MEP change, I used SPSS (Version 25.0, IBM) to analyse a 2 x 7 repeated measures ANOVA (**Experiment 5.1**) and a 3 x 7 repeated measures ANOVA (**Experiment 5.2**) with the factors tDCS-type (**Experiment 5.1**; fixed and individualised, **Experiment 5.2**; fixed, individualised, sham) and time point (block: during 1-5, post 1-2). Variability of MEP change was calculated as the deviation of MEP's from the block's mean after each block has been normalised to the baseline block. Normalising to the baseline block transforms the variables to MEP change from baseline.

ANOVAs with repeated measures (within-subject factors) are particularly susceptible to the violation of the assumption of sphericity. Violation of sphericity occurs when there are no equal variances between the differences of related group combinations. The sphericity of the data was tested using a Mauchly's test. Mauchly's test of sphericity was insignificant for all statistical analyses, thus, we assume that the variance of the comparisons between all combinations of related groups are equal (Abdi, 2010).

2.6.3 Paired sample t-tests for session baseline differences

In **Chapter 5,** paired sample t-test (**Experiment 5.1**) and RM-ANOVA (**Experiment 5.2**) compared MEP baseline averages, within-subjects, at different sessions (**Experiment 5.1**; individualised-, and fixed- dose tDCS, **Experiment 5.2**: individualised-, fixed- and

sham- tDCS). If baseline MEPs differed, I normalised the remaining blocks to baseline. Baseline MEP differences correlate to the electric field in the motor cortex which is correlated to tDCS outcomes (Laakso et al., 2019; Mikkonen et al., 2018), thus all our analysis continue with baseline as a covariate to account for baseline effects on tDCS change. If neuronavigation error was significantly different at baseline or accounted for MEP variability, it was also added as a covariate in the repeated measure ANCOVA to account for variance caused by baseline differences and coil errors.

2.6.4 Neuronavigation coil error analysis

A multiple regression of MEP amplitude against the four coil errors was performed for each subject to assess whether coil errors accounted for MEP variance. To account for potential outliers, a robust regression based on iteratively reweighted least squares with a bisquare weighting function was used. To account for MEP variability explained by coil error on a subject-level, it was added as a covariate in further analysis.

2.6.5 Responders vs non-responders

In **Chapters 3** and **5** I looked at group specific responders. To assess group specific responders, i.e which percentage of our subjects responded with facilitatory or inhibitory responses in each group, I clustered subjects according to their individual grand average MEP change and variability in response to the type of tDCS they received (grand average >100% indicating facilitation; <100% indicated inhibition) and a frequency spectrum was calculated (Wiethoff et al., 2014).

2.6.6 Electric field analysis

In **Chapter 4**, electric field intensities and distribution were compared between HD-tDCS and PA-tDCS. Firstly, electric field intensities were extracted in the LM1 region of interest. I then assessed the stimulator output required so that both montages deliver comparable electric fields in the region of interest and further compared electric field distribution between montages. In **Chapter 5,** I determined the desired electric field intensity at the cortical target site and calculated the individualised tDCS output

intensity to be applied in the experimental session for each participant (see section 2.3.2 for details on the process).

Region of interest (ROI): In order to extract the electric field values from LM1, the eigenvariates from a spherical volume of interest (ROI; 5mm radius) centered at LM1 (MNI: -38, -20, 50; (Eickhoff et al., 2009) were extracted, using the MarsBar toolbox (Brett, Anton, Valabregue, & Poline, 2002). Here, the LM1 ROI was selected as it is often targeted with tDCS in order to quantify its impact on corticospinal excitability (Di Lazzaro et al., 1999, 1998; Di Lazzaro & Rothwell, 2014). Additionally, electric field intensities in a larger ROI (10mm radius; see Appendix D) were quantified. In this case, the electric field was, on average, greater compared to our initial 5mm ROI, however, numerically, this difference was too miniscule to have any substantial effect in our data.

Electric field intensities in LM1: In **Chapter 4**, electric field intensities in LM1 were compared between fixed-dose HD-tDCS (HD-tDCS$_{fixed}$) and fixed-dose PA-TDCS (PA-tDCS$_{fixed}$). The average electric field intensity (V/m) achieved in LM1 across our sample population when PA-tDCS$_{fixed}$ was applied (0.185 V/m) was used as the target intensity for individualised-dose PA-tDCS (PA-tDCS$_{indiv}$). Similarly, the average electric field intensity achieved in LM1 when HD-tDCS$_{fixed}$ (0.128 V/m) was applied, was used for individualised-dose HD-tDCS (HD-tDCS$_{indiv}$). These average intensities were used as target intensities for **Chapter 5**.

Stimulator output trade-off: The average electric field intensity achieved in LM1 with PA-tDCS$_{fixed}$ is substantially higher than that of the HD-tDCS$_{fixed}$ montage with small electrode sizes (Mikkonen et al., 2020). In **Chapter 4**, in order to calculate the percentage difference in tDCS stimulator output intensity (in mA) required to deliver comparable LM1 electric fields (in V/m) with HD-tDCS$_{indiv}$ as produced with PA-tDCS$_{fixed}$, the average PA-tDCS$_{fixed}$ LM1 intensity was used as the target intensity for HD-tDCS$_{indiv}$ (0.185 V/m).

Spatial distribution of electric fields: In **Chapter 4** I also assess whether HD-tDCS retains its focality advantage when delivering matching electric fields as PA-tDCS. I first assessed the distribution of electric fields produced with a fixed dose (1mA) for both montages. Brain areas with electric fields significantly above zero were identified with t-tests, using

the normalised, smoothed electric fields images. Only electric field distribution intensities thresholded at 0.08 V/m are presented. As all values above zero are displayed, this threshold was chosen for visualisation purposes of relative electric fields produced by both montages in LM1. Next, the cortex-wide electric field distribution was compared when HD-tDCS$_{fixed}$ and PA-tDCS$_{fixed}$ montages were simulated. I then compared electric field distribution between HD-tDCS$_{indiv}$ and PA-tDCS$_{indiv}$ montages, when LM1 target intensity was set at the average electric field intensity achieved by PA-tDCS$_{fixed}$ (0.185V/m). Electric field clusters that were significantly higher in intensity when individualised-dose (tDCS$_{indiv}$; to 0.185 V/m) was applied for both montages, were identified using paired t-tests in SPM.

Chapter 3 The effects of tDCS current flow direction on the excitability of the primary sensory and motor cortices

3.1 Abstract

Research utilising tDCS has only recently considered the putative sources of tDCS variability. To gain an in depth understanding of tDCS neuromodulation effects and their variability, it is important to consider the topography of the human brain and how different current flow directions alter membrane polarisation. In this chapter, I investigated the impact of different tDCS directions on the excitability of the primary sensory (S1) and motor (M1) cortices by applying tDCS directed predominantly perpendicularly through the central sulcus with posterior-anterior and anterior-posterior tDCS, versus current directed along the central sulcus in a mediolateral orientation. The impact of stimulation on the left M1 and S1 through MEPs and SEPs was measured, respectively. 54 right-handed participants between the ages of 18-40 were recruited for the study and separated in 3 groups of different tDCS current directions in a between-subjects design. Opposite polarisation trends of MEPs and SEPs indicated that tDCS provides consistent and differential excitability after-effects when current flow is directed across rather than along the central sulcus. However, reversing current flow direction did not reverse the direction of tDCS polarisation on MEPs/SEPs but may affect one bank of the central sulcus more than the other and subsequently influence the impact of cortico-cortical projections of S1-M1 and M1-S1. Acute effects of tDCS on corticospinal excitability show that the largest change happens at the first instance of stimulation, however incomplete evidence from combined protocols may be a contributing factor to the observed effects and should be controlled for. Future research is required in the underlying mechanisms of tDCS and S1-M1 connectivity to improve tDCS efficacy. Factors such as tDCS dose and combined protocol methodology should be considered for the optimisation of tDCS application.

3.2 Introduction

The effects of tDCS on CSE are often variable (Li et al., 2015; Price, McAdams, Grossman, & Hamilton, 2015; Wiethoff et al., 2014), and limit its use as a clinical therapeutic tool. As the effect of tDCS on membrane polarisation depends on the orientation of the external electric field with regards to the orientation of the pyramidal neurons in cortex (Bestmann & Walsh, 2017; Bikson et al., 2004; Dissanayaka et al., 2017; Esmaeilpour et al., 2018; Miranda et al., 2013; Paulus et al., 2013; Radman et al., 2009; Rahman et al., 2013; Reato, Rahman, Bikson, & Parra, 2013), the lack of control of tDCS current flow direction for stimulation of a targeted region may be a vital source of this variability. Thus, to improve the application of tDCS, we first need to understand how current flow direction influences excitability changes based on the topography of a targeted region.

The conventional tDCS montage for modulating corticospinal excitability places one electrode over the hand location of the central sulcus, and another electrode over the contralateral orbit (Lefaucheur et al., 2017; Nitsche & Paulus, 2000). As outlined in **Chapter 1**, conventional tDCS montages for stimulation of M1 do not explicitly control the direction of current flow in the target region. The effect of tDCS on membrane polarisation depends on the orientation of the external electric field with regards to the orientation of the pyramidal neurons in cortex (Bestmann & Walsh, 2017; Bikson et al., 2004; Dissanayaka et al., 2017; Esmaeilpour et al., 2018; Miranda et al., 2013; Paulus et al., 2013; Radman et al., 2009; Rahman et al., 2013; Reato et al., 2013). As illustrated in animal studies, stimulation with the anode over the cortical target is expected to produce an inward flow of current (relative to the cortical surface) and depolarise the soma of a pyramidal neuron whilst hyperpolarising other neuronal sections and neighbouring cells (Bikson et al., 2004; Radman et al., 2009; Rahman et al., 2013). Changes in membrane polarisation of a single neuron are induced mainly by the field component aligned parallel to the neuronal axis (Jackson et al., 2016). Thereby, the extent and the direction of polarisation of different compartments of pyramidal neurons depend on the direction of current flow, which in turn, relatively influence net changes in excitability (Rahman et al., 2013). Despite compelling evidence from animal studies, until now, tDCS applications rarely control for the direction of current flow in the cortex (Bestmann & Walsh, 2017). In humans, the folding of the cortex means that the

direction of current flow relative to the neuronal orientation is more complex (Dmochowski et al., 2012). Thus, understanding how current flow direction influences excitability changes based on the topography of the primary motor cortex (M1) might be relevant for optimising the application of tDCS.

Recently, Rawji and colleagues (2018) found that changes in CSE depend on the direction of current flow between two electrodes with respect to the principal axis of central sulcus (Rawji et al., 2018). Whilst current flowing parallel to the wall of the precentral gyrus (i.e. in medio-lateral orientation) did not affect CSE, current flowing perpendicularly through the wall of central sulcus (i.e. in posterior-anterior direction), did reliably alter CSE. This may be because the primary motor cortex is located in the anterior wall of the central sulcus, and optimal polarisation of pyramidal neurons in this region should occur when current flows along the principal dendritic axis of these neurons i.e, perpendicularly with regards to the cortical surface (de Berker et al., 2013; Esmaeilpour et al., 2018; Rahman et al., 2013; Rawji et al., 2018).

The primary sensory cortex (S1) is located on the posterior wall of the central sulcus. Given that the orientation of pyramidal neurons is opposite for the posterior and anterior banks (Rahman et al., 2013), current flowing through the central sulcus may modulate excitability changes in M1 and S1 differentially (see Figure 3.1; acknowledging that there are also intimate connections between M1 and S1, which may further complicate matters).

I predict that applying tDCS with current flow (mainly) perpendicular through the pre- and post-central sulcus may lead to opposite polarisation effects in M1 and S1, respectively. Based on the topography of the sensory and motor cortices, and the readouts of their excitability that one can obtain in humans, these areas may provide an ideal testbed in humans to investigate the impact of current direction on cortical excitability.

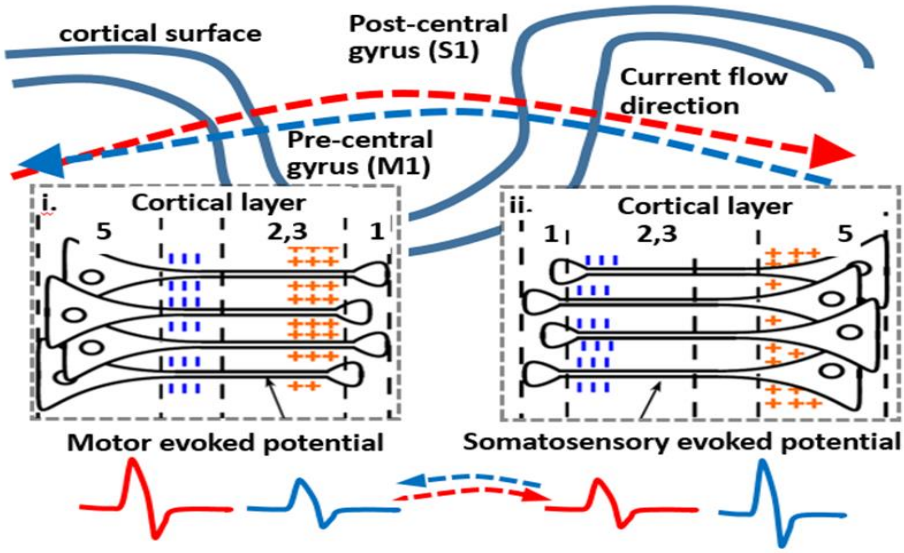

Figure 3.1. Orientation of pyramidal neurons on the opposite banks of the central sulcus and DCS effect.

This figure illustrates the orientation of pyramidal neurons in the banks of the pre- and post-central sulcus. The red arrow illustrates the anterior to posterior tDCS current (AP-tDCS) whereas the blue arrow illustrates the tDCS current flowing from posterior to anterior (PA-tDCS). As described in the hypothesis, I expect that PA-tDCS and AP-tDCS will have opposite polarisation effects.

Here I examined this hypothesis by applying tDCS current directed predominantly perpendicularly through the central sulcus, versus current directed along the central sulcus in a mediolateral orientation. I then measured the impact of stimulation on the left M1 and S1 through MEPs and SEPs, respectively (acknowledging that MEPs reflect corticospinal excitability, not just corticomotor excitability and SEPs reflect spino-cortical projections). To my knowledge, there is currently no study investigating how different directions of tDCS current flow influence excitability changes in S1 and M1. This is relevant as net tDCS effects commonly observed in MEP amplitude may derive from specific polarisation changes in both M1 and S1. Yet, little is known about the tDCS effects on S1 polarisation, and how this might interact with M1 to influence MEP change.

3.3 Methods

All study procedures were pre-registered on the Open Science Framework (OSF) prior to the execution of this study (https://osf.io/bvzwp).

3.3.1 Participants

54 right-handed participants between the ages of 18-40 were recruited from the UCL Psychology and Institute of Cognitive Neuroscience (ICN) subject pools. As detailed in **Chapter 2** section 2.9, sample size calculations were conducted to determine the minimum number of subjects (18 in each group), based on a previous study (Rawji et al., 2018), to recruit for adequate study power (see Figure 3.2; Rosner, 2011). Right-handed participants were used as there have been observable differences in tDCS effects on cortical excitability between left- and right-handed individuals (Guadalupe et al., 2014; Schade et al., 2012). There are known changes in cortical excitability and motor learning in healthy ageing, and our age range of 18-40 minimizes variance arising from these changes (Seidler et al., 2010). All participants gave their written informed consent and were screened for tES eligibility. An information sheet was provided explaining all the procedures of the study and all procedures were explained prior to the beginning of the experimental session. This study has been approved by the UCL research ethical committee and conformed to the requirements of the new GDPR data protection policy.

$$k = \frac{n_2}{n_1} = 1$$

$$n_1 = \frac{(\sigma_1^2 + \sigma_2^2/K)(z_{1-\alpha/2} + z_{1-\beta})^2}{\Delta^2}$$

$$n_1 = \frac{(0.121^2 + 0.121^2/1)(1.96 + 0.84)^2}{0.112^2}$$

$$n_1 = 18$$

$$n_2 = K * n_1 = 18$$

Figure 3.2 Sample size calculation

This figure shows the calculations and formulae used for assessing the number of subjects that needed to be enrolled in this study in order to have sufficient statistical power for the detection of tDCS effects (18 in each Group). Here, Δ = |μ2-μ1, corresponds to the absolute difference between two means. σ1 and σ2 are the expected variance of the mean (expected means and variance based on a previous study by Rawji et al., 2018). n1 and n2 is the sample size recommended for each group. 'A' corresponds to the probability of type I error (usually 0.05) and β corresponds to the probability of type II error (usually 0.2). z = critical Z value for a given α or β and k stands for the ratio of sample size for group 2 to group 1 (Rosner, 2011).

3.3.2 TDCS montages of different current flow directions and expected outcomes

Participants were divided into 3 groups of different tDCS current directions. Group 1 received posterior-anterior tDCS (PA-tDCS), where the anode was positioned 7cm posterior- and the cathode 7cm anterior- to the hand representation of the precentral gyrus (5.5cm from the hotspot to the electrode edge). The hand representation of the precentral gyrus was determined as the area that induced most consistent 1mA TMS-MEPs (see section 3.2.4). The electrodes were placed in a right angle to the presumed orientation of the precentral sulcus at approximately 45 degrees with respect to the central fissure. This angle was determined by the orientation of the TMS coil that induced consistent MEPs at the LM1 hotspot.

Group 2 received anterior-posterior tDCS (AP-tDCS), with the anode and cathode in reversed positions to Group 1. Finally, medio-lateral tDCS (ML-tDCS) was applied for Group 3 with the anode positioned 7 cm medial and the cathode 7 cm lateral (5.5 cm from the hotspot to the electrode edge) to the hand representation of the cortical

surface of the precentral gyrus (Rawji et al., 2018), in parallel to the presumed orientation of the central sulcus at 45 degrees with respect to the central fissure (see Figure 3.3).

I hereby compared the effect of different tDCS montages which manipulated the direction of current flow within the target region. My prediction was threefold: (1) Given that the orientation of pyramidal neurons is opposite for the posterior and anterior banks of the central sulcus (Rahman et al., 2013), current flowing perpendicularly through the central sulcus (i.e., with electrodes placed anteriorly and posteriorly to the presumed M1 hand representation) may modulate excitability changes in M1 and S1 differentially, and would produce opposite effects on M1 and S1 excitability. As PA-tDCS has shown to decrease corticospinal excitability (Rawji et al., 2018), we would expect somatosensory excitability to increase. (2) As such, reversing the direction of current flow through the central sulcus (by positioning the anode anterior and posterior, respectively, to the central sulcus), would reverse polarisation of pyramidal neurons, and hence reverse the observed excitability effects from motor and sensory regions. In this case, AP-tDCS would increase corticospinal excitability and decrease somatosensory excitability. (3) As optimal polarisation of pyramidal neurons in this region should occur when current flows along the principal dendritic axis of these neurons (Rawji et al., 2018), current directed predominantly along the central sulcus (i.e., parallel to the cortical surface in pre- and postcentral sulcus), with electrodes placed medially and laterally of the presumed M1 hand representation, would not lead to consistent changes in M1 and S1 excitability.

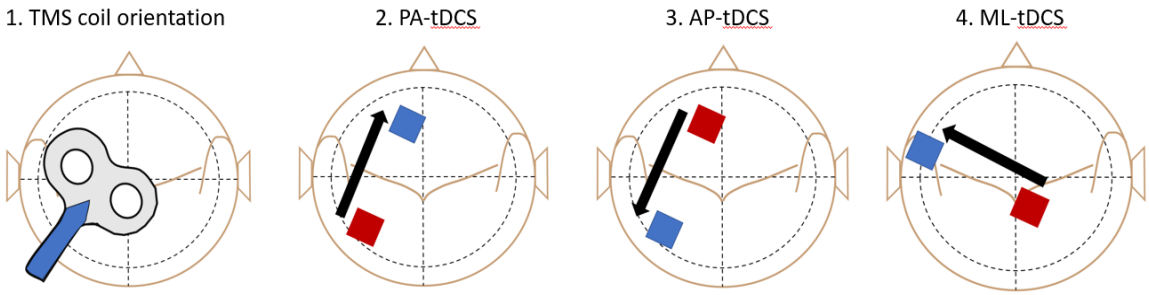

Figure 3.3. Transcranial Direct Current Stimulation electrode montage
TDCS electrodes on the head as applied across (2; PA-tDCS) the central sulcus, with anode 7 cm posterior (red) and cathode (blue) 7cm anterior to the motor hotspot (5.5cm from electrode edge) in the orientation of the TMS coil (1; left). Electrodes were reversed for the AP-tDCS (3; AP-tDCS) current flow montage. ML-tDCS (4; ML-tDCS) directed the current along the central sulcus with the tDCS anode (red) 7cm medial and the cathode (blue) 7cm lateral.

3.3.3 Experimental design

A between-subjects design was used to test the effects of different tDCS conditions (PA-tDCS, ML-tDCS, and AP-tDCS) on motor and sensory excitability. The effects of tDCS were assessed before, during, and after tDCS. MEP and SEP measurements were separated in time and applied in alternating blocks (see Figure 3.4). Each TMS-MEP block consisted of thirty-seven trials (TMS pulses applied at 0.2 Hz), and each Median-Nerve-Stimulation inducing SEPs (MNS-SEP) block consisted of six-hundred trials (MNS pulses applied at 5Hz). One block of MEPs and one block of SEPs were collected before tDCS (see Figure 3.4; Phase 1) that lasted approximately 5 minutes (2.1 minutes for each SEP Block; 2.6 minutes for each MEP block). TDCS was then applied for 20 minutes; throughout this phase, 4 blocks of SEPs and 4 blocks of MEPs were measured, in alternating fashion (see Figure 3.4; Phase 2). TDCS intensity will ramp up/down for 30seconds at the beginning/end of stimulation. 5 minutes following tDCS stimulation I recorded 2 blocks of MEPs and 2 blocks of SEPs (see Figure 3.4; Phase 3).

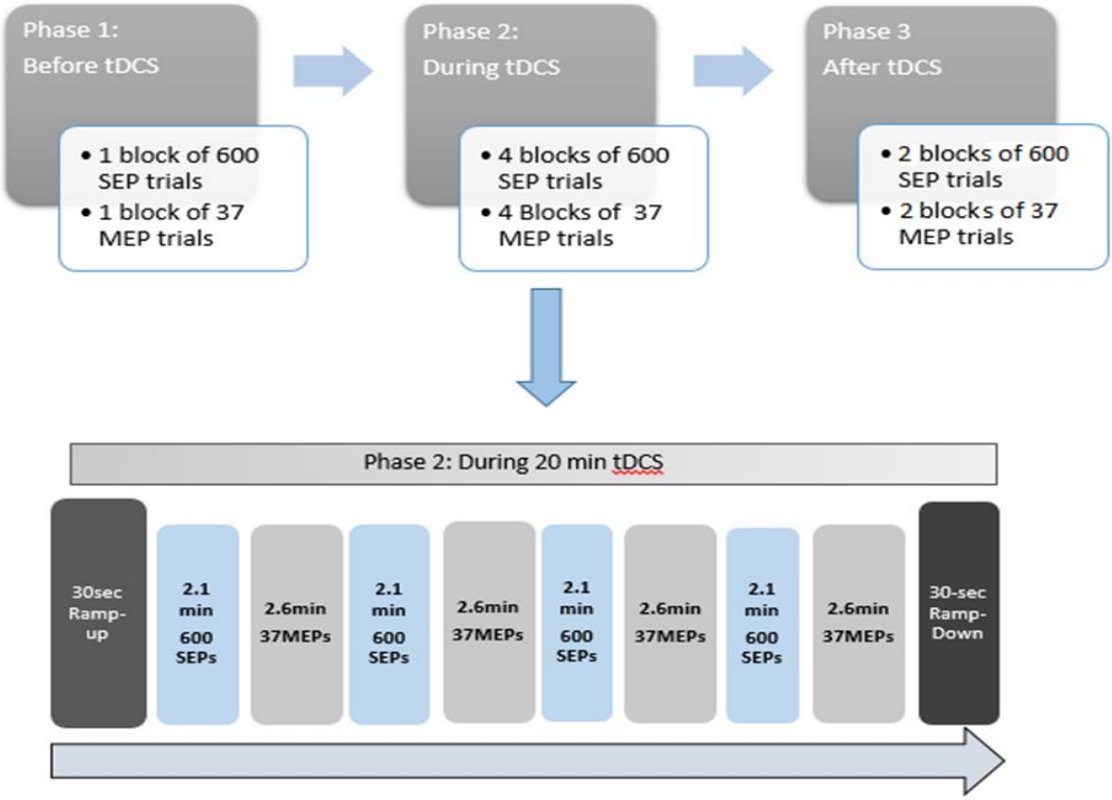

Figure 3.4. The experimental protocol

The effects of tDCS were assessed Before- (Phase 1), during- (Phase 2), and after- (Phase 3) tDCS. MEP and SEP measurements were separated in time and applied in alternating blocks. Each TMS-MEP block consisted of 37 trials and each MNS-SEP block consisted of 600 trials. 1 block of MEPs and 1 block of SEPs were collected before tDCS (Phase 1) that lasts approximately 5 minutes (2.1 minutes for each SEP Block; 2.6 minutes for each MEP block). TDCS was then applied for 20 minutes; throughout this phase, 4 blocks of SEPs and 4 blocks of MEPs were measured, in alternating fashion (Phase 2). TDCS intensity ramped up/down for 30seconds at the beginning/end of stimulation. 5 minutes following tDCS stimulation I recorded another block of MEPs and SEPs. 2 blocks of MEPs and 2 blocks of SEPs will be collected post-tDCS (Phase 3).

3.3.4 Transcranial Magnetic Stimulation induced Motor Evoked Potentials

TMS is a form of non-invasive brain stimulation used to stimulate targeted cortical regions by directing a magnetic field over the scalp and inducing a current in the brain that modulates corticospinal excitability (Rothwell, 1997; Rothwell et al., 1999). Please refer to **Chapter 1** section 1.4.1 for details about the role of M1 and index of corticospinal excitability. Furthermore, refer to **Chapter 2** section 2.2.7 for details on TMS-MEP procedures. Single-pulse TMS was applied over the primary motor area to elicit MEPs in the contralateral FDI muscle during and after tDCS stimulation. TMS was administered using a MagStim BiStim2 (The Magstim Co. Ltd). A standard figure of eight (Magstim, 70mm) alpha coil was placed over the motor area in approximately 45-degree angle perpendicular to the central sulcus, with the coil handle pointing backwards from the midline for posterior to anterior stimulation. I determined the location (motor hotspot) for activation of the FDI muscle by moving the coil in 1 cm steps around the presumed M1. TMS was delivered at 0.2 Hz and EMG signals were digitized at 5 kHz (CED Power 1401; Cambridge Electronic Design, Cambridge, UK). In order to avoid the coil pressing on the EEG scalp electrodes which may cause electrode movement or push out the electrode paste, I designed a TMS Tool for Electrode Movement Prevention (TEMP) made out of foam core (AIREX T10) 5x1x1.5 (Length x width x breadth), fixed at the center of the TMS coil where the current is strongest.

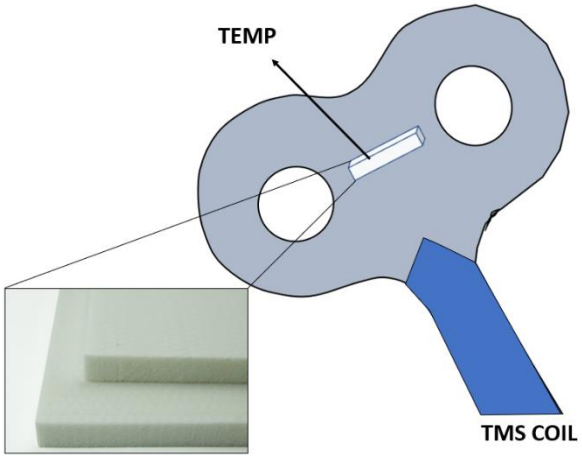

Figure 3.5. Custom-made Tool for Electrode Movement Prevention (TEMP)
TEMP was added at the center of a figure 8 TMS coil to avoid the coil pressing on the EEG scalp electrodes. TEMP is made of foam core material (bottom left) with 5x1x1.5 (Length x width x breadth) dimensions.

As detailed in **Chapter 1** section 1.4, TMS-MEPs are thought to index changes in corticospinal excitability, and thus provide a direct read-out of the impact of tDCS at the time of TMS application (Bestmann, 2012; Bestmann & Krakauer, 2015). TMS-evoked MEPs were measured via EMG electrodes (19 x 38 mm Ambu, WhiteSensor) with an active electrode placed on the FDI muscle of the contralateral hand, and the reference proximal to the interphalangeal joint (see Figure 3.6). The ground electrode was placed on the nasion since there was one ground for both EMG and EEG electrodes. Signals were amplified (gain; 1000) and band-pass filtered (5-2500) (Digitimer D360). MEP data was stored on a computer for offline analysis (Signal Version 6.0, Cambridge Electronic Design, UK).

3.3.5 Median Nerve Stimulation (MNS) induced somatosensory evoked potentials (SEPs)

MNS-SEPs were recorded as measurements of somatosensory excitability. Electrical stimulation was applied to the right median nerve at the wrist at 5 kHz frequency (Digitimer DS7-A). The anode was placed just proximal to the palmar crease, and the cathode was placed between the tendons of the palmaris longus muscle, 3cm proximal to the anode (Legatt, 2014). The intensity of stimulation was fixed just below the threshold for evoking muscle movement in the hand. To do this, I first ramped up the intensity where I saw muscle movement in the hand and then lowered the intensity to the amplitude where I no longer observed a muscle twitch. This was checked throughout the course of the experiment, by monitoring the evoked EMG response of the FDI muscle. At this stimulus intensity, SEPs are usually submaximal in amplitude. There have been a number of studies reporting the effect of stimulus intensity on SEPs and it is generally agreed that the amplitude of scalp recorded SEPs reach plateau with the intensity of stimulus at 1.5 times the motor threshold or 2-3 times the sensory threshold (Huttunen, 1995; Shiga et al., 2001). In order to quantify the impact of tDCS on MEPs, uncontaminated by overt muscle movement during MNS stimulation, I applied subthreshold MNS intensities.

The primary sensory cortex contralateral to MNS is the main generator of both the cortical early (20–40 ms) and late (60–120 ms) responses to stimulation (Baumgärtner

et al., 2010). This brain region is approximately underneath the CP3 electrode position in the 10-20 EEG system (Rehmann et al., 2016). Thereby, SEPs were recorded from two scalp electrode (3mm Ag/AgCl disposable cup electrodes) positions (see Figure 3.6). One electrode was placed at 3 cm posterior from the motor hotspot in the orientation of the TMS coil. Based on my pilot measurements, this electrode location corresponds to approximately CP4-5 standard EEG electrode position (depending on the individual). The other electrode was positioned 3cm posterior to the motor hotspot (not in the orientation of the TMS coil) at the 10-20 system location of CP3 approximately above the primary sensory area whereby the SEP N20 component is greatest in size. Both scalp electrodes were referenced to Fz (Kirimoto et al., 2011; Legatt et al., 2016). A bandpass filter of 3Hz to 3 kHz was used with a Digitimer D360 amplifier (Gain: 100.000x).

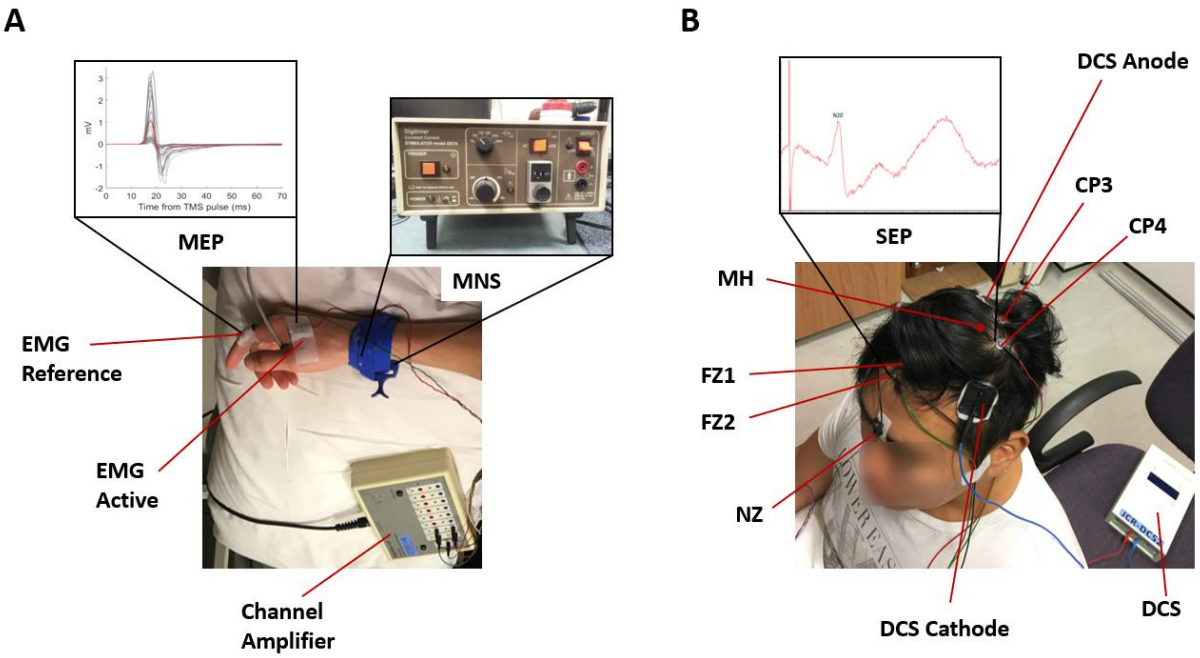

Figure 3.6 Experimental setup

This is an example of electrode placement for a participant part of the ML-tDCS group. ***A)*** *Motor evoked potentials (MEP; top left) were recorded via two electromyographic electrodes; an active electrode placed on the FDI muscle (EMG Active) of the contralateral hand (to the TMS stimulated region, LM1) and a reference electrode placed proximal to the interphalangeal joint (EMG Reference). Median nerve stimulation was applied with the anode of the peripheral nerve stimulator placed just proximal to the palmar crease, and the cathode placed between the tendons of the palmaris longus muscle, 3cm proximal to the anode (MNS; top right).* ***B)*** *Sensory evoked potentials (SEPs; top left) were recorded with from two scalp electrodes positions. One electrode was placed at 3 cm posterior from the motor hotspot (MH) in the orientation of the TMS coil which corresponds to approximately CP4 standard EEG electrode position (depending on the individual). The other electrode was positioned 3cm posterior to the motor hotspot (MH), not in the orientation of the TMS coil, at the 10-20 system location of CP3 approximately above the primary sensory area. Both scalp electrodes were referenced to FZ1 and FZ2, respectively. The ground electrode was placed at the nasion (NZ).*

3.3.6 Transcranial Direct Current Stimulation Protocol

TDCS electrode, amplitude and orientation parameters were replicated from Rawji et al., 2018. During tDCS, 1mA of current strength was applied for 20 minutes with a pair of 9 cm^2 (3 x 3 cm) rubber electrodes evenly applied with conductive paste (Ten20; Weaver & Co., Aurora, USA). After identifying the left hemispheric TMS motor hotspot, the tDCS electrodes were placed 7cm anterior and posterior to the motor hotspot (PA/AP tDCS), as described in section 3.2.2. The tDCS electrodes for ML-tDCS were

placed 7cm medial and lateral to the motor hotspot. The electrodes were applied in the orientation of the TMS coil at about 45° with respect to the midline. This corresponds to how the central sulcus is generally aligned from the midline.

