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AARON KORTTEENNIEMI

Safety and metabolic effects of transcranial electrical stimulation

SAFETY AND METABOLIC EFFECTS OF TRANSCRANIAL ELECTRICAL STIMULATION

Aaron Kortteenniemi

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ABSTRACT

Transcranial electrical stimulation (tES) is a relatively new neuromodulation method, which has been investigated in the treatment of depression and substance dependence, among other conditions. In general, it has been regarded as safe, with very few serious adverse effects (AEs) described in the literature. Additionally, tES has been described to both alter central metabolism and affect peripheral circulating compounds.

Despite the general safety of the tES methods, various mild AEs have been described, ranging from headache to skin lesions under the stimulation electrodes. Despite being considered mild, these AEs are of practical importance, because they dictate the tolerability of stimulation and may lead to treatment cessation. The factors modifying these mild AEs have only been cursorily explored in the literature. Of particular interest is the effect of consecutive stimulations, which could result in intensified AEs. Regarding the metabolic effects, some of the changes observed in central metabolism could lead to peripheral alterations via the blood–brain barrier. Although blood sampling might offer an enticing alternative to expensive and labour-intensive magnetic resonance spectroscopy as a way to investigate tES-induced metabolic changes, the effects of tES on peripheral metabolites have not been thoroughly investigated. In fact, to my knowledge, only a few compounds have been studied.

To investigate these issues, we obtained two samples. The first sample consisted of 82 males, split into two groups, one receiving transcranial direct current stimulation and the other sham stimulation for five consecutive days in a double-blind setting. Blood samples were obtained on days one and five and analysed with mass spectrometry to determine the metabolomic reading for a total of 102 metabolites. The second sample consisted of 60 males and females, each receiving

transcranial random noise stimulation and sham stimulation in a cross-over study setting. Data on AEs, as well as data regarding lifestyle factors, were collected via questionnaires. Appropriate statistical methods were employed to analyse the data, and a computer cluster environment was used to perform power calculations.

We observed no impact of lifestyle factors on tES AEs. Skin redness (estimated on a scale of 0–100 by visual inspection) did not intensify over five consecutive stimulation sessions, and none of the analysed lifestyle factors were statistically significant predictors for AEs. Additionally, our models investigating the effects of tDCS on peripheral metabolites did not reach statistical significance. However, we performed extensive power calculations to estimate the sample sizes necessary for metabolomic studies.

Our findings further support the view of tES as a safe form of treatment. In addition, our findings may suggest that lifestyle factors do not modify tES AEs, although we cannot rule out the possibility of simply lacking the power to detect such effects. Our power calculations will provide a general estimation of a necessary sample size for any future researchers interested in examining the effects of tES on peripheral metabolites.

Keywords: Transcranial Direct Current Stimulation; Metabolomics; Safety; Double-Blind Method; Cross-Over Studies

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TIIVISTELMÄ

Aivojen heikkovirtastimulaatio on melko uusi neuromodulaatiomenetelmä, jota on tutkittu muun muassa masennuksen ja päihdehäiriöiden hoidossa. Sitä pidetään yleisesti turvallisena, ja kirjallisuudessa on kuvattu vain yksittäinen vakava, mahdollinen haittavaikutus. Lisäksi stimulaation on kuvattu muokkaavan keskushermoston metaboliaa että vaikuttavan perifeerisen verenkierron hormonitasoihin ja metaboliatuotteisiin.

Vaikka menetelmää pidetään turvallisena, kirjallisuus tuntee useita lieviä haittavaikutuksia päänsärystä elektrodien alle syntyneisiin iholeesioihin. Vaikka nämä haittavaikutukset ovat luonteeltaan lieviä, ne ovat erittäin merkittäviä heikkovirtastimulaatiohoitojen siedettävyyden näkökulmasta. Näihin haittavaikutuksiin vaikuttavia seikkoja on kuitenkin tähän mennessä tutkittu verrattain vähän. Erityisen kiinnostavaa on toistuvien, perättäisten stimulaatiokertojen mahdollinen vaikutus haittavaikutuksiin, sillä toistuvat stimulaatiokerrat voivat johtaa haittavaikutusten voimistumiseen. Myös heikkovirtastimulaation mahdolliset metaboliset vaikutukset voivat välittyä veri-aivoesteen yli perifeeriseen vereen. Vaikka verinäytteiden ottaminen voisi olla huomattavasti magneettiresonanssispektroskopiaa käytännöllisempi ja edullisempi menetelmä mahdollisten heikkovirtastimulaation aiheuttamisen metaboliamuutosten mittaamiseen, stimulaation vaikutuksia perifeerisiin metaboliitteihin ei ole juurikaan tutkittu.

Hyödynsimme kahta aineistoa selvittääksemme heikkovirtastimulaation haittavaikutuksia ennustavia tekijöitä sekä heikkovirtastimulaatioon mahdollisesti liittyviä perifeerisiä metabolisia muutoksia. Ensimmäinen aineisto koostui 82 miehestä. Miehet oli satunnaistettu kahteen ryhmään, joista toinen sai tasavirtastimulaatiota ja toinen lumestimulaatiota yhteensä viiden perättäisen

päivän ajan. Tutkimus oli kaksoissokkoutettu. Verinäytteet otettiin ensimmäisenä ja viidentenä päivänä, ja analysoitiin massaspektrometrialla. Toinen aineisto koostui yhteensä 60 vapaaehtoisesta miehestä ja naisesta, jotka saivat sekä kohinavirtastimulaatiota että lumestimulaatiota cross-over -asetelmassa. Tieto sivuvaikutuksista ja elämäntapatekijöistä kerättiin kyselyillä. Analyysiin käytettiin soveltuvia tilastollisia menetelmiä. Lisäksi käytössämme oli tietokoneklusteri voimalaskelmien tekemistä varten.

Tuloksemme jäivät enimmäkseen negatiivisiksi. Ihon punaisuus ei lisääntynyt viiden perättäisen stimulaatiosession aikana, ja yksikään tutkituista elämäntapatekijöistä ei merkittävästi ennustanut sivuvaikutuksia. Lisäksi havaitsimme, että aivojen tasavirtastimulaatio ei muuttanut perifeerisen veren metaboliittipitoisuuksia lumestimulaatioon verrattuna. Tulevan tutkimuksen tukemiseksi suoritimme lisäksi voimalaskelmia selvittääksemme tarvittavan otoskoon tuleviin metabolomiikkatutkimuksiin.

Tuloksemme vahvistavat vallalla olevaa käsitystä siitä, että aivojen heikkovirtastimulaatio on turvallinen menetelmä. Lisäksi elämäntapatekijöillä ei vaikuta olevan merkitystä stimulaation haittavaikutuksiin, joskaan emme voi sulkea pois mahdollisuutta siitä, että tutkimuksemme tilastollinen voima oli riittämätön vaikutusten havaitsemiseen. Voimalaskelmamme voivat olla jatkossa hyödyksi otoskoon valinnassa tutkijoille, jotka haluavat tutkia aivojen tasavirtastimulaation vaikutusta perifeeriseen metaboliaan.

Avainsanat: aivot; hermosto; transkraniaalinen tasavirtastimulaatio; haitat; sivuvaikutukset; turvallisuus

I dedicate this work to my friends
– you are, in every way that matters,
my family. I love you.

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Science has long since ceased to be a field of solitary hermits locked in their chambers and is now very much a team sport. This work, too, would not have been possible without my colleagues. I offer my humblest thanks to our research group and all the members of it – without you all, I would have lacked not only wisdom and advice, but the very data I was working with.

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To my friends, my chosen family: you make the life a journey worth experiencing, and I can never thank you enough for being in my life. You accept me, make me smile, and support me when my step falters. You laugh with me when I'm happy and wipe my tears when I'm mournful. You are the reason I live.

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Kuopio, 28th September 2020
Aaron Kortteenniemi

LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following original publications:

I Kortteenniemi A, Javadi AH, Wikgren J and Lehto SM. Progression of adverse effects over consecutive sessions of transcranial direct current stimulation. Clinical Neurophysiology 128.12: 2397-2399, 2017.

II Kortteenniemi A, Lehto SM and Javadi AH. Delayed, distant skin lesions after transcranial direct current stimulation. Brain Stimulation 12.1: 204-206, 2019.

III Kortteenniemi A, Ortega-Alonso A, Javadi AH, Tolmunen T, Kotilainen T, Wikgren J, Lehto SM. The impact of lifestyle factors on the intensity of adverse effects in single and repeated session protocols of transcranial electrical stimulation. Submitted for publication.

IV Kortteenniemi A, Ortega-Alonso A, Javadi AH, Tolmunen T, Ali-Sisto T, Kotilainen T, Wikgren J, Karhunen L, Velagapudi V, Lehto SM. Anodal tDCS over the left prefrontal cortex does not cause clinically significant changes in circulating metabolites. Frontiers in Psychiatry 11:403, 2020.

CONTENTS

ABBREVIATIONS

AE	Adverse effect
AUDIT-C	Alcohol use disorders identification test C
BBB	Blood-Brain barrier
CFS	Cerebrospinal fluid
DBS	Deep brain stimulation
DLPFC	Dorsolateral Prefrontal cortex
ECOG	Electrocorticography
ECT	Electroconvulsive therapy
EEG	Electroencephalography
fNIRS	Functional near-infrared spectroscopy
GEE	Generalised estimating equations
HD-tDCS	High-definition transcranial direct current stimulation
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MST	Magnetic seizure therapy
PMC	Primary motor cortex
SAM	Sympatho-adreno-medullary systems
tACS	Transcranial alternating current stimulation
tDCS	Transcranial direct current stimulation
tES	Transcranial electrical stimulation
tRNS	Transcranial random noise stimulation
WFSBP	World Federation of Societies of Biological Psychiatry

1 INTRODUCTION

Despite its apparent safety, transcranial electrical stimulation (tES) has a number of minor adverse effects (AEs, Moffa et al., 2017). TES comprises a range of painless, non-invasive techniques using weak electric currents to modulate neuronal resting potentials via electrodes placed on the scalp (Woods et al., 2015). During recent decades, tES has moved from the dusty laboratories of university researchers into bustling clinics for clinical testing. It has been studied for and suggested to be effective in the treatment of various conditions such as depression (Lefaucheur et al., 2017) and substance craving (Lefaucheur et al., 2017). The tES methods are non-invasive, cheap, simple to apply, and generally considered safe. In all of the current literature, very few serious AEs have been linked to tES (Rossi et al., 2009).

tES has also been suggested to modify central metabolism (Hunter et al., 2015). As the brain is the control hub of the body, metabolic or other changes in brain functions could also lead to changes in peripheral metabolism. Any changes in central metabolism might also be detectable in the peripheral circulation, as metabolic products move and are transported over the blood–brain barrier (Binkofski et al., 2011). Measuring the effects of tES on peripheral metabolites could both offer insights into the mechanisms of tES and potentially lead to new areas of utilization. Nevertheless, the effects of tES on peripheral circulating compounds have only been investigated in a few studies.

The reported AEs include symptoms such as headache and skin erythema (redness caused by increased blood flow in the capillaries) (Rossi et al., 2009). Recently, however, the lack of systematic research regarding these AEs has been pointed out (Brunoni et al., 2011). Moreover, while the use of longer, multi-week stimulation protocols is increasing in clinical studies (for example, see Loo et al., 2012; Rosset-Llobet & Fàbregas-Molas, 2017), the effect of consecutive stimulations on AEs has, to my knowledge, only been sparsely studied (Nikolin et al., 2018a). In this investigation, we aimed to increase knowledge of the factors affecting the adverse effects by examining the effect of lifestyle factors, namely alcohol use, exercise habits and smoking, as well as the effects of repeated tES sessions on the severity of the minor AEs. These factors were chosen because they are commonly recorded and discussed in clinical settings.

Investigating factors that could modify the intensity of mild AEs, such as lifestyle factors and the repetition of stimulation sessions, which in some cases result in treatment cessation, could lead to both better patient preparation and better patient selection for tES. Moreover, if peripheral metabolite changes are observed,

new insights into the mechanisms of action would be provided, and a new way of peering into the intracerebral changes already observed would be possible.

In summary, the main questions I sought to answer in this research were whether lifestyle factors modify tES AEs, whether these AEs intensify with consecutive stimulations, and whether tES has measurable effects on a panel of peripheral circulating metabolites.

2 REVIEW OF THE LITERATURE

2.1 TRANSCRANIAL ELECTRICAL STIMULATION

tES is a group of non-invasive brain stimulation methods based on the utilisation of weak, sub-threshold currents. They are generally considered safe and very few serious AEs have been reported (please see section 2.4.3 for one report of epileptic seizures potentially linked to tES, Bikson et al., 2016). They work by introducing an electrical field into the brain via electrodes placed on the scalp. The most common, and oldest, of these methods is transcranial direct current stimulation (tDCS). It uses a direct current to generate an unchanging (apart from the ramp-up and ramp-down periods at the beginning and end of the stimulation) electric field in the cerebral tissue. Other tES methods comprise transcranial alternating current stimulation (tACS), which generates an electric field with a cycling potential, and transcranial random noise stimulation (tRNS), which uses electrical random noise

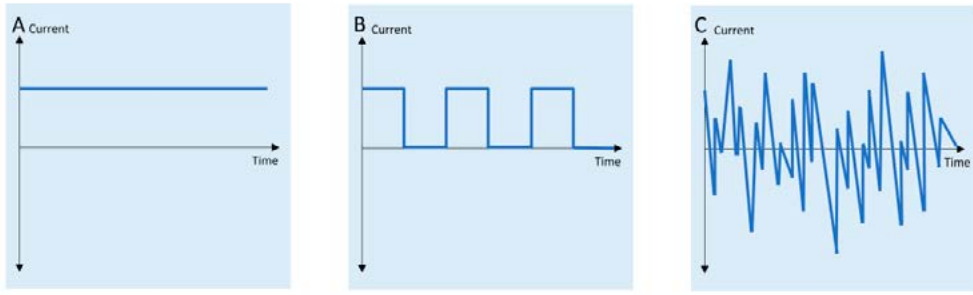

Figure 1. Transcranial electrical stimulation waveforms: A) tDCS, B) tACS and C) tRNS.

with a pre-determined frequency and voltage characteristics to modulate neural function. All of these function by affecting the membrane potentials of the neurons. Please see Figure 1 for an illustration of the waveforms in different types of tES.

2.1.1 Equipment

The equipment necessary for tES is quite simple and moderately priced. The most important piece of equipment is the stimulator, which is a precise current source. It should be programmable for different stimulation durations and types of current (DC, AC or random noise) and have safety features that discontinue the stimulation if the impedance rises too high. The stimulators also differ in terms of factors such

as blinding options, price and compatibility with magnetic resonance imaging (MRI). It is crucial that the stimulator delivers a precise current (Woods et al., 2015).[1] Most of the commercially available stimulators are CE certified (or a comparable national standard). [2] Please see Figure 2 for an example of a tES stimulator, and Table 1 for the specifications of three research stimulators.

Table 1. Three examples of research tDCS stimulators

Manufacturer	**neuroConn**	**Sooma**	**Soterix**
Device	DC Stimulator plus	tDCS stimulator	1x1 tES
Stimulation modes	tDCS, tACS, tRNS	tDCS	tDCS, tACS, tPCS, tODCS, tRNS
Current limits	±4,500 µA	3 mA	2 mA
Study mode	Yes	Yes	Yes
MRI capable	Yes (with addons)	Not specified	Yes (with addons)

In addition to the stimulator, electrodes are needed. The most commonly used electrode assembly consists of a conductive rubber core surrounded by saline-soaked sponges. As the electrode is a site for electrochemical reactions, an electrolyte, commonly saline, is necessary as a buffer (Woods et al., 2015). However, oversaturating the sponge can lead to imprecise application of the current due to saline leaking outside the sponges and creating an uncontrolled, expanded contact area (Woods et al., 2015).

Another electrode option, commonly used with high-definition tES, is silver–silver chloride electrodes (e.g., Sreeraj et

Figure 2. Example of a tDCS stimulator by Sooma Medical. The image belongs to Sooma Medical, used with permission.

[1] To my knowledge, no exact definition for "precise" exists for this in the literature.