3.3.7 Data pre-processing

Peak-to-peak amplitudes of MEPs were measured after excluding trials with artefacts (see Figure 3.7). A window of 100ms before the TMS trigger was analysed to identify pre-contraction in the FDI muscle. To do this, I calculated 2x the standard deviation of the pre-TMS (100ms) EMG signal, and any signal exceeding this was ruled out. I excluded the first 5 MEPs from every TMS Block to ascertain that the TMS coil can be positioned in the same location throughout the experiment (Julkunen et al., 2012). Trials with TMS-evoked MEP amplitudes less than 0.1 mV amplitude were discarded (Mars et al., 2009).

Averaging SEPs across multiple trials increases the signal-to-noise ratio that is typically low for SEPs. With more trials, spontaneous waves in EEG disappear and the evoked potential becomes more prominent (Akay, 2012). 600 SEP trials per block were used to reduce SEP noise through averaging (600 trials per block) (see Figure 3.7; B). This trial number is comparable to previous studies using similar methodologies that used between 300-500 trials (Kirimoto et al., 2011; Matsunaga et al., 2004).

All MEP trials were combined to extract the mean amplitude of MEPs for each block (see Figure 3.7; A). For S1 excitability, I measured the mean amplitude of the N20 component of SEPs. Post-tDCS MEP and SEP block means were presented as a fraction % of their baseline across groups and conditions. I did this to allow for comparison of two factors that are on different scales, and scale differentially (MEP: mV and SEP: microvolts).

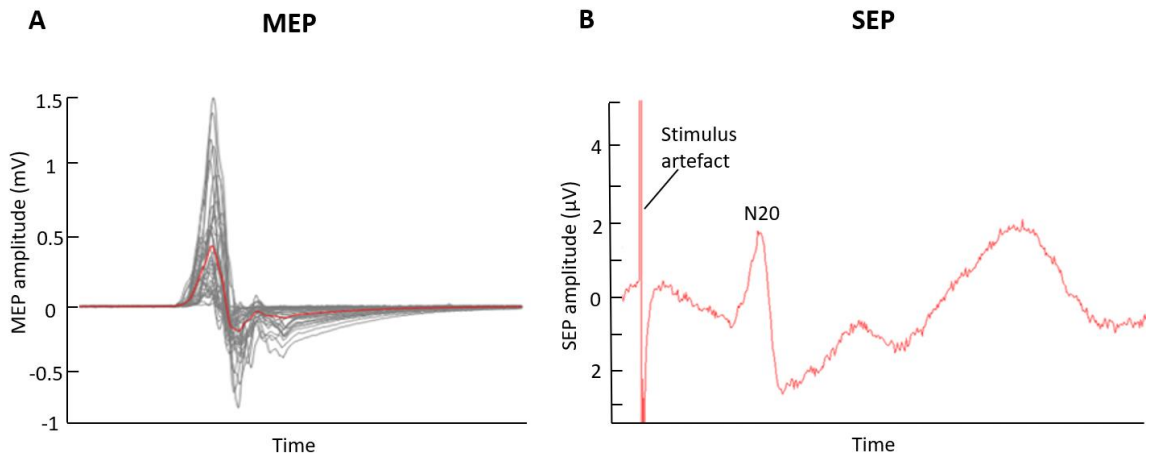

Figure 3.7 Representative participant for visualisation of mean MEPs and SEPs
A*. Representation of mean MEP (Red line; left) across single trials (Grey lines; left).*
B*. Representation of mean SEP (red; right) after 600 trials with N20 peak and stimulus artefact.*

3.3.8 Analyses

To investigate pre- and post- tDCS effects of different current flow directions on S1 and M1 excitability, a two-way repeated measures ANOVA was conducted to analyse the mean differences of the dependent variables' 'biomarker' (SEP/MEP) peak to peak amplitudes between two factors 'current direction' and 'time' and their interaction. One factor is a between-subjects factor; 'current direction' (AP-tDCS, PA-tDCS and ML-tDCS) and the other, are two within-subjects factors; 'biomarker' (MEP/SEP) x 'time' (before- and after- tDCS).

To check for time-dependent acute effects over the 20mins of tDCS, a repeated measures ANOVA with 'time' (before-, during- and after- tDCS), 'current direction' (PA/AP-tDCS or ML-tDCS) and 'biomarker' (MEPS only) interactions was used. ANOVAs with repeated measures (within-subject factors) are particularly susceptible to the violation of the assumption of sphericity. The sphericity of the data was tested using a Mauchly's test. Mauchly's test of sphericity was insignificant for all statistical analyses (with the exception of one; see Results section 3.4), thus, we assume that the variance of the comparisons between all combinations of related groups are equal (Abdi, 2010). Here, I used the standard criteria (p < or equal to 0.05) for determining if the ANOVA

results are significantly different from those expected if the null hypothesis were correct.

Moreover, I examined differences in variability of MEPS and SEPs across the different current direction conditions. Variability was quantified by the MEP and SEP coefficient of variance (CV) for each subject in each group and compared across groups using a multivariate ANOVA (MANOVA). Additionally, I assessed group specific responders, i.e which percentage of our subjects responded with facilitatory or inhibitory responses in each group, by clustering subjects according to their individual grand average MEP change in response to PA-, AP- and ML- tDCS directions (grand average > 100 % indicating facilitation; < 100 % indicated inhibition). This was based on previous research on responders and non-responders analysis (Bashir et al., 2019; Horvath et al., 2015a; Parkin, Ekhtiari, & Walsh, 2015; Roche, Lackmy, Achache, Bussel, & Katz, 2011).

3.4 Results

To assess the effect of different tDCS directions on MEP and SEP excitability, I conducted a RM ANOVA with factors 'biomarker' and 'time' for different current directions (PA-tDCS, AP-tDCS, ML-tDCS). None of the participants reported any adverse effects before, during or after stimulation by tDCS/TMS or MNS application, with the exception of a slight tingling sensation under the tDCS electrodes and drowsiness. The EEG signal during tDCS was contaminated with noise. Due to the TMS stimulator's high input signal, this caused saturation of the EEG signal exceeding the bandpass filter used (3Hz – 3kHz). Following multiple SEP trials after the TMS block, the signal would eventually return to the bandwidth frequency expected, however this affected a substantial amount of (> 80) SEP trials. Additionally, tDCS exacerbated EEG noise arising from various artefacts (such as eye movements and muscle movements) which would be more prominent in some subjects than others. This caused the EEG signal to saturate for some trials and contained a DCS drift at ~2Hz on average. Indeed, it has been recently highlighted that the frequency and magnitude of physiological artifacts is largely dictated by montage-specific DC offset, such that physiological artifacts are more prominent near stimulation electrodes and the clarity of EEG signal is dependent on DC intensity, polarity, or

electrode position (Gebodh et al., 2019). The latter two factors were different between groups. Due to large inconsistencies between usable and non-usable SEP data between and within participants during tDCS, SEPs were not used for comparison to MEPs during tDCS. Pre- and post- tDCS MEPs and SEPs were compared in the RM ANOVA only.

3.4.1 Opposing MEP and SEP effects following TDCS.

Repeated measures ANOVA ('biomarker' x 'time' x 'current direction') on post-tDCS blocks 1 and 2 normalised to baseline, revealed no significant main effects ($p > .05$) of 'time' (F [2,34] = .733, $p > .05$) or 'biomarker' (F [2, 34] = 2.39, $p > .05$) .This indicates that MEPs and SEPs did not differ in size and did not significantly change over time. Additionally, there were no interaction effects of 'time' and 'current direction' (F [4, 68] = .295, $p > .05$) . 'Biomarker' and 'time' interaction effects (F [2, 34] = 3.178, $p = .053$, η^2 = .055) approached significance which indicates a possible differential change of post-tDCS MEP and SEP amplitudes from baseline. The differential change in time between SEPs and MEPs showed a strong significant linear trend ($p = .007$, η^2 =.133) (see Figure 3.8). 'Biomarker' and 'current direction' interaction effects were not significant (F [2, 34] = .702, $p > .05$] suggesting that changing the tDCS current flow direction does not affect MEPs/SEPs differently. Finally, there were no significant interaction effects of 'time', 'biomarker' and 'current direction' (F [4, 68] = .706, $p > .05$) indicating that any changes in time of MEP and SEP amplitudes were not due to reversing the current direction.

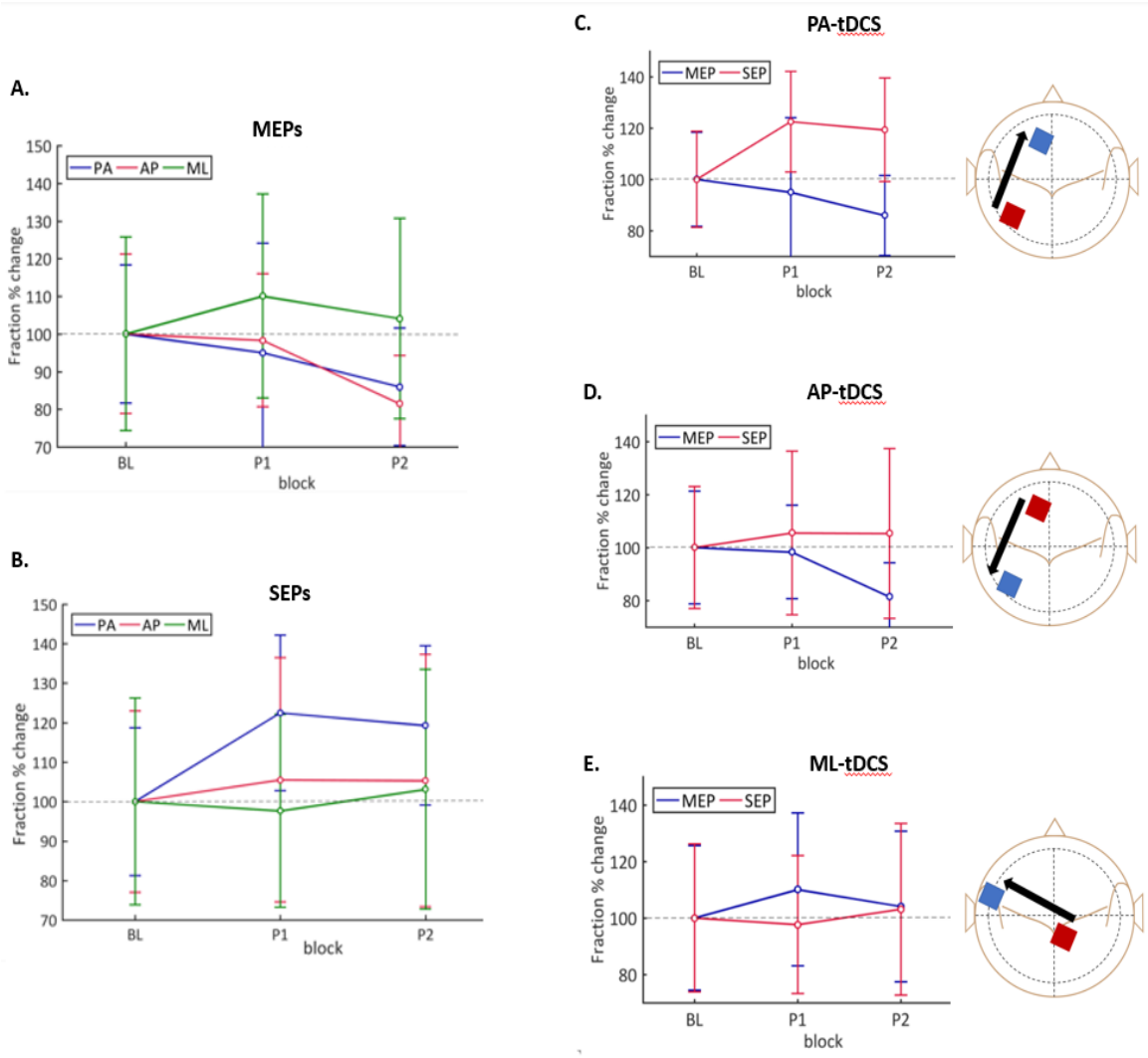

Figure 3.8. Current direction effects on motor and sensory evoked potentials normalised to baseline

This figure shows amplitude changes of MEPs following different tDCS current flow directions in 2 ways (Left; A-B and Right; C-E). A and B (Left), show MEPs (A) and SEPs (B) presented before- (BL), 1st block after- (P1) and 2nd block after- (P2), different tDCS current directions; PA-tDCS (Blue), AP-tDCS (Red) and ML-tDCS (Green). C-E (Right), show MEPs (Blue) and SEPs (Red) in separate graphs based on each current flow direction, PA-tDCS (C), AP-tDCS (D) and ML-tDCS (E). Baseline values were transformed to 100% and post-tDCS MEP/SEP blocks are % change as fractions of the baseline. Error bars represent the standard deviations. Note: As the data was transcribed to reflect a fraction of the baseline, i.e the mean CSE across the population is 100 at baseline, there is variability across subjects at baseline even when normalising to baseline.

3.4.2 Qualitative observations of M1/S1 excitability after-tDCS

Inspection of Figure 3.8 revealed that the post-tDCS N20 increased whereas post-tDCS MEPs decreased from baseline in PA/AP groups. However, the ML group showed no

difference from baseline. The largest change of MEPs was induced by AP-tDCS, which decreased MEPs by ~19% from Baseline. For PA-tDCS, MEPs decreased by ~ 14% (see Table 3.1). On the contrary, PA-tDCS induced the largest SEP difference across all groups, increasing the SEP N20 by ~20% in the second block following tDCS, whereas AP-tDCS only increased SEPs marginally by ~5%. Lastly, ML-tDCS effects on M1 and S1 were largely absent. Opposite to my expectations, reversing the direction from PA to AP did not reverse the direction of MEP/SEP polarisation. I expected this to be the case because the orientation of pyramidal neurons is opposite for the posterior and anterior banks of the central sulcus (Rahman et al., 2013) and current flowing perpendicularly through the central sulcus would be expected to modulate excitability changes in M1 and S1 differentially. Nevertheless, the above qualitative observations are similar to previous findings' (Rawji et al., 2018) that PA-tDCS decreases MEPs and reversing the direction of current will not reverse the effects on MEPs. However, as the effects have not shown any level of significance, the same conclusions cannot be drawn from this study.

Table 3.1. Mean changes of MEP/SEP expressed as fraction of baseline for following tDCS.

	Mean MEP (%)		**Mean SEP (%)**	
tDCS Direction	**B1**	**B2**	**B1**	**B2**
PA-tDCS	95.00 ± 63.34	85.93 ± 34.07	122.47 ± 42.91	119.28 ± 43.95
AP-tDCS	98.27 ± 37.47	81.42 ± 27.13	105.49 ± 65.70	105.30 ± 67.9
ML-tDCS	104.03 ± 57.33	97.49 ± 55.89	97.63 ± 51.91	103.09 ± 64.45

Baseline is set at 100% across all tDCS directions. Values are expressed as mean +- SD % change as fractions of the baseline.

3.4.3 Acute tDCS effects on MEPs

MEPs were assessed during stimulation to measure any differences in immediate effects of different tDCS directions. MEPs were recorded during the application of tDCS to investigate acute effects. Mauchly's test showed that sphericity could not be assumed for the interaction effects of 'current direction' and 'time' (X^2 [35] = .011, p = .003). In this case, degrees of freedom were corrected according to Greenhouse-Geißer estimate of sphericity (ε = .443). Repeated measures ANOVA indicated no statistically significant

interaction effects of 'current direction' x 'time' (F [3.54, 60.19] = .305, p = .853, η^2 = 0.011) on MEP amplitudes. Additionally, there were no significant effects of 'current direction' (F [2, 34] = .324, p > .05) suggesting that quantitatively, different current flow directions did not differentially modulate MEPs. However, there was a significant main effect of 'time' (F [4, 68] = 3.41, p = .014, η^2 = .279), indicating a definite change of mean MEP amplitudes across all time points, non-specific to current flow direction. Qualitatively, the largest % change was observed in the first block during tDCS with the largest dip in MEP change induced by AP tDCS with ~30 % decrease from baseline (see Figure 3.9). PA-tDCS followed with ~20 % dip in MEPs and the least percentage decrease was induced by ML-tDCS (~13 % change). As aforementioned, AP-tDCS had a larger effect on MEPs at all blocks after-TDCS.

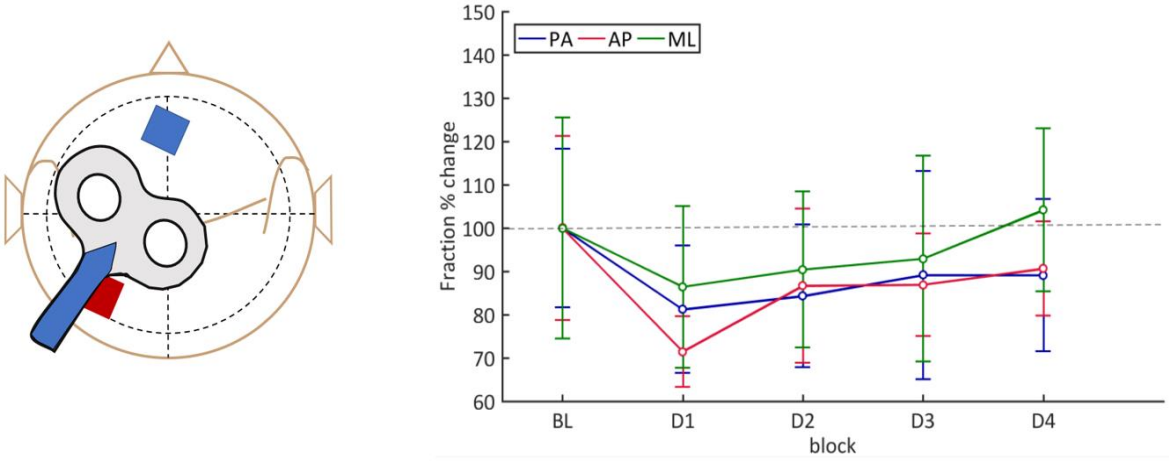

Figure 3.9. Motor-evoked potentials during tDCS
This figure presents the graphical representation of group means based on the different tDCS time points (D1-4), relevant to baseline (BL), during concurrent tDCS and TMS (Left; example of concurrent TMS and PA-tDCS). The coloured lines (Right) correspond to the mean % change of MEPs as a fraction of the baseline (normalised to 100 %), induced by PA-tDCS (Blue), AP-tDCS (Red) and ML- tDCS (Green), respectively.

3.4.4 Qualitative observations of acute tDCS effects on MEP change

Qualitatively, Figure 3.9 shows a similar trend across groups illustrating that tDCS effects on MEPs over time was not due to controlling for different current flow directions. Here, there was a reduction of MEP excitability during the first block of tDCS application. Following the first block of tDCS, MEPs started retreating to baseline until the last block during tDCS. This highlights the importance of observing acute effects of MEPs in order to gain an in-depth understanding on the different parameters of stimulation, or other parameters such as brain state during tDCS, that may illustrate differential trends than those observed before or after the protocol (Pozdniakov, Vorobiova, Galli, Rossi, & Feurra, 2021; Schestatsky, Simis, Freeman, Pascual-Leone, & Fregni, 2013).

Overall, results show that there was MEP change in all conditions, though this was qualitatively larger for AP/PA tDCS than ML-tDCS. Both AP/PA tDCS decreased MEPs during and after stimulation, thus indicating more consistent responses than ML-tDCS.

3.4.5 Variability of different tDCS directions

Next, I examined whether there were any differences in variability of MEPS and SEPs across the different current direction conditions. This variability was quantified by the MEP and SEP coefficient of variance (CV) for each subject in each group. Mean CV values are listed in table 3.2.

Table 3.2 Mean CVs for MEP and SEP biomarkers between groups

TDCS Direction	Mean MEP (CVs)	Mean SEP (CVs)
PA-tDCS	37.61*± 16.89	18.89 ± 12.76
AP-tDCS	29.88 ± 11.53	16.49 ± 9.69
ML-tDCS	26.24*± 8.82	21.66 ± 14.08

**indicates significant difference of mean CV values between groups at p < .05 level.*

A multivariate ANOVA (MANOVA) with 'tDCS direction' (PA-, AP, ML- tDCS) as between-subject factor and MEP and SEP CVs as within subject dependent variables, yielded significant between subject effects of tDCS direction for MEP variability (F [2, 52] = 3.73, p = .03) This indicates that MEP variability differed across different tDCS directions. On the contrary, SEP variability was not significantly different across conditions (F [2, 52] =

.79, p = .46). Post-hoc Bonferroni tests of multiple comparisons revealed that PA-tDCS (M = 37.6 %) produced 11.36 % greater MEP variability (p = .03) than ML-tDCS (M = 26.24 %). There were no significant differences of MEP variability between AP-tDCS (M = 29.88 %) and the rest of the conditions (p > .05). See Figure 3.10 for a graphical representation of MEP and SEP CVs.

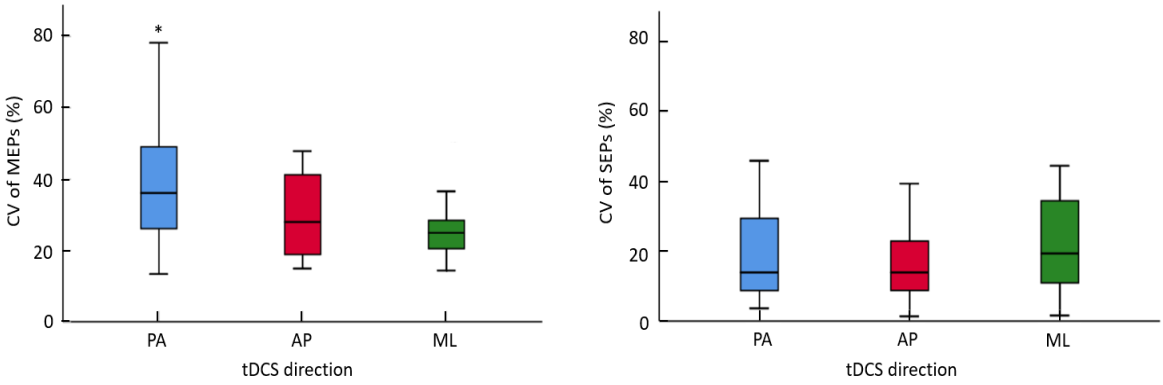

Figure 3.10. Variability comparison of MEPs and SEPs
Boxplot graphs show coefficient of variance (CV) of MEPs (Left) and SEPs (Right) for different current flow directions; PA-tDCS (Blue), AP-tDCS (Red), and ML-tDCS (Green). PA-tDCS showed significantly greater (*) variability of MEPs than ML-tDCS.

As MEP variability differed between subjects on a group level, this could have masked individual responses on a subgroup level of responders and non-responders. I thereby classified group responders (facilitatory) and non-responders (inhibitory) based on their grand average MEP change to reveal any tDCS direction effects on a subgroup level. This analysis was based on previous research work on cluster (Bashir et al., 2019; Horvath et al., 2015a; Parkin et al., 2015; Roche et al., 2011). Grand average analysis on subgroups of responders and non-responders, revealed that ~45 % of AP-tDCS participants responded with an overall facilitatory response. The PA-tDCS condition included less subjects that responded with a facilitation in MEPs (~32%) than AP-tDCS (~45%), however a paired-test showed that this difference was non-significant (p > 0.05). ML-tDCS (~56 %) and AP-tDCS (~45%), showed less consistent effects than PA-tDCS, as approximately half of the participants responded with facilitatory effects (see Figure 3.11).

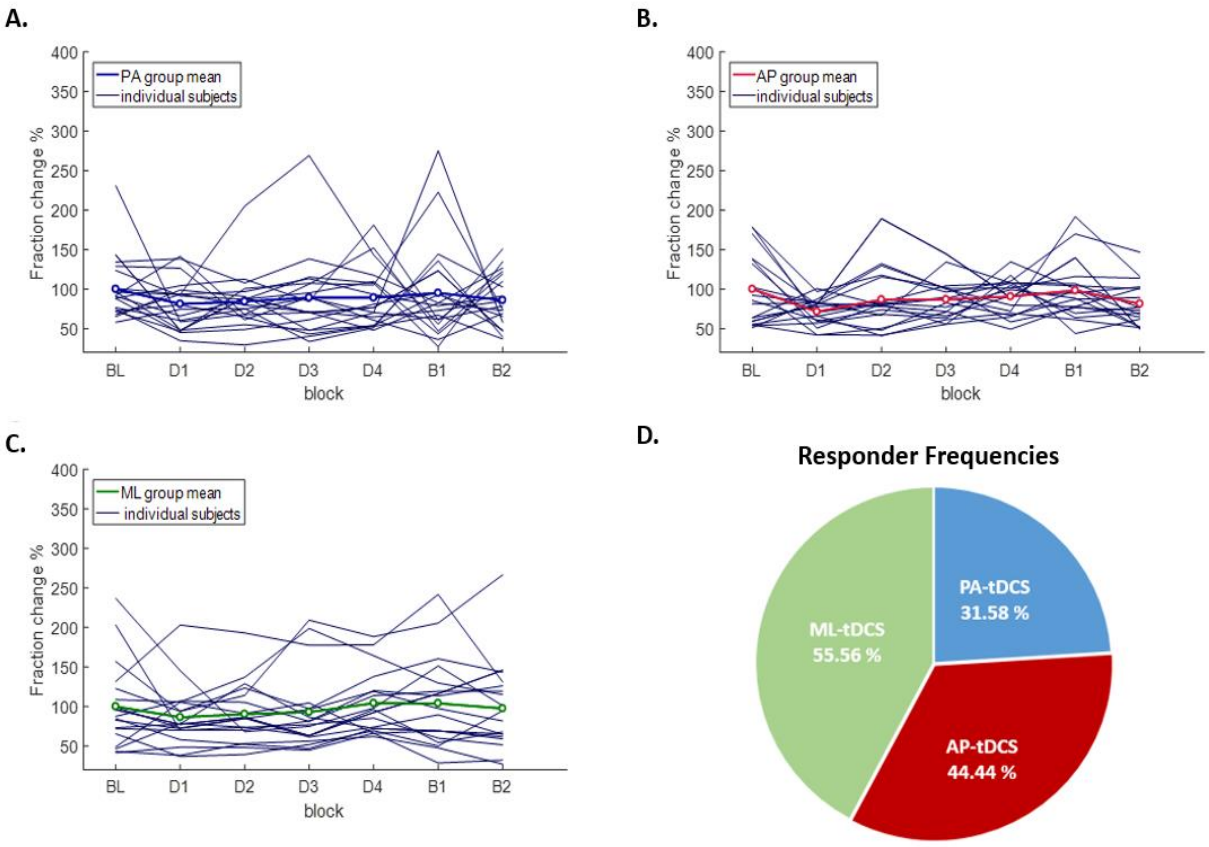

Figure 3.11 Variability of MEPs induced by different tDCS current flow directions
Mean and individual MEP responses for each current direction group (A: PA-tDCS, B: AP-tDCS, C: ML- tDCS) at all time points; Baseline (BL), During (D1-D4) and blocks 1 and 2 after tDCS (B1/B2). D shows the percentage of participants in each group that responded with a facilitatory response.

Overall, variability of tDCS effects across different directions differed only for MEPs. This difference was due to significantly higher variability of PA-tDCS MEPs than ML-tDCS MEPs. Although MEPs were less variable for ML-tDCS, grand average analysis on subgroups of responders and non-responders revealed that ML-TDCS produced the most inconsistent MEP facilitatory/inhibitory effects across groups.

3.5 Discussion

In line with previous research (Bestmann & Walsh, 2017; Rawji et al., 2018) and current flow modelling studies (Dmochowski et al., 2011; Esmaeilpour et al., 2018; Miranda et al., 2006), the effects of tDCS on corticospinal excitability depend on the current flow direction of electric fields. As the orientation of pyramidal neurons is opposite in the two banks of a sulcus (Rahman et al., 2013), directing the current across the central sulcus should affect S1 and M1 excitability differentially. I here examined this by controlling for the direction of current flow across or along the central sulcus and asked if different current flow directions impact MEPs and SEPs differentially. My main findings were: i) firstly, tDCS did not provide differential excitability after-effects when current flow was directed across rather than along the central sulcus,ii) secondly, reversing current flow direction did not reverse the direction of tDCS polarisation on MEPs/SEPs. However, based on qualitative observations, directing the current across the central sulcus (PA/AP tDCS) may affect one bank of the central sulcus more than the other iii) Lastly, the largest tDCS effects on MEPs occurred during active stimulation. Inspections of MEP excitability change during tDCS indicated mixed effects of ML tDCS and portrayed an effect of active tDCS despite direction. Indeed, subgroup analysis of responders and non-responders revealed that ML-tDCS had the most inconsistent MEP effects, despite producing significantly lower MEP variability. In sum, these results show that due to cell morphology and orientation of pyramidal neurons in the two banks of the central sulcus, directing tDCS current flow across the central sulcus (PA- and AP- tDCS) does not differentially modulate MEPs and SEPs. Additionally, reversing the direction of tDCS current flow from anterior to posterior or vice versa, does not reverse the polarisation effects, instead it may affect one bank of the sulcus more than the other as observed qualitatively. Possible explanations for these findings are discussed below.

3.5.1 Lack of differential effects of current flow direction on S1 and M1 excitability

The orientation of pyramidal neurons is approximately opposite in the two banks of a sulcus (Rahman et al., 2013). However, my findings do not validate the notion that directing the current across the central sulcus affect S1 and M1 excitability differentially. PA/AP and ML tDCS had no differential impact on MEP and SEP excitability after tDCS. This contradicts previous research that suggest that more consistent modulation of pyramidal neurons in the motor cortex occurs when current flow is directed along the principal dendritic axis of neurons in this region (Dmochowski et al., 2011; Miranda et al., 2006; Rahman et al., 2013; Rawji et al., 2018).

Contrary to previous findings (Rawji et al., 2018), I show that PA-tDCS had no inhibitory effect on corticospinal excitability. SEPs showed trends of increased excitability when MEPs decreased with PA-tDCS inducing current flow from posterior to anterior direction across the central sulcus. However, as effects were not significant, conclusions cannot be drawn that this effect occurred due to differential current flow directions. The observed trends however are in line with a previous study (Kirimoto et al., 2011) demonstrating that conventional application of tDCS, with the anode over the motor association cortex, increased SEPs and decreased MEPs. Such bidirectional modulations may be similar to SEP gating mechanisms, whereby an inhibitory SEP response occurs when M1 is activated for movement (Ogata, Okamoto, Yamasaki, Shigeto, & Tobimatsu, 2009). Nevertheless, my findings here are qualitative observations of MEP and SEP trends and did not reach significance. Similarly, several studies have not found opposite effects of MEPs and SEPs when applying conventional tDCS over the somatosensory cortex (Dieckhöfer et al., 2006; Matsunaga et al., 2004; Wolters et al., 2005). Reasons for the lack of significance in my findings despite the observed trends may be due to the high interindividual variability of tDCS effects and the effect of peripheral nerve stimulation on S1 and M1 cortico-cortical projections as described below.

3.5.1.1 Lack of significant effects due to high interindividual variability subsection

High inter-individual variability of responses to stimulation is potentially the main reason for the observed lack of significant differential tDCS effects on group level. Since variability was significantly higher for PA-tDCS than ML-tDCS, but was comparable to

that observed in previous studies (Kirimoto et al., 2011; Rawji et al., 2018), this study faces issues provoked by high inter-individual variability. As demonstrated by studies investigating individuals' responses to tDCS, effects of stimulation can be occluded on group level by high inter-individual variability; and subgroups who show effects in the expected direction ("responders") may be lost in group-level analyses (Chew et al., 2015; Laakso et al., 2019; López-Alonso et al., 2014; Strube et al., 2016; Wiethoff et al., 2014). This might be why the observed qualitative trends of differential excitability effects did not reach significance. Indeed, grand average analysis on subgroup of responders and non-responders revealed subgroups reacting differentially to tDCS. PA-tDCS had the most consistent and reliable tDCS effects as 68% of the participants responded with reduced MEP excitability. On the contrary, approximately half of the participants with AP-tDCS (45%) and ML-tDCS (55%) had inconsistent facilitatory and inhibitory MEP effects. This is in line with recent evidence that corticospinal excitability changes following tDCS indicate weak or non-significant effects at group-level which may be due to substantial inter-individual differences in the response to tDCS (Horvath et al., 2016) with responders making up 20 to 61 % of subjects (Chew et al., 2015; López-Alonso et al., 2014; Wiethoff et al., 2014). Reasons for this variability could be differences in current intensity of tDCS-induced electric fields in the brain across individuals due to anatomical differences (Bestmann, 2015; Bestmann & Walsh, 2017; Bikson, Rahman, & Datta, 2012; Datta, Truong, Minhas, Parra, & Bikson, 2012; Horvath et al., 2014; Opitz et al., 2015). For the same stimulator output intensity, electric field intensities reaching a cortical target substantially vary across individuals (Evans et al., 2019; Laakso et al., 2019, 2015). This may lead to variable tDCS effects across a cohort with fixed stimulation. As this study used a between-subjects design, the variability between the different groups may thereby be a result of variable electric field intensities reaching the cortical targets between subjects in the different current flow direction groups. As such, individualizing current intensity based on current flow models so that each individual receives comparable electric fields in a target (Bikson, Rahman, & Datta, 2012; Esmaeilpour et al., 2018; Evans et al., 2019), and using a within subjects design to test different current flow directions, is a reasonable step forward to control for interindividual variability of tDCS effects.

3.5.1.2 The impact of peripheral electrical stimulation on differential effects of S1 and M1 cortico-cortical projections

Another reason for the lack of significant effects of different tDCS directions may be due to the sub-motor threshold (sensory) intensity of the peripheral electrical stimulation (PES). PES at sub-motor threshold (sensory) intensities decrease corticomotor excitability (Schabrun, Ridding, Galea, Hodges, & Chipchase, 2012; Tsuiki et al., 2019). As M1 and S1 are connected (Aronoff et al., 2010; Kinnischtzke, 2013; Petrof, Viaene, & Sherman, 2015; Vaseghi, Zoghi, & Jaberzadeh, 2015) the net effects of tDCS and PES might be similar in S1 and M1 despite opposite actions. Cortico-cortical connections between M1 and S1 have long been evident in both animals (Brochier, Boudreau, Paré, & Smith, 1999; Hiraba, Yamaguchi, Satoh, Ishibashi, & Iwamura, 2000; Lin, Murray, & Sessle, 1993; Pavlides, Miyashita, & Asanuma, 1993) and humans (Gandolla et al., 2014; Kirimoto et al., 2011; Matsunaga et al., 2004). S1 stimulation can induce excitatory/inhibitory modulation of motor cortical synapses possibly through altered effects of intracortical interneurons (Sakamoto, Porter, & Asanuma, 1987) and similarly, stimulation of M1 can induce excitatory and inhibitory effects at multiple levels of the somatosensory system (Jiang, Chapman, & Lamarre, 1990; S. Lee, Carvell, & Simons, 2008; Shin & Chapin, 1989; Zagha, Casale, Sachdev, McGinley, & McCormick, 2013).The connectivity mechanisms of S1-M1 and M1-S1 may underpin the co-modulation of S1 and M1 observed here. As pyramidal neurons in the two banks of the central sulcus face in opposite directions (Rahman et al., 2013), decreased MEPs as a result of PES may have increased SEPs due to the cortico-cortical projections between S1 and M1 (Kaneko, Caria, & Asanuma, 1994a, 1994b) which supports previous findings (Kirimoto et al., 2011) of differential excitability of S1 and M1. As such, this observed effect may have been due to PES and not different tDCS directions. To further clarify this mechanism, future studies should seek to examine intracortical inhibitory and facilitatory networks in response to PES at various stimulus intensities and examine the correlation between M1 and S1 changes. For alternative use of this study's design to avoid impact of PES on M1 one can obtain sensory and motor measures separated in sessions or time so that PES can be applied at above motor threshold intensities, after tDCS delivery and then compare to the combined effects. This would prevent measuring net effects of CSE or

S1-M1 and M1-S1 cortico-cortical interactions (see section below) as a result of combined protocols, whilst quantifying the difference.

3.5.2 TDCS direction may impact the central sulcus banks differentially

In this study, reversing the direction of current did not reverse the polarisation of SEPs and MEPs. A possible explanation could be the impact of PES (here MNS) on M1 and S1 cortico-cortical connectivity (Schabrun et al., 2012) as described above. More specifically, as MNS was used in all conditions, and thus may have decreased M1 excitability and via cortico-cortical connections increased S1 excitability, it may explain the reason that reversing the direction of current did not reverse the polarisation of SEPs and MEPs.

Instead of reversed polarisation effects as expected, reversing the direction of current flow through the central sulcus from PA to AP may have impacted one bank of the central sulcus more than the other. Here, results indicated that PA-tDCS induced the largest SEP difference across all groups, increasing the SEP N20 by ~20% and decreasing MEPs by 14%. On the contrary AP-tDCS induced the largest MEP difference across all groups, decreasing MEPs by 19%, whereas SEPs had no substantial change (marginal increase by ~5%). As PA tDCS had a larger effect on SEPs than AP-tDCS and on the contrary, AP-tDCS had a larger effect on MEPs than PA-tDCS, this may imply that PA-tDCS may affect the post-central bank of the central sulcus (S1) more than the pre-central bank (M1), and AP-tDCS current flow may affect the precentral bank (M1) of the central sulcus more than the post-central bank (S1). A possible explanation for the observed effects could be that PA-tDCS increases M1-S1 impact whereas AP-tDCS increases S1-M1 impact. Indeed, as aforementioned (see section 3.4.1.2) there is a strong evidence suggesting S1-M1 and M1-S1 co-modulation.

Nevertheless, the magnitude and direction of this action, ie amount of M1 excitability change and subsequent impact on S1 and vice versa has not yet been investigated. Doing so may prove challenging; Firstly, there are a lot of factors that may impact S1-M1 and M1-S1 connections such as widespread tDCS currents affecting untargeted cortical areas (Datta et al., 2012; Evans et al., 2019; Mikkonen et al., 2020). As M1 receives direct and

indirect inputs from multiple cortical and thalamic areas, including premotor, executive, and sensory areas (Asanuma & Hunsperger, 1975; Cicirata, Angaut, Cioni, Serapide, & Papale, 1986; Horne & Tracey, 1979; Muakkassa & Strick, 1979; Porter & White, 1983; Reep, Goodwin, & Corwin, 1990), widespread tDCS current may impact such areas and further complicate co-modulation of S1 and M1. Net effects observed at the MEP and SEP level therefore do not reflect the direct impact of tDCS on S1 and M1 excitability. Secondly, S1 is interconnected with other primary sensory cortices including visual (V1) and auditory (A1) areas (Borich, Brodie, Gray, Ionta, & Boyd, 2015). Multisensory integration processes occur via cortico-cortical projections between S1 and V1 (Cappe & Barone, 2005; Ro, Ellmore, & Beauchamp, 2013) and through S1 modulation in response to external stimulation (Liang, Mouraux, Hu, & Iannetti, 2013) such as acoustic (Murray et al., 2005) and visual (Meyer, Kaplan, Essex, Damasio, & Damasio, 2011) information. Further subcortico-cortical connections relay information on various sensory modalities to non-matching primary sensory areas (Henschke, Noesselt, Scheich, & Budinger, 2015). As such, experimental designs should consider the impact of visual and auditory stimuli when assessing somatosensory and sensorimotor outputs in response to tDCS and design the study so that such stimuli are absent. For example, such studies should use noise cancelling earbuds to avoid noise impact on outcomes caused by the TMS 'click' sound which can reach 140 dB (Peterchev, Murphy, & Goetz, 2015).

Furthermore, with the lack of studies modelling current flow through the brain across different montages and investigating how this impacts neural circuits (Bestmann, 2015), it is impossible to predict the relationship of tDCS currents on cortical and cortico-cortical interactions (Bikson & Rahman, 2013). Moving forward, future studies utilising current flow models (Huang et al., 2019) to optimise the delivery of current flow direction within M1 and S1 whilst assessing effects on sensorimotor physiology and controlling for other possible influencing factors such as visual and auditory stimuli could provide novel insight into the impact of current flow direction on S1-M1 interactions. Additionally, and as aforementioned in the previous section, one can obtain sensory and motor measures separated in sessions or time.

3.5.3 Immediate effects of corticospinal excitability during tDCS

Corticospinal excitability was measured during tDCS to assess the immediate effects of tDCS. This study found significant decreased corticospinal excitability during tDCS, irrespective of tDCS direction. This contradicts a previous study that found no corticospinal excitability changes during tDCS application and instead reported that offline tDCS modulated CSE of subjects at rest (Santarnecchi et al., 2014). Indeed, results here also indicated post-tDCS corticospinal excitability effects specific to current direction. Immediate tDCS effects observed here could be due to the impact of PES effects during tDCS on cortico-cortical S1-M1 connectivity as aforementioned in section 3.4.1.2. Another explanation could be the combined effects of tDCS and TMS, however, previous research indicated no significant differences on CSE between combined TMS-tDCS, TMS alone, tDCS alone and tDCS with Sham TMS (Bae, Lee, & Song, 2020). Furtermore, ML-tDCS had a smaller effect during tDCS but not after, which indicates inconsistent effects (Bikson & Rahman, 2013) of ML-tDCS. This validates the notion that current flow across the central sulcus and along the axons of pyramidal neurons has long term potential-like effects on cortical excitability as opposed to stimulating across the axonal orientation of neurons in the brain (Dmochowski, Bikson, Datta, et al., 2012), however, only for the after-effects of tDCS.

Studies report immediate post-tDCS changes induced by different durations of stimulation (Nitsche et al., 2001; Nuzum, Hendy, Russell, & Teo, 2016; Woods et al., 2016), however, there is lack of evidence on the combined TMS-tDCS protocols for assessment of immediate physiological effects of tDCS on corticospinal excitability (Pozdniakov et al., 2021). TDCS effects are commonly reported offline both in motor and cognitive domains (Samaei, Ehsani, Zoghi, Hafez Yosephi, & Jaberzadeh, 2017; Wessel & Hummel, 2018) and whether tDCS exerts its effect during or right after its delivery remains a matter of debate (Schestatsky et al., 2013).

3.5.4 Limitations

As aforementioned in section 3.4.1.2, PES (at subthreshold intensity) is known to have effects on M1 excitability (Schabrun et al., 2012; Tsuiki et al., 2019). However, there is mixed evidence of the immediate effect of concurrent TMS-tDCS protocols (see section

3.4.3 for details). Thus, the combined protocol of TMS, MNS, tDCS may produce net effects that are not directly reflective of tDCS current direction effects on MEPs and SEPs. This study's design applied inter-stimulus intervals of MNS and TMS stimulation during and after tDCS application. Alternatively, one can obtain sensory and motor measures separated in sessions or time so that PES can be applied at above motor threshold intensities, after tDCS delivery and then compare to the combined effects. This would prevent measuring net effects of CSE or S1-M1 and M1-S1 cortico-cortical interactions as a result of combined protocols whilst quantifying the difference.

Furthermore, the combination of multiple neurostimulation techniques with measurement electrodes on the scalp such as TMS/tDCS and EEG here, saturated the EEG signal during tDCS application, and thus SEPs were not calculated during tDCS. The TEMP used in this study (see section 3.2.4) prevented the TMS coil from coming in to contact with the EEG electrodes that could have impacted the EEG signal, however, the electric current delivered by tDCS produced excessive noise and saturated the EEG signal during tDCS. Indeed, there are a number of challenges that can be faced with concurrent EEG and tDCS (Gebodh et al., 2019). As EEG involves recording electrical activity on the surface of the head, and tDCS involves applying electrical current on the surface of the head, these techniques can interfere with each other. One issue faced in simultaneous tDCS-EEG recordings is that the tDCS applies substantially stronger currents (in magnitude and frequency) than the signals recorded with EEG which results in saturation of an EEG recording (Accornero et al., 2014). Noise can also be introduced by the tDCS system during the EEG recording, as seen in this study. Specifically, the tDCS system contains an electronic circuit that can be the source of external noise, and this noise can be recorded in the EEG (Woods et al., 2016). To avoid the challenges presented in this study with concurrent tDCS-EEG a set of procedures to evaluate tDCS equipment/stimulation parameters in the context of the EEG system are recommended which involve recording EEG both without tDCS and during application of tES using a phantom head, thereby eliminating the influence of biological artifacts. The EEG recordings can then be compared between tDCS/no-tDCS sessions to identify unwanted artifacts or noise in the recorded signals with tDCS (Woods et al., 2016).

Another limitation of this study is the inter-individual variability of electric fields across subjects. Here, a between-subjects design was used which may have exacerbated the issue of variability between groups. As such, future studies investigating the effects of tDCS in different conditions would benefit from a within subject's design. However, as described above (see section 3.4.1.1) this does not solve the problem of inter-individual differences of electric field intensities reaching targeted cortical areas across subjects which can vary the effect of tDCS (Bestmann, 2015; Bestmann & Walsh, 2017; Bikson, Rahman, & Datta, 2012; Datta et al., 2012; Evans et al., 2019; Horvath et al., 2014; Laakso et al., 2019, 2015; Mikkonen et al., 2020; Opitz et al., 2015). The use of current flow models (Huang et al., 2019) and individualising-dose (Evans et al., 2019) are suggested ways to control for such variability (Bestmann, 2015; Bestmann & Ward, 2017; Esmaeilpour et al., 2018; Evans et al., 2019). Also, studies are advised to experimentally consider and control for factors that vary tDCS effects between subjects and sessions as much as possible such as genetics, gender, age, hormone level, time of day, parallel motor activity and attention level (Chew et al., 2015; Huang, Datta, et al., 2017; Wiethoff et al., 2014), especially when comparing effects across studies that apply tDCS in different populations (B. Krause & Kadosh, 2014; Li et al., 2015; Terranova et al., 2019).

3.5.5 Conclusion

Directing tDCS current flow across or along the central sulcus did not differentially modulate MEPs and SEPs. Based on the direction of current flow (PA/AP) across central sulcus, trends could indicated that one bank of the central sulcus may be modulated more than the other and reversing the current direction will not necessarily reverse the net polarisation effects. Instead, it may influence the impact of cortico-cortical projections of S1-M1 and M1-S1. However future research is needed to examine this alternate hypothesis. Acute effects of tDCS on corticospinal excitability show that the largest change happens at the first instance of stimulation, however incomplete evidence from combined protocols may be a contributing factor to the observed effects and should be controlled for. Future research is required in the underlying mechanisms of tDCS and S1-M1 connectivity to improve tDCS efficacy. Additional factors such as tDCS

dose and combined protocol methodology should be considered for the optimisation of tDCS application.

Chapter 4 Electric-field Variability of HD-tDCS

4.1 Abstract

Inter-individual differences in the intensity of electrical current reaching a cortical target likely contribute to variable tDCS effects. Variability in electric fields can be reduced by individualising tDCS parameters using current flow modelling, which is critical for the optimisation of tDCS in basic science and translational applications. Dose-control, however, does not minimise variability of brain-wide electrical fields. This, in principle, can be accomplished with high-definition tDCS (HD-tDCS), which delivers relatively focal stimulation in comparison to bipolar montages, however, HD-tDCS produces weaker and more variable electric fields in comparison to more widely used bipolar tDCS montages. Here, through the use of current flow models, I examine whether HD-tDCS remains focally more advantageous than a bipolar montage when delivering matching electric field intensity and variability to a cortical region.

In 50 MRI scans, I estimated electric fields in the left primary motor cortex (LM1) delivered by either posterior-anterior bipolar tDCS and HD-tDCS, and when delivered with either a fixed-dose across individuals, or an individualised-dose, using Realistic Volumetric Approach to Simulate Transcranial Electric Stimulation (ROAST).

Fixed-dose HD-tDCS consistently produced weaker (*M*: 0.128 V/m) and more variable electric field intensities (*range*: .069 - .198 V/m; ~300 % inter-subject variability) in LM1, compared to fixed-dose bipolar tDCS (*M*: .185 V/m, *range*: .125 - .249 V/m, ~100 % inter-subject variability). To deliver comparable electrical field strengths with HD-tDCS, compared to bipolar tDCS, a ~52 % increase in stimulation intensity was required. Despite this increase in stimulation intensity, and the inevitably larger spatial extent of electrical fields, HD-tDCS retains its focality advantage over bipolar tDCS. This confirms its utility for more focal applications of tDCS, but also cautions against simplistically applying it without considering the impact on electrical field intensity. Future work could explore how this impacts physiological and behavioural outcome of different stimulation protocols.

4.2 Introduction

A major part of variability in the effects of tDCS arises from inter-individual differences in the intensity and spatial distribution of the electric field reaching the brain (Bestmann, de Berker, & Bonaiuto, 2015; Bestmann & Ward, 2017; Feng et al., 2018; Laakso et al., 2019, 2015; Li et al., 2015; Price et al., 2015; Wiethoff et al., 2014). The characteristics of the induced electric field are determined by how tDCS is applied (electrode montage and stimulator output intensity) (Dmochowski et al., 2011) and inter-individual differences in anatomy, such as skull thickness (Bestmann, 2015; Bestmann & Walsh, 2017; Bikson, Rahman, & Datta, 2012; Bikson, Rahman, et al., 2009; Chew et al., 2015; Datta et al., 2009; Datta, Bikson, & Fregni, 2010; Evans et al., 2019; Horvath et al., 2015a; Opitz et al., 2015). The difficulty of standardising tDCS-induced cortical electric field changes makes it difficult to optimize stimulation protocols and compare results across studies (Bestmann & Ward, 2017; Chew et al., 2015; Evans et al., 2019; Mikkonen et al., 2020; Opitz et al., 2015).

One way of addressing variability in electric fields is with the use of current flow models. Current flow modelling can provide individualised numerical estimates of electric fields across the brain for a given tDCS montage and stimulation intensity (Bikson et al., 2015; Huang et al., 2019; Laakso et al., 2015; Opitz et al., 2016). In principle, this approach can be used to determine individual stimulation parameters that result in a desired electrical field intensity at a specific cortical location for any given electrode montage (Evans et al., 2019). However, although electrical field intensity can be controlled at a specific cortical location, there is no control over its spatial distribution (Evans et al., 2019).

Spatial distribution of the induced electrical field may be constrained by using more focal montages, e.g. HD-tDCS, which uses four electrodes positioned radially around a central electrode with opposite polarity. HD-tDCS delivers peak electric fields under the central electrode, whilst constraining current flow within the montage's radius (Alam, Bikson, & Truong, 2014; Caparelli-Daquer et al., 2012; Datta et al., 2009, 2012; Dmochowski et al., 2011; Edwards et al., 2013; Karvigh et al., 2017; Shekhawat et al., 2016). While delivering spatially more constrained current, HD-tDCS does not help the problem of variable intensity. In fact, compared to widely used bipolar electrode montages, HD-

tDCS increases electric field variability across subjects and for the same stimulator output delivers weaker electrical fields (Mikkonen et al., 2020).

The question that I address here is whether reduced variability in both intensity and spatial distribution can be achieved with HD-tDCS, through the use of current flow models. In this study, I ask the following:

(1) What are the electric field intensity and variability differences between HD-tDCS and PA-tDCS when applying a fixed-dose and individualised-dose to the LM1?

(2) What stimulator output intensity is required to deliver comparable electric field intensity to LM1 with HD-tDCS as with PA-tDCS?

(3) Does HD-tDCS, under these conditions, still produce more focal spatial distribution than PA-tDCS?

Given that HD-tDCS delivers weaker and more variable electric field in a target (Mikkonen et al., 2020), I expect that current flow modelling will allow to deliver a fixed electric field at LM1 with both HD-tDCS and PA-tDCS montages whilst requiring a substantial increase in stimulator output for HD-tDCS.

4.3 Methods

4.3.1 Structural MRIs

I used 50 structural MRI scans of healthy adults (aged 22-35, 23 males, 27 females) from the Human Connectome Project (HCP) database (https://ida.loni.usc.edu/login.jsp); courtesy of the Laboratory of Neuro Imaging and Martinos Center for Biomedical Imaging, Consortium of the Human Connectome Project (Van Essen et al., 2012). The database's images were obtained using a 3.0 T Siemens Connectome Skyra scanner with a standard 32 channel Siemens receiver head coil. The selected T1-weighted scans are identical to ones used in our previous study (Evans et al., 2019).

4.3.2 Current Flow Modelling

For details on current flow models and their use see **Chapter 1** section 1.6.6. I assessed current flow throughout the brain using the Realistic Volumetric Approach to Simulate Transcranial Electric Stimulation (ROAST) version 2.7.1 (Huang, Datta, et al., 2017). ROAST processes individual MRIs and creates a 3D model of EF distribution, based on user-defined tDCS protocols. ROAST segments 1x1x1 mm isotropic voxel size resolution scans using SPM12 (https://www.fil.ion.ucl.ac.uk/spm/) into grey matter, white matter, cerebrospinal fluid (CSF), bone, skin, and air cavities. The segmentations are corrected for any incongruities that may interfere with the model using morphological operations and simple heuristics (detailed in Huang, Datta, et al., 2017). The model then places electrodes at assigned 10-10 EEG coordinates and an Matlab-based mesh generation and processing toolbox (Iso2Mesh; Fang & Boas, 2009) is used for the computation of the Finite Element Model (FEM). The FEM model is further solved for voltage and EF distribution using a finite element solver (getDP; Dular et al., 1998). The assigned conductivity values for each tissue type are (in S/m): grey matter: 0.276; white matter: 0.126; CSF: 1.65; bone: 0.01; skin: 0.465; air: 2.5×10^{-14}; gel: 0.3; electrode: 5.9×10^{7} (Datta et al., 2009; Wagner et al., 2007).

Following current flow modelling, EF intensities and distribution were extracted by pre-processing the EF images produced by ROAST on SPM12. I spatially normalised structural

and EF images (resampled to 2 x 2 x 2mm voxels) into Montreal Neurological Institute (MNI) space and smoothed the normalised images using a Gaussian filter with a 4 x 4 x 4mm smoothing kernel. Images were smoothed to improve group-level assessment of electric fields and checked that this process did not substantially change EF values (see Appendix D; Table A1 and Figure A2). I applied an explicit binary mask computed of grey and white matter using the normalised structural images to confine analysis to voxels within the mask. See Figure 4.1 for the current flow modelling pipeline & procedure (adapted from Evans et al., 2019).

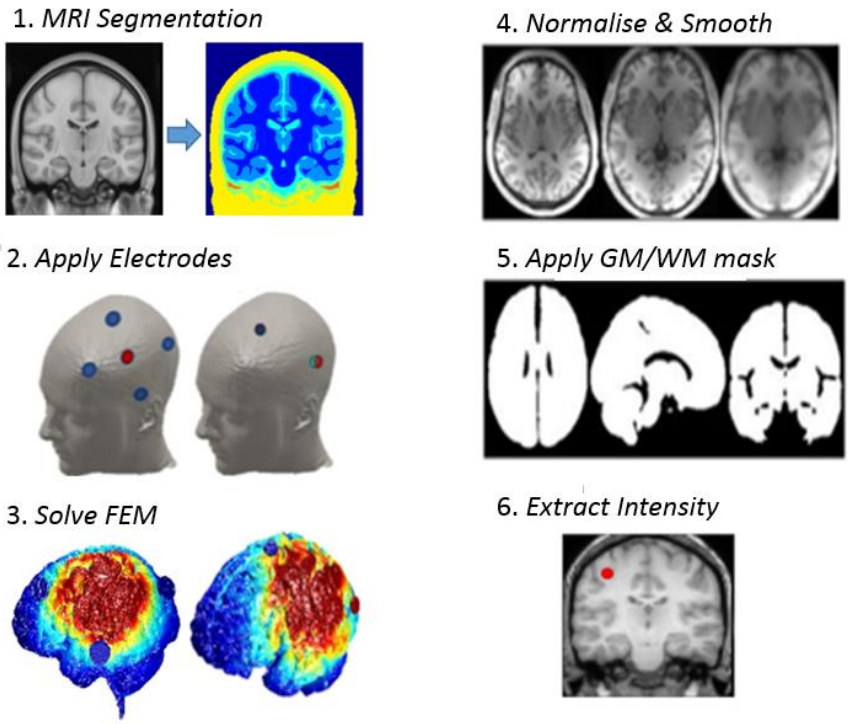

Figure 4.1. Current flow modelling pipeline
Steps 1-3 are generated by ROAST v2.7.1. Steps 4-6 are generated using SPM12. T1 weighted structural MRI is used and segmented in different tissue types (1), the pre-defined electrode montage is applied (2) and the finite element model is solved (3). SPM 12 is used to normalise and smooth the structural MRI and EF images (4), create a grey and white matter explicit mask (5) and then applied to the EF to limit analyses within the cortex (6). Step 6 shows an example of the region of interest used in this study; the LM1 (red).

4.3.3 Current flow modelling for PA-tDCS & HD-tDCS montages

Two tDCS montages with disc electrodes (6mm radius, 2mm height) were simulated (HD-tDCS and PA-tDCS). Electrode positions for both montages were selected to target

the LM1 hand region. For the bipolar montage, I used a posterior-anterior bipolar tDCS montage (PA-tDCS) that controls for the direction of current flow across primary motor cortex (i.e., traversing central sulcus in posterior-anterior direction), which recently has been shown to produce consistent corticospinal excitability changes (Hannah et al., 2019; Rawji et al., 2018). For HD-tDCS, the electrode positions were C3 (Anode), F3, P3, T7 & Cz (Cathodes), respectively, with approximately 7.5 cm ring-radius distance as typically applied (DaSilva et al., 2015). For PA-tDCS, the electrode positions were CP5 (Anode) and FC1 (Cathode). For comparable results, PA-tDCS electrode dimensions and locations were replicated from our previous study (Evans et al., 2019). This montage directs current in a posterior-anterior direction across the central sulcus and at either side of LM1 (Rawji et al., 2018).

To compare electric field intensities and variability between montages, electric fields produced by PA-tDCS and HD-tDCS were modelled and compared using fixed and individualised doses of stimulator output as explained here:

Fixed-dose ($tDCS_{fixed}$): For $tDCS_{fixed}$, the simulated output intensity was fixed at 1mA for both montages, which is commonly used in tDCS applications (Laakso et al., 2018; Nitsche & Paulus, 2000; Rawji et al., 2018; Truong, Magerowski, Blackburn, Bikson, & Alonso-Alonso, 2013).

Individualised-dose ($tDCS_{indiv}$): To individualise the dose of EFs reaching the target, the mean EF intensity reaching LM1 across subjects, produced by 1mA stimulator output, was set as the target intensity (in V/m) for the $tDCS_{indiv}$ condition. As such, for $tDCS_{indiv}$, each subject received an individualised tDCS output intensity (see formula below) which delivered the EF target intensity in LM1. The target EF intensity (i.e the group's average EF intensity produced by $tDCS_{fixed}$) for HD-$tDCS_{fixed}$ was 0.128 V/m and for PA-$tDCS_{fixed}$ was 0.185 V/m. In order to deliver this target intensity to LM1 in each individual, the stimulator output intensities were adjusted using the following formula:

$$\text{Individualised-dose } (tDCS_{indiv}) = (M1_{TI}/M1_{AI}) \times \text{Fixed-dose } (tDCS_{fixed})$$

where '$M1_{TI}$' is the target intensity of the EF in LM1 across the whole sample, '$M1_{AI}$' is the actual EF intensity when administering a fixed-dose ($tDCS_{fixed}$) of 1mA for each

individual, and 'Fixed-dose ($tDCS_{fixed}$)' is the fixed stimulator output intensity that corresponds to the 'Individualised-dose ($tDCS_{indiv}$)'.

4.3.4 Data Analysis

Current flow modelling and statistical analyses as described above were conducted using ROAST v2.7.1, and SPM12 on MATLAB version 2016b (The MathWorks, Inc., Natick, MA, USA). The alpha level was set at 0.05 and analyses were corrected for multiple comparisons using family-wise error correction (FWE).

Region of interest (ROI): In order to extract the EF values from LM1, I extracted the eigenvariates from a spherical volume of interest (VOI; 5mm radius), centered at LM1 (MNI: -38, -20, 50; (Eickhoff et al., 2009). Here, I selected the LM1 ROI as it is often targeted with tDCS in order to quantify its impact on corticospinal excitability (Di Lazzaro et al., 1999, 1998; Di Lazzaro & Rothwell, 2014). Additionally, different sized VOIs may provide differential EF averages that could affect the outcomes of this study, I thereby quantified EF intensities in a larger VOI (10mm radius; see Appendix D) to assess the difference in EF intensities between different sized VOIs. In this case, the EF was, on average, greater compared to the initial 5mm VOI, but note that numerically, this difference was miniscule (< .01 V/m for HD-$tDCS_{fixed}$; <.025 V/m for PA-$tDCS_{fixed}$) to have any substantial effect in the data. Thus, the specific method of ROI selection does not affect the qualitative conclusions of this study.

EF intensities in LM1: EF intensities in LM1 were compared between HD-$tDCS_{fixed}$ and PA-$tDCS_{fixed}$. The average EF intensity (V/m) achieved in LM1 across the sample population when PA-$tDCS_{fixed}$ was applied (.185 V/m), was used as the target intensity for PA-$tDCS_{indiv}$. Similarly, the average EF intensity achieved in LM1 when HD-$tDCS_{fixed}$ (.128 V/m) was applied, was used as the target intensity for HD-$tDCS_{indiv}$.

Stimulator output trade-off: The average EF intensity achieved in LM1 with PA-$tDCS_{fixed}$ is substantially higher than that of the HD-$tDCS_{fixed}$ montage with small electrode sizes (Mikkonen et al., 2020). In order to calculate the percentage difference in tDCS stimulator output intensity (in mA) required to deliver comparable LM1 EFs (in V/m)

with HD-tDCS$_{indiv}$ as produced with PA-tDCS$_{fixed}$, I used the average PA-tDCS$_{fixed}$ LM1 intensity as the target intensity for HD-tDCS$_{indiv}$ (.185 V/m).

Spatial distribution of EFs: In order to assess whether HD-tDCS retains its focality advantage when delivering matching EFs as PA-tDCS, I first assessed the distribution of electric fields produced with a fixed dose (1mA) for both montages. Brain areas with EFs significantly above zero were identified with t-tests, using the normalised, smoothed EF images. I show the distribution of EF intensities thresholded at 0.08 V/m. As all values above zero are displayed, a threshold was chosen for visualisation purposes of relative EFs produced by both montages in LM1. The intensity of the second to lowest average EF reaching LM1 between the two montages was 0.08 V/m, thereby this threshold provided adequate visualisation of relative EFs for both montages. Next, I compared the cortex-wide EF distribution when HD-tDCS$_{fixed}$ and PA-tDCS$_{fixed}$ montages were simulated (Figure 4.6; A). I then compared EF distribution between HD-tDCS$_{indiv}$ and PA-tDCS$_{indiv}$ montages, when LM1 target intensity was set at the average EF intensity achieved by PA-tDCS$_{fixed}$ (.185 V/m), (Figure 4.6; B). To identify EF clusters that were significantly higher in intensity when tDCS$_{indiv}$ (to .185 V/m) was applied for both montages, paired t-tests were conducted on the smooth, normalised electric field images. Paired t-tests identified brain regions where the electric field was significantly higher for one group than the other. As such 2 contrasts were compared: (1) EF clusters that were greater for HD-tDCS$_{indiv}$ (to .185 V/m) than PA-tDCS$_{indiv}$ (to .185 V/m), and (2) EF clusters that were greater for PA-tDCS$_{indiv}$ (to .185 V/m) than HD-tDCS$_{indiv}$ (to .185 V/m) (see Figure 4.5).

4.4 Results

4.4.1 EFs are weaker and more variable with HD-tDCS$_{fixed}$ compared to PA-tDCS$_{fixed}$.

EF intensities within LM1 delivered with HD-tDCS$_{fixed}$ and PA-tDCS$_{fixed}$ montages were highly variable across the 50 individuals assessed in this sample.

In LM1, HD-tDCS$_{fixed}$ produced an average EF of 0.128 V/m with a variation of nearly 300% across individuals (*range*: .069 - .198 V/m). For PA-tDCS$_{fixed}$, EFs were, on average, 0.185 V/m which constitutes 31% higher intensity than with HD-tDCS$_{fixed}$, with a variation of ~100% across individuals (*range*: .125 - .249 V/m; see Table 4.1). A paired t-test conducted in SPM showed that HD-tDCS$_{fixed}$ produced significantly higher EF only directly under the Anode electrode, relatively close to the cortical surface (T [1, 49] = 3.27, $p < .05$).

Table 4.1. Descriptive statistics for EF intensities (V/m) in LM1
EF intensities produced by HD-tDCS and PA Bipolar-tDCS montages when applying fixed-dose and individualised-dose. Stimulator output (S/O) intensities for each target are also shown.

	HD-tDCS					PA Bipolar-tDCS		
	Fixed-dose 1mA	**Target 0.128 V/m**	**S/O**	**Target 0.185 V/m**	**S/O**	**Fixed-dose 1mA**	**Target 0.185 V/m**	**S/O**
Mean	0.128	0.128	1.051	0.186	1.520	0.185	0.184	1.027
SD	0.028	0.003*	0.247	0.004*	0.357	0.033	0.003*	0.187
Min	0.069	0.121	0.647	0.176	0.936	0.125	0.176	0.750
Max	0.198	0.135	1.852	0.194	2.677	0.249	0.190	1.475

**residual variance in EF intensities caused by rounding individualised-dose and repeating current modelling*

As expected, HD-tDCS$_{indiv}$, effectively eliminated the variation of EF intensities in LM1 with target intensity of 0.128 V/m (M = .128 V/m ± .003; Table 4.1). This was also the case for PA-tDCS$_{indiv}$ with target intensity 0.185 V/m (M = .184 V/m ± .003; Table 4.1).

Figure 4.2 illustrates the EF intensities in LM1 when applying $tDCS_{fixed}$ (1 mA) and $tDCS_{indiv}$ for both montages.

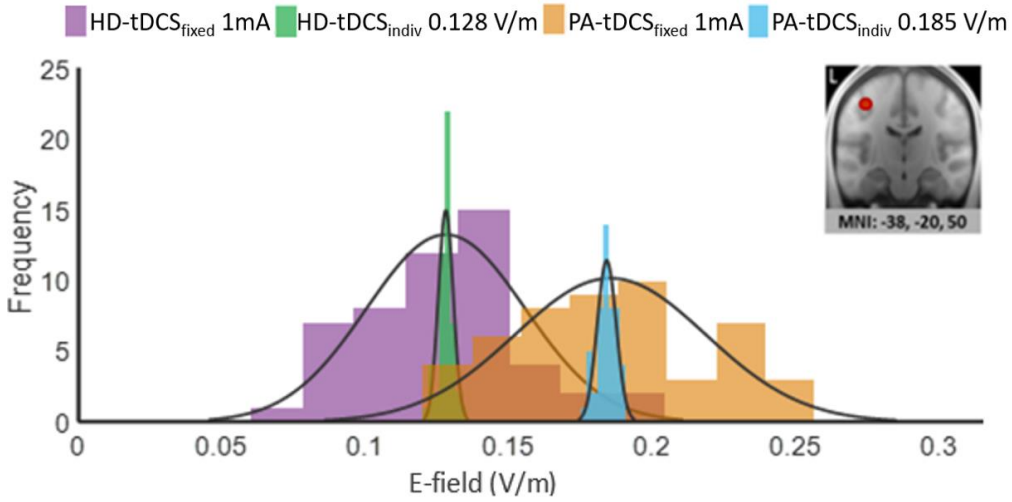

Figure 4.2. Distribution of EF intensities in LM1 with HD-tDCS and PA-tDCS montages
Data are shown for 50 participants when applying a fixed-dose of 1mA HD-tDCS (Purple; HD-$tDCS_{fixed}$ 1mA) and PA-tDCS (Orange; PA Bipolar-$tDCS_{fixed}$ 1 mA), and individualised-dose HD-tDCS to deliver a target intensity of 0.128V/m (Green; HD-$tDCS_{indiv}$ 0.128 V/m), and individualised-dose PA-tDCS to deliver a target intensity of 0.185V/m (Blue; PA-$tDCS_{indiv}$ 0.185 V/m) in LM1. Coronal section of the brain marks ROI (MNI: -38, -20, 50) in LM1 (Red).

4.4.2 HD-tDCS requires substantial increase in stimulator output to deliver comparable EFs to PA-tDCS

As outlined above, HD-$tDCS_{fixed}$ produces comparatively lower EFs (*M* = .128 V/m) in the LM1, compared to PA-$tDCS_{fixed}$ (*M* = .185 V/m). I next determined the required change in stimulator output to deliver comparable EFs to LM1 with HD-$tDCS_{indiv}$ (Figure 4.3) using the aforementioned formula (Evans et al., 2019). Thus, I individualised the stimulator output intensity for HD-$tDCS_{indiv}$ to deliver the same average EF intensity at LM1 as delivered by PA-$tDCS_{fixed}$ (i.e. .185 V/m). This required increasing stimulator output for HD-$tDCS_{indiv}$ by ~52 %, with a substantial range of stimulator intensities across individuals (*M* = 1.52 mA ± .357; *range*: .936 - 2.677 mA). Consequently, in some individuals one would need to deliver more than twice the stimulator output with HD-$tDCS_{indiv}$ in order to deliver EFs to the target are that are comparable to (on average) PA-$tDCS_{fixed}$.