[2] Meaning that the manufacturer states that the devices comply with all the EU regulations pertaining to them, or comply with a similar national standard outside of the EU

al., 2018). As saline sponges are more difficult to use with smaller electrodes, electrically conductive paste (such as the paste designed for use with electroencephalography [EEG] applications) or electrically conductive gel is used. However, these electrodes tend to be more expensive than saline sponge electrode assemblies.

To secure the electrodes to the scalp, several methods are used. The most frequently used solutions consist of either elastic rubber straps or caps like those utilised for EEG recording.

In addition to the scientific and medical equipment described here, there is a market for home-use tDCS devices, with several companies providing such equipment. In addition to the commercial home stimulators, plans for DIY devices also circulate on Internet message boards.

2.1.2 Targeting

The most simplistic method for targeting tES is as follows: the electrodes are placed on the scalp based on, for example, the EEG 10-20 system without imaging the underlying brain, with the electrode placement selected under the assumption that the stimulation targets the brain tissue under the electrodes, and the effect is independent of the position of other electrode(s). These assumptions, however, have not proven to be accurate. Nitsche & Paulus (2000), for example, have demonstrated that the effects of anodal stimulation are dependent on the location of the cathode, most likely explained by different a field geometry influencing different neuronal populations. Woods et al. (2016), on the other hand, demonstrated that even a 1 cm change in electrode position can drastically change the results, highlighting the usefulness of brain imaging in planning tES electrode montages.

Simulation studies have also suggested that the voltage distribution is rather diffuse and imprecise with tES methods (Datta et al., 2010). Indeed, in the same study (Datta et al., 2010), the peak intensity of the electric field was not under the anode at all. To address these issues related to the use of tES, it has been suggested that after the target brain areas have been identified, the optimum montage should be worked out *a priori* with computer modelling to improve focality and intensity (Dmochowski et al., 2011).

Examples of software modelling packages used for this purpose include simNIBS (Saturnino et al., 2019) and ROAST (Huang et al., 2019). Stimulation can be planned using a single brain model or by using individual brain scans from each participant. The latter could be argued to be more accurate, as individual differences in anatomy can affect the resulting electric fields (Opitz et al., 2015). This could be particularly

important when disease-induced anatomical changes are present. Current research suggests this is not an issue with major depressive disorder (Csifcsák et al., 2018), but could be a problem with stroke (Minjoli et al., 2017).

2.1.3 Electrode montages

Several different kinds of montages have been used. Please see Figure 3 for examples of electrode montages. The most basic montage utilises two equally sized electrodes on the scalp (described, for example, in Brunoni et al., 2016). Some use a smaller electrode to better focus the current, and a large reference electrode to decrease the cathodal current intensity and thus dilute unwanted effects in the areas under the return electrode (Boggio et al., 2009). Others, for the same purpose, place the reference electrode on, for example, the shoulder of the subject (Powell et al., 2019). Of particular interest is high density (HD)-tDCS, where an array of small electrodes is used to better focus the current (Wang et al., 2018). For example, one anode might be surrounded by a ring of cathodes, allowing the stimulation of just one region, without any unwanted stimulation of remote areas. Such a stimulation protocol has been used, for example, by Sreeraj et al. (2018).

Brain areas have different functions, and several electrode montages have been developed in order to target different brain areas and achieve different desired effects. For example, stimulation of the primary motor cortex (PMC), with the cathode on the contralateral supraorbital area, has been used to treat neuropathic pain (Fregni et al., 2006). As the right dorsolateral prefrontal cortex (DLPFC) has been associated with decision making and anodal tDCS over it has been observed to decrease risk taking, placing the anode over the right DLPFC and the cathode over left has been used to reduce substance craving (Fecteau et al., 2014). The treatment of major depressive disorder has been attempted, for example, with the anode over the left DLPFC and the cathode on the lateral aspect of the contralateral orbit (Loo et al., 2010). Please see Figure 3 for examples of tDCS montages used for different purposes.

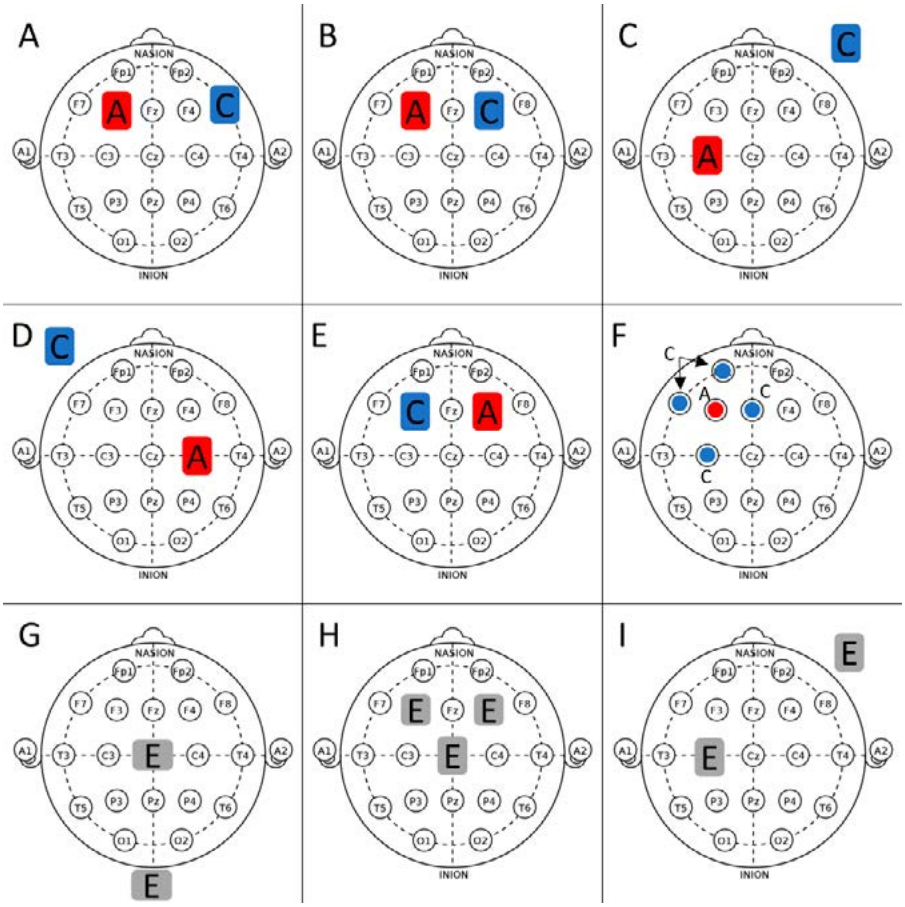

Figure 3. Examples of tES montages. The 10-20 background image is from Wikimedia Commons, by user トマトン124, public domain. Red/A is the anode, blue/C is the cathode, grey/E is a nonspecific electrode.
A) Depression: tDCS, the anode over the left dorsolateral prefrontal cortex (DLPFC) and the cathode over the lateral right frontal area. The treatment target was depression. (Loo et al., 2018)
B) Depression: tDCS, the anode over the left DLPFC and the cathode over the right DLPFC. (Brunoni et al., 2013a)
C) Pain after spinal cord injury: tDCS, the anode over the left primary motor cortex and the cathode over the right supraorbital area. (Fregni et al., 2006)
D) Reduction of blood glucose levels: tDCS, the anode over the right primary motor cortex and the cathode over the left supraorbital area. (Kistenmacher et al., 2017)
E) Alcohol dependence: tDCS, the anode over the right DLPFC and the cathode over the left DLPFC. (Klauss et al., 2014)
F) Improving working memory: HD-tDCS, the anode over the left DLPFC and cathodes surrounding it. (Hill et al., 2017a)
G) Improvement of mental rotation performance: tACS, electrodes on top of the head and over the occipital prominence. (Kasten & Herrmann, 2017)
H) Depression: tACS, smaller electrodes over both DLPFCs and the return electrode over the vertex. (Alexander et al., 2019)
I) Increasing whole-brain excitability: tRNS, electrodes over the left primary motor cortex and the right supraorbital area. (Terney et al., 2008)

2.1.4 Dosage

As AEs are related to the cumulative effect of the current, the current density, obtained from the stimulation current and electrode surface as $\frac{Current}{Area}$, is considered to best describe the delivered stimulation dose (Bikson et al., 2016). However, some authors have argued that the charge density (a measure obtained from the stimulation time, current and electrode surface area as $\frac{Current * Time}{Area}$) is a better option, as it takes into account the time of stimulation, allowing for cumulative effects (Chhatbar et al., 2017).

For treatment applications, a single measure of the dose has not been established, as the parameters of the stimulation are complex enough they are not easily distilled into a single value (Woods et al., 2015). However, a dose–response relationship has been suggested with charge density and current density (Chhatbar et al., 2016), as well as the number of stimulation sessions (Folmli et al., 2018). Nevertheless, the dose–response curve, at least when treating tinnitus, does not appear to be linear (Shekhawat & Vanneste, 2018).

None of the previously mentioned dosage measurements takes into account individual variability in anatomy and/or susceptibility. Given that anatomical variability, both natural (Opitz et al., 2015) and acquired (Minjoli et al., 2017), can affect the resulting electric fields, the dosage could possibly be calculated (via computational modelling) for the targeted brain area, not the electrode surface. However, to my knowledge, no such work has been done.

2.2 EFFECTS OF TES

2.2.1 Neurophysiological effects of electrical fields at the cellular level

Neurons maintain a tightly controlled homeostasis of ions, creating a concentration gradient of ions such as sodium, potassium, calcium, magnesium and potassium over the cell membrane. This equilibrium is maintained by ion pumps, and at rest hovers around -70 mV. This balance is disturbed by any incoming signals, which can be inhibitory or excitatory, respectively lowering or raising the voltage. If the voltage near the axon exceeds the threshold value, a rapid, cascading depolarization (called

the "action potential[3]") is triggered, sending a nerve impulse racing along the axon (Bear, M. F., Connors, B. W., & Paradiso, 2007).

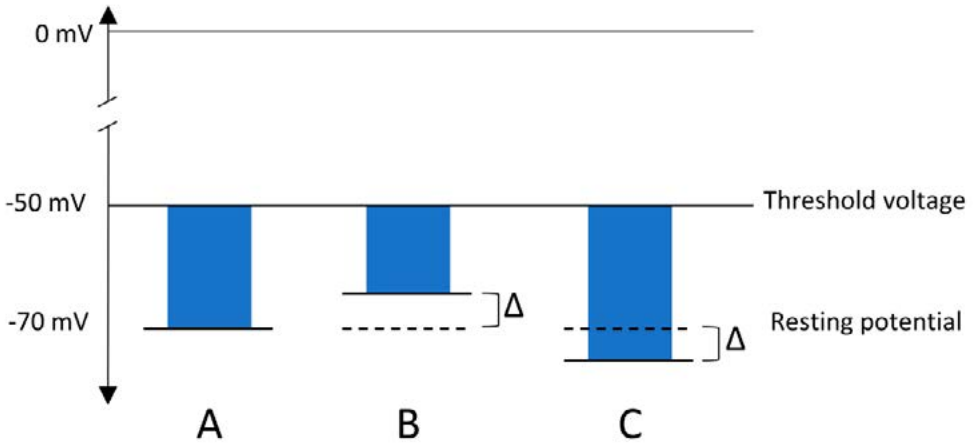

Figure 4. Illustration of the effects of tES on resting potentials. A = no stimulation, B = anodal stimulation, C = cathodal stimulation. The blue bars represent the stimulation necessary to reach the threshold voltage, Δ represents the effect of an external electric field, caused by stimulation, on the resting potential.

As the threshold voltage is dependent on voltage-gated ion channels, it is not easily changed by external sources. In order to fire, the neuron needs to raise its membrane potential from the resting voltage to the threshold voltage. Thus, the sensitivity of the neuron is dependent not only on the threshold voltage, but also on the resting voltage. If an electric field modifies the resting voltage, either lowering or raising it, it in turn either desensitizes or sensitizes the neuron to incoming excitatory signals. When a neuron is placed in an electric field, the external field alters the distribution of intracellular ions, altering the membrane potential (Radman et al., 2009). Please see Figure 4 for a simplified, exaggerated illustration of the effect[4]. However, in reality, the effect of stimulation is more complex, with factors such as cell–field interactions modifying the simple pattern of polarization (Ye &

[3] The action potential cascades through the axon, which ends in an synapse. Upon reaching the synapse, the action potential causes the release of a neurotrasmitter into the synaptic gap. This, in turn, can either affect another neuron (either by depolarizing or hyperoplarizing the cell membrane) or affect another tissue (for example, by causing muscle contraction).

[4] Bikson et al. (2004) and Fröhlich & McCormick (2010) report a resting potential change of 0.1–0.2 mV/V/m. Modelling studies, such as Miranda (2013), suggest that the peak electric field produced by 1 mA stimulation is around 0.38 V/m. This would equate to a change of 0.038–0.076 mV in the membrane potential. Such a small delta would be almost invisible in Figure 4, and thus the magnitude of the effect is exaggerated for visual purposes.

Steiger, 2015). An example of a cell–field interaction could be the external field affecting the cell, causing the cell to actively transport electrolytes over the cell membrane, which would affect the electric field caused by the cell, and which in turn is then superimposed onto the external field. The brain is not just a sum of simple, inert conductors, but responds to external stimuli by, for example, generating electric fields of its own.

Electric fields have been shown to affect the membrane potential of the cell membrane *in vitro* (Bikson et al., 2004). The effect is dependent on the direction of the electric field (Bikson et al., 2004): As the voltage generated is calculated as $\frac{Strength\ of\ the\ electric\ field}{Length\ of\ the\ conductor}$, the voltage generated by an electric field going across the length of the neuron is miniscule, and so is the effect of it. Conversely, if the electric field is directed along the long axis of the nerve cell (determined by its axon and dendrite configuration), the generated voltage is much higher.

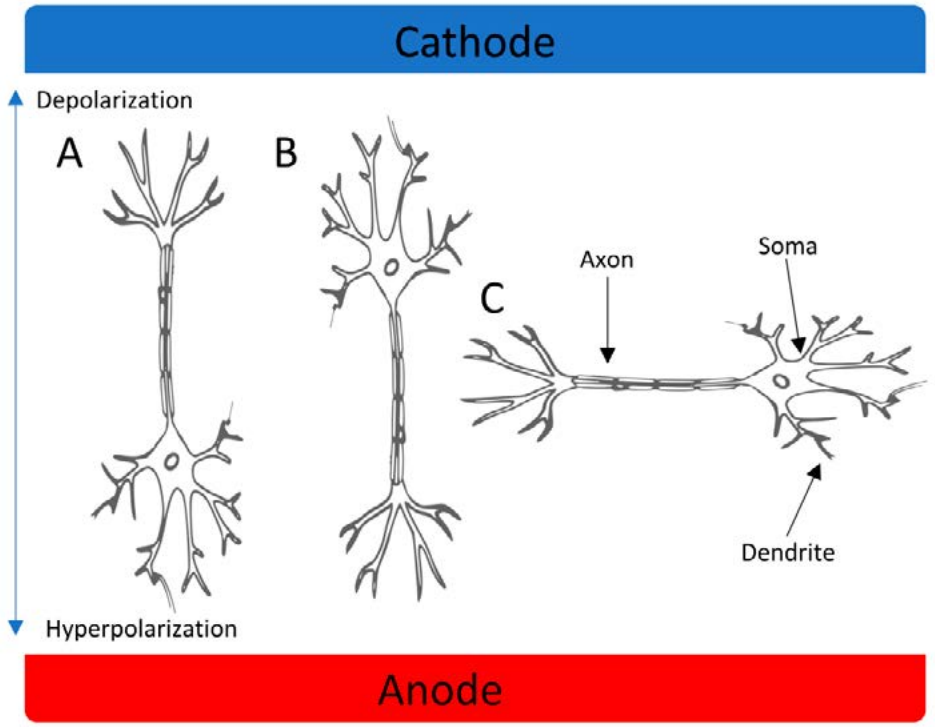

Figure 5. A = The soma towards the anode will hyperpolarize, decreasing excitability. B = The soma towards the cathode will depolarize, increasing excitability. C = electric field perpendicular to the neuronal axis, resulting in no changes in excitability. Image of the neuron by pixabay.com, used with permission.