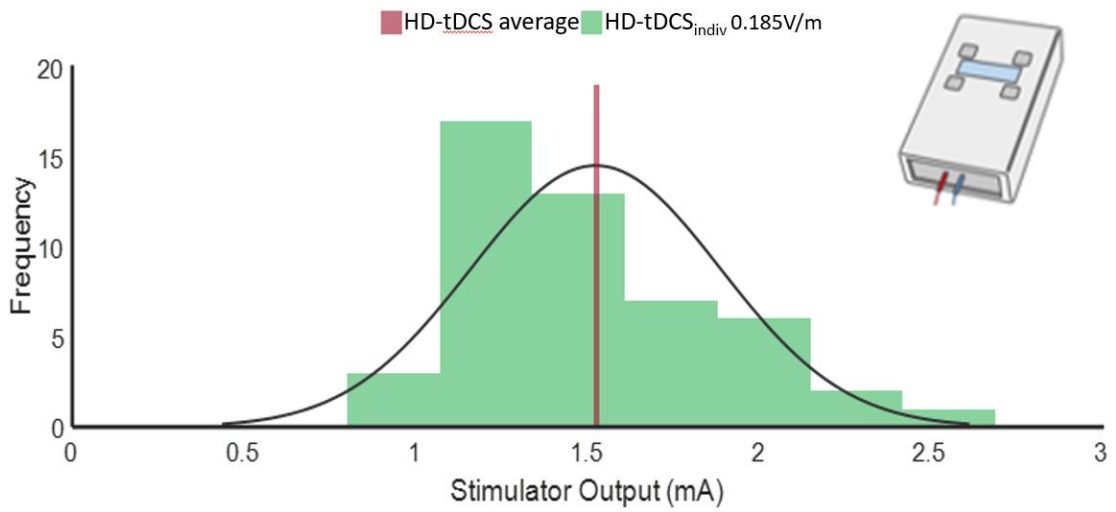

Figure 4.3. Stimulator output (mA) intensities to obtain 0.185V/m at left M1 with HD-tDCS$_{indiv}$ *Stimulator output average (Red) and variability of individual output intensities (Green) are shown for HD-tDCS$_{indiv}$ when the target EF intensity in LM1 is 0.185 V/m.*

4.4.3 HD-tDCS$_{indiv}$ maintains focality advantage whilst delivering comparable EFs as PA-tDCS$_{indiv}$.

To assess the topography of EFs across individuals, I conducted one-sample t-tests on the subjects' smoothed, normalised EF images (V/m) for both montages. Figure 4.4 shows the EF distribution for HD-tDCS$_{fixed}$ and PA-tDCS$_{fixed}$ montages. While for both montages, current is delivered to several cortical regions, EF (exceeding .08 V/m) dispersed more widely for PA-tDCS$_{fixed}$ than for HD-tDCS$_{fixed}$.

T-test results for HD-tDCS$_{fixed}$ revealed that EFs above 0.08 V/m were largely confined to parietal and pre-frontal regions, with marginal extension to temporal regions, and did not extend to the contralateral hemisphere (Figure 4.4; A). By contrast, t-test results on EF images for PA-tDCS$_{fixed}$ indicated that EF intensities above 0.08 V/m extended from parietal to frontal and temporal regions, and into subcortical structures. While EFs above this threshold were predominantly restricted to the left hemisphere, right hemisphere white matter structures also exhibited supra-threshold EFs (Figure 4.4; B). Qualitatively, I found that EFs produced by HD-tDCS$_{fixed}$ are more focal than PA-tDCS$_{fixed}$ (Figure 4.2; C).

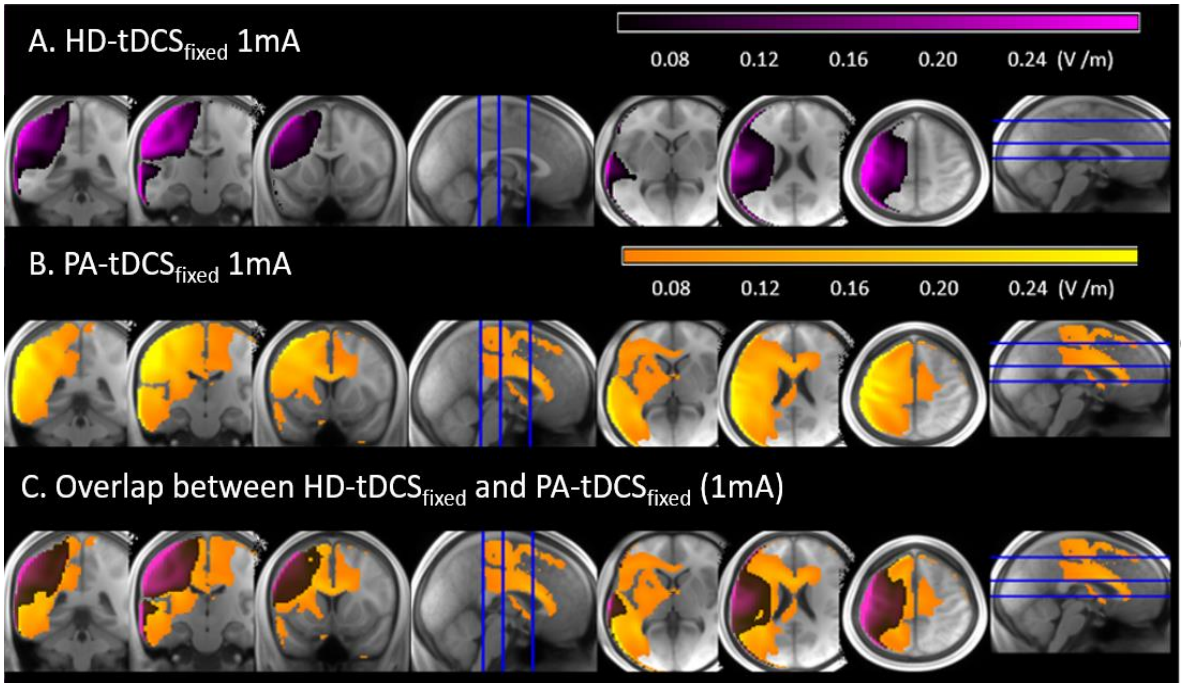

Figure 4.4. EF distribution for HD-tDCS$_{fixed}$ and PA-tDCS$_{fixed}$ (1mA) montages
EF (V/m) distribution of 50 individuals for HD-tDCS$_{fixed}$ 1mA (A; Violet) and PA-tDCS$_{fixed}$ 1mA (B; Orange) in coronal and axial orientations, respectively. C: overlap of EFs for HD-tDCS$_{fixed}$ & PA-tDCS$_{fixed}$ montages. EF threshold set at: 0.08 V/m

Next, I determined whether increasing stimulator output for HD-tDCS$_{indiv}$, so that it matches EFs for PA-tDCS$_{indiv}$, impacts on the advantage of spatial focality for HD-tDCS.

Paired t-tests in SPM showed that HD-tDCS$_{indiv}$ (to .185 V/m) produced significantly higher EF intensities in areas concentrated at LM1, reaching depth up to the body of the caudate nucleus. Prefrontal, parietal and temporal clusters were significantly higher for HD-tDCS$_{indiv}$ directly below the positions of the cathode electrodes (Figure 4.4; A). By contrast, when PA-tDCS$_{indiv}$ to the average EFs found in the population for tDCS$_{fixed}$ delivery (.185 V/m), significantly higher EFs occurred in the rest of the brain, especially the right hemisphere (T [1, 49] = 3.28, $p < .05$), see Figure 4.5; B.

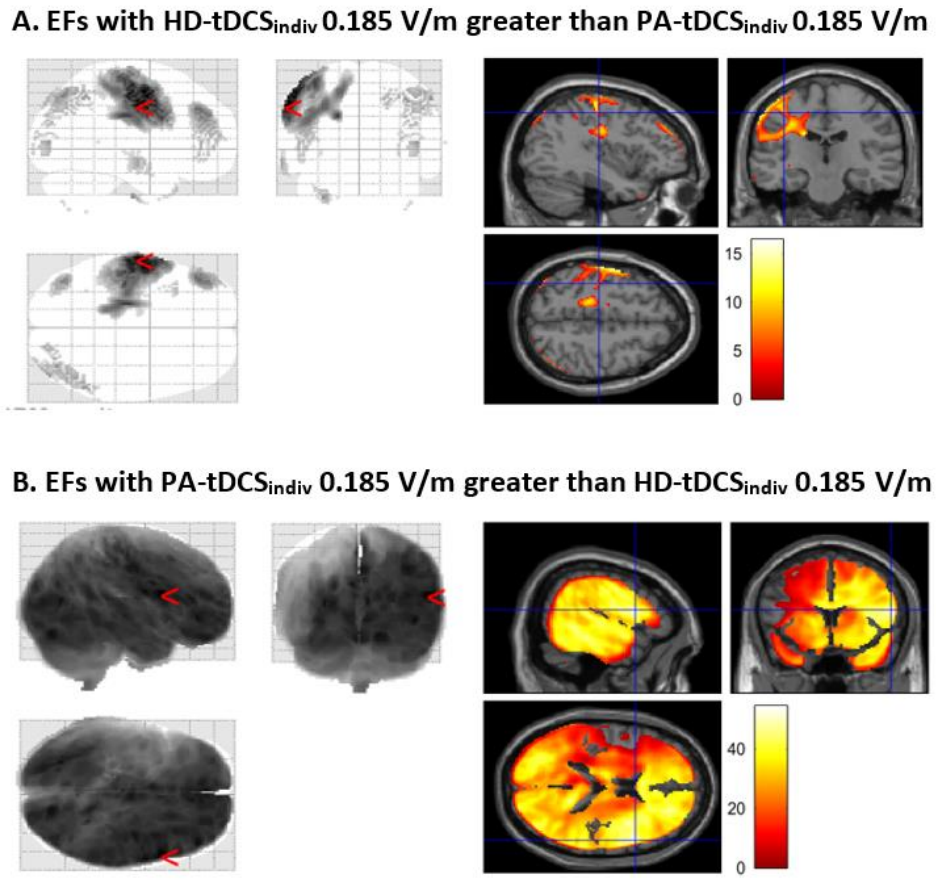

Figure 4.5. Comparison of EFs for HD-tDCS$_{indiv}$ and PA-tDCS$_{indiv}$ to 0.185 V/m
A. *Contrast 1: EF clusters that are significantly higher for HD-tDCS$_{indiv}$ vs PA-tDCS$_{indiv}$.* TDCS$_{indiv}$ was set to deliver 0.185 V/m to LM1.
B. *Contrast 2: EF clusters that are significantly higher for PA-tDCS$_{indiv}$ vs HD-tDCS$_{indiv}$. TDCS$_{indiv}$ was set to deliver 0.185 V/m to LM1.*
Red arrows and crosshairs show the location of the peak t-score. Right-hand panel: Contrast image of significant EF differences between montages. Figure legends indicate magnitude differences of electric field intensities whereby red reflects smaller differences in electric fields magnitude and yellow indicates the greatest difference in magnitude of electric fields between montages. Red arrow (Left) and crosshair (Right) point to LM1 fixed MNI coordinates: -38 -20 50.

As expected, when delivering 0.185 V/m with both HD-tDCS$_{indiv}$ and PA-tDCS$_{indiv}$, EFs above 0.08 V/m extended further than EFs produced with HD-tDCS$_{fixed}$ (Figure 4.6). EFs extended into the left superior and middle temporal lobe, external capsule and prefrontal to frontal lobes. However, and critically, compared to PA-tDCS$_{indiv}$ with the same average EF intensity in LM1 (.185 V/m), HD-tDCS$_{indiv}$ maintained its focality advantage, even though higher stimulator output intensities were needed to achieve the same EF in LM1 (Figure 4.6; B). Moreover, for HD-tDCS$_{indiv}$ with 0.185 V/m intensity,

no EFs above 0.08 V/m were observed in the right hemisphere, the inferior temporal and internal capsule of the left hemisphere (Figure 4.6; B).

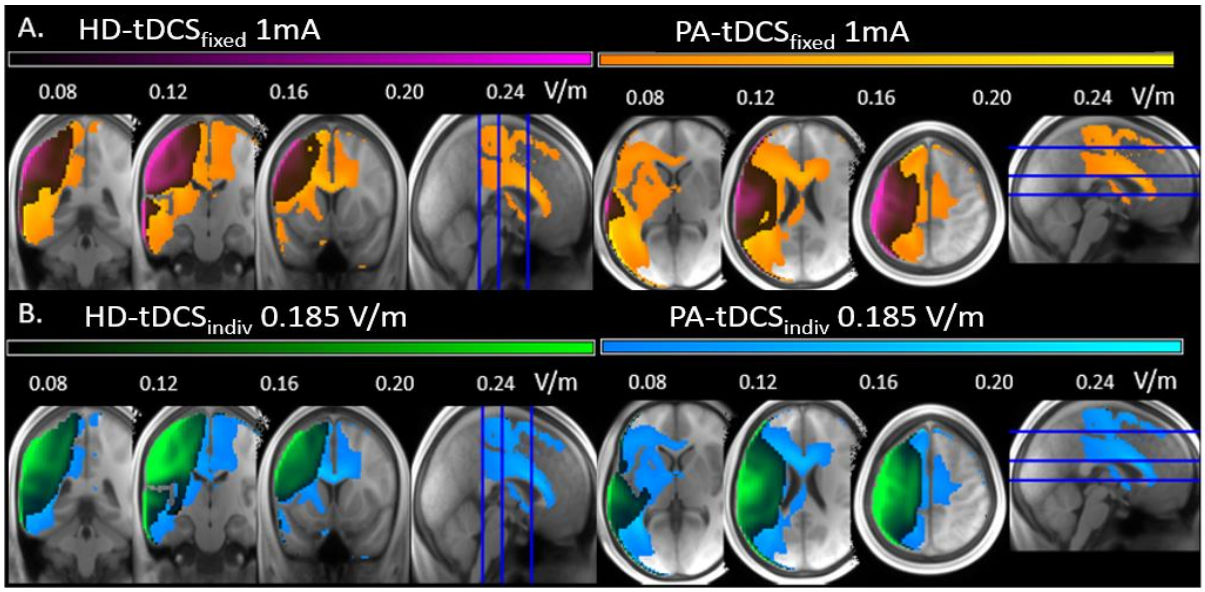

Figure 4.6. Electric field distribution overlap for fixed and individualised tDCS montages.
A. *The overlap of electric field distribution for 1mA HD-tDCS$_{fixed}$ (violet) & PA-tDCS$_{fixed}$ (orange).*
B*. Electric field overlap between HD-tDCS$_{indiv}$ to 0.185V/m (green) vs PA-tDCS$_{indiv}$ to 0.185 V/m (blue below green), for matched EFs delivered to LM1. EF threshold set at: 0.08V/m*

4.5 Discussion

Conventional methods of tDCS application produce substantial inter-individual variation in EF intensity and distribution throughout the brain (Bestmann, 2015; Bestmann & Walsh, 2017; Bikson, Rahman, & Datta, 2012; Chew et al., 2015; Datta et al., 2009; Dmochowski, Bikson, & Parra, 2012; Evans et al., 2019; Feng et al., 2018; Horvath et al., 2015a; Laakso et al., 2015; S. Wagner et al., 2014). Inter-individual EF variability in the brain likely contributes to the known variability in the effects of tDCS, limiting its efficacy and reliability (Bestmann & Ward, 2017). HD-tDCS$_{fixed}$ allows for more localised delivery of current (Caparelli-Daquer et al., 2012; Datta et al., 2012) and may thereby provide more consistent targeting across individuals, though, this comes with higher inter-subject variability of EFs (Mikkonen et al., 2020). Montage comparison effects on behavioural and physiological outcomes can only be reliably assessed when delivering comparable electric field strengths in a target. Doing so, however, would influence the brain-wide electric field distribution. Despite the increased interest in HD-tDCS, formal comparison of a tDCS$_{fixed}$ and tDCS$_{indiv}$ targeting of LM1 between HD-tDCS and other montages, under such conditions, is lacking. Addressing this is vital in the identification of an optimal montage and method for delivering reduced variability in both intensity and spatial distribution, that in turn could provide more consistent outcomes.

In a large population, I here utilised current flow models to examine whether HD-tDCS remains focally advantageous in comparison to PA-tDCS when delivering fixed electric field intensities in LM1 with both montages. Thus, I first formally assessed and compared electric field intensity and variability between these montages when applied with a fixed or individualised dose of current. I next determined the required trade-off stimulator output for HD-tDCS to deliver matching electric fields to PA-tDCS. Lastly, I assessed whether HD-tDCS retains its focality advantage when delivering comparable electric fields as PA-tDCS. I report three main findings. First, I confirm previous research findings, that HD-tDCS produces weaker and more variable electric fields than PA-tDCS (Mikkonen et al., 2020), even in a small cortical target, LM1, and current flow modelling can be used to individualise and eliminate EF variance in different montages (Evans et al., 2019). Second, HD-tDCS$_{indiv}$ requires ~52% higher stimulator output to match the EFs achieved by PA-tDCS$_{fixed}$. Third, HD-tDCS$_{indiv}$ maintained its focality advantage when

stimulator output was increased to achieve comparable EF intensities in LM1 as those seen with PA-tDCS$_{indiv}$.

4.5.1 Current flow models can address montage differences between EFs produced by HD-tDCS$_{fixed}$ compared to PA-tDCS$_{fixed}$

When applying HD-tDCS$_{fixed}$ (1mA) centrally over LM1, across the population, the EF intensities in LM1 are substantially weaker (*M* = 0.128 V/m) than with a PA-tDCS$_{fixed}$ (1 mA) montage (*M* = 0.185 V/m). This is consistent with studies that have found that weaker peak EFs are delivered to the cortical surface with radially inward currents (as with HD-tDCS) than tangential currents (as with PA-tDCS) (Datta et al., 2009, 2012; Dmochowski et al., 2011; Truong et al., 2013). The variability of EFs across individuals however is substantial (Mikkonen et al., 2020), and in the present population was around 300 %. This variance may contribute to variable neuromodulatory effects, since one individual may receive substantially lower or higher EF intensities in LM1 than another. While PA-tDCS$_{fixed}$ suffers from the same issue, it compares favourably to HD-tDCS$_{fixed}$ when using a fixed stimulator output (~100 % variance across individuals) (Evans et al., 2019; Laakso et al., 2015). Differences in inter-individual variability of EFs may occur due to slight anatomical differences, such as skull thickness, and their relation to the montage's directional currents (Evans et al., 2019; Mikkonen et al., 2020; Saturnino, Antunes, & Thielscher, 2015). Bipolar montages produce more consistent electric fields in the cortical area between the stimulating electrode positions (Hannah et al., 2019). Additionally, more focal electric fields such as those of HD-tDCS, are largely affected by individual anatomy than those of a more diffuse bipolar montage (Mikkonen et al., 2020).

If one assumes that there is a direct relationship between EF intensity reaching the LM1 and subsequent increase or decrease in physiological and behavioural excitability, HD-tDCS$_{fixed}$ delivering weaker EFs than PA-tDCS$_{fixed}$ may render direct comparison of the physiological and behavioural effects of these montages difficult. One way to address this is by increasing the radius of the 4x1 electrode arrangement (Datta et al., 2012; Edwards et al., 2013; Mikkonen et al., 2020; Saturnino et al., 2015). Datta and colleagues (2012) discuss that 2 mA HD-tDCS with 3cm ring radius electrode separation is

comparable to EFs delivered with 1 mA bipolar tDCS, whilst extending the 4x1 ring radius from 3cm to 6cm can increase the EF magnitudes to be proportional to those induced with a conventional bipolar montage (with electrodes at C3 and Fp2). However, manipulating electrode size and ring radius distance does not necessarily control for the inter-individual variance of EFs reaching a target site, and sacrifices the advantage of focality of HD-tDCS. I here controlled for the EF intensity delivered with HD-tDCS to a cortical target by individualising tDCS output intensities to obtain a target EF intensity (V/m) comparable to PA-tDCS. This effectively eliminates EF variance in LM1, as also previously shown with a bipolar montage (Evans et al., 2019), but increases the required stimulator output intensity.

4.5.2 HD-tDCS$_{indiv}$ EF distribution compares favourably to PA-tDCS$_{indiv}$ when delivering equivalent EFs in a target.

HD-tDCS$_{fixed}$ produced EFs distributed predominantly in parietal and pre-frontal regions with marginal extension to temporal regions. EFs were constrained to the left hemisphere surrounding the LM1. This is in line with previous work that HD-tDCS$_{fixed}$ delivers more localised current, constrained within the ring-radius (Datta et al., 2009; Dmochowski et al., 2011; Shekhawat et al., 2016). I here show that across a population, EFs delivered by the PA-tDCS$_{fixed}$ montage spread further than with HD-tDCS$_{fixed}$, including temporal regions and even contralateral regions. The wider EF distribution for bipolar tDCS montages is expected, but highlights that EFs can potentially reach physiologically effective values even in remote, subcortical structures (Bikson, Rahman, & Datta, 2012; Dmochowski, Bikson, & Parra, 2012; S. Wagner et al., 2014), and that HD-tDCS$_{fixed}$ benefits from delivering more focal stimulation that minimizes such remote effects (Datta et al., 2009; Dmochowski et al., 2011).

To deliver comparable EFs for both montages, increasing stimulator output intensities by ~52% for HD-tDCS$_{indiv}$ is required. This, however, will also increase the area of cortex to which EFs above a specific threshold are delivered, and thus reduces the effective focality of HD-tDCS$_{fixed}$ (Evans et al., 2019). My results suggest that with increased stimulator output for HD-tDCS$_{indiv}$, EFs extend into the left superior and middle temporal

lobe, external capsule and prefrontal to frontal lobes, but overall, still compare favourably to PA-tDCS$_{indiv}$ in terms of their spatial spread.

While HD-tDCS$_{indiv}$ may deliver more focal EFs compared to PA-tDCS$_{indiv}$, additional parameters including the direction of current flow, spatial distribution and the neuronal gradient of polarisation across the cortical surface also contribute to the net physiological impact of stimulation (Jackson et al., 2016; Rawji et al., 2018). Selection of electrode sizes, shape, and surface area distance significantly alter EF depth and distribution (Mikkonen et al., 2020; Saturnino et al., 2015) and could also potentially be explored as a way of optimising comparable EFs in a target across different montages. Additional impact of underlying brain-state (Li et al., 2019), its changes through ageing (Ciechanski, Carlson, Yu, & Kirton, 2018) or pathologies such stroke (Datta et al., 2011) further complicate the mapping between delivered tDCS dose and the physiological consequences of this stimulation.

In this work, I show that EFs in a cortical target, while hugely variable, can be controlled for by using current flow modelling. Models of current flow as a method to address the variability of tDCS effects are on the rise (Reinkensmeyer et al., 2016), but, whether this is sufficient for optimised tDCS delivery and rehabilitation is the subject of future studies.

Recently, studies have indicated that current flow models can predict outcomes responses to tDCS (Caulfield et al., 2020; Kasten, Duecker, Meiser, & Herrmann, 2019; Laakso et al., 2019), but there remains a dearth of such validation (Bestmann & Ward, 2017). Paired with the use of current flow models to identify the optimal montage for targeting a specific cortical region (Dmochowski et al., 2011, 2013; Huang, Thomas, Datta, & Parra, 2018), the control of tDCS dose may help to boost the efficacy and reproducibility of tDCS. As such, to determine whether current flow models are indeed useful, translation to increased physiological and behavioural consistency outcomes is required. Given, that the intensity of stimulation affects the extent of membrane polarisation (Batsikadze et al., 2013; Wiethoff et al., 2014), one would expect that controlled delivery of electric field intensity and distribution to a cortical region, would in effect reduce the inter-subject variance in the effects of tDCS. This could in turn make tDCS effects more reliable which is crucial if tDCS is to be used for therapeutic purposes.

4.6 Conclusion

HD-tDCS provides more focal stimulation compared to PA bipolar tDCS, but, given the same stimulator output, the EF intensities reaching LM1 are weaker and vary excessively across a population compared to PA-tDCS. With the use of the individualised-dose pipeline and current flow models, one can eliminate variance and assess the individualised-dose to apply comparable EFs in a target, across a population and across differential montages. By individualising stimulator output to make EFs comparable in a target across both montages, I reveal that HD-tDCS sustains its focality advantage over PA-tDCS at a two-fold trade-off of increased stimulator output intensities. Future research investigating the physiological and behavioural effects of individualised-dose tDCS is vital to determine the utility of current flow models for optimising tDCS application.

Chapter 5 Physiological variability of dose-controlled tDCS

5.1 Abstract

To assess whether current flow models and dose-control are useful, demonstration that they translate in reduced variability of tDCS effects on physiology is required. Here I examine whether individualising tDCS output (dose), reduces inter-individual variability of the response to tDCS. Two experimental studies addressing this question were conducted separately with differential tDCS montages. **Experiment 5.1** examined the effects of 20 minutes of fixed-dose (1 mA) and individualised-dose posterior-anterior tDCS (PA-tDCS) on corticospinal excitability and variability. **Experiment 5.2** examined the effects of 20 minutes fixed-dose (1 mA), individualised-dose and sham high-definition tDCS (HD-tDCS) on corticospinal excitability and variability. TMS-evoked MEPs were used as a measure of corticospinal excitability and their change in mean absolute deviation from baseline was used as a measure of variability. Neuronavigation was used to control for coil movements and their impact on MEPs. Neither fixed- nor individualised-dose tDCS significantly changed corticospinal excitability. In **Experiment 5.1**, individualised-dose PA-tDCS did not significantly reduce inter-individual variability of responses compared to fixed-dose tDCS. On the contrary, individualised-dose HD-tDCS, increased the variability of tDCS responses (**Experiment 5.2**). Analysis of condition-based responders and non-responders indicated that individualised-dose PA-tDCS reduced corticospinal variability in comparison to fixed-dose tDCS, exclusively for subjects that were responders in both conditions (**Experiment 5.1**), however a larger study would need to validate this on a group-level. Controlling the electric field intensity with the method adopted in this study is therefore not sufficient for improving reliability of tDCS effects. Responders losing their effect due to differences in dose when individualising and montage differences may be potential reasons for the insignificant effects. The findings highlight the need to optimise the criteria for dose-controlled tDCS by considering factors such as functional targeting of cortical locations and longer post-stimulation periods whilst controlling for other possible sources of variability.

5.2 Introduction

TDCS effects often show low reliability and efficacy, with several studies failing to replicate expected outcomes (Galli et al., 2019; Hordacre et al., 2017; Horvath et al., 2015b, 2016; Tremblay et al., 2016). This calls into question the utility of tDCS as a research tool and limits its therapeutic potential (Bestmann & Walsh, 2017; Terranova et al., 2019).

Current flow models are valuable for predicting electric current flow and magnitude through the brain and individualising tDCS dose, however, are they useful? In order to demonstrate the importance of current flow models, we need to show that controlling for the effective electric field dose reaching a cortical target (Evans et al., 2019; Mikkonen et al., 2020) can lead to a reduction in variance of tDCS effects.

Current flow modelling helps guide tDCS protocols to deliver less variable tDCS current to a cortical target, interindividually (Evans et al., 2019). Modelling current flow in 50 individuals revealed that electric fields induced by individualised stimulator output reach the desired intensity and are consistent at the cortical target site (Evans et al., 2019). However, it is still unknown whether delivering less variable tDCS current leads to improved effect sizes and reduced inter-subject variability in physiological outcomes. Making that connection is an important step forward to optimise tDCS application and increase its adoptability in a therapeutic setting.

It seems logical that the stimulation of a system as complex as the brain does not lead to simple results at the physiological level (Laakso et al., 2018). One study investigated whether the EF was related to the after-effects of tDCS (Laakso et al., 2019). By first calculating the electric fields and then applying 1 mA conventional tDCS with the anode over the target for 20 min on the right M1, they demonstrated that a large part of inter-individual variability in tDCS-induced MEPs are due to differences in the electric fields and suggested that electric field dosimetry could be useful for controlling the neuroplastic effects of tDCS.

Verification of electric field magnitude and focality has been linked to evoked muscle responses (Edwards et al., 2013). So far, however, no studies have validated whether

controlling electric field variability with the use of current flow models, indeed translates to reduced inter-individual variability of corticospinal excitability responses (Bestmann & Walsh, 2017). Such demonstration would bring a number of advantages. If individualising tDCS proves to be effective, it will make tDCS effects more reliable across individuals, which is imperative if tDCS is to be used for therapeutic purposes. Furthermore, reduced inter-individual variability will also improve the efficacy of tDCS on a group level, making it more useful as a research tool. Eliminating variance in effective tDCS dose in a target will allow the identification of other variables that determine an individual's response to tDCS, such as genetics, or attention level (Bestmann & Ward, 2017). Moreover, controlling for the dose of tDCS in a target may provide insight to the indetermined dose-response relationship that can further inform decisions concerning stimulation protocols for clinical applications (Bestmann & Walsh, 2017). However, as corticospinal excitability modulation differs between different montage types (Kuo et al., 2013), individualising dose for different montage types may influence tDCS effect sizes and variability. Thus, complicating montage selection.

Conventional tDCS montages deliver a wide-spread distribution of electric field in comparison to more focal montages (Alam et al., 2014; Bortoletto et al., 2016; Edwards et al., 2013; Morya et al., 2019). On the other hand, as also shown in **Chapter 4**, more focal montages produce weaker and more varying electric fields than conventional montages (Mikkonen et al., 2020). Possible differences in interindividual variability and effect of stimulation, in corticospinal excitability, between the different montage types when individualising dose, have not yet been fully addressed. Thus, one needs to consider the dose-response relationship between the electric field reaching a cortical target site and subsequent changes in variance of corticospinal excitability for varying montages.

The aim of experimental **Chapter 5** is therefore to test if individualising tDCS dose with the use of current flow models can reduce the inter-individual variability of tDCS effects for two different montages targeting the left M1. To this end, I investigated the effects of 20-minutes of tDCS fixed-dose, individualised-dose (**Experiment 5.1**) and sham (sham tDCS was only used in **Experiment 5.2**) on corticospinal excitability (measured with TMS-

evoked MEPs) using a posterior-anterior tDCS montage (**Experiment 5.1**) and an HD-tDCS montage (**Experiment 5.2**). Additionally, TMS coil positioning was monitored with a neuronavigation system to ensure MEPs are consistently evoked from the same cortical location and ensure that any variability induced by TMS coil position is accounted for. Based on previous studies using the PA-tDCS montage (Rawji et al., 2018), corticospinal excitability is expected to decrease with fixed- and individualised- dose tDCS (**Experiment 5.1**). On the contrary, based on previous studies using HD-tDCS montages (Caparelli-Daquer et al., 2012; Kuo et al., 2013), corticospinal excitability is expected to increase following active (fixed- or individualised- dose) compared to sham tDCS (**Experiment 5.2**). Importantly, individualising tDCS dose at a target is expected to reduce inter-individual variability of tDCS effects, evident by less variable corticospinal excitability changes following individualised-dose tDCS than fixed-dose tDCS.

5.3 Methods

5.3.1 Participants:

25 (**Experiment 5.1**) and 16 (**Experiment 5.2**) subjects were recruited from the UCL Psychology subject pools and reimbursed £10 per hour. Following preliminary outlier data analysis, 22 (**Experiment 5.1**) and 14 (**Experiment 5.2**) participants' data was used. Subjects were required to have undergone an MRI from the Wellcome Trust Centre for Neuroimaging at UCL and provide a T1 structural scan. All subjects completed a safety screening for transcranial electric stimulation (Appendix; B) and gave informed consent for the study (Appendix; E), which was approved by the UCL Research Ethics Committee (approval ID: 12011/001).

5.3.2 Experimental design:

Two experiments were conducted independently. In **Experiment 5.1**, each participant took part in two, two-hour sessions. In each session, they received either fixed- or individualised- dose tDCS. In **Experiment 5.2**, each participant took part in three two-hour sessions. In each session, they received either fixed-, individualised- dose or sham tDCS. In this experiment, the order in which participants went through tDCS conditions was fully counterbalanced and double-blinded. After the last session, blinding effectiveness was confirmed with a questionnaire (Appendix; A).

For both experiments, the stimulation protocol consisted of a baseline block of TMS (BL) to record pre-tDCS MEPs, followed by 20 minutes of tDCS during which five more blocks of TMS where administered (during-tDCS, D1-5). Immediately and five minutes after the end of tDCS, two more blocks of TMS were applied (post-tDCS, P1-2). This amounted to a 2 x 8 (**Experiment1**) and 3 x 8 (**Experiment 5.2**) 'tDCS-type' x 'time', within-subject design with the dependent variable corticospinal excitability (See figure 5.1; B, for details of the stimulation protocol). Sessions were separated by at least 48 hours in order to avoid carry-over effects of brain stimulation (Dissanayaka et al., 2017).

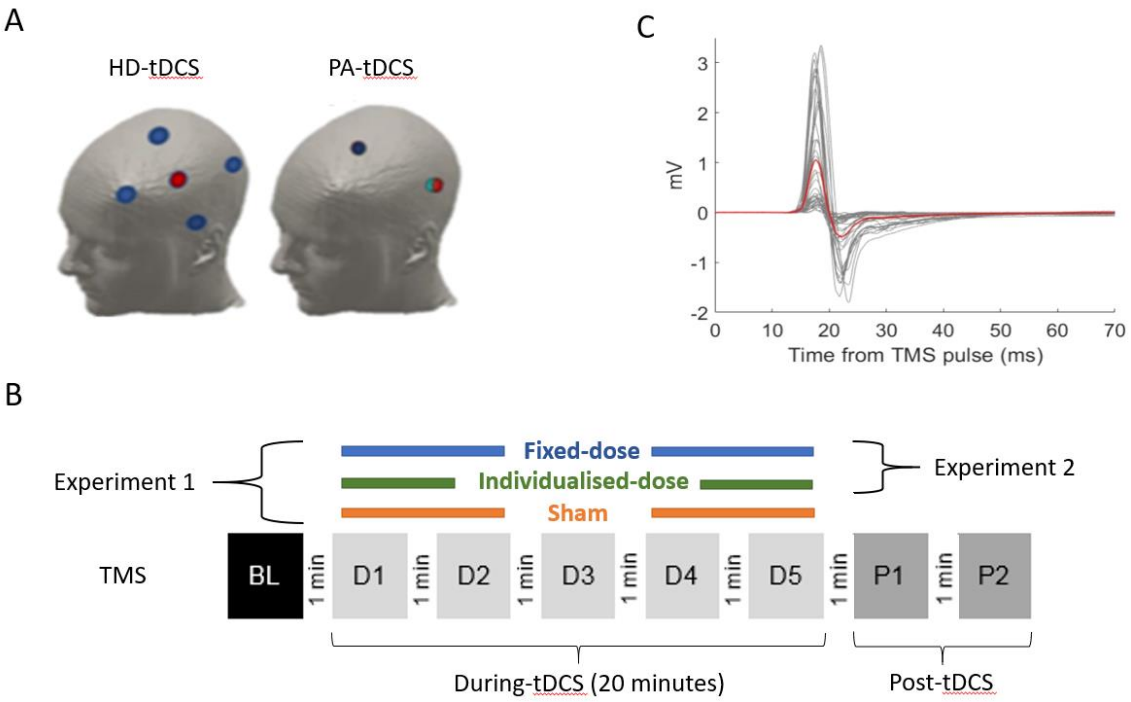

Figure 5.1. Details of the stimulation protocol
A. *4x1 HD-tDCS (left;* ***Experiment 5.1****) montage with anode over C3, and cathodes over F3, Cz, P3 and T7 and PA-tDCS (right;* ***Experiment 5.2****) montage with anode over CP5 and cathode over FC1.*
B. *Stimulation protocol consisting of a block of baseline MEPs (BL) followed by 20 min of tDCS in 2 sessions of different tDCS conditions for* ***Experiment 5.1*** *(fixed-dose and individualised-dose) and 3 sessions for* ***Experiment 5.2*** *(fixed-dose, individualised-dose and sham). During tDCS, five blocks of MEPs (D1-5) were recorded, followed by two blocks of MEPs at 0 and 5 min post-tDCS (P1-2).*
C. *Exemplary block of 37 MEPs (grey: individual MEPs; red: block average).*

5.3.3 Current flow modelling

Prior to the first testing session for each experiment, current flow modelling was performed on each participant's structural MRI scan to estimate the electric field intensity at the cortical target site (LM1) induced by 1 mA fixed-dose tDCS. Based on this intensity, each subject's individualised tDCS output intensity was then determined.

Details on estimating electric field intensity at LM1 using ROAST can be found in **Chapter 2** section 2.3.1. Briefly, ROAST (Huang et al., 2019) segments the structural MRI image into different tissue types. It then places virtual electrodes on the scalp according to user-defined 10/10 coordinates. It then generates and solves the finite element model to estimate current flow resulting from the user-defined tDCS output intensity. To

estimate electric field intensities in a target region across participants, the 3D image of electric field intensities extracted by ROAST was normalised to MNI space using SPM12 and the electric field intensity at a selected target location (here LM1 defined as a sphere of 5mm radius around coordinates [-38 -20 50] was extracted using MarsBar toolbox (Brett, Anton, et al., 2002).

Details on individualising tDCS output intensity can be found in **Chapter 2** section 2.3.2. To individualise the tDCS output intensity, first the desired electric field intensity at the cortical target site had to be determined, i.e. the mean electric field at LM1 that our fixed-intensity montage (1-mA PA-tDCS for **Experiment 5.1** and 1 mA HD-tDCS for **Experiment 5.2**) typically induces. To this end, the current flow induced by 1 mA PA-tDCS and 1mA HD-tDCS was modelled on 50 subjects from the Human Connectome Project revealing an average electric field of 0.185 V/m for PA-tDCS and 0.128 V/m for HD-tDCS at LM1. Next, for each subject, the individual tDCS intensity required to induce an electric field of 0.185 V/m (**Experiment 5.1**) and 0.128 V/m (**Experiment 5.2**) at LM1 was determined. This was done based on the electric field intensity observed in the subject's LM1 when modelling the fixed-intensity tDCS, according to a previous study's formula (Evans et al., 2019):

$$\text{Individualised-dose } (tDCS_{indiv}) = (M1_{TI}/M1_{AI}) \times \text{Fixed-dose } (tDCS_{fixed})$$

where '$M1_{TI}$' is the target intensity of the EF in LM1 across the whole sample, '$M1_{AI}$' is the actual EF intensity when administering a fixed-dose ($tDCS_{fixed}$) of 1mA for each individual, and 'Fixed-dose ($tDCS_{fixed}$)' is the fixed stimulator output intensity that corresponds to the 'Individualised-dose ($tDCS_{indiv}$)'.

5.3.4 TDCS montages

A NeuroConn DC-STIMULATOR MC (Brainbox Ltd., Cardiff, UK) was used to administer 20 minutes of tDCS, plus ramp-up and ramp-down periods of 30 seconds at 1 mA or the subject's individual intensity. For **Experiment 5.2**, sham stimulation involved ramping up and down for 30 seconds at the beginning and end of 20 minutes, with no stimulation in between. Rubber electrodes (diameter 2 cm, height 1 mm; Brainbox Ltd.) were applied to the scalp with conductive paste (Ten20; Weaver & Co., Aurora, USA). Both

montage electrode positions were replicated from my previous study (**Chapter 4**). For PA-tDCS, the anode was placed at the 10/10 location CP5 and the Cathode was placed at FC1 (Figure 5.1; A, Right). For HD-tDCS, the centre anode was placed over left M1, at C3 according to the 10/10 electrode system, surrounded by four cathodes at Cz, F3, T7 and P3 (Figure 5.1; A Left). Correct positioning of the electrodes was ensured with the neuronavigation software Brainsight® TMS Navigation (Brainbox Ltd.) to replicate the montage used with current flow models as accurate as possible (see section 5.3.6 below for details on this procedure). Mean impedance was kept below 5 kΩ in **Experiment 5.1**. For **Experiment 5.2**, mean impedance per electrode was reported at 13.58 kΩ, SD = 7.36.

5.3.5 TMS-induced MEPs

Single-pulse TMS was delivered using a figure-of-eight alpha coil at a frequency of 0.2 Hz in blocks of 37 pulses. The coil was positioned over the left M1, at the location where stimulation consistently induced 1 mA MEPs in the contralateral first dorsal interosseous (FDI) muscle. MEPs were recorded with electromyographic (EMG) electrodes. The reference was placed proximal to the interphalangeal joint and the ground on the head of the ulna bone. The electromyographic signals were amplified (1000x), band-pass filtered (5-2500 Hz; D360, Digitimer, Welwyn Garden City, UK), digitised at 5 kHz (CED Power 1401, Cambridge Electronic Design Ltd, UK) and viewed online using Signal (Version 6.0, Cambridge Electronic Design Ltd).

5.3.6 Neuronavigation

For details on the process of monitoring the position of the TMS coil using Brainsight® neuronavigation see **Chapter 2** sections 2.2.10 and 2.2.11. Correct positioning of tDCS electrodes was ensured with neuronavigation software (Brainsight®) to replicate the montage used in the individuals' current flow models as precisely as possible (see Figure 2.6).

To achieve this, before the experimental session, MRI segmented images of the subject's skin and electrodes were imported to Brainsight and the positions and dimensions of electrodes were coregistered. Next, six landmarks were selected (nasion, inion, left and

right pre-auricular, tip of the nose and philtrum (above lip) for registration of the scan to the subject's head. I then attached a tracker on the TMS coil and calibrated the coil for its exact position tracking, using the calibration block in the view of the camera (Polaris tracker). The Polaris optical position sensor used with Brainsight™ requires there is no obstruction between its line of sight and the trackers on the coil, pointer and subject. To achieve this while allowing free movement around the subject requires planning, and thus, the TMS device, chair and trackers were set-up prior to the subject's arrival. The Polaris tracker was positioned above the subject's head in a diagonal view looking down at the entire area surrounding the subject, minimizing the chances of standing between the camera and a tracker.

During the experiment, subjects wore tracking glasses and sat comfortably at a lower diagonal view of the Polaris Tracker. The subject tracker (tracking glasses) was fixed onto the subject's head with tape so it would not move during the study, nor interfere with the TMS coil or any other objects on the subject's head. I then registered the virtual pre-assigned landmarks to the subject's head, using the pointer tracker. To do this, the pointer is used to identify and sample the location of the landmarks on the subject. Following registration of the scan to the subject, the pointer was then used to trace the subject's head to assess whether registration was optimal as indicated by the distance of the pointer to registered head on the neuronavigation system. Registration was assessed when there were less than 2mm gaps between the individual's head and the pointer position. Less distance reflected more accurate scan registration. I then traced the location of the tDCS electrodes on the subject's scalp and added a marker to indicate the centre position of that electrode. This was done for all electrode positions.

Following TMS coil positioning, the targeted coil position was saved on the Brainsight system as a reference position for the entirety of the experiment and for all sessions. For each TMS pulse, Brainsight® records the displacement of the coil from the targeted stimulation site, yielding four coil errors: 1) 'Target' error, the shortest distance between the hotspot and the vector projecting into the head from the centre of the coil; 2) 'Angular' error, the tilt of the coil with respect to the target trajectory; 3) 'Twist' error, the coil's rotation within in the plane perpendicular to its target trajectory; and 4)

'Distance to Target' error, the Euclidean distance between the coil's centre and the hotspot. Figure 5.2 illustrates the neuronavigation process.

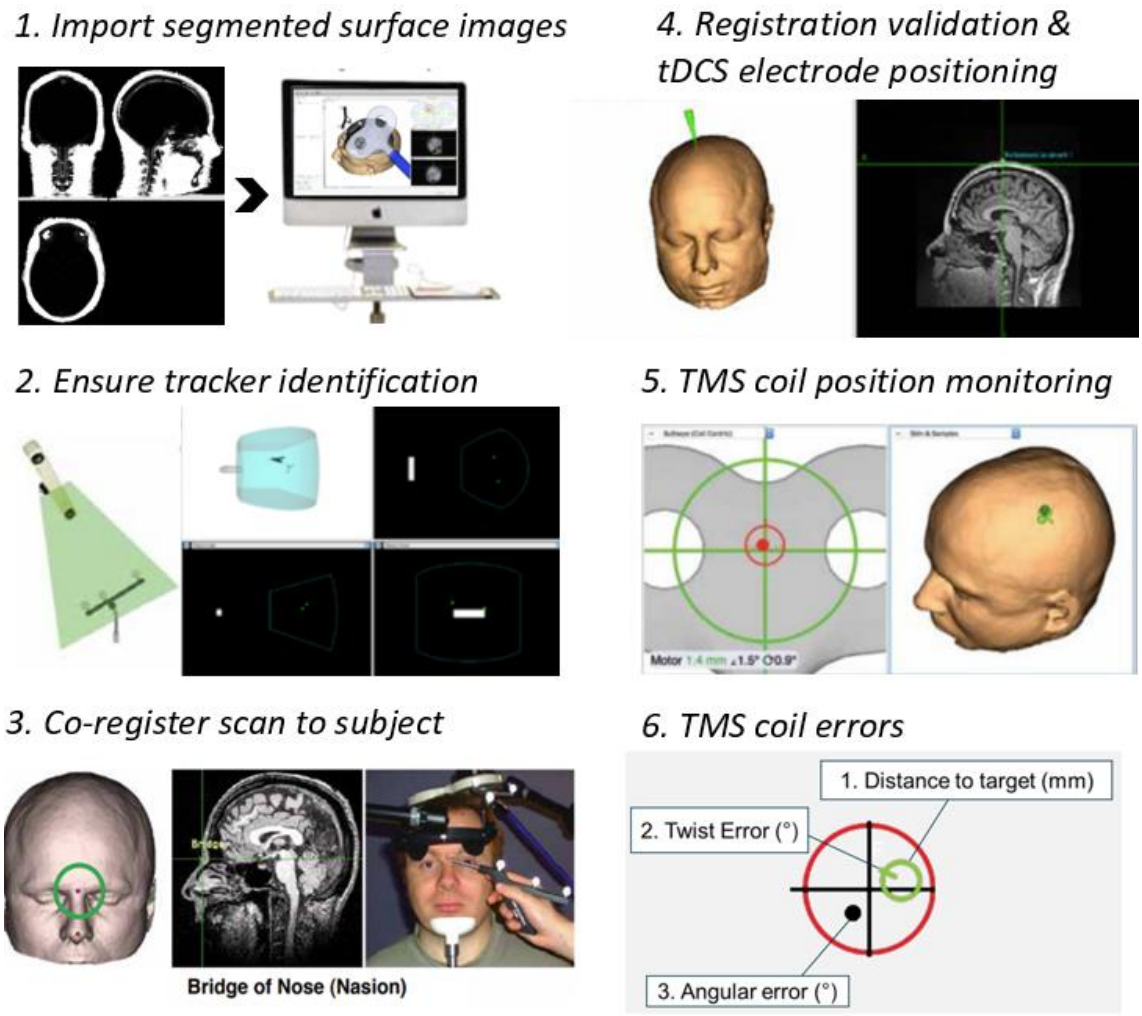

Figure 5.2. Neuronavigation procedure for placement of tDCS electrodes and tMS coil monitoring

1. Imported and coregistered segmented MRI surface images (skin and electrode images) of subject's scan to the Brainsight software. 2. The subject was then seated with the tracking glasses in a diagonal point of view under the Polaris tracker. No obstruction between the polaris line of sight and the trackers on the coil, pointer and subject was ensured in a 3-dimensional view from the tracker. 3. Imported MRI surface images were co-registered to subject's head using six pre-assigned landmarks. The image here shows an example of registering 1 landmark; the Nasion. 4. The pointer was then used to trace the subject's head to a) assess whether registration was accurate enough as indicated by less than 2mm distance between the pointer to the registered scan and b) identify the location of the tDCS electrodes on the subject head as indicated by the registered electrode scan on the neuronavigation system. A marker was added on the subject's head to indicate the centre position of each electrode. 5. The TMS hotspot was identified functionally, and the position was saved on the neuronavigation system for monitoring and consistent coil positioning. 6. Four TMS coil errors were monitored, 'Distance to Target', 'Twist', 'Angular' and 'Target' errors.

5.3.7 Data analysis

The procedure for data analysis in both experiments was as follows: MEPs were pre-processed and regressed against the four coil errors to investigate whether coil movement explained part of MEP variability. A multiple regression of mean MEPs across mean errors in each condition was used to investigate group level effects. Next, baseline differences in mean MEPs were compared using paired t-tests for **Experiment 5.1** and an analysis of variance (ANOVA) for **Experiment 5.2** to investigate any group differences of MEPs at baseline. If baseline differences were significant, further analyses included baseline as a covariate to account for baseline effects on tDCS change. Additionally, any significant differences in coil errors between conditions were also added as a covariate in further analysis to account for variance caused by coil errors.

Furthermore, to investigate tDCS-effects on corticospinal excitability change, a repeated measures ANOVA was used with factors 'tDCS-type' and 'time'. To analyse whether different tDCS conditions affected corticospinal excitability change, a RM ANOVA with factors 'tDCS-type' and 'time' was used on the absolute values of mean MEP deviation change from baseline. To investigate the relationship between the amount of electric field at the target and changes in corticospinal excitability, I regressed the individual electric field values against the mean changes in MEPs from baseline for fixed-intensity tDCS. The difference between electric field intensity reaching a target between fixed- and individualised- dose was also regressed against the difference between MEP change for fixed- and individualised- dose to understand how the change in electric field reaching a target impacted corticospinal excitability between conditions. Finally, to investigate condition-based facilitatory and inhibitory effects between responders and non-responders and how this impacts variability in each condition, I conducted a grand average analysis on condition-based responders.

5.3.7.1 Pre-processing

Electromyographic data was converted from Signal (.csf) to Matlab (.mat) format, and peak-to-peak amplitudes were assessed. Neuronavigation coil errors were imported from BrainSight text files to Matlab variables. The first 5 MEP trials of each Block were discarded (Julkunen et al., 2012). Furthermore, MEPs were discarded if the following

criteria were met: 1. Pre-stimulus (100 ms prior to the TMS pulse) muscle pre-contractions exceeded 0.1 mV; 2) MEPs were smaller than 0.1 mV; 3) MEPs were larger than the block's mean MEP + 2 SD.

5.3.7.2 Neuronavigation coil error analysis

A multiple regression of MEP amplitude against the four coil errors (Distance-to-target, target, angular, and twist errors) was performed for each subject on a trial-by-trial basis to assess whether coil errors accounted for MEP variance. To account for potential outliers, a robust regression based on iteratively reweighted least squares with a bisquare weighting function was used. One-sample t-tests were performed on the resulting β weights to determine if any of the coil errors significantly explained MEP variability on a population level. Furthermore, paired t-tests (**Experiment 5.1**) and repeated measures ANOVA (**Experiment 5.2**) was used to determine any differences between coil errors in each condition on a group-level. To account for MEP variability explained by coil errors in further analyses, any significant coil error effect was added as a covariate.

5.3.7.3 Analysis of tDCS effect and effect variability

Using SPSS (Version 25.0, IBM), a repeated measures ANOVA with factors 'tDCS-type' and 'time' was used on corticospinal excitability change from baseline to assess any condition-based differences in CSE change. The variability of tDCS effects was assessed with the absolute deviation from the blocks mean normalised to baseline. I chose absolute values of deviation change from baseline as we are interested in the overall amount of variability and how it changed for each tDCS condition. Absolute deviation provides a measure of variability that is relative to the mean, thus allowing comparisons across different data sets (Lund & Lund, 2020; Navarro, 2020). To assess whether individualising tDCS reduces the variability of its effects, a 2 x 7 RM ANOVA (**Experiment 5.1**) and a 3 x 7 RM ANOVA (**Experiment 5.2**), with factors 'tDCS-type' (**Experiment 5.1**: fixed- and individualised- dose; **Experiment 5.2**; fixed-, individualised-, and sham- dose) and 'time' (D1-5, P1-2) was conducted on the mean absolute deviation of MEP change from baseline.

5.3.7.4 Electric field at the target and CSE change

The target electric field intensity in LM1 for individualised-dose PA-TDCS was 0.185 V/m as previously predicted with a posterior-anterior montage (CP5-FC1; Evans et al., 2019). For HD-tDCS (**Experiment 5.2**), the targeted electric field intensity at LM1 was 0.128 V/m based on results from **Chapter 4**). For this study current flow modelling was conducted sequentially on subjects as they participated in the study (see limitations section 5.5.4 in discussion for further details). This could mean that the actual average electric field intensity at target LM1 differs from the expected intensity. This was tested by recalculating the average electric field across this cohort to compare to our initial target from the average of 50 MRI scans calculated in **Chapter 4**. To investigate whether electric field at LM1 was correlated to change in corticospinal excitability in each condition, I regressed electric field values at the target against corticospinal excitability change from baseline.

5.3.7.5 Responders vs non-responders

Group specific responders and non-responders were analysed to investigate effects at a sub-group level. To assess group specific responders, i.e which percentage of subjects responded with facilitatory or inhibitory responses in each group, I clustered subjects according to their individual grand average MEP change in response to the type of tDCS they received (grand average >100% indicating facilitation; <100% indicated inhibition) and a frequency spectrum was calculated (Wiethoff et al., 2014). The % of responders in each condition who had an overall reduction in variability of CSE with individualised-dose and fixed-dose tDCS was then calculated.

5.4 Results

5.4.1 Preliminary analysis

Participants reported a tingling or itching sensation at the electrode positions during tDCS, which vanished within a couple of minutes, consistent with experiences commonly described for tDCS (Turski et al., 2017). For **Experiment 5.2**, the stimulation conditions (fixed- and individualised- dose vs. sham) were guessed correctly in 68.69 % of sessions.

For both experiments, no block contained less than ten MEPs after exclusion, which yields reliable TMS measures according to Bastani and Jaberzadeh (2012). For **Experiment 5.1**, applying the exclusion criteria resulted in discarding 4.52 MEPs (*SD* = .43) on average per block for the individualised-dose group and 3.95 MEPs (*SD* = .84) on average for the fixed-dose group. Data of one participant, whose Brainsight file could not be used was excluded from the analysis, since coil errors could not be used for regression against their MEP data. Any outlier MEP values that exceeded the block's mean ± 2 SD were removed as they were considered outliers. In total, 7 of 176 mean MEP blocks were removed in the fixed-dose condition and 7 of 176 in the Individualised-Dose condition. 1 outlier participant was detected as 5 out of 8 blocks had to be removed for the fixed-dose condition due to large MEPs (> 3 mV) exceeding 2 SD of the block's mean. Another outlier participant was removed as most trials were contaminated with pre-stimulus muscle activity. From the 25 participants, removal of the above participants resulted to the usage of data from 22 subjects.

For **Experiment 5.2**, applying the exclusion criteria resulted in discarding 2.78 MEPs (*SD* = 3.43) on average per block. In total, 3 of 112 mean MEP blocks were outliers in the fixed-dose condition and 2 of 112 in the individualised-dose condition. Data of one participant, whose mean baseline MEPs exceeded 3 mV in one session, was excluded from analysis. An additional participant was further excluded as 6 out of 8 blocks were outliers in the individualised-dose condition due to large MEPs on average (> 3 mV) that exceeded the blocks mean by 2 SD. Removal of the above subjects resulted to the data usage of 14 participants.

5.4.2 The relation between neuronavigated coil movements and MEP amplitude.

A robust regression of the MEP amplitudes against the four TMS coil errors for each subject indicated that at least one of the coil errors explained a significant amount of MEP variability for most of the participants (**Experiment 5.1**; 19 of 22 subjects, **Experiment 5.2**; 12 of 14 subjects). In **Experiment 5.1**, the 'Distance to Target' and 'Twist' errors were significant in most of these cases (11 of 22 subjects), whereas in **Experiment 5.2**, the 'Distance to Target' error alone was significant in most of the cases (10 of 14 subjects). In **Experiment 5.1** this was followed by 'Angular' (10 of 22 subjects) error, whereas in **Experiment 5.2**, both 'Twist' and 'Angular' errors (6 of 14 subjects) followed. Results from both experiments indicated that 'Target' error was the least significant coil error among subjects (**Experiment 5.1**: 9 of 22 subjects; **Experiment 5.2:** 4 of 14 subjects). On a group level, in both experiments, this effect was not consistent. One-sample t-tests on the β weights of each coil error showed that, none of the β weights differed significantly from zero. This indicates that there is no consistent relation between any single coil error and MEP amplitude. In other words, MEP changes at the group level are not significantly explained by coil movement.

Assessment of coil error differences between sessions was conducted using paired sample t-tests in **Experiment 5.1** and an ANOVA for **Experiment 5.2**. In **Experiment 5.1**, paired samples t-tests on the mean coil errors between conditions revealed a significant difference of 'Distance to Target' error between sessions (T (21) = 2.242, p = .036), but none of the other errors were significantly different (p > .05). This indicates that the 'Distance to Target' error varied across the fixed-dose and individualised-dose sessions. Thus, further analyses continued with 'Distance to Target' error as a covariate. In **Experiment 5.2**, an ANOVA of the mean coil errors across groups revealed that error types were not significantly different across different sessions (F [2, 24] = .443, p = .647; Table 5.1) nor interaction effects of session x error-type (F [6, 72] = 1.243, p = .295; Table 5.1). See Figure 5.2 for distributions of each error type from an exemplary participant.

Table 5.1 Mean errors for each tDCS-type and experiment

tDCS-type	Experiment	Distance to Target	Target	Angular	Twist
Fixed-dose	1	1.13 ± 0.43	0.55 ± 0.12	1.73 ± 0.73	0.81 ± 0.33
	2	1.01 ± 0.65	0.65 ± 0.61	2.12 ± 1.33	1.00 ± 2.62
Individualised -dose	1	1.04 ± 0.35	0.56 ± 0.11	1.83 ± 0.75	0.80 ± 0.30
	2	1.47 ± 1.19	0.62 ± 0.44	1.94 ± 1.53	0.61 ± 1.03
Sham	2	1.10 ± 0.62	0.53 ± 0.12	2.00 ± 0.54	0.42 ± 0.32

All values represent mean error ± SD in mm. No significant differences between error types except for Distance to Target in ***Experiment 5.1.***

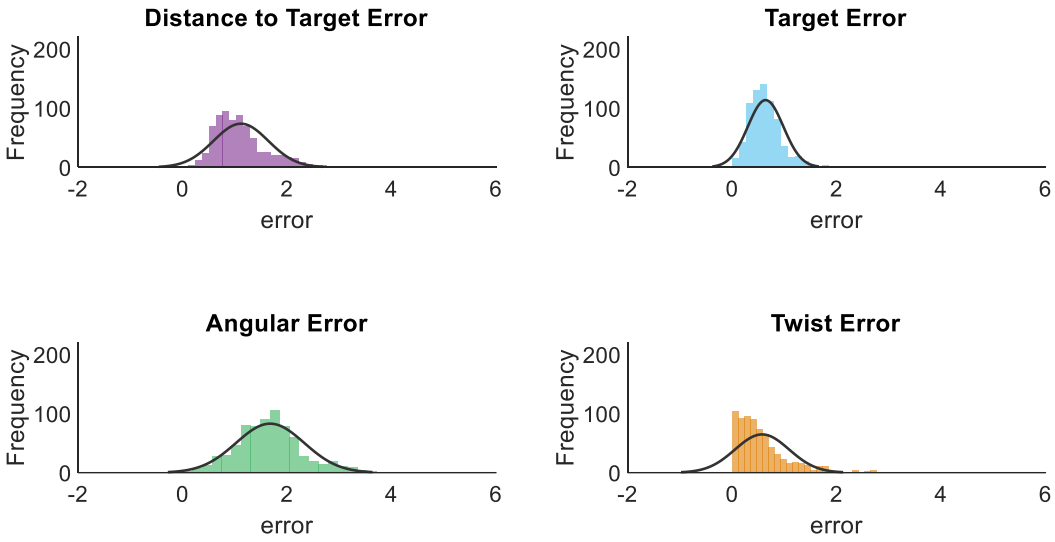

Figure 5.3. Normal distribution of coil errors from an exemplary participant
This figure shows normal distribution plots of the four coil errors ('Distance to Target' error; Purple, 'Target' error; Blue, 'Angular' error; Green, 'Twist' error; Orange) in mm from an exemplary participant. As the twist error contained negative values dependent on the direction of the coil's deviation from the target, twist coil errors were transposed to the absolute values.

5.4.3 Baseline differences

For both experiments, mean baseline MEPs were ~1 mV on average (**Experiment 5.1**, *range*: .46 – 2.12 mV; **Experiment 5.2**, *range*: .30 – 1.9 mV).

In **Experiment 5.1**, a paired sample t-test compared baseline MEP means from the same participants at different sessions (individualised- and fixed- dose tDCS). Mean baseline MEPs (T (21) = 2.352, p = .029; Table 5.2) differed between sessions, thus, to account for baseline effects on corticospinal excitability change, analysis continued with baseline as a covariate. The remaining blocks were normalised to baseline for all analyses. TMS intensities (T [21] = 1.727, p = .203; Table 5.2) did not differ between sessions.

In **Experiment 5.2,** one-way repeated measures ANOVA revealed no significant differences of baseline MEPs between sessions (F [2, 24] = .281, p = .757; Table 5.2) or TMS intensities (ANOVA, F [2, 24] = 2.16, p = .14; Table 5.2), confirming the conditions' initial comparability.

Table 5.2. Mean baseline MEP amplitudes and TMS intensities for each tDCS-type

tDCS-type	Experiment	Baseline MEP (mV)	TMS intensity (% MSO)
Fixed-dose	1	1.12 ± 0.46	55.73 ± 6.78
	2	1.11 ± 0.28	52.50 ± 8.19
Individualised-dose	1	0.84 ± 0.27	54.86 ± 7.02
	2	1.09 ± 0.41	54.14 ± 9.49
Sham	2	1.14 ± 0.43	53.36 ± 9.57

*Note. Mean baseline MEPs differed between conditions in **Experiment 5.1** (Paired t-test p < .05) thus all remaining analyses included baseline as a covariate. TMS intensities did not differ between conditions (Paired t-test p > .05). Mean baseline MEPs and TMS intensities in **Experiment 5.2** did not differ between conditions (one-way repeated measures ANOVAs, p > .05). Values presented as mean ± standard deviation. MSO: maximum stimulator output.*

5.4.4 Effects of tDCS on corticospinal excitability change

Group specific corticospinal effects were investigated to assess if there were differences between tDCS conditions. In **Experiment 5.1**, I looked at group specific corticospinal effects of individualised-dose tDCS versus fixed-dose (1 mA) tDCS whilst accounting for session differences in baseline MEPs and 'Distance to Target' error. Based on previous reports (Rawji et al., 2018) and findings from **Chapter 3,** I would expect that fixed-dose tDCS will decrease MEP amplitude. Participants' change in corticospinal excitability over time was represented by the mean MEPs normalised to baseline. Figure 5.3 (A-B) shows

the time course of excitability changes compared to baseline for the two stimulation conditions (fixed- and individualised- dose tDCS) in **Experiment 5.1**.

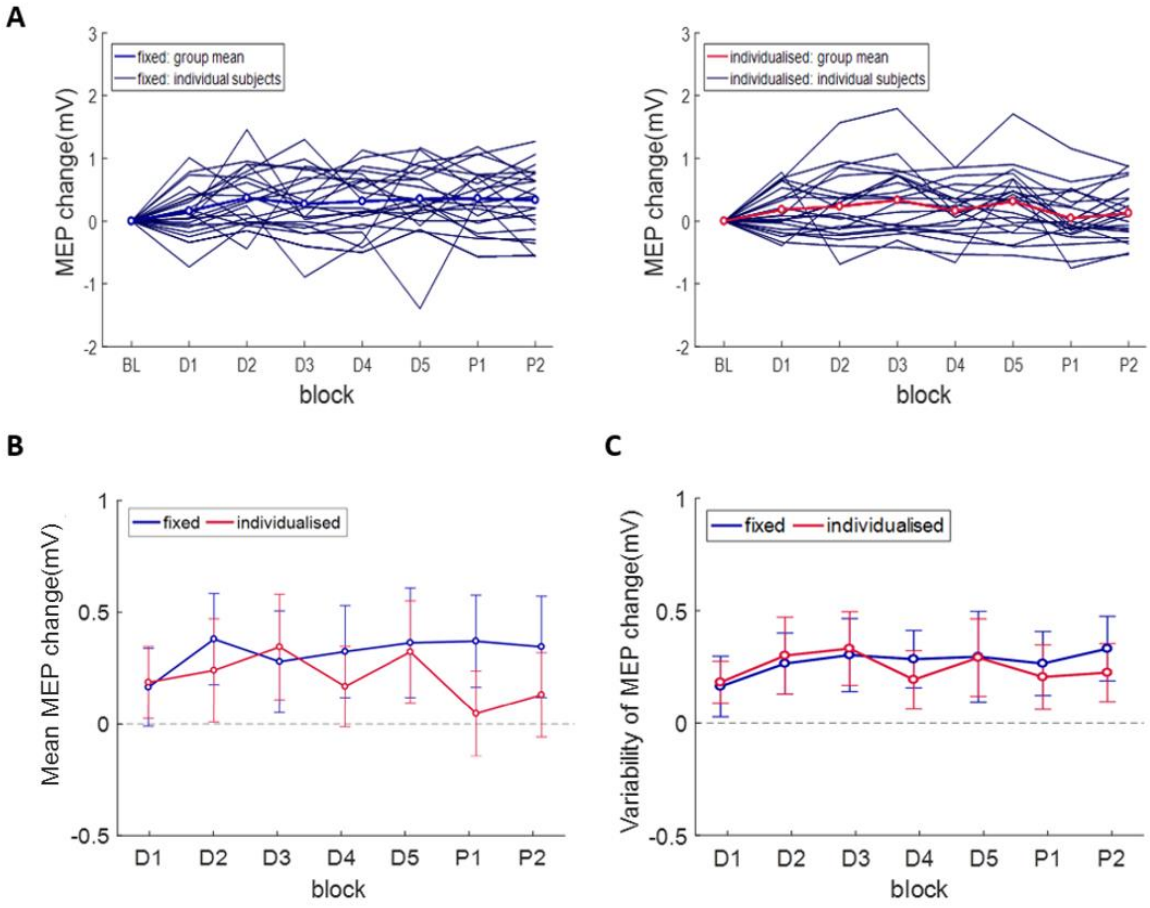

Figure 5.4. PA-tDCS mean motor excitability and variability change
A*. Mean and individual MEP responses for each condition (Fixed-dose tDCS; Left, Individualised-dose tDCS; Right) at all time points; Baseline (BL), During (D1-D4) and blocks 1 and 2 Post tDCS (P1/P2).*
B*. Mean MEP changes relative to baseline for each condition (Fixed-dose tDCS; Blue, Individualised-dose tDCS; Red).*
C*. Mean variability of MEP change relative to baseline for each condition (Fixed-dose tDCS; Blue, Individualised-dose tDCS; Red). Variability change was calculated as the absolute values of MEP deviation change from baseline. Error bars: + 2 standard error of the mean*

Following normalisation to baseline, repeated measures ANCOVA with factors (tDCS-type (2) x time (7), **Experiment 5.1**) and baseline MEP and 'Distance to Target' error covariates was conducted to assess statistically significant differences between conditions and time whilst accounting for variance cause by differences in baseline MEP

and ‘Distance to Target’ error. Sphericity could be assumed for both factors ‘time’ and interaction between ‘time’ and ‘tDCS-type’ as indicated by Mauchly’s test of sphericity (‘time’: X^2 [20] = 29.23, p = .09; ‘tDCS-type x Time’: X^2 [20] = 22.47, p = .33). Hence, I did not use any correction estimate.

The RM ANCOVA indicated no main effect of the ‘tDCS-type’ factor (F [1, 18] = .14, p = .72). Thus, mean MEP change between conditions was not significantly different (M difference: .110mV) when using individualised versus a fixed stimulator output. Furthermore, the results revealed no significant main effect of ‘time’ (F [6, 108] = .34, p = .92) nor ‘tDCS-type x time’ interaction effect (F [6, 108] = .47, p = .83), indicating that there was no significant change in corticospinal excitability from baseline between conditions when accounting for variance from ‘baseline’ MEPs and ‘Distance to Target’ error between session differences.

Fixed-dose tDCS revealed a steady increase of mean MEP change from baseline that resulted in .485 mV increase to the last block during stimulation D5 (M = .485 mV), peaking at D4 (M = .495 mV). For individualised-dose tDCS, mean MEP change shows increased excitability from baseline to the last block during tDCS D5 (M = .319 mV) and peaking at the second block during tDCS D2 (M = .335 mV). Corticospinal excitability change drastically declined following stimulation (P1; M = -.060 mV) and approached baseline (P2; M = -.012 mV). For fixed-dose tDCS, corticospinal excitability marginally declined following stimulation (P1; M = .320 mV) but sustained increased motor excitability change from baseline (P2; M = .365 mV).

Contrary to our hypothesis that PA-tDCS would decrease corticospinal excitability for PA-tDCS, our results show no significant tDCS effect on MEP change. There was a trend of increased corticospinal excitability during stimulation, which may indicate that the utmost changes in excitability occur during stimulation. Following stimulation, individualised-tDCS effects on motor excitability changed direction, dipping back to baseline immediately after stimulation. This was in antithesis with fixed-dose tDCS effects which were sustained following stimulation. However, these findings were not robust enough to reach significance.

In **Experiment 5.2**, condition-specific corticospinal effects were assessed for individualised-dose, fixed- dose (1mA), and sham tDCS. Based on previous reports (Caparelli-Daquer et al., 2012; Kuo et al., 2013), we would expect that fixed-dose tDCS will increase MEP amplitude. Participants' change in corticospinal excitability over time was represented by the mean MEPs normalised to baseline. Figure 5.4 (A-B) shows the time course of excitability changes compared to baseline for the 3 stimulation conditions.