The end of the neuron oriented towards the anode will depolarize, while the end oriented towards the cathode will hyperpolarize. The location of the soma determines the effect of the stimulation: if the soma is hyperpolarized, the threshold for firing is increased, and if it is depolarized, the reverse is true. If the axis of the cell is perpendicular to the electric field, no net polarization of the soma occurs. Thus, the orientation and morphology of the cell relative to the electric field determine the effect (Bikson et al., 2004). In practice, it is usually assumed that the target of tES is pyramidal neurons, which are assumed to be perpendicular to the surface. Thus, as the orientations of the cortex change in the sulci and the gyri, the effects of tES could be affected by the macroanatomy of the brain. Please see Figure 5 for an illustration of the effect of the neuronal axis on the response to external electric fields caused by electric fields.

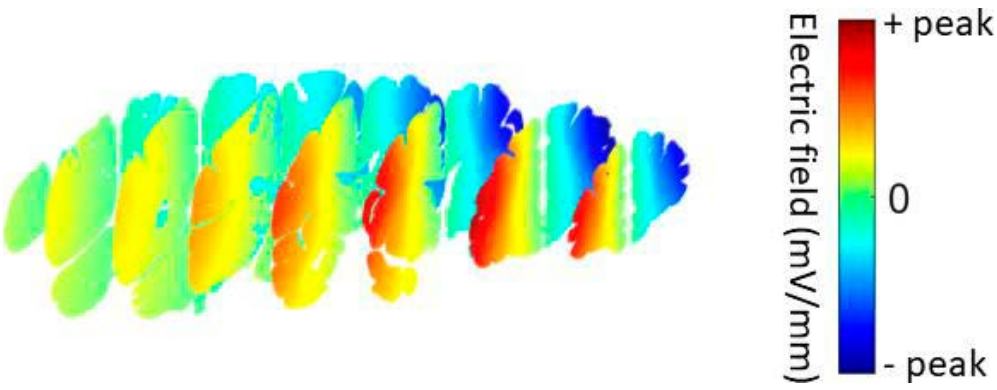

Figure 6. An example of electric field modelling from our substudy III. Note how the stimulation is quite spread out. Previously unpublished figure by Amir-Homayoun Javadi.

The ROAST fully automated open-source pipeline was used to simulate the current flow (Huang et al., 2019). Two 5 × 5 cm^2 virtual electrodes were placed over F3 and F4. The New York head model was used for the simulation (Huang et al., 2016). ROAST has been shown to have strong conformity with other available modelling systems such as commercial FEM software (Huang et al., 2018).

The intracranial electric field is not a simple set of field lines gently curving inside the cranium, but much more complex. The head is a complex object formed of multiple tissues with differing electrical properties, such as skin, skull bone, cerebrospinal fluid, grey matter and white matter. In addition, the conductance of the brain tissue is dependent on the orientation of the axons, and this

inhomogeneity and anisotropy results in complex field morphologies (Rampersad et al., 2014). However, recent research has suggested that while individual anatomy greatly influences the resulting fields (mostly by the curvature of the cortex resulting in different orientations of the neurons in relation to the electric field), the anisotropy might not (Huang et al., 2017). Please see Figure 6 for an example of electric field modelling.

While a strong enough electric field, such as the one generated by transcranial magnetic stimulation, can depolarize the membrane enough to trigger the neuron to fire, tES it not powerful enough to do so. Indeed, the fields caused by tES measured in humans have been up to 0.5 mV/mm (Opitz et al., 2016) in human studies using a 1 mA current. This effect is small compared to the resting potential (-70 mV) and threshold potential (-50 mV). Likewise, the electric field strengths from simulated experiments have ranged from 0.2 mV/mm to 3.0 mV/mm using a 1 mA current, which is quite a low current for current stimulation protocols (Parazzini et al., 2012). While rat studies suggest that an electric field of 1 mV/mm is required to affect neuronal spiking activity (Vöröslakos et al., 2018), this does not necessarily mean that the same applies for human neural networks. Indeed, as measured before, the measured electric field strengths in humans are below this

2.2.2 Neurophysiological effects of electric fields in the central nervous system

Neurons are arranged into complex neural networks, constantly either exciting or inhibiting each other. In addition, the neurons are oriented differently, both due to macroanatomy (the brain is roughly a ball-shaped object, causing linear electric fields to interact with different parts of the brain at different angles), local anatomy (the cortex at the peak of the gyri is oriented at ninety degrees to the cortex at the walls of the sulci), and cellular anatomy (for example, some interneurons are oriented at the plane of the cortex, while pyramidal cells have their dendrites in the top layers, and their axons move downwards). Thus, the effects of tES cannot solely be explained by local effects on an individual neuron, as even a small effect could change the function of a neuronal network.

An often-measured variable in tES studies is motor evoked potentials (MEPs), which are thought to be a passable proxy for brain excitability. MEPs are recorded by first attaching surface electrodes on the skin over the target muscle and then stimulating the corresponding area in the primary motor cortex with a transcranial magnetic stimulator. This causes a signal to be sent to the target muscle, which is then recorded as a MEP. When measuring changes in brain excitability, the baseline

MEP is first recorded, stimulation is administered, and the MEP is recorded again. The amplitude of the recorded MEPs is then compared to identify any changes (Nitsche & Paulus, 2000b).

2.2.3 tDCS

Online effects, or effects that happen during the stimulation, of tDCS on the cortex seem to be solely based on the modulation of the membrane potential. An excitability increase resulting from anodal stimulation (as measured by MEPs), for example, is reduced by the calcium channel blocker flunarizine and abolished by the sodium channel blocker carbamazepine (Nitsche et al., 2003). Based on the fact that neither drug had any effect on the excitability decrease resulting from cathodal tDCS, the authors hypothesized that the hyperpolarization inactivates the voltage-gated channels (which open with membrane depolarization), so administration of the channel blocking drugs would have no effect (Nitsche et al., 2003).

Long-term potentiation, unlike short-term effects, is not only dependent on voltage-gated sodium and calcium channels (Nitsche et al., 2003), but also on NMDA receptors (Monte-Silva et al., 2013) and protein synthesis (Huang et al., 2004). The effects are dose-dependent: while anodal stimulation is generally considered to be excitatory and cathodal stimulation inhibitory, this effect may flip when the stimulation dose is increased. For example, a study by Monte-Silva (2012) demonstrated that while 13 minutes of tDCS of the primary motor cortex increased the amplitude of motor evoked potentials (MEPs), suggesting increased excitability of the cortex, two 13-minute blocks back to back resulted in diminished MEPs. This effect was abolished by administration of the calcium-channel blocker flumazenil (Monte-Silva et al., 2013).

In the same study, following repeated 13-minute blocks administered not back to back but with the second stimulation a few hours after the first, excitability was increased for up to 24 hours after the second stimulation. This long-term potentiation cannot be explained by changes in membrane potential, which only occur during the stimulation. This was corroborated by the long-term potentiation being blocked by the NMDA-receptor antagonist dextromethorphan (Monte-Silva et al., 2013).

In addition to the effects on cortical excitability, anodal tDCS has also been shown to affect the neurotransmitter concentration in the brain tissue. Magnetic resonance spectroscopy (MRS) measurements have demonstrated that GLX (a combined measure of glutamate and glutamine) concentrations increase (Clark et al., 2011) and GABA concentrations decrease after anodal tDCS (Bachtiar et al., 2015; Stagg et al., 2009).

Aftereffects of cathodal stimulation also seem to be blocked by dextromethorphan (Nitsche et al., 2003). In MRS imaging, glutamate concentrations decrease (Stagg et al., 2009) and GABA concentrations increase (Stagg et al., 2009) after tDCS. However, unlike anodal stimulation, where increasing the stimulation current from 1 mA to 2 mA seems to increase the effect, with cathodal stimulation, a 2 mA current has been reported to lead to *increases* in cortical excitability (Batsikadze et al., 2013). The mechanism behind this phenomenon was unclear, but the authors suggested increased calcium flux as the mechanism. The sample size was small (twenty one), but the study was single-blind and sham-controlled, increasing its credibility.

Anodal tDCS has also been shown to affect local perfusion as measured by functional near-infrared spectroscopy (fNIRS, Merzagora et al., 2010) and functional MRI (fMRI, Stagg et al., 2013; Zheng et al., 2011). However, Paquette et al. (2011) suggested that while tDCS affects the magnitude of the task-induced increase in perfusion, it might not affect the baseline blood flow. In addition to increased blood flow, tDCS has been demonstrated to lead to a decrease in high-energy phosphate compounds in the brain tissue, suggesting increased metabolic activity (Binkofski et al., 2011).

As no brain area works in isolation, stimulating the target area *and* its network, identified by changes in resting state functional connectivity MRI, can increase the effect of the stimulation. This was demonstrated by Fischer et al. (2017) when identifying the resting state network of the left primary motor cortex (brain regions whose activity at rest was correlated with that of the left primary motor cortex) via fMRI and designing a 5-anode, 6-cathode montage using a finite-element model. The excitability increase was more than doubled compared to unifocal stimulation. Likewise, Antonenko et al. (2017) demonstrated that tDCS can alter the function of brain networks, in their study by specifically reducing age-induced interhemispheric connectivity and functional coupling in older adults.

2.2.4 tACS

The electric potential of a single neuron is rather challenging to measure *in vivo,* requiring equipment such as deep brain stimulation electrodes, and the measurement of a single neuron rarely tells us anything useful about the function of the relevant neuronal network. However, neurons are often arranged in neuronal networks with synchronized activity. When a population of neurons with similar spatial orientations fire in synchrony, the potentials generated by them sum up and can be measured with EEG or electrocorticography (ECOG). Features of these

rhythms, such as the phase and relative amplitude of different frequency bands, also correlate with cognitive phenomena such as navigation, memory retrieval, motor preparation and working memory (Buzsáki, 2006; Donner & Siegel, 2011; Wang, 2010).

Entrainment is a phenomenon where the frequency of an oscillator, such as a neural network, tends to adapt to and follow another, interacting oscillator that is very near to it in frequency (Thaut et al., 2015). If both oscillators are able to adapt, they tend to synchronize with each other. By changing the stimulation frequency over time, the oscillations of the neuronal networks can be entrained, or changed. A distinct feature of tACS over other forms of tES is the ability to manipulate and entrain the oscillatory activity of neuronal networks (Antal & Paulus, 2013; Thut et al., 2011). This is usually done with sinusoidal waveforms, but other forms, such as square waves, can be used.

tACS has been suggested to modulate both endogenous (voluntary) and exogenous (involuntary) attention when stimulating in 40 HZ and 10 Hz ranges, respectively (Hopfinger et al., 2017), and 40 Hz tACS has also been demonstrated to affect speech perception (Rufener et al., 2016). First determining the individual alpha frequency of the subject, and then stimulating at that frequency, seems to improve mental rotation performance during *and after* the stimulation (Kasten & Herrmann, 2017). Motor learning (Antal & Paulus, 2013) and motor memory (Lustenberger et al., 2016) have also been experimentally improved with appropriate tACS application.

Working memory seems to be linked to brain wave activity, and an enhancement of working memory was seen by manipulating theta- and gamma-wave interactions with tACS (Alekseichuk et al., 2016: 47 participants, sham-controlled design). In another study, the authors hypothesized that working memory capacity is determined by the ratio of theta to gamma frequencies, and they were able to increase working memory performance by slowing down the cortical theta frequency (Wolinski et al., 2018: 32 participants with a single-blind sham control).

tACS has also been used to enhance other psychological phenomenon. In lucid dreaming, dreamers are aware that they are dreaming and can potentially control the course of the dream. This ability can be of use if an individual tends to dream of boring subjects, such as school and shopping, and would prefer grand dreams of being an astronaut, or playing board games with Norse gods. Thankfully, tACS has also been suggested to increase self-awareness and the lucidity of dreams (Voss et al., 2014), potentially helping in achieving lucidity.

2.2.5 tRNS

Stochastic resonance is a phenomenon where adding random noise to a system can boost an undetectable signal to a detectable level. However, adding too much noise drowns the signal (Figure 7, Miniussi et al. [2013]). This is thought to be the effect behind tRNS, where both electrodes (they are functionally identical) appear to be excitatory (Terney et al., 2008). An appropriate amount of noise would primarily affect the neurons that are nearer to their threshold potential, so the effect would be network activity-dependent. The dose–effect curve is expected to be an inverted U-shape, with the effect increasing as a function of the dose until the ideal amount of noise is reached, but thereafter, increasing the stimulation intensity starts to drown the signal instead of boosting it.

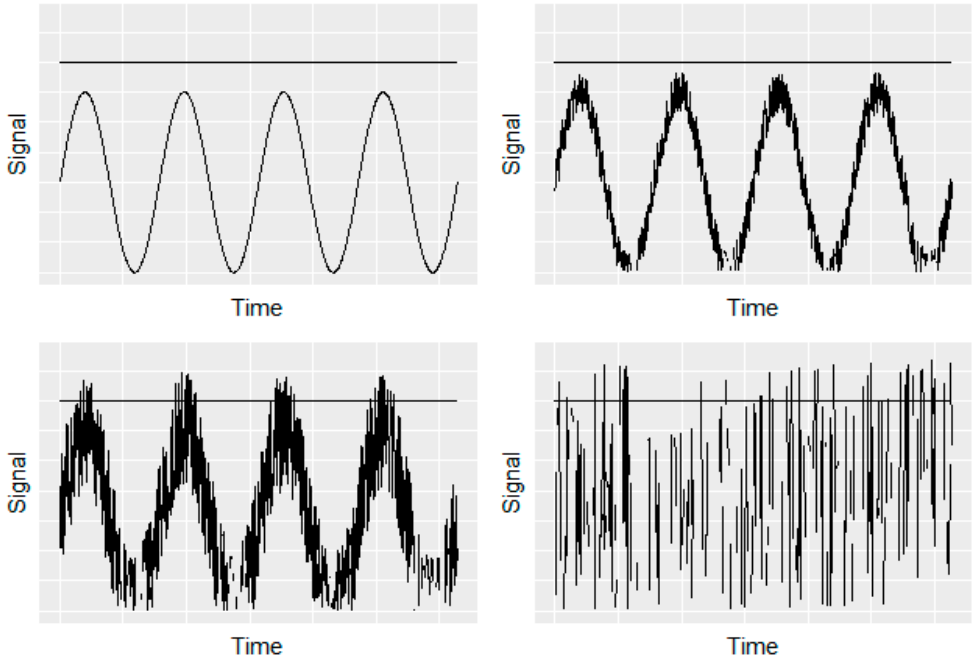

Figure 7. Adding noise to a sub-threshold signal can boost it over the detection threshold. From left to right, top to bottom: no noise, a little noise, an optimal amount of noise raising the signal to the detection threshold, and too much noise, drowning the signal. Figure modified based on one published by Miniussi et al., 2013.

Sometimes, a neuron receives almost but not quite enough stimulation to go over the threshold potential and fire. If a spike in voltage caused by tRNS happens at the same time, some of these almost-firing events become firing events. On the

contrary, if an inhibitory spike coincides, nothing is changed from the un-stimulated state. This explains the generally excitatory effects caused by the noise introduced by tRNS (Miniussi et al., 2013).

At the molecular level, high-frequency stimulation has been demonstrated to cause inward-going sodium currents and weak depolarization *in vitro* (Schoen & Fromherz, 2008). Based on this, Chaieb et al. (2015) demonstrated that tRNS-induced excitability enhancements (measured as MEP changes) are significantly reduced by the sodium-channel blocker carbamazepine in humans. In the same study, the NMDA blocker dextromethorphan had no effect, suggesting that the mechanism behind tRNS is different from that behind tDCS.

tRNS has been suggested to increase cortical excitability, at least in the motor areas (Terney et al., 2008). It has also been suggested to offer stronger improvements in perceptual learning than tDCS (Fertonani et al., 2011). A study by van der Groen and Wenderoth (2016) demonstrated the necessity of finding an optimal amount of noise: visual perception followed an inverted U-curve, improving up to a point with an increasing stimulation current, but then starting to fall again as the visual system began to be overwhelmed by noise (van der Groen & Wenderoth, 2016).

2.2.6 Neurophysiological effects of electric fields at the periphery

The effects (which are further described in the following paragraphs) of tES at the periphery could be caused by several mechanisms. The brain exerts direct control over many of the processes of the body, either through neuronal or humoral pathways. The brain also excretes compounds, either for humoral signalling or simply to remove metabolic waste products. Another, indirect pathway also exists: Direct control of peripheral processes could lead to effects further downstream.

2.2.7 tDCS

Anodal tDCS to left DLPFC has been demonstrated to reduce the stress response, as measured by reduced salivary cortisol (stress induced by images of negative emotional valence (Brunoni et al., 2013b) and mathematical tasks given to an individual with math anxiety (Sarkar et al., 2014)), as well as increased heart rate variability (Brunoni et al., 2013b). Salivary cortisol is thought to indicate the state of the hypothalamic–pituitary–adrenal (HPA) system at a given moment, and heart rate variability is similarly considered to reflect the activity of the sympatho–adreno–medullary (SAM) system (Brunoni et al., 2013b). Interestingly, the salivary cortisol response to stress (induced by the Trier social stress test, Allen et al., 2017) was also

reduced by anodal stimulation of the *right medial* prefrontal cortex (given the low locality of tDCS, stimulation of the right medial prefrontal cortex may not differ much from stimulating the right DLPFC, and having the same effect as stimulating the same area on both sides is somewhat surprising) (Antal et al., 2014). Brunoni et al. (2013b) argued that their results (which were similar to others cited in this paragraph) demonstrated that tDCS can induce transitory, top-down modulatory effects on the HPA and SAM systems. They further pointed out that such modulation is interesting in the context of the treatment of mood and anxiety disorders, in which these systems do not work optimally.

In a study by Binkofski et al., (2011: 15 participants, sham-controlled cross-over design), it was demonstrated that anodal stimulation of the primary motor cortex not only reduced circulating cortisol levels, but also lowered both diastolic and systolic blood pressures and increased systemic glucose uptake under the euglycemic-hyperinsulinemic clamp[5]. The authors argued that while the hypothalamic stress systems were clearly affected, they may not have caused the changes in glucose metabolism, as glucose metabolism returned to the baseline level before the other measurements did. Expanding on this, Kistenmacher et al. (2017: 14 participants, sham-controlled cross-over design) measured the effect of tDCS on blood glucose levels during 8 days of stimulation under physiological conditions and found an increasingly powerful effect of lowering blood glucose via insulin-independent mechanisms. The authors proposed that since insulin-dependent glucose transporters prevail in the periphery, whereas glucose-independent transporters occupy the blood–brain barrier, the effect might be due to increased glucose consumption in the brain. They did not find an effect on circulating cortisol, although they measured baseline cortisol, not the stress response directly. The study further points to the cerebral uptake of glucose as a mechanism behind the decrease in circulating glucose levels.

Anodal tDCS has also been suggested to improve muscle endurance, but not the maximal strength produced (Cogiamanian et al., 2007: 24 participants, no sham control). The authors suggested that this might be due to increased excitability of the primary motor cortex, reduced muscular pain or more optimal synergistic muscle activation. Indeed, a previous study by Power et al. (2006: 10 participants with sham control) demonstrated that tDCS could increase muscle coherence. It has

[5] In the euglycemic-hyperinsulinemic clamp, the plasma insulin concentration is raised and held constant with a constant infusion of insulin, after which the plasma glucose concentration is maintained with a variable-rate glucose infusion. After a steady state is reached, the rate of glucose infusion equals the combined glucose uptake rate of all tissues

to be noted that the studies mentioned above had small sample sizes and/or no sham control, so the level of evidence is low.

In summary, published effects of tDCS in the periphery mostly consist of modulation of the stress response and increased glucose uptake. The latter might be of use in clinical practice when treating diabetes, although this is far from clinical reality.

2.2.8 tACS & tRNS

As newer stimulation paradigms, tACS and tRNS have received far less research attention in general. To my knowledge, no research focusing on the effects of tACS or tRNS on the periphery has been published.

2.3 COMPARISON WITH OTHER BRAIN STIMULATION METHODS

tES can easily be confused with various other methods that involve electrical or other stimulation of the brain and/or other areas. In the following section, several methods commonly associated or mixed with tES are discussed.

2.3.1 Electroconvulsive therapy (ECT)

ECT has long been used to treat a variety of conditions (Singh & Kar, 2017), and has historically gathered notoriety on par with horrors such as ice pick lobotomy and forced cold baths. However, unlike the latter, ECT still has a place in today's clinics as an excellent treatment for conditions such as treatment-resistant depression and catatonia (Weiner & Reti, 2017). It has supporting evidence and recommendation as both an add-on treatment and monotherapy for schizophrenia, although the quality of evidence for monotherapy is low[6]. In addition, it is considered an important modality for treating catatonia[7] and psychotic and/or treatment-resistant

[6] Category C evidence and a grade 4 recommendation as an add-on to pharmaceutical treatment, category D and grade 5 as stand-alone treatment from the World Federation of Societies of Biological Psychiatry (WFSBP, Hasan et al., 2012). Category C evidence from Finnish Current Care Guidelines as an addon to pharmaceutical therapy (Schizophrenia: Current Care Guidelines, 2020)

[7] Category C evidence and a grade 4 recommendation from WFSBP (Hasan et al., 2012)

depression, as well as situations where rapid relief from depression is necessary, such as refusal of food and water [8].

In the past, patients with epilepsy were observed to have a higher number of glial cells compared with patients with schizophrenia. This observation created an idea of inducing seizures to treat psychiatric conditions, and research on ECT began (Singh & Kar, 2017). Currently, the exact mechanism of action of ECT remains unknown, but various theories have been proposed, from changes in blood flow (Takano et al., 2011) to altered gene expression (Kaneko et al., 2015).

As opposed to tES, where there is no need for anaesthesia due to the painlessness of the procedure, in ECT the patient is anesthetized, and a high current is delivered through the brain. Unlike in tES, the current is short-lasting and powerful enough to depolarize the neurons in its path, which tES cannot do. And while tES aims to modify intrinsic brain activity, ECT is considered to reset it.

The indications of use differ: while tES has been suggested to be helpful in the treatment of various conditions, such as non-treatment-resistant depression and neuropathic pain, ECT is primarily used to treat severe mental illnesses, such as psychotic depression, acute manic episodes, catatonia, schizophrenia and bipolar disorder (Shekhawat & Vanneste, 2018).

The risks also differ. Very few serious AEs have ever been reported with tES (one case of epileptic seizures has been observed during a series of tDCS treatment sessions in a patient previously diagnosed with epilepsy; please see section 2.4.3), but while ECT has become quite safe, risks are still involved. Mortality is about 2.1 per 100 000 treatments. This is, however, relatively low, as the respective figure for general anaesthesia used with surgical procedures is 3.4 per 100 000 (Tørring et al., 2017). Other AEs associated with ECT include mild headaches, postictal confusion and transient anterograde amnesia (Kalisova et al., 2018).

2.3.2 Transcranial magnetic stimulation (TMS)

As opposed to previous methods, which all rely on an electric current, TMS relies on magnetic fields. Specifically, it uses a rapidly changing magnetic field to induce a current into a conductor, in this case a neuron. As opposed to tES, TMS can induce action potentials, which can be observed, for example, as muscle twitches or

[8] Category C evidence and a grade 4 evidence from WFSBP (Bauer et al., 2013). Category A evidence for severe or psychtic depression, category C evidence for treatment-resistant moderate depression from the Finnish Current Care Guidelines (Depression: Current Care Guidelines, 2020).

phosphenes (Valero-Cabré et al., 2017) when stimulating the motor or the visual cortex, respectively.

TMS can be split into two sub-techniques: single-pulse TMS and repetitive TMS, in which a series of pulses is delivered. In research, single-pulse TMS is useful to establish links between cortical regions and their functions, and to map network connectivity. In diagnostics, it has been used to probe the remaining connectivity after brain lesions. Repetitive TMS, on the other hand, has been suggested to be useful in areas ranging from stroke rehabilitation (by increasing plasticity) to depression (Valero-Cabré et al., 2017). Repetitive TMS has been included in treatment guidelines for conditions such as schizophrenia (although the evidence is limited)[9] and depression (especially treatment-resistant depression)[10] both in Finland and internationally.

The procedure is rather painless, although the stimulation can induce twitches in muscles under the stimulation coil, which can feel somewhat unpleasant. In addition, the stimulation coil can be rather noisy, potentially reaching unsafe sound levels (Rossi et al., 2009). The equipment is a lot more expensive than tES equipment, and if holding arms are not used, an operator is necessary to hold the coil in place.

Safety considerations are similar to those for tES (except for those related to the skin–electrode interface), although more care needs to be taken, as the high energy fields can damage or heat implants. Moreover, as TMS can cause action potentials, there is a greater possibility for adversely affecting brain function. Unlike tES, TMS can induce epileptic seizures if safety limits are not followed (Rossi et al., 2009).

2.3.3 Deep brain stimulation (DBS)

DBS is another way to electrically stimulate the brain. However, instead of using electrodes on the scalp, DBS uses surgically implanted electrodes deep in the brain tissue, making the procedure highly invasive. In addition, the electrodes cannot be easily adjusted after implantation. However, DBS stimulation is precise and can target subcortical structures, such as the globus pallidus internus and the

[9] Category D evidence, a grade 5 recommendation from WFBSP for the treatment of both positive and negative symptoms (Hasan et al., 2012). Category C evidence from the Finnish Current Care Guidelines for treatment-resistant auditory hallunications. (Schizophrenia: Current Care Guidelines, 2020.)

[10] Category A evidence from the Finnish Current Care Guidelines for both normal and treatment-resistant depression (Depression: Current Care Guidelines, 2020). No recommendation from WFBSP, although the guidelines are from 2013.

subthalamic nucleus in Parkinson's disease, and the thalamic region in refractory Tourette's syndrome (Almeida et al., 2017; Farmer et al., 2016). Furthermore, once the patient has recuperated from the procedure, the stimulation can be delivered as programmed without hindering the daily life of the patient. The main use for deep brain stimulation is in the treatment of Parkinson's disease (Almeida et al., 2017). The main risks with the procedure are associated with the surgery, and are generally considered manageable (Fenoy & Simpson, 2014).

Despite the generally invasive nature of the treatment, DBS has found its way into the treatment guidelines for Parkinson's disease[11]. It is especially useful for cases where more conventional therapy is no longer effective.

2.3.4 Magnetic seizure therapy (MST)

MST is similar to ECT in that it induces seizures in the brain. It works by inducing electrical activity in the brain via rapidly changing magnetic fields (similar to TMS, but with higher intensity) up to the point where a spontaneus seizure is generated (Engel & Kayser, 2016). As magnetic fields, unlike direct current, are not diffused through the brain, the resultant stimulation, and the resulting seizures, are more localized (Hoy & Fitzgerald, 2010), potentially sparing, for example, the hippocampus and other areas involved in memory. This could, in turn, reduce amnesia compared to ECT (Hoy & Fitzgerald, 2010).

MST is still a novel treatment method, with relatively few studies conducted. However, there is preliminary evidence for its antidepressive effects (Engel & Kayser, 2016). Nevertheless, MST has fewer adverse effects than ECT (Lisanby et al., 2003), and the patients' orientation recovers more quickly (Lisanby et al., 2003). To my knowledge, MST has not been included in any treatment guidelines.

2.3.5 Vagus nerve stimulation

Vagus nerve stimulation has been used to treat treatment-resistant depression and treatment-refractory epilepsy (Wheless et al., 2018; Yuan & Silberstein, 2016). It has been included in the treatment guidelines for depression by WFSBP and the Finnish Current Care Guidelines[12], and in the treatment guidelines for treatment-resistant

[11] Category A evidence from the Finnish Current Care Guidelines (Parkinson's disease: Current Care Guidelines, 2019).

[12] Category D evidence, a grade 5 recommendation from WFSBP (Bauer et al., 2013). Category C evidence from the Finnish Current Care Guidelines (Depression: Current Care Guidelines, 2020).

epilepsy with focal to bilateral seizures, when surgery is not an option according to the Finnish Current Care Guidelines[13].

Currently, vagus nerve stimulation is mainly applied with devices implanted under the skin of the chest, although non-invasive variants have been developed. Online devices that modulate the stimulation based on, for example, heart rate, have been developed with the aim of detecting the start of an epileptic seizure and suppressing it. Voice alterations, cough and dyspnoea are risks for vagus nerve stimulator surgery, in addition to conventional surgical risks (Yuan & Silberstein, 2016). Compared to tDCS, vagus nerve stimulators use a pulsating or alternating current (Farmer et al., 2016).

2.3.6 Galvanic vestibular stimulation

Galvanic vestibular stimulation, or transcranial vestibular stimulation, uses principally the same equipment as tES, but has a different target: instead of cerebral tissue, the vestibular organ is targeted. The method uses a variety of waveforms, from white noise (Fujimoto et al., 2018) to a stepped direct current (Wardman et al., 2003). The goal is to introduce false sensations of rotation, to investigate the function of the vestibular system, or to potentially treat problems within the same system. Initially, 2-pole stimulation (electrodes on mastoid processes) was discovered to induce false sensations of roll (Fitzpatrick et al., 1999), and this was later expanded into a 4-pole stimulation method (additional electrodes on the forehead) capable of inducing false sensations of roll, pitch and yaw (Aoyama et al., 2015). The technology has potential in virtual reality (Preuss & Ehrsson, 2019), investigation of the functioning of a diseased brain (Panichi et al., 2017) and the treatment of balance disorders (Fujimoto et al., 2018). To my knowledge, galvanic vestibular stimulation has not been included in any treatment guidelines.

As the protocol is quite similar to tES, apart from the montages, the safety and adverse effect profile should be quite similar. Specifically, the observed adverse effects include itching and tingling, but no nausea (Utz et al., 2011). However, as an electric field spreads in the brain in tES studies, it is expected that brain tissue could also accidentally be stimulated with vestibular stimulation protocols.

[13] Category B evidence from the Finnish Current Care Guidelines (Epilepsy: Current Care Guidelines, 2020).

2.4 AREAS OF APPLICATION

The chief use of tDCS and tRNS in research is in investigating the functions of the cerebral or cerebellar cortex. Specifically, tES allows either excitation (anodal TDCS and tRNS) or inhibition (cathodal tDCS) of the underlying cortical region, and observation of the behavioural changes arising from the modification of brain activity (Pleger & Timmann, 2018). This allows an inspection of the causal roles the brain regions may play, unlike purely observational methods, such as fMRI.

tACS, on the other hand, can be used to entrain the naturally occurring oscillations of a given cortical area (Tavakoli & Yun, 2017). This allows it to be used to investigate how the properties of these oscillations affect the function of the area. In addition, amplitude-modulated tACS can be used to modify the amplitude of various oscillations in lieu of the frequency, in order to investigate its function (Witkowski et al., 2015).

2.4.1 Stimulation of the healthy brain

tDCS has been demonstrated to improve (anodal stimulation) and hinder (cathodal stimulation) visual contrast sensitivity in humans (Antal et al., 2001), although the study was small (15 participants) and had no sham control. tDCS has also been shown to affect motion perception in an interesting manner: with no distracting visual stimulus, anodal stimulation improved performance in a motion perception task, but with distractors present, cathodal stimulation improved it instead (Antal et al., 2004: 12 subjects, no sham control). According to the authors, this was explained by cathodal stimulation improving the signal-to-noise-ratio with distractors present, while anodal stimulation increased neuronal activation, allowing for improved performance when a noiseless stimulus was presented.

Of special interest are two studies by the same group (Antal et al., 2004: 12 subjects, no sham control; Kruse et al., 2004: 42 subjects, no sham control), in which tDCS improved performance in a visual tracking task in a learning-dependent manner. Specifically, when the task was novel and still being learned, anodal stimulation improved it, but when the task was overlearned, cathodal stimulation enhanced performance. Again, this was speculated to result from anodal stimulation enhancing the functioning of still new neural connections, while cathodal stimulation was not able to impair the solidified system. On the contrary, cathodal stimulation was interpreted to reduce concurrent activation, thereby improving the signal-to-noise ratio and the function of the solidified system.

The brain has been described as a self-organizing system (Singer, 1986), developing based on external stimuli to meet the needs of the organism. Such a system should, in theory, achieve an optimal signal-to-noise ratio. However, the needs of the organism are varied, and while the brain might self-optimize for a complex set of functions (such as both learning new skills and utilizing already learned ones efficiently), the end result might not be optimized for any one of these, but for the combination. In this case, tES could tune the neuronal population to the target task, while at the same time inhibiting other functions. Another possibility of the perceived inefficiency of the neuronal population might be a local maximum. Optimization commonly becomes stuck on a local peak, even if higher peaks exists, as reaching them would require going downhill for a while.