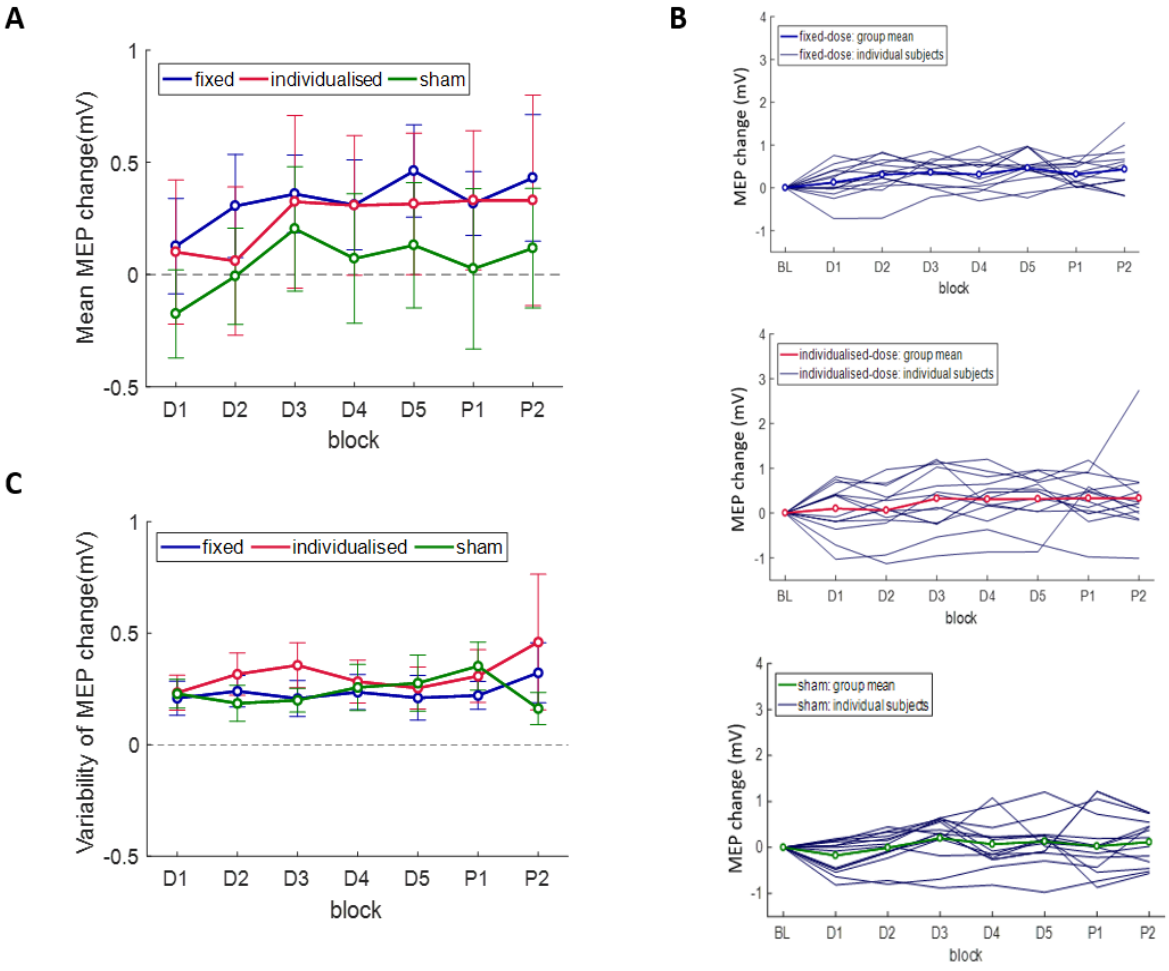

Figure 5.5. HD-tDCS Mean Motor Excitability and variability change from Baseline
***A**. Mean MEP changes relative to baseline for each condition (Fixed-dose tDCS; Blue, Individualised-dose tDCS; Red).*
***B**. Mean and individual MEP responses for each condition (Fixed-dose tDCS; Top-right, Individualised-dose tDCS; Middle-right, Sham; Bottom-right) at all time points; Baseline (BL), During (D1-D4) and blocks 1 and 2 Post tDCS (P1/P2).*
***C**. Mean variability of MEP change relative to baseline for each condition (Fixed-dose tDCS; Blue, Individualised-dose tDCS; Red, Sham; Green). Variability was calculated as the absolute values of MEP deviation change from baseline. Error bars: + 2 standard error of the mean*

Following normalisation to baseline, one-way repeated measures ANOVA with factors (tDCS-type (3) x time (7)) was conducted to assess statistically significant differences between the different conditions over time. Sphericity could not be assumed for the interaction between time and tDCS-type as indicated by Mauchly's test of sphericity ['time x tDCS-type': X^2 (77) = 142.065, p = .001], therefore, degrees of freedom were corrected according to Greenhouse-Geißer estimates of sphericity (ε = .442).

The RM ANOVA revealed no main effect of tDCS-type (F [2, 24] = 1.52, p= 0.24), indicating that CSE changes did not differ between stimulation conditions when collapsing across all time points. There was, however, a main significant effect of time (F [6, 72] = 3.10, p = .009), with a strong effect size (η^2 = .205), indicating there was a change in MEP excitability from baseline. Fixed- and individualized- dose tDCS types, had the largest mean increase of 0.33 mV and 0.25 mV, respectively. Sham-tDCS remained relatively (M = .05 mV) close to baseline. The greatest difference between groups stood between sham and fixed-dose tDCS (M difference= .278 mV) which approached significance (p = .06). Importantly however, the RM ANOVA indicated no significant interaction effect between 'tDCS-type' x 'time' (F [5.31, 63.67] = .42, p = .96), suggesting that any changes in excitability over time did not depend on tDCS type.

Overall, in **Experiment 5.2**, corticospinal excitability significantly increased over time, irrespective of stimulation condition. The increased excitability was driven by significant change in MEPs induced by fixed-dose tDCS, and near-significant increases of individualised-dose TDCS MEPs. This may indicate a main effect of active tDCS versus sham. However, this effect did not reach significance in direct comparison between sessions, thus I could not conclude that active HD-tDCS conditions indeed increased excitability in comparison to sham stimulation.

5.4.5 Variability of tDCS effects on corticospinal excitability

The hypothesis that individualising tDCS dose would reduce the inter-individual variability of responses was tested by comparing variability of corticospinal excitability changes following individualised- and fixed- dose tDCS (**Experiment 5.1)** and

individualised, fixed and sham tDCS (**Experiment 5.2)**. To measure variability, I first transformed MEP data to absolute deviation of MEPs from the blocks mean. MEP data was then normalised to baseline transforming the variable to change of MEP deviation in each condition. To analyse the effects of each tDCS condition on variability change, a RM ANOVA with factors 'tDCS-type' and 'time' was used on the variability metric.

In **Experiment 5.1**, sphericity of data could be assumed as Mauchly's test was not significant for time or tDCS-type x time factors $p > 0.05$. RM ANOVA (tDCS-type (2) x time (7)) revealed no 'tDCS-type' (F [1, 21] = .116, p = .737), 'time' (F [6, 126] = 1.235, p = .293) or 'tDCS-type x time' (F [6, 126] = .465, p = .833) interaction effects. This indicates that variability of tDCS was not different due to different tDCS conditions. Figure 5.3 (C) supports this conclusion as there seem to be no differences in variability change for both conditions.

In **Experiment 5.2**, Mauchly's test showed that sphericity could not be assumed for time (X^2 [20] = 41.51, p = .01) nor 'tDCS-type x time' interaction (X^2 [77] = 184.17, p = .01). In these cases, degrees of freedom were corrected according to Greenhouse-Geißer estimates of sphericity (ε = .45; ε = .34, respectively*)*. The ANOVA revealed significant 'tDCS-type' differences (F [2, 19] = 4.18, p = .04) with a small (Lakens, 2013) effect size (η^2 = .26) indicating that variability of tDCS effects marginally differed between conditions. Pairwise comparisons revealed that this effect was driven between reduced variability for fixed-dose tDCS (M = .235 mV) in comparison to individualised-dose tDCS (M = .315 mV) with a mean difference of -.08 mV (p = .04). Although this difference was small (see Figure 5.4; C), it was robust enough to reach significance. As opposed to my hypothesis, this result indicates that individualising-dose marginally increased variability of tDCS effects.

Furthermore, there were no significant differences between fixed-dose tDCS and sham tDCS (M difference= -.01, p = .93), nor individualised-dose tDCS and sham tDCS (M difference= .08 mV, p = .06) indicating that the use of active tDCS did not affect the variability of corticospinal excitability change. Lastly, the RM ANOVA revealed no significant differences in time (F [6, 32] = .89, p = .45) nor 'tDCS-type x time' interaction effect (F [4, 49] = 1.50, p = .22). This demonstrated that variability did not change over time and there were no tDCS-type dependent changes in variability over the various

time points. Nevertheless, a significant quadratic trend of tDCS-type (F [1, 12] = 5.55, p = .04, η^2 = .316) was present, indicating fluctuations in variability over time.

Overall, there were mixed effects across the two experiments. **Experiment 5.1** revealed no significant differences in variability between fixed-dose and individualised dose tDCS. However, and in contradiction to my hypothesis, for HD-tDCS in **Experiment 5.2**, individualised-dose tDCS increased effect variability in comparison to fixed-dose-tDCS. This effect was marginal, yet robust enough to reach significance.

5.4.6 The relationship between electric field and corticospinal excitability change

In **Experiment 5.1** stimulator output intensities were individualised for the individualised-dose tDCS condition so that all subjects receive 0.185 V/m, as previously predicted with a posterior-anterior montage (CP5-FC1; Evans et al., 2019), at left M1. I ran current flow modelling on subjects sequentially as they participated in the study (see section 5.5.2 in **discussion** for further details). In this cohort, 1 mA fixed stimulation produced an average electric field of .159 V/m in the left motor cortex with .023 V/m variation across participants that ranged from .117 V/m to .204 V/m. The average electric field reaching the left M1 (.159 V/m) was therefore 13.5% lower than the expected electric field intensity (.185 V/m), individualised to. Thus, for the individualised-dose condition, participants received on average an 18.5% increase in stimulator output (M = 1.185 mA, SD = .178 mA) than the fixed-dose condition (M= 1 mA, SD = 0). See Table 5.3 for details.

Table 5.3 Mean electric fields with fixed-dose tDCS and mean stimulator output for individualised-dose tDCS.

	Experiment	Electric field at LM1 (V/m)	S/O (mA) for target (V/m)
Average ± SD	1	.159 ± .023	1.185 ± .178 (.185)
	2	.124 ± .028	1.016 ± .232 (.128)
Range	1	.117 – .204	.900 – 1.575 (.185)
	2	.078 – .182	.659 – 1.537 (.128)

*Values in last column (Right) represent stimulator output (S/O) in mA required for targets (.185 and .128 V/m) in **Experiments 5.1** and **5.2**, respectfully.*

In **Experiment 5.2,** stimulator output intensity was individualised for each subject so that they receive .128 V/m target electric field intensity at LM1, as previously predicted in **Chapter 4** with an HD-tDCS montage. 1 mA fixed-dose stimulation produced an average electric field of .124 V/m in LM1 with .028 V/m variation across participants that ranged from .078 V/m to .182 V/m. The average electric field reaching LM1 (.124 V/m) was therefore 3.2 % lower than the expected electric field intensity (.128 V/m). Thus, the individualised-dose condition produced on average 1.6 % stronger stimulation (*M* = 1.016 mA, *SD* = .232 mA) than the fixed-dose condition (*M* = 1mA, *SD* = 0). See Table 5.3 for details.

Furthermore, to investigate any correlation between the amount of electric field reaching a target during tDCS (D1-5) and subsequent changes to corticospinal excitability, the electric field values for each participant were regressed across their mean MEP changes during tDCS. In **Experiment 5.1**, there was a positive correlation (p = .04) between the electric field intensity at LM1 and mean MEP change during tDCS induced by the fixed-dose tDCS condition. This indicated that higher electric field intensities at LM1 with fixed-dose tDCS resulted to greater MEP change during tDCS. However, this was not significant with variability change during and after tDCS ($p > 0.05$), indicating that electric field differences in a target do not impact differences in variability change across participants. Regression of electric field for individualised-dose across MEP changes was not correlated to corticospinal excitability change ($p > 0.05$).

To understand how individualising dose, thereby changing tDCS output intensity, affected corticospinal excitability change, the differences in electric field change from fixed-dose to individualised-dose tDCS conditions were regressed against the differences in corticospinal excitability change between fixed- and individualised- dose. In **Experiment 5.1**, the regression revealed that individuals requiring larger increases in electric fields (thus more mA in stim O/p) when individualising-dose, projected greater changes in corticospinal excitability change (p = .03). On an individual level, all 4 participants receiving less stimulation output when individualising-dose (*M* = .90 mA),

lost their effect (see Figure 5.5; PA-tDCS, A-B). Furthermore, changing electric field at a target did not correlate with differences in variability change ($p > .05$).

In **Experiment 5.2**, there were no significant correlations between electric field at a target and MEP changes for either fixed-dose and individualised dose tDCS ($p > 0.05$) (see Figure 5.5; HD-tDCS, A). Regression of the difference in electric field at a target across the difference in MEP changes did not reveal any significant effects between the two conditions ($p > .05$) (see Figure 5.5; HD-tDCS, B). A possible reason for this may be that HD-tDCS produces lower electric-field intensities on average than PA-tDCS (see **Chapter 4**). Lower electric-field intensities may not be directly related to MEP change as there may be an individual threshold for each subject to be a responder, which on average, may be higher than intensities reaching LM1 with HD-tDCS (see discussion for details).

Overall, the average intensity reaching a target was lower for **Experiment 5.1** than the target intensity (.185V/m). Thereby subjects received higher stimulator output on average when individualising dose. On the contrary, this effect was only marginally different for **Experiment 5.2**. Furthermore, the electric field reaching a target with PA-tDCS was directly correlated with corticospinal excitability change during tDCS when fixed-dose tDCS was used. However, this was not the case for individualised-dose tDCS, possibly due to responders losing their effect when individualising dose. Nevertheless, this was not consistent for HD-tDCS. In **Experiment 5.2**, tDCS types showed no correlation between electric field intensities at a target and CSE change, and at an individual level, responders did not lose their effect when individualising dose. To understand condition-based responders further, I ran a grand average analysis (next section) on responder and non-responder subgroups.

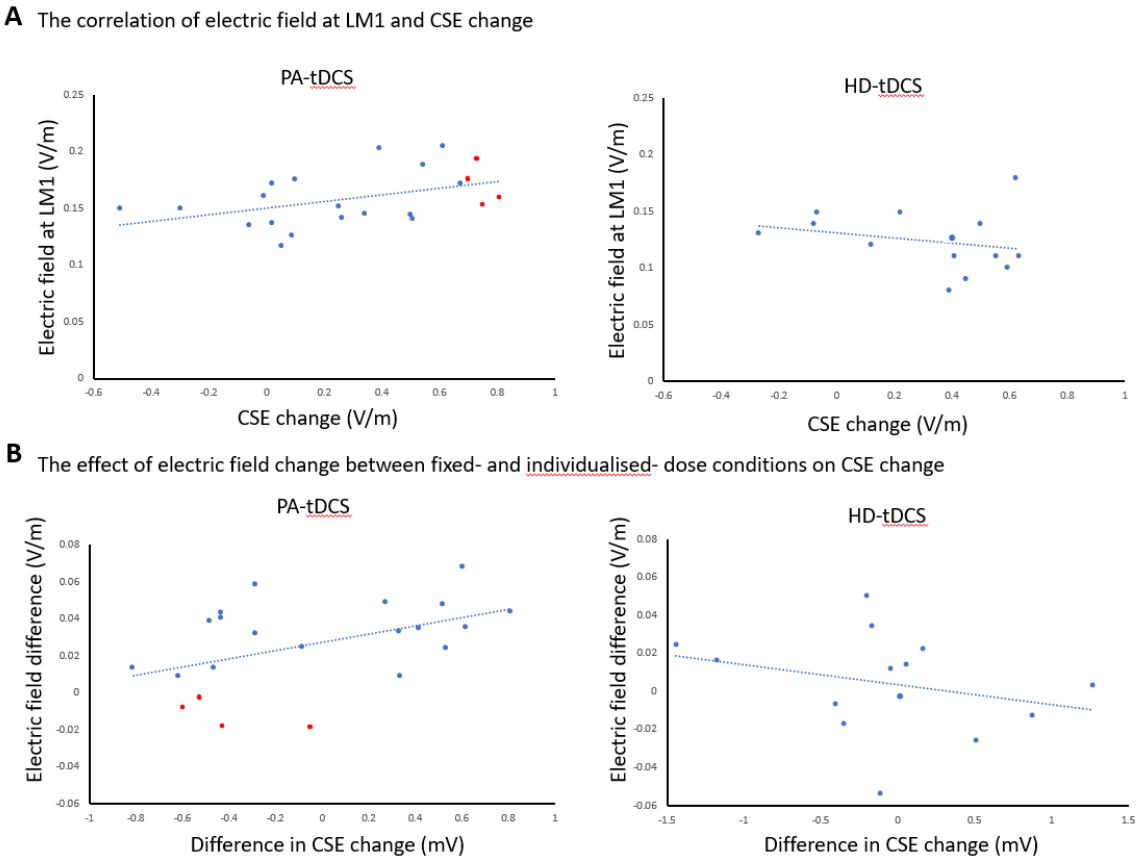

Figure 5.6. Correlation of electric field and electric field difference between conditions with corticospinal excitability change

A. *Electric field intensities at LM1 regressed against corticospinal excitability (CSE) change for each experiment (PA-tDCS; Left, HD-tDCS; Right).*

B. *Difference in electric fields at LM1 between conditions (fixed- and individualised- dose tDCS) regressed against the difference in corticospinal excitability change for each experiment (PA-tDCS; Left, HD-tDCS; Right).*

Red data points (PA-tDCS; A) correspond to the strongest responders with fixed-dose tDCS, and red data points (PA-tDCS; B), correspond to the same responders, showing loss of CSE effect following individualised-dose. This was not the case for HD-tDCS.

5.4.7 Responders versus non-responders analysis

To investigate which percentage of our subjects responded with facilitatory or inhibitory responses in each group and how this impacted variability in each condition, grand average analysis was conducted on responders and non-responders in each condition and their variability of corticospinal excitability change.

In **Experiment 5.1**, grand average analysis of the condition fixed-dose tDCS revealed 82% responders. This indicates that the majority of subjects that received fixed-dose tDCS had facilitatory effects (Figure 5.6; A, left). On the other hand, grand average analysis on the individualised-dose condition showed that 64% were responders, indicating that individualising-dose may have made responders lose their facilitatory effects (Figure 5.6; A, right).

Furthermore, the % of subjects that had an overall reduction in variability with individualised-dose tDCS was analysed and compared against fixed-dose tDCS. This indicated that 64 % of subjects had overall less variability with individualised-dose tDCS (Responders: *N* = 14; non-responders: *N* = 8). 55% (*N* = 12) of participants had consistent excitability effects across groups (increased MEP change) for both conditions (see Figure 5.6; B). Of these participants, 92 % (*N* = 11) had less variability with individualised-dose tDCS. This may indicate that individualised-dose tDCS reduces variability only for responders. All individuals with increased corticospinal excitability change during fixed-dose tDCS and decreased CSE change with individualised-dose tDCS (27 %; *N* = 6), had increased variability with individualised-dose tDCS. Whereas subjects with increased CSE change during individualised-dose tDCS (*N* = 2) had increased variability in both sessions. Since this was only 2 subjects, it is difficult to make any inference.

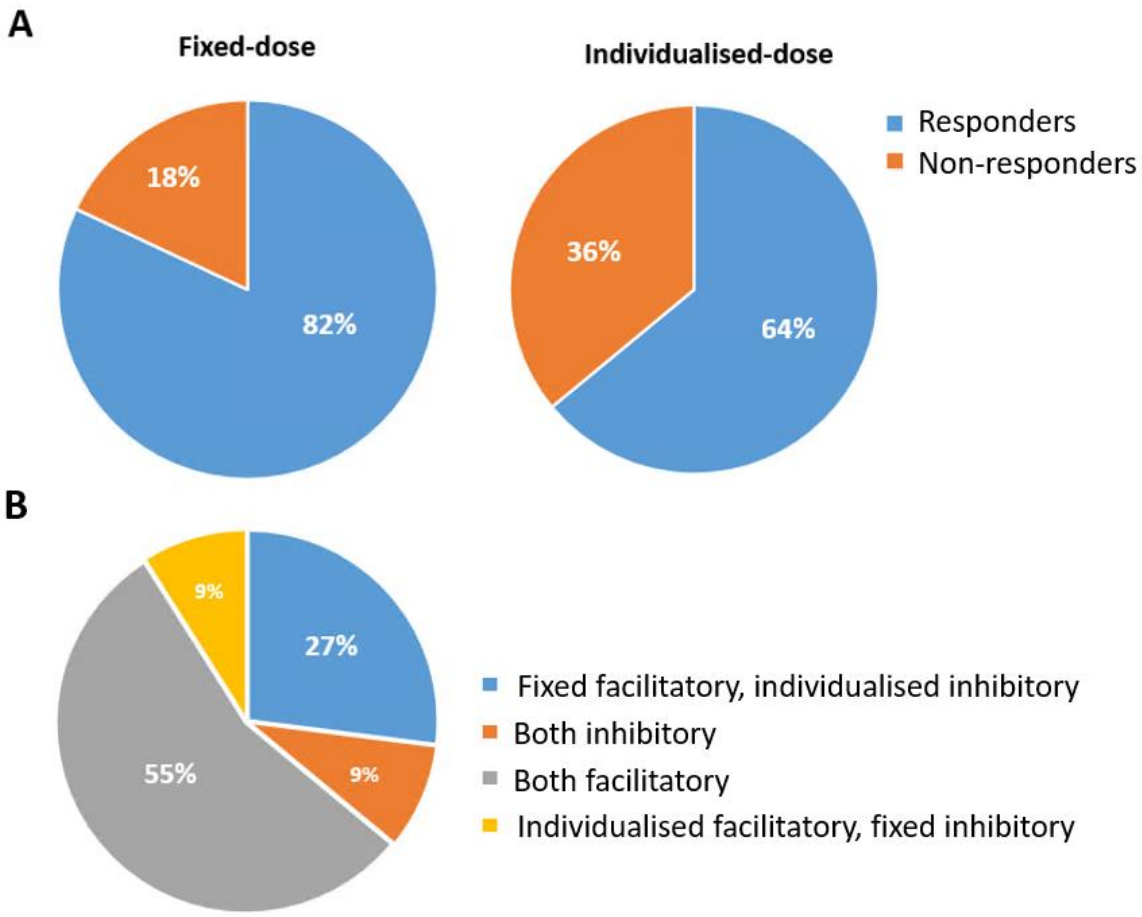

Figure 5.7. PA-tDCS condition-based responders versus non-responders.
A. *The percentage of participants in each condition that responded with a facilitatory response (Responders; Blue) or an inhibitory response (Non-responders; Orange).*
B. *The percentage of condition based responders and non-responders that had facilitatory response with fixed-dose tDCS and inhibitory response with individualised-dose tDCS (light blue), inhibitory responses under both conditions (Orange), facilitatory responses under both conditions (Grey), and facilitatory responses with individualised-dose tDCS and inhibitory with fixed-dose tDCS (Yellow).*

In **Experiment 5.2**, grand average analysis revealed that both fixed- and individualised-dose conditions had 77 % responders, and sham-tDCS had 62 % responders (see Figure 5.7; A). This indicates that most of the sample responded with a facilitatory change in CSE with active tDCS than sham. In this experiment, only 3 people (21 %) had less variability with individualised-dose, confirming the RM ANOVA results that individualising dose did not reduce variability, but on the contrary increased it for HD-tDCS. In this experiment, only 23% of subjects responded with facilitatory effects with active tDCS and inhibitory effects with sham-tDCS. The majority of responders (31%) had

facilitatory effects with all conditions, including sham (see Figure 5.7; B). As also indicated in Figure 5.4 A, excitability was marginally higher from baseline for sham-tDCS. This indicates the importance of using sham-tDCS as a comparison to active tDCS to understand the differences in excitability provoked by active tDCS versus fluctuation in excitability that may occur due reasons other than tDCS stimulation. As only a small number of participants had a reduction in variability with individualised-dose (*N* = 3), no further inference could be made on condition-based responders and non-responders with regard to variability change. Figure 5.7 B, shows the percentage of participants that had facilitatory or inhibitory responses under each condition (fixed-dose, individualised-dose or sham tDCS)

Overall, results from **Experiment 5.1** revealed a reduction in variability for people who have consistent effects across both conditions (responders in both conditions). In other words, variability of MEP change more consistently decreases for people who have consistent increase in PA-tDCS induced MEP change across groups (i.e for responders). On the contrary this could not be validated for HD-tDCS, as only a minority had reduced variability in the individualised-dose condition.

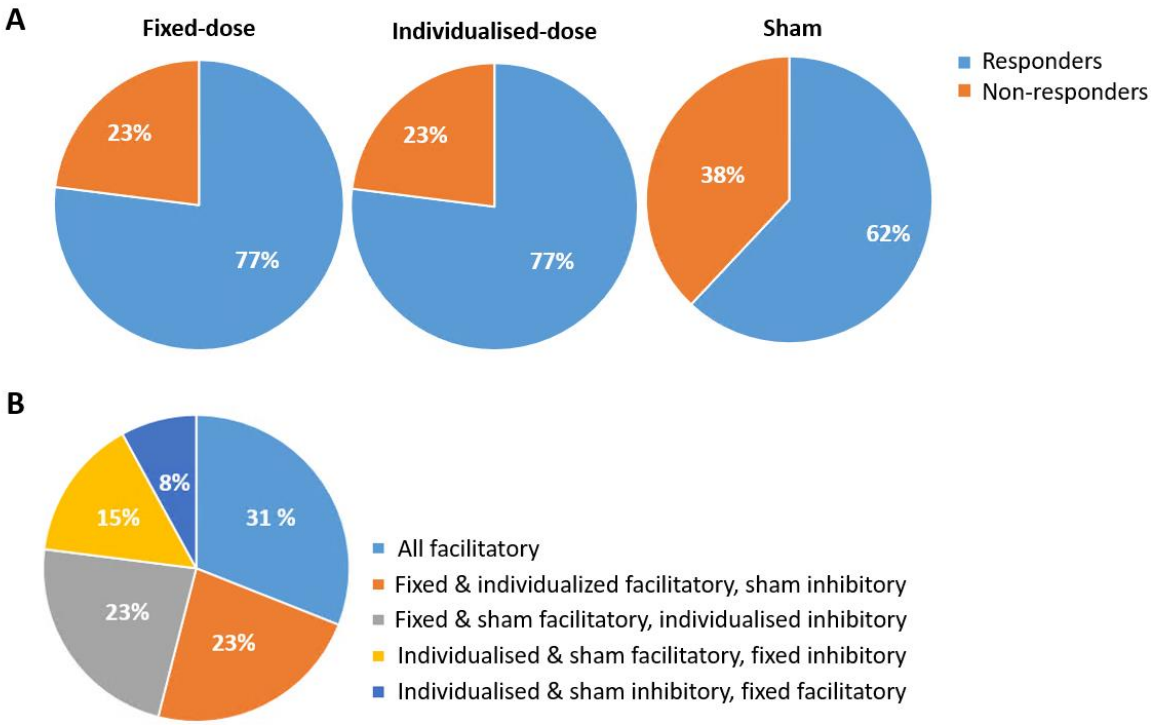

Figure 5.8. HD-tDCS condition-based responders versus non-responders

A. *The percentage of participants in each condition that responded with a facilitatory response (Responders; Blue) or an inhibitory response (Non-responders; Orange).*

B. *The percentage of condition-based responders and non-responders that had facilitatory responses in all conditions (Light blue), facilitatory responses with fixed and individualised dose tDCS, and inhibitory responses with sham (Orange), facilitatory responses with fixed-dose and sham tDCS, and inhibitory with individualised-dose (Grey), facilitatory responses with individualised-dose and sham tDCS, and inhibitory with fixed-dose tDCS (Yellow), and lastly, facilitatory responses with fixed-dose tDCS and inhibitory responses with individualised-dose and sham tDCS (Dark blue).*

5.5 Discussion

TDCS has shown great promise in both basic research and clinical applications due to its neuromodulatory effects (Bestmann & Walsh, 2017). Despite this, tDCS is limited due to large variability of its effects across subjects and studies (Bestmann & Ward, 2017; Datta et al., 2012; Esmaeilpour et al., 2018; Laakso et al., 2015; Price et al., 2015). One factor affecting this variability may be the inter-individual anatomical differences (Bestmann, 2015; Bestmann & Walsh, 2017; Bikson et al., 2009; 2012; Chew et al., 2015; Datta et al., 2009; Evans et al., 2019; Horvath et al., 2015a; Opitz et al., 2015) that influence the magnitude of electric field reaching a targeted cortical area. Individualising dose reaching a target across a population has been suggested to reduce the variation of electric fields reaching a target, which may lead to less variability in tDCS effects (Bestmann & Ward, 2017; Esmaeilpour et al., 2018). The aim of this study was to test whether individualising tDCS stimulation intensity so that each participant receives the same electric field intensity at the cortical target site, meaningfully reduces the inter-individual differences of responses to tDCS, compared to conventionally applied fixed-dose tDCS. This was tested in two experiments using different montages targeting the left M1 (**Experiment 5.1**; PA-tDCS, **Experiment 5.2**; HD-tDCS). My main findings were 3-fold:

(1) In **Experiment 5.1**, there were no significant differences in corticospinal excitability changes between fixed- and individualised- dose tDCS. This contradicts my hypothesis that active stimulation would lead to decreased corticospinal excitability changes. In **Experiment 5.2**, neither fixed- nor individualised- dose HD-tDCS to M1 significantly affected corticospinal excitability compared to sham, contrary to the hypothesis that active stimulation would lead to an increase of excitability. Thus, on a group-level, no effect of tDCS could be observed. Grand average analysis on responders and non-responders revealed the expected facilitatory effects of HD-tDCS in comparison to sham, however this also revealed increased excitability effects for PA-tDCS which contradicts previous findings (Hannah et al., 2019; Rawji et al., 2018).

(2) The magnitude of electric field intensities reaching LM1 were correlated with changes in corticospinal excitability with fixed-dose PA-tDCS (**Experiment 5.1**) and

individualising dose made responders lose their effect. However, this was not the case for HD-tDCS (**Experiment 5.2**), indicating montage-based differences.

(3) Contrary to expectations, the inter-individual variability of responses to both PA-tDCS and HD-tDCS was not reduced following individualised-dose tDCS; in fact, changes in corticospinal excitability were significantly more variable when using individualised-dose than fixed-dose HD-tDCS. This indicates that the method of dose-control employed in the present study, i.e. controlling for electric field intensity, did not effectively reduce variability of tDCS responses, but instead had opposite effects with HD-tDCS. Further analyses of responders and non-responders revealed a reduction in variability for people who were responders in both conditions for PA-tDCS, however this could not be validated with HD-tDCS.

Potential explanations for the lack of tDCS variability reduction, as well as implications of these findings and suggestions for further research is discussed below.

5.5.1 Lack of corticospinal excitability changes of PA-tDCS and HD-tDCS

Changes in corticospinal excitability induced by PA-tDCS were not robust enough to reach significance. However, mean differences of CSE change from baseline indicated a trend of increased corticospinal excitability change induced by PA-tDCS. This contradicts previous findings that PA-tDCS reduces corticospinal excitability (Rawji et al., 2018), and my findings from **Chapter 3**. HD-tDCS had no substantial effect of active tDCS on motor excitability compared to sham. Active stimulation did elicit increases of excitability relative to baseline that could not be observed with sham stimulation, which might hint at an increased CSE effect of HD-tDCS, in line with previous findings (Caparelli-Daquer et al., 2012; Kuo et al., 2013). This effect, however, was not robust enough to reach significance in direct comparison of stimulation conditions. The lack of expected excitability change from both PA-tDCS and HD-tDCS is in line with the notion of low reliability of tDCS effects across studies of tDCS (Horvath et al., 2015b; Huang, Lu, et al., 2017; Ridding & Ziemann, 2010). Several reasons may explain the lack of effect of PA-tDCS and HD-tDCS in these experiments, these include high inter-individual variability,

responders losing their effects with individualised-dose tDCS and the short post-tDCS measurements (see next subsections for details).

5.5.1.1 High inter-individual variability

High inter-individual variability of responses to stimulation is a potential reason for the observed lack of tDCS effects on a group level. Since variability was not reduced by the attempted method of individualised-dose tDCS, but instead was comparable to that observed in previous studies (see review by Caparelli-Daquer et al., 2012; Horvath et al., 2015b), this study faces the same issues that are carried by high inter-individual variability. Group level tDCS effects may be overridden by high inter-individual variability, such that responders that show effects in the expected direction, may lose their effects in group-level analyses (Chew et al., 2015; Laakso et al., 2019; López-Alonso et al., 2014; Strube et al., 2016; Wiethoff et al., 2014). This may be one reason for the increased excitability trends observed during and after tDCS for fixed- and individualised- dose in both experiments, which, due to high variability, did not reach significance.

5.5.1.2 Responders lost effect when individualising dose

To avoid the problem of high inter-individual variability that may have masked condition-based responses, a grand average analysis on responders and non-responders was used to reveal any differences of subgroups reacting differentially to tDCS conditions (Wiethoff et al., 2014). In **Experiment 5.2**, a possible effect of active versus sham HD-tDCS, in line with previous findings (Caparelli-Daquer et al., 2012; Kuo et al., 2013) was detected. Analysis on HD-tDCS responders versus non-responders indicated that subjects responded equally with facilitatory effects in both fixed- and individualised- dose conditions (77 %). In comparison, for sham tDCS, less subjects responded with facilitatory effects, as expected (62 %). In **Experiment 5.1**, faciliatory effects of PA-tDCS were evident by the majority of subjects (82 %), which contradicts previous research (Rawji et al., 2018). The individualised-dose tDCS condition had less subjects responding with facilitatory effects (64 %) indicating that individualising dose made responders lose their facilitatory effect. Indeed, 4 subjects with the greatest CSE

change, had all become non-responders and lost their effect when individualising dose (**Experiment 5.1**; PA-tDCS). As previously shown (Laakso et al., 2019), individually calculated electric fields were correlated to inter-individual differences in the responses to tDCS, such that greater electric fields related to greater CSE changes when applying 1mA fixed-dose tDCS. Thereby, the loss of CSE changes when individualising dose may be due to subjects receiving lower stimulation (< .9 mA) when individualising dose. Although the effects of tDCS are not necessarily linear (Benwell et al., 2015; Bonaiuto & Bestmann, 2015; Esmaeilpour et al., 2018; Learmonth et al., 2017), each individual may have a specific threshold of electric field required for them to become responders which may also be reliant on factor affecting tDCS effects such as brain-state, age, gender, hormone levels, time of day and attention (Chew et al., 2015; Ridding & Ziemann, 2010). In **Experiment 5.1**, applying less stimulation output to responders with the highest CSE change when individualising-dose has made them lose their effect, which in turn, affected group level effects. On the other hand, this could not be validated with HD-tDCS as individualising-dose did not make responders lose their effect. This may be because individualising dose increased corticospinal variability in comparison to fixed-dose tDCS (see section 5.5.3 lack of variability reduction). It may also be related to differential electric fields reaching a target when individualising dose (for details, see section 5.5.2).

5.5.1.3 Timing of post-tDCS excitability measures

The observed lack of tDCS effects may also be due to measuring corticospinal excitability changes only up to five minutes after stimulation. Maximum increase of excitability tends to be around 15 minutes post-tDCS (López-Alonso, Fernández-Del-Olmo, Costantini, Gonzalez-Henriquez, & Cheeran, 2015). HD-tDCS in particular shows delayed effects, with peak excitability at 30 minutes post-tDCS (Kuo et al., 2013). This is also the case for PA-tDCS which shows peak excitability at 30 minutes after tDCS (Rawji et al., 2018). Thus, more distinct excitability changes may be observed when measured for longer periods post-stimulation. Additionally, other measures, such as short interval intracortical inhibition (SICI), might have been more sensitive to detect neurophysiological changes induced by tDCS (Biabani et al., 2018).