Anodal tDCS applied to the left primary motor cortex has also been demonstrated to improve motor performance (Heise et al., 2009: 10 subjects, cross-over design with double-blind sham control). Interestingly, the effect was more powerful in older subjects, which the authors surmised might mean that tDCS has negligible effects on an optimally working motor system, but can improve the function of a non-optimised system. Anodal, but not cathodal, tDCS has also been shown to improve the formation and retention of simple motor memories (Galea & Celnik, 2009: 9 subjects, no sham control). However, in a similar experiment, while the stimulation was only applied during the last five minutes of the 30-minute learning session, stimulation of either polarity hindered learning (Rosenkranz et al., 2000: 6 subjects, no sham control). This may suggest that the timing of the stimulation is critical for the outcome.

Regarding motor adaptation, anodal tDCS applied to the primary motor cortex has been shown to improve the retention of a novel visuomotor transformation, but to not have an effect on the acquisition of it (Galea et al., 2011: 72 subjects with a double-blind sham control). In contrast, anodal tDCS applied to the ipsilateral cerebellum improved the acquisition of the new model, but did not affect retention, which the authors interpreted as two different parts of the brain being responsible for different facets of motor adaptation.

Anodal tDCS of the DLPFC improved memory encoding and recovery (Javadi & Walsh, 2012: 32 subjects, double-blind sham control). Interestingly, the emotional load of the images appears to interact with stimulation. Right anodal fronto-temporal stimulation improved the recall of pleasant images, while left anodal fronto-temporal stimulation improved the recall of unpleasant images, which might have an effect in the treatment of, for example, patients with depression (Penolazzi et al., 2010: 12 subjects with a sham control). In a multi-day stimulation study, where subjects received tDCS on M1 while training for a sequential visual isometric pinch

task, stimulation improved the between-day progress (offline effect), but not the within-day effect (online effect) or the rate of forgetting the newly acquired skill (Reis et al., 2009: 24 subjects with a double-blind sham control). Both anodal and cathodal stimulation of the left DLPFC improved sustained alertness over sham stimulation (Warm et al., 2014: 25 subjects with a double-blind sham control).

tDCS also either impaired or enhanced numerical cognition in a polarity-dependent manner (Cohen Kadosh et al., 2010: 15 subjects with a sham control). The study remarkably demonstrated that the improvement was still present 6 months after the training, showing that the effects of tDCS may be very long lasting.

While the previously mentioned findings might be argued to fall under "research use", in principle, tDCS could be used to enhance the performance of the healthy brain outside of the laboratory. These possibilities are further explored under the heading "Future perspectives". However, it has to be mentioned that most of the studies discussed here had rather small sample sizes, many of them were without a sham control, and many of them were the only report of the particular effect. Perhaps the best-established effect is the improvement of memory function, with several sham-controlled studies having reasonable sample sizes. Other effects discussed here need to be verified with randomized, sham-controlled, double-blind studies having decent sample sizes to rule out false positive results, and most of the results discussed here should only be considered as preliminary findings to guide future research, not as definitive truths. In conclusion, stimulation of the healthy brain currently serves mostly as an investigative tool in neuroscience, allowing the exploration of causal relationships between brain activity and behaviour, although there are indications it could be used in the future for neuroenhancement.

2.4.2 Clinical applications

As tES can be used to direct attention to either side of the body in a healthy population (Ni et al., 2019), it has been investigated in the treatment of spatial neglect. Spatial neglect is a condition where the patient fails to attend to or orient to a stimulus contralateral to the brain lesion. Often, extinction is observed, where the contralateral stimuli are ignored only in the presence of ipsilateral stimuli. Indeed, tDCS has been used to improve the function of the lesioned side, alleviating the symptoms of neglect (Ko et al., 2008). The study had a small sample size of fifteen, but had a double-blind, sham-controlled cross-over design.

In an excellent analysis by Lefaucheur et al. (2017), studies on the clinical use of tDCS were analysed and recommendations drawn. In conclusion, the authors could offer a level B (probably effective) recommendation for the treatment of

fibromyalgia-related pain, major depressive disorder without drug resistance, and the treatment of substance addiction. A level C (possibly effective) recommendation was offered for the treatment of lower limb neuropathic pain secondary to a spinal cord lesion. More recently, Fregni et al. (2020) gave tDCS a level A (definitively effective) recommendation for the treatment of depression, and a level B recommendation for neuropathic pain, migraine, fibromyalgia, post-operative pain, Parkinson's disease, motor stroke, epilepsy, schizophrenia and alcohol addiction.

Substance craving was reduced by tDCS for smoking (Boggio et al., 2009; Fecteau et al., 2014) and crack-cocaine use (Batista et al., 2015). With alcohol use, tDCS improved the quality of life, but did not affect craving scores (Klauss et al., 2014). However, at the six-month follow-up, the number of alcohol-abstinent patients was greater in the stimulation group. Of special note, these studies all used a montage with the anode on the right DLPFC and the cathode on left DLPFC, which is the mirror image of the montage commonly used for depression treatment. This could potentially be a complication for patients with co-morbidities, as depression and substance dependence can occur together. This group of patients would seem to require stimulation with two diametrically opposite montages, and the effects of such a treatment are unknown.

While tDCS has been investigated in the treatment of various kinds of pain, literature on two types of pain in particular has accumulated. Fibromyalgia pain was eased more with active tDCS than sham (Fagerlund et al., 2015; Valle et al., 2009), and the effects remained significant after 21 (Fagerlund et al., 2015) and 30 days (Valle et al., 2009). Neuropathic pain secondary to spinal injury was relieved by tDCS in studies by Fregni et al. (2006a) and Soler et al. (2010); the latter study also demonstrated that efficacy persisted for 12 weeks after treatment with a combination of tDCS and visual illusions. The former study, however, failed to demonstrate any benefit after 14 days.

Depression has been attempted to be treated with various electrode montages, but two in particular have been popular: the anode on the left DLPFC and the cathode over the right occipitofrontal cortex, and the anode on the left DLPFC and the cathode on the right DLPFC. The previously mentioned study by Lefaucheur et al. (2017) summed up recent analyses and found support for a level B recommendation (probable efficacy) for the former, but not the latter, for the treatment of non-treatment-resistant depression. Both of the montages aim to correct the observed imbalance between the left and right DLPFC (the right being overactive compared to the left, when compared to a healthy brain), and it could be hypothesised that the anode on the left DLPFC and the cathode on the right DLPFC could be more efficient, affecting both relevant brain areas. While a

recommendation for this montage could not be given, this might be because of the smaller number of studies. In another study, Brunoni et al. (2013a: 120 participants in a sham-controlled, double-blind trial) determined that sertraline (a selective serotonin reuptake inhibitor that stops neurons from recovering serotonin from the synaptic cap, increasing its concentration in the synaptic cleft) and tDCS have similar efficacies, but the combined therapy is more efficacious than either treatment alone. This demonstrates that tDCS may not only be a potential replacement, but an addition to conventional treatment. However, in another study, Brunoni et al. (2016) detected no additional cognitive improvement with tDCS vs. conventional sertraline treatment of depression. In addition, not all studies have shown an effect of tDCS on depression, perhaps due to multiple variables intrinsic in tDCS (such as the stimulation montage, the current used, and the number and distribution of stimulation sessions) and the heterogeneity of depression as a disease (Lefaucheur et al., 2017).

As tDCS (anode over the primary motor cortex, cathode on the left forehead) has been shown to induce widespread brain activity, which leads to the depletion of high-energy compounds stored *in situ* and an increase in glucose uptake (Binkofski et al., 2011), tDCS has been postulated to be a potential treatment for diabetes mellitus. Based on this, Kistenmacher et al. (2017: 14 participants with a single-blind, sham-controlled, cross-over study design), using the above-described electrode montage, tested the effects of one week of repeated tDCS stimulation on blood glucose and insulin levels. The results were encouraging, as insulin levels remained constant, but glucose levels were significantly lower, with the effect persisting at least 8 days after stimulation. The authors suggest that tDCS might be an effective adjuvant therapy to DM2 with a low side-effect profile. However, while the experimental design was rather sound, the sample size was quite small, so the finding needs to be replicated in a larger sample.

Stroke is not uncommon, and as survival rates increase, the aftereffects of stroke are becoming increasingly common in society. Deficits in motor control, memory and language, among other effects, can drastically reduce the quality of life and the capability for independent life of the sufferers. Motor functions, and associated activities of daily living, have been improved with tDCS (Hummel, 2005: 6 patients, double-blind, sham-controlled, cross-over design), especially when coupled with motor training (Zimerman et al., 2012: 12 patients, double-blind, sham-controlled, cross-over design). Language recovery studies have showed similar results: improvements with tDCS (Baker et al., 2010: 6 patients, double-blind, sham-controlled design) enhanced by concurrent training (Fridriksson et al., 2011: 8 patients, double-blind, sham-controlled, cross-over design) and results visible for at

least 3 weeks. It should be noted, however, that the results of the studies in this area have been highly variable, and there is no definitive evidence on which type of stimulation montage best fits certain types of patients. The previously mentioned studies have had sound designs, but small sample sizes.

As tDCS has been demonstrated to affect memory, Alzheimer's disease has also been suggested as a potential target for tES treatment. However, even though early results were encouraging with both anodal and cathodal stimulation (Khedr et al., 2014), more recent, large studies have found no effect (Suemoto et al., 2014). This might, however, be explained by the different outcome measures used by the studies, namely MMSE and apathy scores. This may indicate that tDCS does not, in fact, treat the underlying process of Alzheimer's disease, but can perhaps help alleviate its different symptoms to various degrees.

To summarize, the applications where tDCS is closest to routine clinical use are in the treatment of depression, substance dependence and neuropathic pain (Lefaucheur et al., 2017). However, the majority of clinical use is still experimental. The Finnish Current Care Guidelines (Depression: Current Care Guideline, 2020), for example, only included a low-level recommendation of tDCS for the treatment of depression in their last revision.

2.4.3 Future perspectives

tES has been combined with EEG measurement to form closed-loop and open-loop feedback-driven systems. The purpose of a closed-loop system is to adapt the stimulation parameters based on measured stimulation effects, while open-loop systems time the stimulation based on the state of the underlying brain networks generating the brain waves (Karabanov et al., 2016). Recently, a proof-of-concept study demonstrated the feasibility of an open-loop system (Nelson & Tepe, 2015), suggesting potential future uses for the technology. In particular, they suggested that EEG could be used to detect the start of a focal epileptic seizure, and then cathodal tDCS could be used to suppress it, while at the same time avoiding both AEs of the stimulation when there is no emergent seizure and potential accommodation of the brain.

Given that the military often requires its personnel to operate at the very peak of human capacity, often with literal lives on the line, it is not surprising that the military has been inventive in their use of potential technologies. As tES has been suggested to improve learning and attention, among other effects, it has also been of military interest. Specifically, tDCS has been investigated in relation to maintaining performance in long-term tasks requiring high levels of attention, as well as recovery from psychiatric and neurological conditions resulting from combat

(Nelson & Tepe, 2015). However, a recent meta-analysis on the effects of tDCS (Chaieb et al., 2019) pointed out that recently, two previous studies have been unable to be replicated, and that task performance does not seem to improve with lessened mind wandering, casting some doubts on the feasibility of this approach. Other areas being proposedly useful for military use are enhancements in training (for example, by enhancing motor learning and decreasing training time) and reaction time, the latter of which could be the dividing line between life and death in firefights and aerial combat (Davis & Smith, 2019).

One of the most interesting areas of research still to be comprehensively investigated is the timing of the stimulation. tDCS shows different effects during the beginning of learning a new skill and after the skill has been established (Kruse et al., 2004). This seems to suggest that the effects of tDCS are dependent on the state of the stimulated system, so it could be that tES also has different effects during different phases of depression recovery, for example, leading to different protocols being optimal in different phases of the disease. In addition, the timing of concurrent therapies for optimal results is still unknown. The combination of tES and pharmacological interventions has been studied with depression (Brunoni et al., 2013a), but while the combination seems beneficial, the interaction of the treatments in different phases of illness and at different levels of severity remain unknown. Even more interesting is the potential interplay of tES and psychotherapy. While the effect of antidepressant drugs can be expected to remain relatively constant over the course of a stimulation regime if there are no changes in dosage, appropriate timing of stimulation sessions relative to psychotherapy sessions might improve efficacy. As the results of tES are dependent on the timing and the neuronal network state (Sriraman et al., 2014), it might be reasoned that tES before, during and after psychotherapy sessions will have different effects, and these in turn might differ during different phases of recovery.

2.4.4 Ethical considerations

Some ethical considerations are warranted in the context of tES. For example, tDCS has been used to increase muscle endurance and decrease fatigue in normal subjects (Cogiamanian et al., 2007). While this might at first glance appear beneficial, and indeed is in a variety of situations, it does raise a concern about doping in the world of professional sports. In addition, it is currently impossible to detect whether tES has been used (Davis, 2013). While even a small gain in performance would be significant at the highest level – for example, the difference between last two world records in the men's 100 m sprint is about 1.1% – it is

unclear whether tDCS increases capabilities or merely optimizes them. It is unclear whether athletes at the very peak of human performance have anything left to optimize, or if they are already functioning at their very best.

While it is far from becoming a reality, and may never reach fruition, similar concerns could be relevant in the context of enhancing learning, for example in entrance exams, especially as those with poorer financial or other resources might not be able to use neuroenhancement, lowering their chances of getting into more competitive fields. This could potentially lead to decreased social mobility. While the problem is essentially the same as with any other resource that one can buy to enhance performance, if neuroenhancement via electrical or other means becomes commonplace, it might be prudent to take measures to either prohibit neuroenhancement, or alternatively make it easily and widely available in society, to prevent social classification.

While the previously mentioned issues are related to effects that so far lack established evidence, and even if true might lack practical significance, "snake oil salesmen" remain a problem. Several companies offer potentially harmful devices with questionable health benefits. These issues are discussed further in section 2.4.7. Nevertheless, the most pressing issue remains with the utilisation of new therapies, such as tES, replacing established, effective treatments when there is still a lack of solid scientific evidence for their efficacy. This issue becomes particularly pressing when seriously ill or otherwise vulnerable groups are being treated. This problem, however, is not inherent to tES, but can be commonly observed when new, less investigated forms of treatment become available.

2.5 SAFETY, ADVERSE EFFECTS AND TOLERABILITY

In this literature review, I have divided AEs into local effects (i.e. AEs affecting the skin) and generalized effects (i.e., more widespread effects). Despite decades of study, no serious AEs have ever been observed from tES, apart from one possible case of epileptic seizure, which is further discussed below (Bikson et al., 2016). However, most tES studies fail to adequately report AEs, so systematic knowledge of these effects is lacking (Aparício et al., 2016).

2.5.1 Mild local AEs

These effects are felt immediately at the site of stimulation, modified by factors such as the conductivity of the electrode–skin interface medium (Woods et al., 2015). As

such, they should be less affected by the location of the electrode than generalized effects discussed later in section 2.4.2.

2.5.1.1 Skin sensations

Skin sensations under the electrodes could be caused by several factors, such as 1) electrochemical reactions (although this is prevented by sufficient electrolyte solution acting as a buffer (Antal et al., 2017)), 2) direct effects of electricity on the nerves under the electrode, or 3) heating of the tissue via a resistive mechanism. They range from mild to intolerable and are most often encountered at the beginning or the end of the stimulation.

Fertonani et al. (2015) administered tES to 531 subjects, who received altogether 693 stimulation sessions (434 tDCS sessions, 109 tACS sessions, 150 tRNS sessions), and asked them to rate itchiness, pain, burning, heat, pinching, iron taste and fatigue, and pooled them all into an aggregate variable for analysis. Thus, the results are not specific to skin sensations, but nonetheless are illustrative, as skin sensations were the most frequently observed AEs. Anodal tDCS led to AEs more often than cathodal tDCS, and a higher stimulation intensity and larger electrode area both resulted in more intense discomfort. The authors suggest that the last, unintuitive finding related to larger electrodes leading to more intense discomfort compared with smaller electrodes is the result of spatial summation, with more cutaneous receptors being stimulated, resulting in a stronger evoked sensation. The same relationship between electrode size and skin discomfort was also observed by Turi et al. (2014). Poreisz et al. (2007) reported that patients (a mix of migraine, tinnitus and post-stroke patients) experienced fewer skin sensations than healthy participants.

In a study focusing on cortical reactivity and working memory, Hill et al. (2017b) observed that HD-tDCS (where multiple small electrodes are used instead of one large electrode in order to better focus the current) induces stronger skin sensations than traditional tDCS. The authors interpreted this as probably being due to the higher current density (the combined electrode area for HD-tDCS electrodes was smaller than that for tDCS electrodes), even though this in contradiction with the previously mentioned results. The explanation for this discrepancy is still unclear.

Kessler and colleagues, when pooling together data collected from a number of tDCS studies performed at the hospital of the University of Pennsylvania, compared AEs between tDCS (n = 183 sessions) and sham groups (n = 94 sessions), and determined that the tDCS group had significantly more intense skin sensations than the sham group (Kessler et al., 2012). This reinforces the notion that the effects were

caused by the tDCS (i.e., electric current) rather than the stimulation protocol in general (i.e. saline, pressure from the electrodes or the straps used to secure the electrodes in place, stress, nocebo).

In an effort to lower the discomfort associated with tDCS, McFadden et al. (2011) successfully investigated the use of local numbing agents (EMLA gel) to reduce pain and discomfort associated with tDCS. Interestingly, and in contrast with previously discussed studies, cathodal stimulation in this study was more poorly tolerated than anodal stimulation.

2.5.1.2 Erythema

Erythema means reddening of the skin immediately under the electrodes. As an AE, it poses a dual problem. On the one hand, when current is applied, for example, to forehead, erythema can pose a cosmetic hindrance, while in research settings, it can prevent successful blinding if the subject can observe their own head (such as when asked to rate their own erythema, from bathroom mirrors, or in multi-day studies, from their own mirrors at home), or when double blinding is desired (Ezquerro et al., 2017). In addition, the study determined that the level of skin redness is significantly lower in the sham group and suggested that the effect is related to vasodilatation. However, DC-iontophoresis-induced contact dermatitis (rash caused by ions pushed into the skin by the electric current used in the stimulation) has also been suggested (Riedel et al., 2012).

Guarienti et al. (2015) investigated the prevention of skin erythema and found that pre-treatment of the skin with 2% ketoprofen significantly reduced redness, at the cost of increased preparation time and possible iontophoresis of the ketoprofen deeper into the tissues. Lidocaine or hydroxyzine had no effects on erythema.

2.5.1.3 Burns

Perhaps the most serious – and disturbing – of the mild AEs is the infliction of electric burns under the electrodes. While such AEs are rare (ranging from 1 in 5[14] (Frank et al., 2010) to none reported in most studies), they are also inconvenient and quite preventable. Previous literature suggests that factors such as using tap water instead of saline, repeated stimulations (Frank et al., 2010; Palm et al., 2008), abrupt

[14] The authors speculated that this relatively large incidence was caused by a combination of using tap water and not replacing the sponges, leading to the concentration of unspecified impurities in the sponges, which in turn caused skin irriation. Saline is normally used, and the sponges are replaced regularly.

current changes and drying electrode gel (Lagopoulos & Degabriele, 2008) can lead to these injuries. Other researchers have suggested that the higher current density at the edges, and especially at the corners, of square electrodes may increase the risk of burns (Turi et al., 2014).

2.5.2 Mild generalized effects

Generalized effects refer to AEs not manifesting directly under the stimulation electrodes, and thus probably not being related to the local electric or electrochemical effects, but to more diffuse changes in the brain and the body.

2.5.2.1 Tiredness

Poreisz et al. (2007) investigated the AEs of tDCS in an pooled dataset of their own studies (most of the included studies had a cross-over design, with the same patients receiving both sham and tDCS), and found that fatigue occurred in 22.6% of the patients after tDCS, and 35.4% of the patients during the tDCS, with the difference being statistically significant, perhaps suggesting that tDCS could cause tiredness during the stimulation. Alternatively, this might be due to the stimulation being a boring activity, but as a sham condition was not compared with stimulation, this is unknown. In addition, one patient reported acute, unspecified sleep disturbances.

However, in most studies, even if tiredness or fatigue has been reported, the significance of the prevalence of AEs compared with sham stimulation has not been statistically analysed, leading to difficulties in separating tiredness directly linked with tES and tiredness linked with, for example, the experimental protocols (which often involve sitting with little activity to occupy the mind). While this may be because most studies did not aim to examine adverse effects, and merely reported them as incidentals, it is nevertheless disappointing how few statistical comparisons have been performed. A study by Gillick et al. (2015), reporting the incidence of fatigue per group, found that the control group experienced more fatigue, perhaps indicating that the study setting, not the tDCS, induced feelings of tiredness, or that tDCS might increase alertness.

Additionally, tDCS has also been suggested to improve attention during long tasks (McIntire et al., 2017; Nelson & Tepe, 2015; Xu et al., 2015), as well as to treat fatigue from multiple sclerosis (Saiote et al., 2014), which might indicate that it can also be used to maintain focus and counteract fatigue in certain tasks. Therefore, the evidence regarding tiredness and fatigue as AEs remains controversial.

2.5.2.2 Headache

Poreisz et al. (2007) also examined the incidence of headache during and after stimulation. It was present in 11.8% of the subjects after the stimulation and 4.9% of the subjects during tDCS. However, the difference during the stimulation, compared with headache after the stimulation, was not statistically significant. This suggests that any headache experienced by the participants may not be related to the stimulation, but rather was either a random effect or an effect related to other aspects of the study protocol, such as ergonomics or pressure from the electrodes, or the systems utilised to secure the electrodes in place. However, the last of these would probably cause headache during the stimulation. Alternatively, any headache caused might last long enough to be unchanging between the time points (during and after the stimulation). The authors did not specify the time between the end of the stimulation and the gathering of the "post-stimulation" AEs, and unfortunately they did not conduct comparisons between tDCS and sham stimulation. Poreisz et al. (2007) also reported that patient populations more frequently experienced headache than healthy participants.

However, tDCS has also been suggested to be a treatment for various types of pain (Lefaucheur et al., 2017). While different types of pain have distinctly different mechanisms (for example, nosiseptic, neural and idiopathic pain all have different mechanisms), and pain can be alleviated via different mechanisms (for example, by repairing the damaged tissue, blocking the transmission of the pain in the nerves, blocking the chemical transmission of the pain, and many others), this might suggest that tES could also be used to alleviate any possible headache. However, the only evidence for tDCS-induced headache is its occurrence in tDCS studies, with little or no statistical analysis carried out.

2.5.2.3 Other AEs

Poreisz et al. (2007) also investigated various other AEs, such as difficulties in concentrating and nervousness. Of these examples, only nervousness displayed significant differences when comparing measurements during and after stimulation, while difficulties in concentrating did not. However, AEs other than those described in previous chapters are rather rare, and thus it is difficult to draw conclusions from the few reports concerning them. For example, Poreisz et al. (2007) had subjects report difficulties in concentrating and visual sensations at the start/end of the stimulation, in addition to the previously listed effects. Neither of these showed statistically significant differences between the time points during and after the stimulation.

2.5.3 Serious adverse effects – seizures

tES is not powerful enough to cause neuronal firing on its own, and indeed has been well tolerated by epileptic patients. Cathodal tDCS has even been reported to reduce seizure frequency in children (Auvichayapat et al., 2013). However, there exists one case of a seizure after consecutive daily tDCS in an 4-year-old boy (Ekici, 2015). The patient had remained seizure-free with topiramate and valproate (anti-epileptic drugs, whose exact mechanism of action is unclear, but may include blocking voltage-gated sodium channels) and confirmed so before initiating tDCS. Stimulation was aimed to reduce the spastic paresis the patient had developed before, and the valproate dose was reduced, topiramate was tapered, and escitalopram (an antidepressive drug of the selective serotonin reuptake inhibitor class) was started.

Four hours after the third stimulation session, the patient experienced an epileptic seizure, which was treated with diazepam and midazolam. No infections or electrolyte imbalances were detected, and the patient remained seizure-free after the cessation of the protocol.

The authors suggest that the modification of antiepileptic drug dosages might have caused the seizure. However, the patient remained asymptomatic when tDCS treatment was stopped. The authors also noted that anodal stimulation, at least in animal studies, appears not to affect the localized seizure threshold.

To my knowledge, this is the only reported instance of a seizure following tES, so the causal link remains unclear. Nevertheless, it is plausible that tES may induce seizures in epileptic patients if, for example, the area whose excitability is increased happens to be the epileptogenic focus. However, as tDCS has been tested as a treatment for epilepsy (San-Juan et al., 2015, 2018), and this is the only report of a tDCS-induced seizure, this would be a relative, not an absolute contraindication.

2.5.4 The unknowns – AE predictors and the effects of repeated stimulations

To my knowledge, very little literature exists on factors predicting the occurrence or severity of the AEs of tES. While the majority of the observed AEs are mild, they can be unpleasant enough that subjects have dropped out of the studies (Paneri et al., 2016). Furthermore, it is an ethical necessity to try to determine which patients would gain the most benefit while experiencing the least harm.

Likewise, while longer protocols with up to six weeks (Martin et al., 2018) of consecutive stimulation have become increasingly common in clinical studies, very

little literature exists on how the AEs progress over time. The effects related to the skin could especially progress, as the possible damage to the skin could accumulate. A study by Paneri et al. (2016) briefly mentions that they observed a trend of a reduced sensation of tingling over multiple sessions, but that the observed trend remained non-significant. The most significant piece of literature on the matter is a meta-analysis by Nikolin et al. (2018a), in which the incidence of adverse effects from the cumulative charge (a product of the stimulation current, stimulation time and number of sessions, $Current * Time\ of\ stimulation * Number\ of\ sessions$) was analysed, and no effect of the cumulative charge on discomfort, erythema, fatigue, headache or paraesthesia was found.

Poreisz et al. (2007) reported that patient populations experience more frequent headache but less intense skin sensations than healthy participants. Additionally, fatigue was more common when administering tDCS to the motor cortex when compared to visual cortex stimulation. Nevertheless, AEs experienced by different patient populations and as a result of different tES montages remain an area with a need for more research.

On the other hand, the tolerability of tES appears to be very good (Fertonani et al., 2015; Paneri et al., 2016; Pondé et al., 2017), so it might be that any combination of potential predictors deeming an individual more vulnerable to tES AEs will not result in an intolerable end result. If this turns out to be the case, such observations will simplify the patient selection for tES, as potential AEs are likely to be minor enough to be ignored in clinical practice and research settings.

2.5.5 Comparison of tDCS, tACS and tRNS adverse effects

Fertonani et al. (2015), in the study already discussed regarding skin sensations, also compared the discomfort caused by tDCS, tACS and tRNS. They determined that among all tES methods, tDCS is most poorly tolerated (taking into account factors such as stimulation intensity and electrode size), followed by tACS, with tRNS being the best tolerated form of stimulation.

While a study by Ambrus et al. (2010) tested perception thresholds but not AEs, it determined that tRNS is more difficult to perceive than either anodal or cathodal tDCS. This is in line with the previously mentioned results.

2.5.6 Tolerability of tES

Poreisz et al. (2007) examined the tolerability of tDCS by measuring various AEs during and after the stimulation. Their study had limitations, namely not measuring the presence of potential AEs before the stimulation, not specifying the time

between the end of tDCS and the collection of the post-stimulation AE data, and only comparing AEs during and after stimulation, not between tDCS and sham. Nevertheless, they determined that the incidence of skin sensations (tingling, itching, burning, pain), fatigue and nervousness was higher during than after the stimulation. However, none of the subjects requested the stimulation to be stopped or needed any medical intervention, despite 567 tDCS sessions being administered. This indicated that tDCS is well tolerated.

Moffa et al. (2017), in a meta-analysis comprising 289 patients, determined that drop-out rates were similar between tDCS and sham stimulation groups, with no statistically significant differences observed. However, they also determined that a higher current density led to higher dropout rates, while being a treatment responder led to lower dropouts. The last observation is unsurprising: if one feels that the treatment alleviates one's suffering, one is less likely to stop the treatment, despite AEs.

Another meta-analysis by Bikson et al. (2016) reported 14 dropouts from a total of 507 stroke patients when utilising tDCS. These also included personal reasons, so not all the reported drop-out cases can be attributed to tES AEs. Of the reported reasons for dropping out of a study, scheduling issues require further discussion. While the subjects might not have found the AEs intolerable, they might find the time commitment to be such. Same remains true with treatment with tES, albeit to a lesser degree, as lengthy questionnaires often associated with research are unnecessary in clinical settings. This issue is being investigated, with at-home treatment devices being an potential answer (Palm et al., 2018).

2.5.7 Risks of home use

The use of tES at home can be divided into medically supervised use, with clear directions and approved equipment, and aimed at alleviating symptoms of a disease, and unsupervised use, in which the equipment can be self-made, protocols extreme, and the aims can be neuro-enhancement or neurodoping, instead of the treatment of a disease.

For obvious reasons, studying the risks of individual people using tES on their own is quite difficult. However, several forums for such hobbyists exist on sites such as Reddit ("r/tDCS," 2019). Such forums contain examples of, for example, skin burns caused by improper electrodes. Self-built machines are of unknown quality and might deliver currents different from those desired. Some companies such as foc.us ("foc.us," 2019) also offer commercial devices for unsupervised home use.

Other than faulty hardware, the risks of do-it-yourself home use include untested protocols, including untested montages and long-term use, all of which carry unknown consequences. Even using tested protocols to self-medicate can carry risks resulting from insufficient monitoring of the treatment effects and AEs.

Medically supervised tDCS at home (either remotely controlled or patient controlled), on the other hand, appears to be safe. Palm et al. (2018), in a meta-analysis of home use of tDCS, reported safety and tolerability similar to laboratory use, with only minor AEs such as one case of unspecified skin irritation and one burn. On the other hand, the analysis highlights the need for sufficient training and support (via phone or video call) from the staff, as the studies with older patients, or with insufficient training, reported up to 50% dropouts. Incorrect electrode placement and mixing of the anode and cathode were also reported.

2.5.8 Blinding

Unblinding of the experimenter can result in unwanted effects, both on measurements by the experimenter and, if/when their demeanor changes, also in the participant. Unblinding of the participant can result in increased placebo and nocebo effects. All of this is problematic in the research setting, and different placebo protocols have been developed to combat this. As the cutaneus sensations thought to possibly reveal the stimulation are the strongest during ramp up and ramp down at the ends of the stimulation, perhaps the most common sham protocol is the delivery of stimulation just during those times for the sham group, which is thought to have neglible neuromodulatory effects. However, the efficacy of such a protocol has recently been questioned (Greinacher et al., 2019; Turi et al., 2019; Wallace et al., 2016). The usage of local anelgesic cream has been suggested as a way to prevent skin erythema and sensations, which are thought to be the cause of accidental unblinding (Ezquerro et al., 2017).

2.6 MEASUREMENT OF PERIPHERAL METABOLIC PROFILES

Metabolomics is a field of study attempting to examine the status of the organism by measuring the end-products of its metabolism. It represents the step between the study of proteins in a given organism, proteomics, and the biological phenotype. Generally, it uses chromatographic and spectrographic means to characterize the metabolites of a sample (Schrimpe-Rutledge et al., 2016).

Targeted metabolomics is hypothesis-driven, focusing on either validating or rejecting a suggested change in the state of the organism. It usually focuses on a

limited set of related end products, and benefits from clear-cut interpretation of the resulting statistics (Schrimpe-Rutledge et al., 2016).

If targeted metabolomics is like a bloodhound, very likely to find what it is meant to (the missing person), but totally ignoring other potentially interesting finds (such as sausages), untargeted metabolomics is like taking a metal detector to a beach – you will most likely find *something*, but while it might be important (for example, an antique coin), it might just as well be insignificant (such as a bottle cap). It focuses on a relative quantification of a wide array of end products, aiming to generate hypotheses for future studies, instead of validating pre-existing ones (Schrimpe-Rutledge et al., 2016).

2.6.1 Methods for measuring peripheral metabolite profiles

Magnetic resonance spectroscopy (MRS) and chromatography-spectroscopy are the most common methods used in metabolomics. MRS uses techniques fundamentally similar to MRI to determine the concentrations of certain molecules in samples. Critically, it does not use ionizing radiation, so can be carried out *in vivo* with minimal risks (Hwang & Choi, 2015).