5.5.2 Expected electric field intensity at target is not consistent

To my knowledge, this study is the first to individualise-dose using the method by Evans and colleagues, (2019), and consequently investigate the effects on corticospinal excitability. Thus, given the same montage was applied for fixed- and individualised-dose, the lack of excitability change differences between these conditions was expected. Although I varied the dose reaching a cortical target in the individualised-dose condition, all subjects were expected to receive the same magnitude at LM1 (**Experiment 5.1**: .185 V/m; **Experiment 5.2**: .128 V/m) that corresponded to the average magnitude produced by fixed-dose tDCS. Thus, on a group level, there were no expected differences between the individualised- and fixed- dose conditions. However, the average magnitude targeted in this study was based on a different population (50 scans from the Human Connectome Project; HCP), and as calculated in previous studies (.185 V/m, Evans et al., 2019; .128 V/m, **Chapter 4**). Target electric field intensities from previous studies were used to avoid inefficiencies of first collecting all participant MRI scans for all conditions to analyse the average electric field before testing. One inefficiency would have been participant drop-outs if the study would take place at a later period than participants signed up for. A second inefficiency would have been the pause in activity in the study for the time it takes to get enough participants signed up. Thereby, testing was conducted sequentially (as participants signed up to the study) and not after acquiring all participant scans. Due to interindividual anatomical differences, 1 mA fixed-dose tDCS could produce a different average electric field in LM1 in one cohort than another (Bonaiuto & Bestmann, 2015; Datta et al., 2011; Laakso et al., 2019; Li et al., 2015; Mikkonen et al., 2020). Indeed, this was the case for **Experiment 5.1** whereby fixed-dose PA-TDCS produced an average electric field of 0.159 V/m than the expected and targeted 0.185 V/m electric field intensity. This meant that on average, 18.5% stronger tDCS stimulation was applied to the individualised-dose group, than required. For HD-tDCS, the average increase of stimulation output (1.2%) was miniscule to have any substantial effect. Nevertheless, applying on average higher stimulation output did not seem to produce any CSE differences between conditions. Even if it may be inefficient, ideally, prior to testing, future research using individualised-tDCS methodologies should

first receive all participant scans and calculate the average EF based on the same cohort being tested in the study.

5.5.3 Lack of Variability reduction of individualised-dose tDCS

Based on the assumption that inter-individual differences of tDCS responses are caused by differences in electric field intensities reaching a targeted cortical region (Bestmann & Ward, 2017), this study investigated if CSE responses could be made more consistent when controlling the electric field intensity reaching LM1. This was attempted by individualising tDCS output intensity based on current flow models (Evans et al., 2019). However, variability could not be reduced with this method of dose-control. Variability was comparable to fixed-dose PA-tDCS (Rawji et al., 2018) and increased in comparison to fixed-dose HD-tDCS (Caparelli-Daquer et al., 2012). Furthermore, frequency spectrum on grand average analysis of the variability in each subgroup of responders and non-responders for each condition, indicated that facilitatory effects of tDCS in both conditions (55% responders for fixed- and individualised- dose tDCS), had reduced variability of tDCS responses when individualising dose in comparison to applying a fixed dose. This was evident in nearly all of the subjects (94%) with consistent effects in both conditions (55%). This finding validates the proposed notion that dose-control may be an effective method for reducing variability of tDCS effects (Bestmann & Ward, 2017; Datta et al., 2011; Esmaeilpour et al., 2018; Laakso et al., 2019). However, this may only be true if individualised-dose tDCS does not make responders lose their facilitatory effect. Possible considerations for improving the current methodological innovation so that responders do not lose their effect are discussed in **Chapter 6** section 6.4. Given that this occurred in 55% of subjects, it would need a larger study to power this comparison adequately. Nevertheless, reduced variability of tDCS responses was evident when using PA-tDCS but not HD-tDCS. Potential explanations why individualising tDCS intensity did not lead to more consistent responses and instead, produced differential outcomes for HD-tDCS and PA-tDCS, are outlined in the following.

5.5.3.1 TDCS dose is not (exclusively) determined by electric field intensity

A potential explanation why individualising tDCS dose did not lead to more consistent responses for both experiments may be that tDCS is not exclusively determined by electric field intensity as previously suggested (Bestmann & Ward, 2017; Datta et al., 2012; Kim et al., 2014; Laakso et al., 2018; Morya et al., 2019). The notion of individualising tDCS intensity based on the electric field reaching a target originates from the assumption that there is a direct relationship between electric field intensity at the cortical target site and changes in excitability at this location (Laakso et al., 2019). Therefore, locally controlling for electric field intensity should lead to more consistent effects of tDCS. However, this study did not confirm these expectations. Indeed, the electric field in **Experiment 5.1** was correlated with CSE change when applying fixed-dose tDCS, however, individualising-dose did not lead to more consistent CSE changes on a group level. Reasonably, there are more decisive factors underlying the magnitude of tDCS effects than just electric field intensity. Thereby, controlling solely for electric field intensity may be insufficient to reduce inter-individual variability of tDCS effects (Bestmann & Ward, 2017).

Potential factors to control for and further optimise the delivery of tDCS such as methodological considerations of individualised-dose, brain-state and other contributing factors to variability such as dose-to-network connectivity, attention and hormone levels are discussed in **Chapter 6**.

5.5.3.2 Lack of current flow direction control may explain increased HD-tDCS effect variability

Opposite to expectations, individualising dose for HD-tDCS (**Experiment 5.2**) significantly increased the variability of CSE in comparison to fixed-dose tDCS, suggesting that individualising dose may apply additional variation of CSE effects with HD-tDCS than PA-tDCS.

A potential reason for this finding is the lack of current flow direction control with HD-tDCS montages than PA-tDCS montages (Mikkonen et al., 2020). Montage differences in the direction of current flow is an important parameter that is seldom considered

(Hannah et al., 2019; Mikkonen et al., 2020) which could explain the differences between experiments. Insufficient control of electric field direction may be a source of increased inter-individual variability of HD-tDCS effects (Hannah et al., 2019) between montages. Evidence for the impact of current flow direction on the effects of electrical stimulation derives from studies of multiple levels of observation. In vitro studies demonstrated that membrane polarisation effects are mainly induced by the electric field component aligned parallel to the neuronal axis (Jackson et al., 2016; Radman et al., 2009; Rahman et al., 2013). In humans, the folding of the cortex means that the direction of current flow relative to the neuronal orientation is more complex (Dmochowski et al., 2013). The direction of current flow with regard to the cortical surface has been shown to significantly impact CSE of PA-tDCS (as seen in Rawji et al., 2018; and **Chapter 3**) as well as behavioural measures such as retention of motor learning (Hannah et al., 2019). HD-tDCS on the other hand uses radial current flow, resulting in multifaceted directional impact relative to targeted neurons underneath the centre electrode than conventional montages (Caparelli-Daquer et al., 2012; Dmochowski et al., 2011; Edwards et al., 2013; Mikkonen et al., 2020). This may be a reason for the differences in variability between the two experiments. As such, going forward, the use of current flow models to optimise and control for electric field direction across subjects may help improve tDCS efficacy.

5.5.4 Limitations

The method of individualising tDCS (Evans et al., 2019) might not have optimally controlled the electric field intensity at the relevant cortical location, and therefore not have reduced variability on a group level. Possible limitations of the study's methodology are worth considering.

Firstly, the target electric field intensity at LM1 was not based on subjects from this study. This occurred as subjects were tested sequentially (at the time they signed up to this study. Thereby, subjects in the individualised-dose conditions for **Experiment 5.1** received on average stronger stimulation than the groups average. However, this difference was miniscule with HD-tDCS in **Experiment 5.2** (see section 5.2.2). This distinction may have influenced responders in one experiment than the other. That

being said, tDCS effects on corticospinal excitability and the variability of effect were not compromised on a group-level. To avoid this, and although inefficient, all subject scans should be acquired and analysed before experimental sessions commence.

Secondly, restricting the post-tDCS MEP recordings to five minutes post-tDCS stimulation may have prevented the detection of robust tDCS effects. As aforementioned in section 5.5.1.3, peak changes may occur up to 30-minutes post-stimulation for both HD-tDCS and PA-tDCS (Kuo et al., 2013; Rawji et al., 2018; respectively). Thus, future research should acquire measurements of post-stimulation tDCS effects for longer periods when using these montages.

Thirdly, region of interest (LM1) was targeted with tDCS using fixed coordinates of the 10/10 system (C3 location) which is based on skull anatomy (Seeck et al., 2017) rather than each individual's motor hotspot as functionally determined by TMS in the majority of studies (e.g Caparelli-Daquer et al., 2012; Hannah et al., 2019; Kuo et al., 2013; Laakso et al., 2019; Nitsche & Paulus, 2000; Rawji et al., 2018). Thereby the region targeted by tDCS may have not corresponded to each individual's motor hotspot. Since the electric field induced by individualised tDCS is constant only at the targeted location when individualising dose, it is just as variable at any other location (Evans et al., 2019). While more time-consuming, localising the hand knob for each subject manually and individualising tDCS based on the electric field intensity at this location, may result in more consistent tDCS effects. See **Chapter 6** section 6.4 for further detail on the optimisation of this methodology.

Lastly, this study did not control for other sources of response variability, such as age, gender, time of day or hormone levels (Ridding & Ziemann, 2010). To further limit the amount of inter-individual variability and bring out the effect of dose-related variability more clearly, these factors should be controlled for. However, since the stimulation conditions in this study were compared within-subject and fully counter-balanced, these factors should not have biased the comparison between fixed- and individualised- dose tDCS, therefore conclusions on these comparisons are valid.

5.5.5 Conclusion

TDCS has become popular due to its neuromodulatory potential in basic and translational research. However, tDCS utility suffers from low efficacy and reliability across subjects and studies due to variability of its effects (Bestmann & Walsh, 2017; Bestmann & Ward, 2017; Datta et al., 2012; Dissanayaka et al., 2017; Esmaeilpour et al., 2018; Evans et al., 2019; Laakso et al., 2017). Utilising current flow models to individualise tDCS output to a target whilst controlling for inter-individual anatomical differences has been suggested (Bestmann & Ward, 2017; Esmaeilpour et al., 2018; Evans et al., 2019; Laakso et al., 2019). This study tested this hypothesis and found no evidence of tDCS effect, nor variability reduction when individualising dose. Further than methodological limitations, I highlight that, responders losing their effect and montage differences may be potential reasons for the insignificant effects. Further research may benefit by optimising methodological criteria such as targeting cortical locations based on a functional approach in the group being tested and acquiring tDCS for longer post-stimulation periods whilst controlling for other possible sources of variability such as attention and hormone levels. This in turn could increase the robustness of observed effects and provide better tDCS control of variability making tDCS more efficacious and reliable, which is crucial for its use in research and therapeutic settings.

Chapter 6 General Discussion

The ability of tDCS to modulate brain activity has vast scientific and therapeutic potential, however, its effects are variable across subjects and studies which limit its utility. In this thesis, I attempted to optimise the delivery of tDCS application by investigating the controlled delivery of current flow direction (**Chapter 3**) and whether, through the use of current flow models, we can individualise tDCS dose and deliver comparable electric fields with reduced variability across differential montages (**Chapter 4**). To assess whether current flow models are useful for the controlled delivery of tDCS, I further investigated if dose-control translates to more consistent physiological outcomes (**Chapter 5**).

Through my research, I demonstrate that the interconnection of the two banks of the central sulcus were not of paramount importance when stimulated by differential current flow directions across the central sulcus (posterior-anterior or anterior-posterior current flow) as opposed to previous findings (Rawji et al., 2019). Furthermore, with the use of dose-control, I demonstrate that HD-tDCS remains focally more advantageous, even with the delivery of comparable electric field intensity and variability as PA-tDCS to a cortical region. Together, the work presented here suggests that current flow models are useful for informing dose-controlled protocols and montage comparisons for optimising tDCS delivery, however, controlling for anatomical differences in the delivery of electric fields to a target is not sufficient to reduce the variability of tDCS effects in physiology. Thus, the methodology for optimised tDCS delivery remains a subject for further improvement and investigation.

Each experimental chapter contains an extensive discussion of the issues pertinent to the above findings. In this chapter, I reflect on the main findings and how they relate to relevant literature, outline the implications of this body of work, and discuss some limitations and potential improvements for future research in this field.

6.1 Key findings, implications, and future directions

The work presented in this thesis is founded upon a large body of physiological, behavioural and current flow modelling studies proposing the potential of optimised tDCS in basic and clinical research. Having investigated various parameters involved in the outcome effects of tDCS, such as different montages and their respective current flow direction, electric field intensity and distribution, the experiments presented in this thesis provide novel insights that advance the field of tDCS. The key findings are discussed below.

6.1.1 Controlling the direction of current flow based on topographical and functional S1-M1 connections

The orientation of the external electric field with regards to the orientation of the pyramidal neurons in the cortex plays a major role for tDCS effects on membrane polarisation and subsequently influence the direction of neuroplastic change (Dissanayaka et al., 2017; Miranda et al., 2013; Paulus et al., 2013). However, conventional methods of tDCS do not explicitly control the direction of current flow (Bestmann & Walsh, 2017; Dmochowski, Bikson, Datta, et al., 2012; Hannah et al., 2019). Findings from **Chapter 3,** did not validate the importance in controlling for electric field direction when targeting these regions. This could be due to high inter-individual variability of tDCS effects that were not controlled for in this study. Future research utilising tDCS should thereby ensure greater sample sizes and controlling for factors influencing the variability of electric fields to ensure greater effect sizes and more consistent outcomes.

Furthermore, and contrary to my hypothesis, I here established that reversing the current flow direction across the central sulcus does not reverse the net polarisation effects of M1 and S1 and instead may have influenced the impact of cortico-cortical projections of S1-M1 and M1-S1. Note, although clear trends were present in the data, these findings were not robust enough to reach significance, possibly due to the high interindividual variability of tDCS effects and the effect of peripheral nerve stimulation on S1 and M1 cortico-cortical projections. More specifically, as pyramidal neurons in the two banks of the central sulcus face in approximately opposite directions (Rahman

et al., 2013), decreased MEPs as a result of peripheral electrical stimulation may have increased SEPs due to the cortico-cortical projections between S1 and M1. As such, to assess whether opposite current flow directions may impact one bank of the central sulcus more than the other, the effect of subthreshold intensity peripheral electrical stimulation should be taken out of the equation. To do this, one can obtain sensory and motor measures separated in sessions or time so that PES can be applied at above motor threshold intensities, after tDCS delivery. This would prevent measuring net effects of CSE or S1-M1 and M1-S1 cortico-cortical interactions as a result of combined protocols. Based on my findings that one bank may be more impacted with one direction than the other, my alternate hypothesis would be that PA-tDCS would have a greater impact on the post-central bank, thereby increasing SEPs at a greater magnitude than AP-tDCS whilst inhibiting MEPs with smaller effect sizes. On the contrary, AP-tDCS is expected to have a greater impact on the pre-central bank and thus produce greater reduction of MEP than PA-tDCS, whilst marginally increasing SEPs via cortico-cortical interactions. Such a demonstration would further our understanding of the effect of current flow direction on the primary sensory and motor areas and how current flow may interact with the underlying cortico-cortical interconnections and functional mechanisms of these cortical areas.

Many tDCS studies primarily focus on the intensity of the electric field, while ignoring its directionality. This occurs regardless of the known effects of tDCS direction (Bikson, Rahman, et al., 2009; Dmochowski, Bikson, Datta, et al., 2012; Hannah et al., 2019; Jackson et al., 2016; Rahman et al., 2013; Rawji et al., 2018). The direction of electric field, as well as its intensity, is influenced by anatomical differences and depends on the tDCS montage (Dmochowski, Bikson, Datta, et al., 2012; Dmochowski et al., 2011). Poor control of electric field direction may therefore be a source of inter-individual variability of tDCS effects (Hannah et al., 2019). As variability of electric field direction may contribute to inter-individual differences in tDCS responses, the use of current flow models to optimise and control for electric field direction across subjects may help improve tDCS efficacy.

6.1.2 Utility of current flow models

A major part of variability in the effects of tDCS arises from inter-individual differences in the intensity and spatial distribution of the electric field reaching the brain (Bestmann et al., 2015; Bestmann & Ward, 2017; Feng et al., 2018; Laakso et al., 2019, 2015; Li et al., 2015; Price et al., 2015; Wiethoff et al., 2014), which varies based on anatomical differences (Bestmann, 2015; Bestmann & Walsh, 2017; Bikson, Rahman, & Datta, 2012; Bikson, Rahman, et al., 2009; Chew et al., 2015; Datta et al., 2009, 2010; Evans et al., 2019; Horvath et al., 2015a; Opitz et al., 2015) and tDCS output intensity and montage (Dmochowski et al., 2011; Laakso et al., 2017; Mikkonen et al., 2020; Moliadze et al., 2010; Price et al., 2015) . Current flow modelling can provide individualised numerical estimates of electric fields across the brain for a given tDCS montage and stimulation intensity (Bikson et al., 2015; Huang et al., 2019; Laakso et al., 2015; Opitz et al., 2016) and can be used to determine individual stimulation parameters that result in a desired electrical field intensity at a specific cortical location for any given electrode montage (Evans et al., 2019). Although electrical field intensity can be controlled at a specific cortical location, there is no control over its spatial distribution (Evans et al., 2019). While HD-tDCS is used to deliver spatially more constrained current (Alam et al., 2014; Caparelli-Daquer et al., 2012; Datta et al., 2009, 2012; Dmochowski et al., 2011; Edwards et al., 2013; Karvigh et al., 2017; Shekhawat et al., 2016), it also increases electric field variability across subjects, and for the same stimulator output, delivers weaker electrical fields than bipolar tDCS (Mikkonen et al., 2020). Thus, the challenge is that selecting one parameter to control for, such as focality with HD-tDCS, may risk changing others, in this case increasing electric field variability, due to their inter-dependencies.

In **Chapter 4**, I illustrate that through the use of CFMs, one can individualise tDCS current flow across montages that produce different electric field intensities and distribution such as HD-tDCS and bipolar PA-tDCSn and deliver comparable electric field intensities at a target. I further reveal, for the first time, that HD-tDCS can still produce more focal electric fields than PA-tDCS even when delivering a two-fold increase of stimulator output to produce comparable electric field intensities at a cortical target. This is an important advancement in the field of basic neuroscience and neuroimaging studies, as montage comparison effects on behavioural and physiological outcomes can only be reliably assessed when delivering comparable electric field strengths in a target. With

this method, future studies could look into the physiological effect sizes and variability between montages when delivering comparable electric fields to a target whilst accounting for electric field variability from inter-individual anatomical differences.

The benefits of delivering more consistent current to a target with different montages include (1) firstly, the ability to identify other factors (whether biological or protocol-dependent, i.e cortico-cortical functional connections or the effect of different current flow directions on a targeted area, respectively) that determine an individual's physiological and/or behavioural response to tDCS, and (2) secondly, protocols producing more reliable and effective outcomes could potentially pave the way for standardising tDCS-induced cortical electric field effects, which could help optimise stimulation protocols and allow more consistent comparison of results across studies (Bestmann & Ward, 2017; Chew et al., 2015; Evans et al., 2019; Mikkonen et al., 2020; Opitz et al., 2015). Nevertheless, there is no consensus as to whether controlling for focality of stimulation versus direction is more beneficial. See next section (section 6.2) for details.

In **Chapter 5,** I investigated the utility of individualising HD-tDCS and PA-tDCS output to a target whilst controlling for inter-individual anatomical differences with current flow models, as previously suggested (Bestmann & Ward, 2017; Esmaeilpour et al., 2018; Evans et al., 2019; Laakso et al., 2019). I found no significant changes in corticospinal excitability for either fixed- and individualised- dose tDCS (**Experiment 5.1**), nor in comparison to sham (**Experiment 5.2**). On the contrary, on a qualitative level, there was a substantial increase from baseline for PA-tDCS which contradicts my hypothesis that active stimulation would lead to decreased corticospinal excitability changes. Furthermore, I found no significant variability reduction when individualising dose for either montage. This outcome was possibly due to methodological limitations, high inter-individual variability, and responders losing their effects when individualising dose as discussed in detail in **Chapter 5**. The sections below highlight the importance of dose-to-network interactions when considering other factors (than just dose-control in a region) such as control of electric field direction and focality. Moreover, section 6.4, provides suggestions for optimising methodological criteria for individualising dose with the use of current flow models to guide future research in this field.

6.2 Controlling for focality of stimulation versus direction

In **Chapter 4**, I highlight that CFMs can be a useful tool for montage comparison and that HD-tDCS may produce more focal electric fields than PA-tDCS. However, this finding does not necessarily imply that HD-tDCS would indeed produce more consistent and effective outcomes in physiology and behaviour when compared to PA-tDCS, as there is no consensus that greater electric field focality provides better outcomes than less focality, or whether controlling for focality is better than controlling for direction of current in a targeted cortical area. For example, pharmacological interventions have long shown great effects and yet, are not focal. This could be attributed to several reasons, as explained below.

Firstly, greater focality with HD-tDCS does not imply isolation of the electric field to the targeted cortical area as done with invasive intracranial electrodes (Huang, Liu, et al., 2017; Jackson et al., 2016; Krause et al., 2017; Radman et al., 2009; Rahman et al., 2013). On the contrary, tDCS current with non-invasive scalp electrodes delivers diffuse current that flows through a range of cortical structures, including the targeted region, and may reach multiple regions in one network, even with more focal HD-tDCS montages (Datta et al., 2009; Dmochowski et al., 2011; Lafon et al., 2017; Rahman et al., 2013). Put simply, the effects of tDCS can never be attributed to stimulation of a single region alone, regardless of montage. Untargeted areas such as the primary sensory and secondary motor areas are still impacted by more 'focal' electric fields produced with HD-tDCS. Given that tDCS has shown beneficial effects with wide-spread distribution of electric fields, it could be better to consider the overall cortical area targeted and the cortical interconnections between the impacted areas than just targeting one area in isolation.

Secondly, stimulation of untargeted brain areas is likely important because brain regions do not work in isolation. Instead, brain regions form interconnected networks, which suggests that stimulating one area might influence, and vice versa, be influenced by other areas of its network (Fischer et al., 2017). For example, stimulation of secondary motor areas modulates M1 excitability (Boros, Poreisz, Münchau, Paulus, & Nitsche, 2008; Kirimoto et al., 2011). Modulation of motor excitability via the premotor cortex has recently shown to produce more reliable outcomes than provided when stimulating

M1 directly (Lefebvre et al., 2019), which highlights the importance of understanding how tDCS electric fields reaching untargeted brain regions may affect motor outcomes, whilst also showcasing the potential of network-targeted tDCS for increasing reliability of effects. In line with previous fMRI (Alon, Roys, Gullapalli, & Greenspan, 2011; Sehm et al., 2012; Stagg et al., 2014), EEG (Polanía, Nitsche, & Paulus, 2011) and *in vitro* studies (Reato et al., 2013) that illustrate tDCS modulation of the functional connectivity of neuronal motor networks, a strong predictor of the effects of tDCS over M1 is the functional connectivity of the motor network (Hordacre et al., 2017). This suggests that the effects of tDCS are not exclusively determined by the electric field intensity at a single target area, but rather depend on interactions with distributed neural networks each montage may modulate. The dependency of tDCS effects on network interactions might explain why controlling electric field intensity at M1 alone did not reduce the variability of tDCS responses in **Chapter 5** for either montage.

The reason more focal stimulation is presumed to be an advantage is that we are not impacting excessively more areas than intending to which may add to the observed net effects of stimulation. However, and as described above, this is untrue for transcranial montages as they are not restricted to one brain region, thereby effects of stimulation remain dependent on functional network interactions and the impact of tDCS on such functional connectivity in targeted and untargeted brain regions. As such, even though HD-tDCS delivers more focal electric fields than PA-tDCS, the cortical impact of the affected areas and the cortico-cortical connections to our targeted area M1 are still likely to add to the net effects of tDCS. Considering these effects when designing tDCS protocols is therefore a major challenge to the field.

6.3 Opposite current flow directions impact differential functional networks

Furthermore, numerous studies state the importance of controlling for current flow direction based on topographical representation of the targeted area, and suggest that poor control of electric field direction may therefore be a source of inter-individual variability (Dmochowski, Bikson, Datta, et al., 2012; Dmochowski et al., 2011; Hannah et al., 2019; Jackson et al., 2016; Radman et al., 2009; Rahman et al., 2013; Rawji et al.,

2018). However, controlling for current flow direction in **Chapter 3** in this thesis did not consider the cortico-cortical projections of the underlying networks, evident in animals (Brochier et al., 1999; Hiraba et al., 2000; Lin et al., 1993; Pavlides et al., 1993) and humans (Gandolla et al., 2014; Kirimoto et al., 2011; Matsunaga et al., 2004), that may directly or indirectly affect the net effects of our measures from M1 and S1 areas. Currently, this is a major challenge that is yet to be solved.

Due to the functional cortical network nature of brain mechanisms, the hypothesis of reversed effects with reversed current flow direction may be logically flawed in humans, considering the diffuse tDCS current that is delivered in humans. For example, PA-tDCS may impact on different neuronal networks and functional connections to M1 and S1 than AP-tDCS, which would not be the case with intracranial electrodes directly targeting the pre- and post- central bank neurons. Thereby, in humans, reversing the current flow direction may not directly reverse the expected function of the impacted cortical areas as shown in animals, and could add to the variability of tDCS effects. As our hypothesis that reversed direction would produce reverse effects was based on animal modelling studies of single neurons, we would ideally need layer-precise current flow modelling, or even models at the cellular level which currently do not exist. This would largely advance our understanding of the effect of electric field direction on physiology and behaviour and could further explain the sources of variability for montages, which may result in more complex directionalities such as those produced by HD-tDCS, which deliver radial current flow relative to targeted neurons underneath the central electrode than conventional montages (Caparelli-Daquer et al., 2012; Dmochowski et al., 2011). Current flow models are limited in their field of observation as they only estimate the electric field in the brain based on anatomical tissue type conductivities and not with regard to different neuronal layers or polarisation of single neurons and their networks. Thus, we are yet to understand how different current flow directions, interact with cortico-cortical interconnections and functional networks.

Considering the above, to increase efficacy and reduce variability of tDCS effects, it therefore seems imperative to improve the dose-control method of tDCS delivered to

brain networks. Recent evidence suggests that the effects of brain stimulation can be enhanced by simultaneously targeting a region and its network (Fischer et al., 2017; Mondino et al., 2020) whilst considering brain-state and polarity of tDCS when delivering stimulation (Li et al., 2019). Resting state functional connectivity MRI (rs-fcMRI) is commonly used to detect functional networks between associated brain areas via synchronous oscillations of spontaneous brain activity (Fox & Raichle, 2007; Fox et al., 2005; Yeo et al., 2011). Cortical activity measured through EEG and fMRI has been used as a biomarker to target cortically active areas with tDCS (Cancelli et al., 2016; Dmochowski et al., 2013; Dmochowski, Koessler, Norcia, Bikson, & Parra, 2017). Network-targeted tDCS with the use of rs-fcMRI to identify LM1 and its associated resting state network, produced a two-fold increase in motor cortex excitability compared to traditional tDCS and a multifocal control (Fischer et al., 2017). This provides a promising step forward to improve the controlled delivery of tDCS to brain networks. Combining tDCS with neuroimaging methods, such as resting-state fMRI or EEG, may shed light on its impact on brain networks (Sehm et al., 2012). However, concurrent use of EEG with tDCS may be limited by stimulation artefacts (Gebodh et al., 2019) and both EEG and fMRI may present challenges for dose-controlled tDCS for future therapeutic purposes as they are monitoring techniques which require a laboratory environment. Additionally, there remains a lack of understanding of the physiological impact of tDCS on various brain networks to effectively individualise tDCS and further research is required prior to leveraging cortical activity as a biomarker for targeted stimulation.

Computational modelling of tES currents on different levels of observation; starting from polarisation of single neurons, over to current flow in the cortex and its impact on cortico-cortical interconnections and interactions with neural networks, to neurophysiological and behavioural outcomes, may provide in the future, a complete picture of the dose-response relationship of tDCS and its effects whilst enabling an individually targeted, dose-controlled delivery of tDCS which could yield reliable and effective results (Bestmann & Walsh, 2017; Bikson et al., 2015; Rahman, Lafon, & Bikson, 2015). However, such models would not be able to currently derive from MRI scans as MRI's do not provide information as described above. Instead, for in vivo information, in vivo studies would be required which are invasive, and mostly performed in animals,

making it difficult to translate to humans. Further improvements to the current methodology of dose-control are discussed below.

6.4 Improving the methodology of individualising dose

While the concept of controlling electric field intensity at a target is promising, the method of individualising tDCS dose adopted in **Chapters 4 and 5** may not have optimally controlled the electric field intensity at the relevant cortical location, and therefore did not achieve reduced variability in physiology (in **Chapter 5**) for either montage (PA-tDCS and HD-tDCS). Methodological issues that may be worth improving in future research are described in the following.

6.4.1 Extracting electric field intensity at fixed cortical target site across subjects

The method of individualising dose in this thesis was based on the individual electric field intensities extracted by the LM1 cortical target site as defined by a fixed set of coordinates previously shown to correspond to LM1 (Koessler et al 2009), in each subject's smoothed and normalised image of electric field estimates. Although electric field intensity had miniscule (.01 V/m) differences between normalised and normalised and smoothed images, the process of normalising images, however results to imperfect alignment across images to standard space (Poldrack, Mumford, & Nichols, 2011). Anatomical differences in size and shape of the head and cortex exacerbate such alignment differences. Therefore, the LM1 region localised with this automated method may have included adjacent CSF tissue in some subjects which has different resistive properties than grey matter. Under these circumstances, the extracted electric field intensities for some subjects may not reflect the exact current flow in the hand knob region of the motor strip. Normalisation may therefore limit the accuracy of dose-control. Whilst it may be more time consuming, manual localisation of the hand knob in the motor cortex for each subject may prove to provide more accurate electric field intensities represented in the LM1. Individualising tDCS according to the electric field intensity at the manually selected region may result in more consistent tDCS effects. However, segmentation inaccuracies and MRI limitations such as differences in the

orientation of the scan across participants be taken in consideration as these can exacerbate incongruities across scans in group analyses.

6.4.2 Correspondence of LM1 targeted by tDCS versus TMS

A further limitation of the dose-control methodology (Evans et al., 2019) that can be improved is the correspondence of the LM1 ROI that tDCS-dose was targeted and controlled for, versus the region targeted by TMS as the motor hotspot. Ideally, the region targeted with tDCS should be the same as the motor hotspot targeted by TMS to ensure that the electric field dose (controlled for this region), impacts the same location from which excitability changes are then measured. Note, that the electric field induced by individualised-dose tDCS is only constant at the targeted region and remains just as variable as without individualisation in any other cortical regions (Evans et al., 2019). By this means, excessive discrepancy between the dose-controlled, tDCS-targeted ROI and the TMS hotspot would defeat the purpose of individualising dose. This discrepancy may have been the case in **Chapter 5**, as the LM1 region targeted with tDCS, was defined by fixed coordinates of the 10/10 system (i.e. C3), which is based on skull anatomy (Seeck et al., 2017), instead of each subject's motor hotspot, as was functionally determined by TMS (Caparelli-Daquer et al., 2012; Kuo et al., 2013; Nitsche & Paulus, 2000; Rawji et al., 2018). Since the motor hotspot, identified functionally, is often not located exactly at anatomical M1, (as defined by the 10/10 system; C3), and may slightly vary across individuals, this might have introduced regional incongruity. Given the above, the efficacy of dose-control in **Chapter 5** may have been compromised because the LM1 was located and stimulated with tDCS using the conventional EEG system, whereas excitability was measured using the functionally defined individual motor hotspot. Future studies employing this methodology could therefore identify the motor hotspot region with TMS, prior to modelling tDCS current flow. This location should then be used as target for current flow modelling. A possible way to do this is to use the subject's individual structural MRI with the neuronavigation system, calibrating the scan to the subject's head and identifying the motor hotspot functionally. When the TMS hotspot is identified, the coil location should be extracted for consistent placement in the upcoming sessions, as well as extracting the regional 3D coordinates of the LM1 hotspot

to be used for current flow modelling. However, this method would inevitably increase the number of sessions participants need to attend and thus requires a lot of time.

6.4.3 ROI selection versus brain-wide electric field

A further limitation of the individualised-dose method (Evans et al., 2019) that could be improved is the selection of the LM1 ROI size or shape. As outlined above, it is important that the region targeted with TMS corresponds to the region targeted with tDCS for current flow modelling, to measure the effects of individualised dose in the relative region. However, tDCS current flow is diffuse (Alam et al., 2014; Bortoletto et al., 2016; Edwards et al., 2013; Morya et al., 2019) and reaches untargeted regions across subjects (Evans et al., 2019; Laakso et al., 2019; Mikkonen et al., 2020). As aforementioned (section 6.2), untargeted brain areas that tDCS impacts play an important role in tDCS outcomes through functional network connectivity to M1 (Boros et al., 2008; Fischer et al., 2017; Kirimoto et al., 2011; Lefebvre et al., 2019; Matsunaga et al., 2004). Not accounting for the electric field intensity and focality of the entire electric field in the brain when individualising dose may still result in non-consistent effects between subjects. Thereby, considering both the entire electric field strength and distribution in the brain is functionally important for individualising dose.

In **Chapter 5**, the lack of effect and variability reduction with individualised-dose tDCS may be a result of individualising electric field in just one target region whilst discounting its variability and effect on untargeted brain regions. To improve individualised-dose methodology, the intensities of the global electric field in the brain should be compared and controlled for across subjects and not just in one region i.e., LM1 as this may prove more effective. To calculate the overall strength and distribution of the electric fields, the median magnitudes and normal components of the electric field over all subject scans should be determined, as previously suggested (see Mikkonen et al., 2020). Thereby, to quantify the focality of the EF on an individual level, the proportion of the surface area of the right hemisphere where the EFs exceeded 50% of the EF magnitude/normal component at the target point should be used (Mikkonen et al., 2020).

Although logically sound, there is currently no research investigating whether individualising dose based on the entire electric field in the brain proves more effective than individualising-dose based on the electric field reaching a specific area of the brain. Adding to this, there is currently no consensus in shape or size selection of the ROI in M1, or whether the entire motor strip should be selected versus the entire electric field volumetric area impacting the brain. Future research in this direction could help improve the methodology of individualising tDCS dose and determine the dose-response relationship, which in turn could help inform future protocols to deliver more consistent outcomes.

6.4.4 Conventional stimulation intensities may be insufficient to induce physiological effects.

While conventional tDCS intensities (1-2mA) may be effective for some individuals, tDCS methodology lacks a simple or inexpensive solution and biomarker to validate that the conventional dosage of tDCS is sufficient to produce any physiological effects in the cortex (Caulfield et al., 2020). Thereby, dose-control may result in some individuals receiving sub-effective doses, contributing to the inconsistent findings in the field (Chew et al., 2015; Horvath et al., 2015a, 2015b, 2016; Loo et al., 2010; López-Alonso et al., 2014, 2015; Wiethoff et al., 2014). There is great interest in establishing a method of dose-control as it would inform the experimental design and interpretation of tDCS research, providing more consistent outcomes and likely improving effect sizes. This would further allow for rigorous clinical and investigational use.

TMS-induced MEPs could be a potential physiological measurement that is sensitive to tDCS dose as one study showed that the TMS output intensity required to induce an MEP of 1 mV, was directly related with electric field produced by 1 mA tDCS (Mikkonen et al., 2018). Following from this, it has been recently shown that tES motor thresholds or reverse-calculation modeling (Caulfield et al., 2020) can estimate individualized tDCS doses. However, when individualizing tDCS dose to deliver 1 V/m to LM1, electric field intensities vary substantially in a 5 x 5 x 5 voxel grid ROI and are above safety guidelines (*M* = 6.03 mA, *SD* = 1.44 mA, *range*: 3.75–9.74 mA). Although, TDCS applied at a uniform 1–2 mA dose may underdose some individuals (Caulfield et al., 2020), perhaps reverse

calculating electric fields whilst participants perform a relative task may lower the tES motor threshold and thereby could potentially make this method applicable under the safety guidelines.

6.5 Methodological innovation

I here outline some conceptual and methodological strengths of the present thesis. To begin with, this thesis draws upon a wide range of research in the field on potential variability sources of tDCS effects. Although there are various sources of inter-subject variability including genetics, gender, age, hormone level, time of day, parallel motor activity, attention level and habituation to the presented task (Chew et al., 2015; Huang, Datta, et al., 2017; Wiethoff et al., 2014), these have not been the focus of this thesis. I here focused on factors more directly related to the delivery of tDCS, such as tDCS montage, direction of current flow, and the delivered electric field intensity and focality.

By considering for current flow direction, focality and intensity and individualising tDCS output to control electric field across subjects with different montages, this thesis utilised the most straightforward concept of dose-control implicated by previous research on the variability of tDCS effects (Bestmann & Ward, 2017; Evans et al., 2019). With the above in mind, this thesis advanced recent research demonstrating: 1) the relationship of electric field direction with the topography of the M1 (Rawji et al., 2018), 2) the relationship of tDCS-induced electric field intensity and changes in corticospinal excitability (Laakso et al., 2019), and 3) the relationship of intensity and focality in different montages (Mikkonen et al., 2020). Prospectively, controlling the electric field intensity, direction and focality, rather than with hindsight correlating it with tDCS outcomes, provides a greater assessment of the impact of current flow on tDCS effects, and paves the way toward identifying and improving criteria for dose-controlled tDCS to develop more effective and reliable stimulation protocols.

Methodologically, for every chapter, this thesis utilised well-controlled experimental designs by administering control groups (ML-tDCS current flow direction control-group, **Chapter 3**; sham-control group, **Chapter 5 Experiment 5.2**), and utilised double-blinded

(**Chapter 5 Experiment 5.2**) and neuronavigated tDCS (**Chapter 5** both experiments) as previously suggested (Horvath et al., 2015a) with within-subject comparison (**Chapter 5** both experiments), and responders grand average analysis (Wiethoff et al., 2014), enhancing statistical power. Additionally, power calculations were conducted to ensure sufficient statistical power for **Chapters 3** and **5**.

Using both an HD montage, which elicits more focal, less heterogeneous current flow than conventional tDCS montages (Datta et al., 2009; Datta et al., 2012), and a bipolar tDCS montage that controls for current flow direction which could potentially produce more consistent effects (Hannah et al., 2019; Rawji et al., 2018; Tremblay, Hannah, Rawji, & Rothwell, 2017), this thesis harnessed the benefits of a newly developed technique (Evans et al., 2019) in the field of brain stimulation and contributed to its validation, utility, and improvement. Neuronavigation was used to ensure that variability of tDCS responses were not caused by inconsistent TMS coil placement (**Chapter 5**). Any remaining MEP variability that could be explained by coil position was statistically accounted for which signifies an advancement from previous neuronavigation research (Herwig et al., 2001; Rodseth, Washabaugh, & Krishnan, 2017; Ruohonen & Karhu, 2010). As tDCS effects also suffer from high inter- and intra- subject variability, outcomes may be masked at a group level. To look at individual tDCS responses to tDCS, grand average analysis of group-specific responders and non-responders was used to investigate effects at a sub-group level, as suggested (Wiethoff et al., 2014).

Finally, I here utilised the open science framework (OSF) for research conducted on behalf of **Chapters 3** and **4** to increase the efficiency and effectiveness of my research whilst promoting transparency and collaboration through sharing and archiving documents and data related to my research projects. Registering research projects on the OSF involves introducing relevant literature that leads to my aims and hypotheses, followed by details of the experimental design and methodology used to answer the research questions. This allows for great planning and transparency to make scientific research and data accessible to all. For example, by registering details of my planned research, this could attract interest from other scientists conducting relevant

experiments which provides collaboration opportunities, sharing of data, and allows for greater reproducibility and transparency of scientific-work underway.

6.6 Clinical implications

Due to the ability of tDCS to modulate brain activity, it provides great promise for therapeutic applications. However, inconsistent results across clinical studies (Elsner et al., 2013; Lüdemann-Podubecká et al., 2014; Marquez et al., 2015) hinder its widespread adoption in clinical settings (Bestmann & Ward, 2017). With a potential dose-control methodology, tDCS could provide more consistent and reliable positive therapeutic outcomes, promoting its use for modulation of motor plasticity and learning in both health and disease, especially stroke (Butler et al., 2013; Chhatbar et al., 2016; Das et al., 2016; Elsner et al., 2016; Jackson et al., 2016; Kang et al., 2016a, 2016b; Kim et al., 2010; Pikhovych et al., 2016; Stagg et al., 2011; Ward, 2016). Currently, there is no tDCS protocol for stroke producing consistent and reliable outcomes and the myriad of combinations of target area, stroke phase, type of stimulation and task make it difficult to compare across studies. What is more, the heterogeneity of stroke type, lesion location and size, chronicity and cortical tract integrity vary the effects of tDCS (Bestmann & Ward, 2017; Feng et al., 2018) and perhaps individualising stimulation to the patient is one way to reduce such variability. The complexity of brain current flow modulation due to the detailed pathologic anatomy suggests that current flow models are vital for the scientific interpretation and design of individualized tDCS stroke-therapy (Datta et al., 2011). Currently, there is lack of control and individualisation of tDCS effects based on current flow models in clinical populations. This may further reduce the largely inter-subject and inter-study variability of tDCS outcomes in motor rehabilitation. However, clinical populations present a set of additional challenges over healthy populations that need to be considered for dose-control as discussed in the following.

6.6.1 Current flow modelling in a lesioned brain

Current flow models assign a set of conductivity values to each tissue type. Lesion sites are usually assigned CSF conductivity values based on imaging and histopathology studies (Datta et al., 2010; Jacobs et al., 2001; T. Wagner et al., 2007). Post-stroke lesioned brains introduce anisotropy by white matter tracts, which connect different areas of grey matter within the brain and carry nerve impulses (action potentials) between neurons. Additionally, post-stroke lesioned brains introduce anisotropy by lesions that vary in size, shape and location, small vessel disease, and a cystic nature of a chronic stroke whereby cysts are filled with CSF enclosed in a membrane, all of which may produce drastic incongruities in the electric field distribution (Datta et al., 2011, 2010; Suh, Lee, & Kim, 2012). Anisotropy by white matter fiber tracts across post-stroke individuals is rarely included in current flow models, which leads to suboptimal and inaccurate modelling of the generated electric field in the post-stroke brain (Lee, Liu, Mueller, Lim, & He, 2009; Metwally, Han, & Kim, 2015). Calculating tissue anisotropy, however, would require using current flow models with regressors from diffusion-weighted images to provide voxel by voxel estimates of anisotropy measures (Feng et al., 2018). Therefore, to provide more accurate individualised estimates of current flow models in clinical populations anisotropy as described above is one factor that may need to be considered. Even if modelling current flow through post-stroke brains is achieved accurately, there is currently no consensus of a tDCS protocol, nor biomarker that would be best suited for use in individualising tDCS for post-stroke patients to evoke consistent and reliable therapeutic outcomes.

6.6.2 Post-stroke cortical excitability differs to healthy populations

There remain fundamental unknowns about the neurophysiological relationship between the electric field and stroke recovery processes. This relationship is highly dependent on functional brain activity occurring in a post-stroke brain which greatly differs to a healthy brain (Feng et al., 2018; Ward, 2017; Ward & Cohen, 2004). There are conflicting views of brain modulation protocols to facilitate stroke recovery (Feng et al., 2018), thus, to understand how to dose-control effectively in a post-stroke brain we first need to understand cortical excitability following a stroke and how this might relate to recovery. Given tDCS can modulate cortical excitability and promote an excitable

brain environment, it thereby has great potential to support motor recovery (Ward, 2016).

Our current understanding partly comes from animal models (Biernaskie, Chernenko, & Corbett, 2004; Krakauer, Carmichael, Corbett, & Wittenberg, 2012; Ward, 2017; Zeiler et al., 2016), which have shown that cortical excitability increases during the first 3 months following a stroke and returns to pre-stroke excitability levels approximately six months post-stroke. This period of increased excitability shares a timeline with a period of rapid recovery seen after stroke in humans, whereby rapid recovery processes are more prominent immediately following stroke and continue up to six months after (Bernhardt et al., 2017; Krakauer et al., 2012; Ward, 2017). Following this period, motor recovery continues, especially with higher-intensity rehabilitation. However recovery is weaker following 6-months post-stroke and may be related to compensatory mechanisms (Ward, 2017; Ward, Brander, & Kelly, 2019). A recent study has validated that human post-stroke excitability indeed follows a similar trajectory to that seen in animals (Hordacre et al., 2021).

As we expect brain state to be different at different stages post-stroke, further research should first identify the time-point that tDCS application is most effective post-stroke which may importantly unravel the relationship between tDCS effects on post-stroke brain state. In order to do so, identification of appropriate biomarkers that bridge the gap between cellular, physiological and behavioural effects of tDCS on cortical function and plasticity in both healthy and post-stroke brain states, would help illustrate the efficacy of therapeutic interventions and aid the improvement of tDCS protocol choices (montage and intensity) for who to treat (type of stroke) and when to treat them (timing post-stroke).

6.6.3 Corticospinal tract (CST) integrity

With the rise in interest of biomarkers that predict patient motor recovery, several anatomical and functional measures have been identified that possess predictive

potential in early clinical assessment of motor impairment, including corticospinal tract intactness (Kwakkel, Kollen, van der Grond, & Prevo, 2003; Prabhakaran et al., 2008). Patients that have intact TMS-induced MEPs show better motor recovery, and on the contrary, patients with more extensive damage of the corticospinal tract typically have worse motor recovery (Bembenek, Kurczych, Karli Nski, & Czlonkowska, 2012; Burke & Cramer, 2013; Stinear, 2010). Although previous research using current flow models shows that electric field in the M1 correlates with motor thresholds in healthy participants (Mikkonen et al., 2018), this may not be the case with post-stroke patients with damaged corticospinal tracts, as the extent of damage may affect physiological outcomes regardless of the amount of electric field reaching a target, yet current flow models do not account for corticospinal tract intactness. Thus, patients with a great level of corticospinal tract damage that do not elicit TMS-MEP measures would need to be excluded from dose-controlled tDCS therapy if the individualised-dose protocol was based on CST integrity. This would limit the potential application of tDCS to patients with less damage of the corticospinal tract.

Incorporating information about cortical and subcortical damage in sensorimotor function in combination with CST damage has shown greater potential for predicting motor recovery (Rondina, Filippone, Girolami, & Ward, 2016). However, as there is currently no single clinical or neurophysiological measure that accurately explains individual recovery potential, the combinations of biomarkers could potentially provide greater insight of the capacity of motor recovery and help identify 'fit-for-purpose' biomarkers to individualise tDCS protocols. Possibly, in the near future, current flow modelling research in this field would adjudicate whether detailed, patient-specific tDCS protocols are important for therapeutic purposes or if the same protocol can be applied to all patients, regardless of lesion location, size, position and brain-state. In essence, we need to understand which inter-individual parameters (for example, lesion size) make a difference in tDCS outcomes in order to individualise tDCS effectively and induce more consistent beneficial effects. Importantly, this effort should be a simple, user-friendly solution that does not add to NHS bottlenecks so that it is adoptable in a clinical environment. Despite all advances, we are still far from a personalized tDCS therapeutic approach that considers patients' pathology.

6.7 Concluding remarks

This thesis portrays the complex relationship between brain physiology and non-invasive brain stimulation methods with the potential use of computational modelling to create an improved, individualised protocol for more consistent and reliable tDCS outcomes. My research and that of others, suggests promising directions to a deeper understanding of the underlying motor physiological mechanisms and their relationship with tDCS-induced electric fields. Although all the work here is based on improving the application of tDCS with the use of current flow models in a laboratory setting, my hope is that advancements in this field through incorporation of various information on the neurophysiological mechanisms in health and disease and their relationship with tDCS electric fields, will lead to an optimised dose-controlled methodology of tDCS, assisting stroke survivors with a more effective and efficient journey to motor recovery.

Appendix

Appendix A:

Blinding questionnaire

Subject number: _____

In which sessions do you believe you received sham, and in which real stimulation?

How confident are you in these guesses?

Session 1	☐ sham stimulation	Not at all		reasonably		very	confident
	☐ real stimulation	☐	☐	☐	☐	☐	
Session 2	☐ sham stimulation	Not at all		reasonably		very	confident
	☐ real stimulation	☐	☐	☐	☐	☐	
Session 3	☐ sham stimulation	Not at all		reasonably		very	confident
	☐ real stimulation	☐	☐	☐	☐	☐	

Appendix B:

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DEPARTMENT OF CLINICAL
AND MOVEMENT NEUROSCIENCE
University College London

Safety Screening Questionnaire for Transcranial Direct Current Stimulation (tDCS) and Transcranial Magnetic Stimulation (TMS)

Participant Pseudonym: _____ **Date:** _____

Current Age: _____ (in years) **Handedness:** Left Right Both

Have you ever had an adverse reaction to tDCS or TMS?	☐Yes	☐No
Do you have epilepsy or have you ever had a seizure/convulsion?	☐Yes	☐No
Have you ever had a fainting spell or syncope? If yes, describe.	☐Yes	☐No
Have you ever had a stroke?	☐Yes	☐No
Have you ever had a serious head injury (with loss of consciousness)?	☐Yes	☐No
Have you ever had neurosurgery of any type (including brain or spinal cord)?	☐Yes	☐No
Do you have hearing problems or ringing in your ears?	☐Yes	☐No
Do you have any metal in your body such as shrapnel, surgical clips, or fragments from welding or metalwork?	☐Yes	☐No
Do you have any implanted devices such as cardiac pacemakers, aneurysm clips, cochlear implants, medical pumps, deep brain stimulators, or intracardiac lines?	☐Yes	☐No
Do you have a medication infusion device?	☐Yes	☐No
Do you suffer from frequent or severe headaches?	☐Yes	☐No
Have you ever had any other brain-related condition?	☐Yes	☐No
Have you ever had any illness that caused brain injury?	☐Yes	☐No
Are you taking any psychiatric or neuroactive medications? (please list)	☐Yes	☐No
Are you taking any other medications or other drugs/substances?	☐Yes	☐No
Are you pregnant or do you have any reason to believe that you may be?	☐Yes	☐No
Do you hold a heavy goods vehicle driving license or bus license?	☐Yes	☐No
Have you consumed alcohol in the past 24 hours?	☐Yes	☐No
Did you have adequate sleep last night?	☐Yes	☐No
Have you participated in a TMS or tDCS study within the past 24 hours?	☐Yes	☐No

Potential Contraindication Drugs

Strong Potential Hazard: imipramine, amitriptyline, doxepine, nortriptyline, maprotiline, chlorpromazine, clozapine, foscarnet, ganciclovir, ritonavir, amphetamines, cocaine, (MDMA, ecstasy), phencyclidine (PCP, angel's dust), ketamine, gamma-hydroxybutyrate (GHB), alcohol, theophylline

Relative Potential Hazard: mianserin, fluoxetine, fluvoxamine, paroxetine, sertraline, citalopram, reboxetine, venlafaxine, duloxetine, bupropion, mirtazapine, fluphenazine, pimozide, haloperidol, olanzapine, quetiapine, aripiprazole, ziprasidone, risperidone, chloroquine, mefloquine, imipenem, penicillin, ampicillin, cephalosporins, metronidazole, isoniazid, levofloxacin, cyclosporin, chlorambucil, vincristine, methotrexate, cytosine arabinoside, BCNU, lithium, anticholinergics, antihistamines, sympathomimetics.

Withdrawl Hazard: alcohol, barbiturates, benzodiazepines, meprobamate, chloral hydrate.

Appendix C:

Participant Information Sheet for Healthy Adult Participants

UCL Research Ethics Committee Approval ID Number: 12011/001

YOU WILL BE GIVEN A COPY OF THIS INFORMATION SHEET

Title of Study: Optimising the application of Transcranial Direct Current Stimulation

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Department: Sobell Department of Motor Neuroscience and Movement Disorders

Name and Contact Details of the Researcher(s):

Name and Contact Details of the Principal Researcher:

You are being invited to take part in a PhD research project. Before you decide to volunteer it is important for you to understand why the research is being done and what participation will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. ***Thank you for reading this.***

1. What is the project's purpose?

The purpose of the research is to investigate how a neuroscientific tool (transcranial direct current stimulation) may be used optimally to promote motor learning and other forms of behaviour, which is of interest for a potential application in neurorehabilitation. The research questions include: Where and how much current is delivered to the brain and how does it affect behaviour? Considering that everyone's brains are different physically and cognitively; what are the optimal parameters of tDCS to bring the most promising results for motor learning in every

individual? To address these questions, we are stimulating and imaging the brains of healthy individuals while they perform different tasks.

2. Why have I been chosen?

You have been chosen because you are a healthy adult between the ages of 18-40 and have been screened for the health and safety questionnaire for Transcranial electrical stimulation. 160 participants are chosen for this study who are all right-handed individuals. No left-handed, vulnerable adults or children will be used for this study.

3. Do I have to take part?

Taking part in the study is entirely voluntary. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason and it will not disadvantage you in any way. If you decide to withdraw you will be asked what you wish to happen to the data you have provided up that point.

4. What will happen to me if I take part?

Before the experiment starts you will be asked a number of questions regarding your physical health, especially any history of neurological disorders suffered by yourself or close family members and the details of any medication you are taking. You will also be asked about any metal apparatus in your body, such as a cardiac pacemaker or implanted medication pumps. If you are pregnant or think you might be pregnant then you should not participate in the study. These questions are solely for ensuring that you will not be exposed to any avoidable risks, and the questions that will be asked solely concern the standard criteria for participating in these kinds of experiments.

How long will the study take?

This depends on the particular experiment in which you are taking part. You will be asked to complete up to (checked option applies):

☐ TDCS Behaviour-only Experiment: 1 session of 1.5-2 hours.

☐ Structural MRI Experiment: 1 session of 1 hours.

▣ Combined TDCS-TMS Experiment: 1 session of 1.5 hours.

For each experiment, you will be reimbursed for your travel and time /per hour.

Experimental Procedures

Safety guidelines will be strictly followed when applying tDCS, TMS and structural MRI. To control MRI, TMS and tDCS risks participants will also undergo a detailed screening to determine their suitability. This study will be conducted by highly trained and professional members of the Institute of Neurology.

As indicated by the checked box, you will undergo one, or a combination of the following experimental procedures, which are indicated below:

☐ **Behavioural task only**

You will be asked to perform some simple behavioural tasks. The specific requirements of each task will be described to you verbally and through written instructions by an investigator. For example, you will be asked to make a button-press response to picture stimuli. None of these tasks carries any risks or discomfort other than potential boredom with the simple task. Regular rest breaks will be offered to avoid fatigue.

☒ **Transcranial Direct Current Stimulation**

TDCS (Transcranial Direct Current Stimulation) is a technique that induces a weak electrical current in a broad area of the brain, using electrodes attached to the scalp safely and painlessly. There might be a tickling sensation due to the skin or the muscles reacting to the weak electrical stimulation. However, the electrodes do not heat up during stimulation.

Are there any risks associated with TDCS?

TDCS (medium-risk): There are reports of transient enhancements in selective cognitive abilities following tDCS. The procedures used in the proposed work lead, however, at best to transient and reversible enhancements.

☐ **Structural MRI Scan**

Magnetic resonance imaging is a painless and safe technique that provides pictures of the brain (as well as other parts of the body). During each MRI session you will be asked to remain still on a comfortable padded table inside a large tube that comprises the imaging magnet. The scanner is loud while it is working and you will wear protective ear plugs. Because of the powerful magnetic field involved, you must not bring any metal into the scanner room. ***If you have any non-removable metal in or on your body, then you can not have an MRI scan.*** If you have any questions about what is allowed, we can help you to determine whether you are eligible. Some of the exclusion criteria include the presence of: cardiac pacemaker, aneurysm clip, cochlear implants, pregnancy, shrapnel, history of metal fragments in eyes, or neurostimulators. Once you are inside the MRI scanner, we will ask you to stay inside for up to 30 minutes. If you suffer from claustrophobia, then being inside the tube of the magnet may be uncomfortable for you and it may not be possible for you to participate. Please discuss this with us if you are concerned about it.

If you have acquired an MRI scan, we will use the scan to model how current would flow through the different tissues of the brain in order to individualise the tDCS current intensity accordingly. This process will be done prior to your participation in the tDCS experiment.

Are there any risks associated with MRI?

Structural MRI (low-risk): at present, MRI carries no known risks of injury or discomfort in participants who answer NO to a set of specified criteria.

☐ **Transcranial Magnetic Stimulation (TMS)**

Transcranial Magnetic Stimulation TMS (Transcranial Magnetic Stimulation) was developed in 1985 and has been widely used as a research tool in neuroscience. In TMS, a figure-of-eight coil delivers a magnetic pulse to the scalp, which induces an electrical current in a small area of brain underneath the coil. The coil is held to the participant's scalp by the experimenter. The technique is not painful but at high stimulation intensities there may be some incidental stimulation of the muscles in your face, which you may experience as a jaw twitch or eye blink. Similarly, the magnetic pulse in the coil makes a loud clicking noise and this becomes louder at

high intensities. These unintended effects can be unpleasant, and if you are experiencing them you should ask the experimenter to stop the experiment. Earplugs are available if you would like to wear these during the experiment to minimise discomfort from noise.

Are there any risks associated with TMS?

TMS (Low-Risk): Some types of TMS carry a small risk of seizures, but this is extremely rare for the type of TMS used in this study (Single-Pulse)

☐ **Electroencephalography (EEG)**

EEG is a painless, non-invasive, and safe technique that records small changes in the electric field surrounding the head. EEG involves placing a set of electrodes on your head. These electrodes are held with a conductive paste to your head. 2 electrodes will be filled with a gel that will make contact between the electrode and your scalp. The fitting of the electrodes and gel will likely disrupt your hairstyle. We will provide you with a place to clean up after the procedure. During the actual recording procedure we will ask you to remain still and minimize blinking and eye movements.

Are there any risks associated with EEG?

EEG is safe and is not known to have any harmful side effects.

☒ **Combined TDCS-TMS**

The details regarding experimental procedures, equipment and safety requirements are identical to those described above separately for TMS and TDCS. There are no increased risks known to be associated with combining EEG, TMS and TDCS.

5. What are the possible benefits of taking part?

It is unlikely that you will benefit personally from participating in this research project, but the results will help our understanding of some basic functions of the healthy human brain. Indeed, we hope that the results of this study will eventually help us to understand impairments in motor functions in certain neurological conditions such as Stroke.

6. What if something goes wrong?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. Contact details are provided at the top of this information sheet. If you remain unhappy and wish to complain formally, you can report this through research-incidents@ucl.ac.uk. All communication will be dealt in strict confidence. You can also contact the Chair of the UCL Research Ethics Committee – ethics@ucl.ac.uk

7. Will my taking part in this project be kept confidential?

All information that is collected from you during the course of the research will be kept strictly confidential, anonymised, and will be collected and stored in accordance with the Data Protection Act 1998. Data will be kept in secured accommodation and on secured computers in the Wellcome Centre for Human Neuroimaging, Institute of Neurology, UCL. The data will be used only for the purpose of informing the research questions in this study, and only accessible by the relevant research teams at the Centre. The data will be retained indefinitely and securely, and may be accessed by the research teams for comparison with future data.

8. Limits to confidentiality

- Please note that assurances on confidentiality will be strictly adhered to unless evidence of wrongdoing or potential harm is uncovered. In such cases the University may be obliged to contact relevant statutory bodies/agencies.
- Please note that confidentiality will be maintained as far as it is possible, unless during our conversation I hear anything which makes me worried that someone might be in danger of harm, I might have to inform relevant agencies of this.
- Confidentiality will be respected subject to legal constraints and professional guidelines.
- Confidentiality will be respected unless there are compelling and legitimate reasons for this to be breached. If this was the case we would inform you of any decisions that might limit your confidentiality.

9. Use of Deception

Research designs often require that the full intent of the study not be explained prior to participation. Although we have described the general nature of the tasks that you will

be asked to perform, the full intent of the study will not be explained to you until after the completion of the study [at which point you may withdraw your data from the study.

10. What will happen to the results of the research project?

Other authenticated researchers will have access to your anonymised or pseudonymous data and it will be retained indefinitely and securely, and may be accessed by the research teams for comparison with future data. Once the study is complete, has been written up, presented within a PhD thesis, peer-reviewed and accepted in a scientific journal, you will be contacted by post with a summary of the findings, and can elect to receive a full copy of the report if you wish. You will not be identified personally in any publication.

11. Data Protection Privacy Notice

All information that is collected during the course of the research will be stored in accordance with the General Data Protection legislation –GDPR.

Notice:

The data controller for this project will be University College London (UCL). The UCL Data Protection Office provides oversight of UCL activities involving the processing of personal data, and can be contacted at data-protection@ucl.ac.uk. UCL's Data Protection Officer is Lee Shailer.

Your personal data will be processed for the purposes outlined in this notice. The legal basis that would be used to process your personal data will be the provision of your consent. You can provide your consent for the use of your personal data in this project by completing the consent form that has been provided to you.

Your personal data will be processed and will be retained indefinitely and securely, and may be accessed by the research teams for comparison with future data. If we are able to anonymise or pseudonymise the personal data you provide we will undertake this, and will endeavour to minimise the processing of personal data wherever possible.

If you are concerned about how your personal data is being processed, please contact UCL in the first instance at data-protection@ucl.ac.uk. If you remain unsatisfied, you may wish to contact the Information Commissioner's Office (ICO). Contact details, and details of data

subject rights, are available on the ICO website at: https://ico.org.uk/for-organisations/data-protection-reform/overview-of-the-gdpr/individuals-rights/

12. Who is organising and funding the research?

This research is organised by the researchers aforementioned in this form and it is funded by the principal investigators and the Biomedical Research Centre (BRC)

13. Contact for further information

Evridiki Gregoriou (PhD Student) Prof Sven Bestmann (Supervisor) Prof Nick Ward (supervisor)

Address: 33 Queen Square, London WC1N 3BG, United Kingdom

Thank you for reading this information sheet and for considering to take part in this research study.

Appendix D:

	V/m With Smoothing	V/m Without Smoothing
Mean	0.185	0.175
SD	0.033	0.034
Range	0.125-0.249	0.120-0.248

Table A.1 Electric Fields at target LM1 with smoothed vs unsmoothed EF images with PA-tDCS$_{fixed}$

The average EF in the LM1 target region with and without smoothing of EF images leads to qualitatively comparable EF estimates. Overall, not smoothing produced .01 V/m less EF strength at the target LM1.

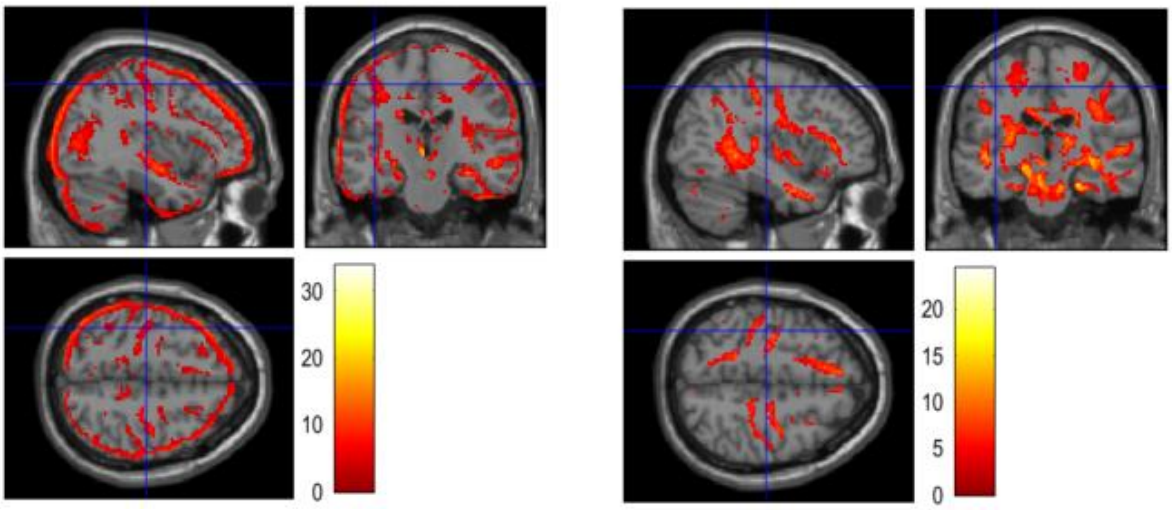

Figure A.1. Smoothing versus non-smoothing electric field images

Clusters that are significantly higher in electric field intensity when smoothing electric field images (left) and without smoothing (right). A paired t-test comparing smoothed vs non-smoothed EF at the whole brain level showed a statistically significant increase (T (51) = 3.26, p=.001) in the clusters shown (red). Crosshair indicates LM1 coordinates (-38 -20 50). Legend indicates the strength of electric field greater than 0 with dark red indicating minor differences in electric field and bright yellow indicating greater differences in electric field. Note that these differences are numerically marginal. Whether smoothing/non-smoothing, normalization/non-normalization is a better or worse method for dose-control is currently unknown, and there is no literature that has identified the 'optimum' process for tDCS$_{indiv}$ output.

	PA-tDCS$_{fixed}$ (1mA)		HD-tDCS$_{fixed}$ (1mA)	
	5mm ROI	**10mm ROI**	**5mm ROI**	**10mm ROI**
Mean (V/m)	0.185	0.210	0.128	0.137
SD (V/m)	0.033	0.034	0.028	0.030
Range (V/m)	0.125-0.249	0.136-0.275	0.069-0.198	0.072-0.208

Table A.2. Difference between EF for 5mm vs 10mm ROI at LM1 for PA-tDCS$_{fixed}$ vs HD-tDCS$_{fixed}$ montages

EF average is greater when increasing ROI from 5mm to 10mm. Differences here are miniscule to infer that a larger spherical ROI would make any significant difference in the data. Currently, there is no scientific indication on which type of ROI should be used, whether that is tissue type i.e grey matter, white matter, the motor strip versus a spherical ROI, or whether the entire electric field projected in the brain should be accounted for.

Appendix E:

CONSENT FORM FOR HEALTHY PARTICIPANTS IN RESEARCH STUDIES

Please complete this form after you have read the Information Sheet and/or listened to an explanation about the research.

Title of Study: "Optimising the application of transcranial direct current stimulation."

Department: Department of Clinical and Movement Neurosciences

Researcher(s): Evridiki Gregoriou

Principal Researcher: Prof Sven Bestmann

UCL Data Protection reference: Z6364106/2017/10/106 health research

This study has been approved by the UCL Research Ethics Committee: Project ID number: 12011/001

Thank you for considering taking part in this research. The person organising the research must explain the project to you before you agree to take part. If you have any questions arising from the Information Sheet or explanation already given to you, please ask the researcher before you decide whether to join in. You will be given a copy of this Consent Form to keep and refer to at any time.

I confirm that I understand that by ticking/initialling each box below I am consenting to this element of the study. I understand that it will be assumed that unticked/initialled boxes means that I DO NOT consent to that part of the study. I understand that by not giving consent for any one element that I may be deemed ineligible for the study.

		Tick Box
1.	*I confirm that I have read and understood the Information Sheet for the above study. I have had an opportunity to consider the information and what will be expected of me. I have also had the opportunity to ask questions which have been answered to my satisfaction *and would like to take part in the above study*	
2.	*I understand that I will be able to withdraw my data up to 4 weeks after my participation in this study	
3.	*I consent to the processing of my personal information *hand movement responses, MRI scan,* for the purposes explained to me. I understand that such information will be handled in accordance with all applicable data protection legislation.	
4.	**Use of the information for this project only** *I understand that all personal information will remain confidential and that all efforts will be made to ensure I cannot be identified. I understand that my data gathered in this study will be stored with a pseudonym (any fictitious name) I select, securely. It will not be possible to identify me in any publications.	

5.	*I understand that my information may be subject to review by responsible individuals from the University (to include Brain Research Trust) for monitoring and audit purposes.	
6.	*I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason, [*without the care I receive or my legal rights being affected*]. If the study involves deception, I have the option to withdraw the data I provide once I find out the true purposes of the research. I understand that if I decide to withdraw, any personal data I have provided up to that point will be deleted unless I agree otherwise.	
7.	I understand the potential risks of participating and the support that will be available to me should I become distressed during the course of the research.	
8.	I understand the direct/indirect benefits of participating.	
9.	I understand that the data will not be made available to any commercial organisations but is solely the responsibility of the researcher(s) undertaking this study.	
10	I understand that I will not benefit financially from this study or from any possible outcome it may result in in the future.	
11	I understand that I will be compensated for the portion of time spent in the study (if applicable) or fully compensated if I choose to withdraw.	
12	I agree that my anonymised research data may be used by others for future research. [No one will be able to identify you when this data is shared.]	
13	I understand that the information I have submitted will be published as a report and I wish to receive a copy of it. Yes/No	

14	I hereby confirm that I understand the inclusion criteria as detailed in the Information Sheet and explained to me by the researcher.	
15	I hereby confirm that: (a) I understand the exclusion criteria as detailed in the Information Sheet and explained to me by the researcher; and (b) I do not fall under the exclusion criteria.	
16	I agree that my GP may be contacted if any unexpected results are found in relation to my health.	
17	I have informed the researcher of any other research in which I am currently involved or have been involved in during the past 12 months.	
18	I am aware of who I should contact if I wish to lodge a complaint.	
19	I have been informed that there is a small risk of seizures in individuals with a family history of epilepsy.	
20	I voluntarily agree to take part in this study.	
21	Use of information for this project and beyond I would be happy for the physiological data (motor -evoked responses) I provide to be archived at UCL Data Safe Haven and the Wellcome Centre for Human Neuroimaging, Institute of Neurology, UCL if I have acquired a head scan. I understand that the data will be used only for the purpose of informing the research questions in this study, and only accessible by the relevant research team.	

	I understand that all information that is collected from me during the course of the research will be collected and stored in accordance with the Data Protection Act 1998. I understand that other authenticated researchers will have access to my pseudonymised data and that it will be retained indefinitely and securely, and may be accessed by the research teams for comparison with future data.	

If you would like your contact details to be retained so that you can be contacted in the future by UCL researchers who would like to invite you to participate in follow up studies to this project, or in future studies of a similar nature, please tick the appropriate box below.

	Yes, I would be happy to be contacted in this way	
	No, I would not like to be contacted	

_____	_____	_____
Name of participant	Date	Signature
_____	_____	_____
Researcher	Date	Signature

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