MRI works by first aligning the spins of atomic nuclei to an external magnetic field and then disturbing the status with a radiofrequency pulse. The nuclei then return to their resting position, emitting a faint but detectable RF signal. While conventional MRI is, essentially, a picture formed from water density data, MRS brings in another dimension by recording the emission spectra, allowing the detection of other molecules besides water. Therefore, while MRI allows for anatomical imaging, MRS allows retrieval of the biochemical status of the sample. The limitations of MRS include the high cost of the equipment and long duration of the measurements. Temporal, spatial and spectrographic resolutions of the measurement are limited by both the measurement time and magnetic field strength. If greater resolution is desired, either the field strength or the measurement time must be increased.

Given the limitations of measuring metabolite concentrations *in vivo*, sample-based analysis methods are needed. The standard way to do this is to use mass spectrometry and either gas (Chanpimol et al., 2017) or liquid chromatography (Gika et al., 2019) in combination.

Chromatography is based on different interactions of various compounds with a mobile and a stationary phase. In metabolomics, it is used as the first phase of separation, with compounds being separated in time. Afterwards, the separate is moved into mass spectrometry. The basic principle is to ionize the analyte,

accelerate it via electric fields (and select only a certain speed of ions to go through to the next stage), and then deflect the ions by using magnetic fields. The force imparted by the magnetic field is related to the charge of the ion, and the acceleration it undergoes is dependent on the force and the mass of the ion. Thus, mass spectrometry sorts ions by their mass/charge ratio.

To aid in the identification of the analyte, *hard ionization*, which in addition to converting the analyte to ions also fragments it into smaller particles, is used (Chong et al., 2018). This is useful, as while two bigger molecules might have the same mass/charge ratio, they most likely have different fragmentation patterns, resulting in a different spectrum from all the fragmentation products. This allows more precise identification of the compounds.

2.6.2 Areas of application

2.6.2.1 Experimental research use

Compared to metabolomics, genetics is static within an organism, epigenetics nearly so, and even proteomics fails to capture the ever-changing nature of the cellular mechanics, as it is restricted in temporal resolution by the time taken for protein synthesis. In contrast, metabolomics captures even the rapid changes in the state of the organism.

This has allowed metabolomics to be used in both an untargeted manner, generating new hypotheses, and a targeted manner, investigating pre-existing hypotheses (Schrimpe-Rutledge et al., 2016). For example, metabolomics has been used to investigate the metabolic changes behind obesity (Daniel et al., 2019) and peer inside cancer cells (Dinatale et al., 2019).

However, while metabolomics has shown great promise, it is still a field in its infancy, with many unanswered questions. In particular, there is no consensus on managing the false discovery rate, and the lack of reference standards for many metabolites hinders accurate identification (Schrimpe-Rutledge et al., 2016). Nevertheless, the authors consider metabolomics as one of the most accurate and promising methods for looking into the mechanisms underlying health and disease, as it is highly correlated with the phenotype.

2.6.2.2 Use in clinical research

Metabolomics is still mostly confined to experimental use, with both the procedures and interpretation of the result quite challenging. Nevertheless, metabolomics is increasingly being used in various areas of clinical medicine.

Metabolomics methods have been used to predict disease development and treatment responses. Cardiovascular disease is one of the most prevalent killers in the developed world, but chemical biomarkers for it are mostly limited to troponins, creatine kinase and brain natriuretic peptides, which are poorly suited to early detection. Metabolomics has recently shown promise in the early detection and characterization of the disease (Dona et al., 2016).

As another example, lipid metabolomics profiles have specifically been reported to differ between cancer patients and healthy controls, potentially allowing the use of metabolomics as a screening tool (Spratlin et al., 2009). Metabolomics has also been reported to be potentially useful in monitoring the response to cancer treatment (Spratlin et al., 2009).

2.7 REPLICATION CRISIS

Lately, there has been growing concern about the inability to replicate a large number of reported effects (Collaboration, 2015). tES especially seems to be a magic bullet, with a wide variety of sometimes contradictory effects being reported. A recent meta-analysis has questioned the efficacy of tDCS in altering physiological measurements other than MEPs (Horvath et al., 2015), although the study has received heavy criticism for methodological shortcomings, such as incorrectly pooling together studies with incompatible stimulation parameters (Antal et al., 2015).

While the general consensus, despite the previously mentioned criticism, seems to be that tES is indeed an effective neuromodulation tool, several specific findings have lately been unable to be replicated (Boayue et al., 2020; Minarik et al., 2016). It stands to reason that many of the previously mentioned, widely varying results would also fail replication, and thus should be taken with a grain of salt. Being an adaptable and rather novel too, the field of tES has an abundance of preliminary, low-power results that should not be taken as definitive.

On the other hand, when trying to replicate results, the relevant parameters have to be copied carefully, including the stimulation current, length, number of sessions, placement of both electrodes, and the handedness and health of the participants. Even minor changes in any of these parameters could change the stimulation results. It has also been suggested that studies should employ *a priori* power analysis to determine a sufficient sample size, and that any datasets gathered should be available for analysis and meta-analysis by other groups (Minarik et al., 2016).

3 AIMS OF THE STUDIES

The aims of this study were twofold: firstly, to examine the factors affecting AEs and the safety of tES, and secondly, to examine the effects of tDCS on peripheral circulating metabolites.

The specific aims of the substudies were to:

1. Determine whether and possibly how multiple consecutive sessions of tDCS modify the AEs. I also aimed to determine the effects of age on AEs;
2. Introduce a novel case of tDCS-induced burns;
3. Determine the effects of various lifestyle factors, as well as the sex of the subjects, on the AEs they experienced from tDCS and tRNS;
4. Investigate how multiple sessions of tDCS potentially affect a large panel of peripheral metabolites, and determine the sample sizes required to detect any changes to assist in planning future studies.

4 MATERIALS AND METHODS

4.1 SUBSTUDY I

4.1.1 Subjects

The subjects were gathered as part of the Optimizing Transcranial Electrical Stimulation for Clinical Applications (OptES) Study. The study protocol was approved by the Ethics Committee of the North Savo Hospital District, Finland. Written informed consent was obtained from all the participants.

Eighty-two healthy, right-handed, tDCS-naïve males aged 18-40 years were recruited from the North Savo region of Finland. Exclusion criteria are explained in Table 2. The last two exclusion criteria are due to the metabolomics determination as part of the same study.

Table 2 Inclusion and exclusion criteria of the tDCS sample

Inclusion criteria	**Exclusion criteria**
Age of 18 to 40 years	Metal implants inside the skull or the eye
Male sex	Severe skin lesions of the stimulation area
Right-handed (1**st** to 10th right decile in the Edinburgh handedness questionnaire)	A pacemaker
No previous tDCS experience	History of epilepsy or seizures
	Intracerebral bleeding within 6 months
	History of any endocrinological conditions
	Self-reported history of substance use/dependency within 6 months

4.1.2 Study Procedures

All participants were asked to abstain from alcohol for 12 hours and consume no more than two doses during the preceding 24 hours, to abstain from caffeine for 3 hours, and abstain from smoking and exercise for one hour before the study appointments. Each participant was asked if they had followed the instructions before the stimulation, and were excluded if the instructions were not followed. Each participant took part in five consecutive stimulation sessions, in which they received either 20 minutes of 2 mA stimulation (with 15 s of ramp up and ramp down) or an equivalent duration of sham stimulation (with the stimulation present only at each end of the session, to induce comparable sensations to the real

stimulation). The stimulator used was a neuroConn DC stimulator (neuroConn GmbH, Ilmenau, Germany).

The electrodes were 5 x 5 cm rubber electrodes placed inside saline-soaked sponge pads. Twelve millilitres of saline was used. The anode was placed at site F3 and the cathode at F4, using the international 10-20 electroencephalography system.

Before the first stimulation, the patients completed a 10-item version of Cohen's Perceived Stress Scale (Cohen et al., 1982), as well as a background questionnaire from which data about sex and age were extracted. After each stimulation, the participants and the experimenter both completed a form rating various AEs (including skin redness) on a scale of 0 to 100. The participants were given a mirror to aid in evaluating the skin that was under the electrodes, and both the participants and the experimenter were blinded to the stimulation condition.

4.1.3 Statistical analysis

The AE data contained excess zeros and were non-normally distributed. Preliminary analysis was conducted using the Mann-Whitney U-test, and more detailed analysis was performed using fixed-effects (to analyse the likelihood of AEs) and mixed-effects zero-inflated Poisson models[15] (to analyse the intensity of the AEs). The analysis was carried out using SPSS 21 and the R scripting language package glmmADMB (Fournier et al., 2012). Skin redness was chosen as the variable of interest due to being common enough for these analyses to be possible.

4.2 SUBSTUDY II

4.2.1 Subjects

In tDCS studies at the University of Kent, UK, the same stimulation protocol has been used over 400 times, resulting in two incidences of skin lesions. Substudy II was a case report describing the observed lesions. The subjects were both female, aged 18 and 19 years. The studies from which these cases were derived were

[15] Zero-inflated models allow for analysis of statistical distributions with excess zeros. They combine two parts into one mode: one generating zeros, and the other, in this case a Poisson distribution, generating other values, some of which may also be zero. Factors affecting the likelihood of being an excess zero and those affecting the Poisson part of the model are modelled separately

approved by the ethics committee of University College London and the University of Kent. Participants gave written informed consent.

4.2.2 Study Procedures

The stimulator used was a neuroConn (Ilmenau, Germany) DC stimulator, and 5 x 7 cm rubber electrodes, enclosed in saline-soaked sponges, were used. A 20-ml pod of saline was used for the two electrodes. The stimulation was 1.5 mA for 15 minutes, with 16 seconds of ramp up and ramp down. The anode was on position F3 and the cathode was placed on the left wrist. The electrodes were held in place with a sports bandage.

4.3 SUBSTUDY III

4.3.1 Subjects

For a description of the tDCS sample, please see section 4.1.1 above. In addition to the tDCS sample, this study included a second sample using tRNS. The tRNS sample was also gathered as part of the OptES Study, among which a total of 60 males and females were recruited from the North Savo region of Finland. Please see Table 3 for a summary of the inclusion/exclusion criteria.

Table 3 Inclusion and exclusion criteria of the tRNS sample

Inclusion criteria	**Exclusion criteria**
Age of 18 to 50 years	Metal implants inside the skull or the eye
Right-handed (1**st** to 10th right decile in Edinburgh handedness questionnaire)	Severe skin lesions of the stimulation area
	A pacemaker
	History of epilepsy or seizures
	Intracerebral bleeding within 6 months

4.3.2 Study Procedures

For a description of the study procedures of the tDCS sample, please see section 4.1.2 above. In the tRNS study, the participants received both tRNS and sham stimulation. The stimulations were given in separate sessions, in a random order, and with at least two weeks between the sessions. The study was double-blinded. After each stimulation, both the participant and the experimenter were asked to rate a number of AEs on a scale of 1 to 100. The difference compared to the tDCS study was that the participants were not asked to rate skin redness.

The stimulation was 2 mA peak-to-peak between +1 and -1 mA, with the current fluctuating between 101 and 640 Hz. In the tRNS study, similarly to the tDCS study, the participants were asked to refrain from alcohol use for 12 hours, to consume at most 2 doses within the preceding 24 hours, to not use caffeinated products within 3 hours, and to abstain from heavy exercise and smoking for 1 hour before the study appointments.

In both samples, before the stimulation, the participants were asked to complete a background questionnaire, from which data about age, alcohol use (utilising the Alcohol Use Disorders Identification Test C (AUDIT-C, Bradley et al., 1998) score), smoking (as years of smoking), exercise (as average hours of exercise per week), quality of sleep of the night preceding the stimulation and sex (in the tRNS study) was extracted. While AUDIT cannot distinguish between different types of healthy alcohol use, it is a readily available measure of alcohol (ab)use in a clinical setting and was thus chosen.

4.3.3 Statistical analysis

To deal with the significant over-dispersion of the AE data, negative binomial regression[16] was chosen. All analyses were run using R, with the package MSME (Hilbe & Robinson, 2018). Potential predictors considered in the analyses were alcohol use (as measured with AUDIT-C score), years of smoking, quality of sleep (on a scale ranging from 1 (very good) to 4 (very poor)), duration of sleep (hours) and weekly exercise (hours). The AEs of interest were feelings of tiredness, sensations under the electrodes, and skin redness under the electrodes.

Since stimulation condition and age could modify AEs, they were included in all the negative binomial models. To allow the models to be used with our limited dataset, a separate model, describing adverse effects with age, stimulation group, predictor variable, and the interaction of group and predictor variable ("Adverse effect ~ Age + Group × Predictor" in R-syntax) was built for each adverse effect–predictor pair. The main outcomes of interest were the Group x Predictor and Predictor coefficients.

In addition to the analyses focusing on potential predictors, both visual inspection of the data and Mann-Whitney U-tests (comparing days 1 and 5 of the tDCS study) were used to investigate the progression of the AEs. These methods were chosen to enable analyses of all the AEs of interest, as the data on AEs other than skin redness were too sparse to enable more sophisticated analysis methods.

4.4 SUBSTUDY IV

4.4.1 Subjects

For a description of the sample, please see section 4.1.1 above. For this substudy, we excluded three individuals from whom we were unable to obtain the needed serum samples. Thus, the final sample was 40 subjects in the tDCS group and 39 in the sham group.

4.4.2 Study procedures

For a description of the study procedures, please see section 4.1.2. In addition, the parts of the procedure relevant only to this study are described here.

[16] The mixed-effects ZIP used in substudy I allowed us to examine within-subjects factors (time), but only skin redness data could be fitted due to rather noisy data. On the other hand, this package and negative binomial models did not allow for mixed effects, but was robust when faced with overdispersion, and allowed other AEs to be analysed.

Venous blood samples were collected by a qualified nurse before and after the first study session, as well as after the fifth session. The samples were left at room temperature for 30 minutes and centrifuged at 2400 g for 10 minutes. The serum was extracted, frozen at -80 °C and shipped for analysis. Ultra-pressure liquid chromatography combined with mass spectrometry, using an ACQUITY UPLC-MS/MS system (Waters Corporation, Milford, MA, USA), was used.

4.4.3 Statistical analysis

In preliminary inspection, 9 out of 102 metabolites were excluded from any further tests due to ≥50% missing values within one or both of the experimental groups to avoid unanticipated biases. Furthermore, all the metabolite variables were analysed for normality using the Shapiro-Wilk test, and were transformed[17] with the function getting them closest to normality (from raw, negative reciprocal, power 2, power 3, power 4, power 1/2, power 1/3, and natural log transformations) to allow for analysis and to reduce the influence of extreme outliers.

The transformation and standardisation not only helped in comparing relative changes across metabolites, but also in establishing the power and sample size calculations. These latter procedures were based on simulated data following a standard normal distribution and replicating the standardised group differences observed in the normalised and standardised metabolite data. Therefore, taking into account the Central Limit Theorem, these computations should hold and be considered valid, although caution would be advisable for researchers aiming to use these estimates in future studies, as they represent the power and/or sample size

[17] Transformation is a mathematical operation that changes the measurement scale of a variable (normally within a model, this would be the dependent variable) to follow a more symmetrical (Gaussian) distribution. This data pre-processing would help in fulfilling certain criteria for more reliable model estimates (e.g. Gaussian distribution of model residuals). Thus, modelling transformed data does not invalidate the statistical procedure, although one needs to be aware that model estimates are based on rescaled data, and this procedure should be used with caution in the case of independent variables with asymmetric distributions that greatly differ from the normal distribution. In addition, clinical decisions need to be taken on model estimates scaled back to "real" units. In addition to the transformation, the variables were also standardised to the "standard normal", so that means would equal 0 and standard deviation would equal 1. This data pre-processing, with specific transformations for each metabolite followed by a standardisation, is occasionally implemented in metabolomics analyses; see, for instance, Vogt et al. (2016).

for variables with such characteristics (that is, researchers in future studies should also aim to normalise and standardise their data to replicate our findings).

Power calculations were conducted using simulation methods in a computer cluster environment. This was done by simulating 10 000 samples with 10 individuals per group, and if the simulated power was <80%, a new set of 10 000 was generated with more individuals added. This process was automatically repeated until the statistical power was >80%, with the final number of individuals noted as a result. This was done for alpha values of 0.05 and 0.0005376. The metabolites with "time x group" coefficients of less than 0.005 (for estimations with an alpha value of 0.0005376) and 0.05 (for estimations with an alpha value of 0.05) were omitted from the analysis. This was in order to avoid excessive computation time, as well as considering the fact that the sample sizes to detect such small effects would be prohibitively large, rendering the information about required sample sizes rather useless.

Longitudinal analysis was performed using Generalized Estimating Equations (GEE) models, which estimate the regression coefficients for both main effects and interactions. To account for within-individual correlations, the variances were then multiplied against a working correlation matrix. The preliminary model was then entered into an iterative least squares process to produce the final fitted model.

The alpha level was determined by dividing the conventional level (0.05) by the product of the minimum number of principal components explaining >95% of the observed variance, and the number of model contrasts. Consequently, the p-value threshold was set at 0.05 / 93 = 0.0005376.

This p-value correction method is explained in full detail within the "Supplementary Material" of the corresponding study. Despite its use not being widespread, we believe it is a statistically valid and accepted procedure of implementing family-wise error correction of p-values, or Bonferroni correction as the most known of them, in situations in which multiple correlated variables are modelled separately within the same experiment. In such a case, each model or statistical test is not fully independent, as dependent variables are not between themselves, thus violating Bonferroni assumptions.

The computation of the principle components (a statistical technique for reducing data dimensionality) from the modelled, correlated variables (the larger set of metabolites) examines of the number of independent variables into which the variability within them can be reduced, thus allowing the use of the Bonferroni method with the pertinent adaptation of the algorithm. This procedure has already been successfully implemented by many researchers, including in the field of metabolomics. Please see, for instance, Kujala et al. (2013).

5 RESULTS

5.1 SUBSTUDY I

Tables 4 and 5 present abbreviated results from substudy I, the results being derived from the fixed effects zero-inflated Poisson (ZIP) and mixed-effects ZIP models, respectively. In almost all of the models, active stimulation was associated with increased skin redness. Age was not a significant predictor in fixed-effect models. However, in the mixed-effect model, which included all the data and as such was considered more powerful[18], a higher age predicted stronger skin redness. Most importantly, the number of stimulations ("Day") was not a significant predictor. Perceived stress was also not a predictor.

[18] The software used when running mixed-effects models did not return coefficients for the zero inflation part. Thus, we ran in both a fixed-effects model (not including the effect of consecutive sitmulations, and having a separate model for each day) to assess the factors influencing the likelihood of being an excess zero, and a mixed-effects mode to assess the factors influencing the intensity of the adverse effect.

Table 4. Abbreviated results of substudy 1: Fixed-effect models, effect of group and participant age on skin redness rated by both the experimenter and the participant, zero-inflation part of the model. Please note that the values here indicate the change from being an excess zero. For example, on day two, the active stimulation group is less likely to be excess zero, and thus more likely to be non-zero. That is, the active group is more likely to experience AEs.

		Participants		**Experimenter**	
		Coefficient	**p-Value**	**Coefficient**	**p-Value**
Day 1	**Group**			-0.392	0.421
	Age			-0.029	0.502
	Group x Age			0.072	0.409
Day 2	**Group**	-1.914	**0.001**	-0.897	0.059
	Age	0.028	0.541	-0.017	0.677
	Group x Age	-0.021	0.835	-0.065	0.452
Day 3	**Group**	-1.589	**0.004**	-1.408	**0.004**
	Age	0.042	0.358	-0.015	0.718
	Group x Age	0.007	0.952	0.024	0.773
Day 4	**Group**	-1.000	**0.037**	-0.388	0.405
	Age	0.008	0.845	-0.046	0.263
	Group x Age	0.059	0.482	0.050	0.555
Day 5	**Group**	-1.829	**<0.001**	-2.316	**<0.001**
	Age	0.040	0.364	-0.018	0.692
	Group x Age	0.045	0.676	-0.067	0.500

Table 5. Abbreviated results of the substudy 1: Mixed-effect models, effect of group and participant age on skin redness rated by both the experimenter and the participant, Poisson part of the model.

	Participants		**Experimenter**	
	Coefficient	**p-Value**	**Coefficient**	**p-Value**
Group	0.205	0.107	0.455	**<0.001**
Day	0.035	0.346	-0.016	0.587
Age	0.038	**<0.001**	0.019	**0.008**
Group x Day	0.063	0.464	-0.042	0.503
Group x Age	0.041	0.055	0.047	**0.005**

5.2 SUBSTUDY II

As this was an observational case report, no statistical analyses were conducted. However, to summarize, we reported two cases of stimulation-induced delayed lesions near the cathode on the wrist of the subject. One was under the electrode, while the other was on the contralateral side of the wrist, under the sports bandage holding the electrode in place. The reported lesions were of a rare type, and no lesions distant from the electrodes have previously been reported. The lesions healed well, although taking several weeks.

5.3 SUBSTUDY III

Table 6 summarizes the most important results from substudy III. As the alpha level of 0.0083 was chosen after Bonferroni correction, none of the models reached significance. In short, we could not detect whether the investigated lifestyle factors modified tDCS or tRNS AEs.

Table 6. Abbreviated results of substudy III: Effects of various lifestyle factors on skin redness, tiredness and sensations under the electrodes. Only the interaction (Group x Predictor) coefficients are shown. The tDCS study was male only, so sex was not analysed.

		tDCS Day 1		tDCS Day 5		tRNS	
		Ratio	**P-Value**	**Ratio**	**p-Value**	**Ratio**	**p-Value**
Redness under the electrodes	**Sex**					3.03	0.640
	Hours of exercise	1.02	0.767	1.04	0.692	1.21	0.430
	AUDIT_C	1.01	0.950	0.89	0.616	0.15	0.047
	Years of smoking	1.77	0.166	1.23	0.328	0.01	0.969
	Sleep duration	1.17	0.664	0.70	0.404	0.28	0.341
	Sleep quality	0.41	0.163	3.46	0.069	4.47	0.468
Tiredness	**Sex**					0.44	0.512
	Hours of exercise	1.68	0.120	1.07	0.812	0.92	0.733
	AUDIT_C	0.91	0.842	1.19	0.767	1.03	0.937
	Years of smoking	9.46	0.221	2.30	0.126	0.95	0.786
	Sleep duration	1.52	0.492	1.37	0.771	1.39	0.602
	Sleep quality	2.97	0.358	3.42	0.455	0.85	0.859
Sensations under the electrodes	**Sex**					0.04	0.076
	Hours of exercise	0.93	0.204	0.97	0.623	3.09	0.065
	AUDIT_C	1.14	0.304	1.12	0.471	0.76	0.678
	Years of smoking	0.93	0.297	1.03	0.809	0.71	0.224
	Sleep duration	0.72	0.201	1.13	0.634	4.83	0.083
	Sleep quality	1.40	0.398	0.98	0.964	0.75	0.878

Abbreviations: AUDIT-C = Alcohol use disorders identification test C; tDCS = transcranial direct current stimulation; tRNS = transcranial random noise stimulation

5.4 SUBSTUDY IV

After correcting for multiple analysis, no models reached statistical significance. However, sophisticated power calculations were performed in order to determine the sample sizes necessary for future studies investigating the same metabolites (Figure 8). The sample sizes required, according to our analyses, range from dozens per group, which is quite manageable, to tens of thousands per group, which is decidedly not.

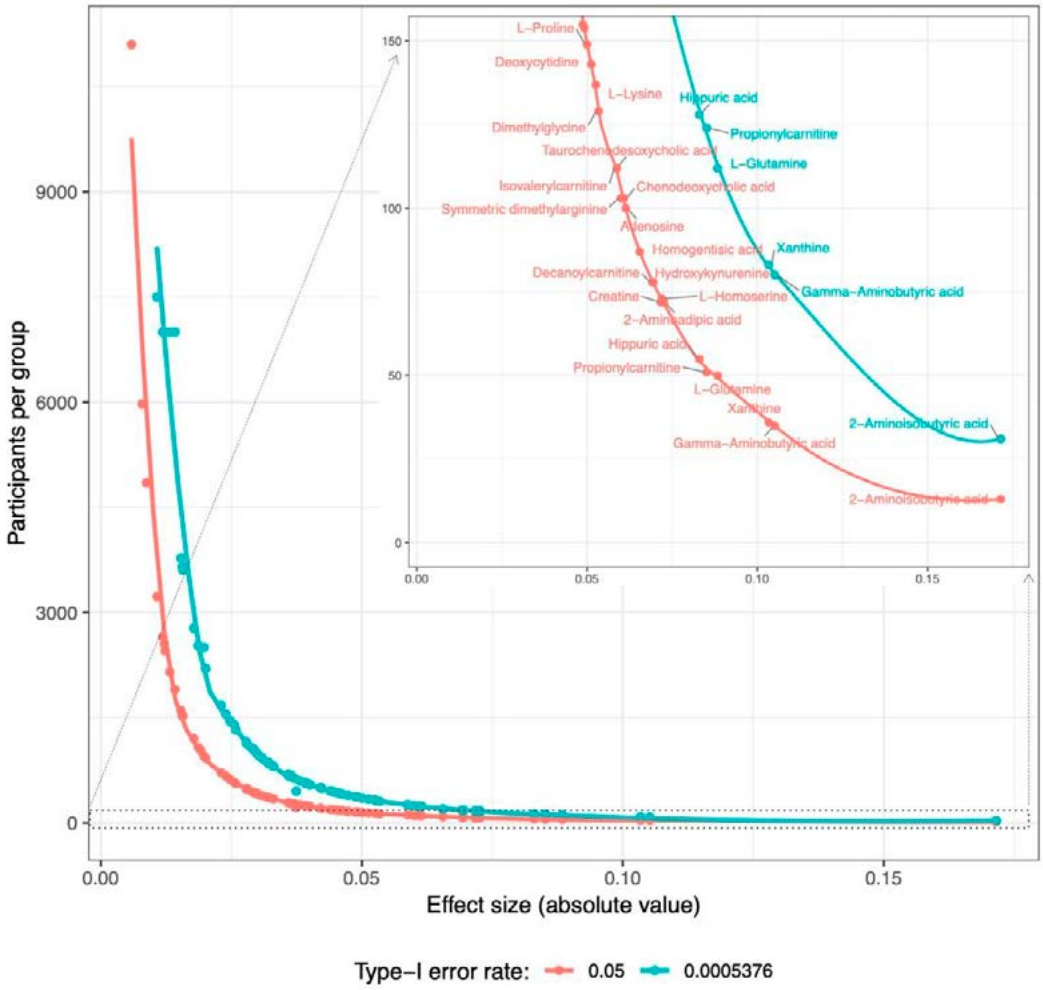

Figure 8. Number of participants per group required to detect changes in selected metabolites by tDCS. The presented values are based on sample size calculations derived from the observed differences between tDCS and sham stimulation groups after five consecutive days of stimulation. Originally published in "Anodal tDCS over left prefrontal cortex does not cause clinically significant changes in circulating metabolites," Kortteenniemi et al., Frontiers in Psychiatry, 2020. Reprinted with permission from Frontiers with minor modifications.

6 DISCUSSION

6.1 MAIN FINDINGS

6.1.1 Substudy I

The main findings of this study could be summarised as follows:

- Active stimulation was associated with more skin redness when compared to sham stimulation
- A higher participant age was associated with more intense skin redness
- Skin redness did not increase as the number of consecutive stimulation sessions increased

Skin redness was used as a proxy for all AEs, as it was the most common phenomenon, and thus the data concerning it were most suitable for the analyses conducted. While to my knowledge there is no evidence suggesting that people who experience more skin redness also experience more adverse effects of other types, skin redness was the only adverse effect from our data suitable for the analysis methods in this study, specifically for analysing the effect of consecutive stimulations.

6.1.2 Substudy II

As an observational case report, substudy II did not have main findings *per se*, merely a report of a pair of tDCS-induced burns, one of them unusually on the opposite side of the wrist from the electrode. However, the observations suggest that measuring the amount of saline used in moistening the sponges is crucial in avoiding AEs.

6.1.3 Substudy III

We did not find any evidence that the investigated lifestyle factors (i.e., exercise, smoking, use of alcohol and sleep) affected tDCS or tRNS AEs. Even though the absence of evidence is not evidence of absence, this may suggest that these factors can be ignored when choosing potential study or patient populations.

6.1.4 Substudy IV

TDCS did not appear to modulate any of the investigated metabolites. However, we ran comprehensive power calculations, which offer an efficient starting point for future studies seeking to determine the required sample sizes for investigations focusing on the same metabolites.

6.2 COMPARISON WITH PREVIOUS LITERATURE

6.2.1 Substudy I

Several previous studies (for example: Antal et al., 2017; Ezquerro et al., 2017; Nikolin et al., 2018a) have previously determined that skin redness is more prevalent in those receiving tES compared to the sham stimulation. The relationship of age with tES AEs has, to my knowledge, not been extensively explored in the literature, although a study by Maizey et al. (2013) suggested that age does not modify TMS adverse effects. However, some studies have been conducted regarding the relationship between subject age and tES treatment outcomes. For example, Málly et al. (2018) found that a combination of tDCS and TMS had a much better effect in slowing the progression of Parkinson's disease in younger patients when compared to older ones. While it is doubtful that the same mechanisms would be behind their findings and ours (as PD affects the CNS, while skin redness is a topical reaction), it follows the general wisdom in medicine: the elderly, or people who are otherwise frail, are often more susceptible to AEs (Beijer & de Blaey, 2002).

Nikolin et al. (2018a) have published a meta-analysis on the effect of consecutive tDCS stimulations on AEs. In their analysis, they used cumulative charge instead of stimulation days to account for variation in stimulation protocols, but mentioned that cumulative charge and the number of stimulation days were highly inter-correlated. Nevertheless, their findings support ours: continued exposure to tDCS in adults does not appear to pose a significant risk from the AE standpoint. However, the previous meta-analysis was criticized for including investigations ranging from RCTs to observational studies (Alwardat, 2018). While the authors defended their methodology convincingly (Nikolin et al., 2018b), the issue highlights the lack of research specifically focusing on tES AEs. Unfortunately, this is the only meta-analysis on the progression of tDCS adverse effects we could find.

6.2.2 Substudy II

In this case report, we described delayed skin lesions following tDCS. Some previous case reports have described a similar phenomenon (Palm et al., 2008; Riedel et al., 2012), with lesions appearing up to 1–2 days after stimulation (Frank et al., 2010). Some of the risk factors suggested are the use of tap water in the electrode sponges (Frank et al., 2010) and degraded electrode sponges (Rodríguez et al., 2014). However, in all the above-mentioned cases the lesion has been under the stimulating electrode, while in one of our cases, the lesion appeared on the side of the palm contralateral to the electrode.

The explanation we proposed was oversaturation of the saline sponge, leading to saline solution leaking out under the sports bandage and forming a conductive path. As overly dry electrodes are also a previously known factor for cutaneous AEs (i.e., insufficient saline can lead to varying resistance over the electrode surface, causing current density to increase in some parts), we concluded that proper measurement of the saline is crucial when utilising tES.

6.2.3 Substudy III

A very limited amount of research is available regarding the possible effects of lifestyle factors on tDCS or tRNS AEs, even though lifestyle data are widely gathered in clinical settings and are known to have widespread effects on disease processes and the efficacy of treatments (Diaz & Shimbo, 2013; Kolb & Martin, 2017; S. Mannu et al., 2013). This might be because tES is generally considered to have minimal AEs.

Some studies have been conducted on the effects of tES on tobacco craving (Coles et al., 2018; Fecteau et al., 2014), but these investigations did not mention AEs at all. Nevertheless, the former study was constructed to measure the effects of tES on tobacco use, and not to measure the effects of tobacco use on tES effects. The state of knowledge is similar regarding tDCS and alcohol use. One previous study focusing on the topic (Klauss et al., 2014) did mention AEs, but simply described how they were similar across the groups.

In conclusion, to the best of my knowledge, there appears to be no previous literature on the effects of lifestyle factors on tES AEs. Even studies involving both tES and lifestyle factors have focused on other aspects of the interaction between the two, not on the possible modulatory effect on AEs.

6.2.4 Substudy IV

Similarly to the case with the above, previous substudies, studies on the effects of tES on peripheral metabolites are scarce. However, a few studies on the topic exist. For example, tDCS has been demonstrated to lower salivary and venous cortisol levels (Antal et al., 2014; Brunoni et al., 2013b; Sarkar et al., 2014), and cortisol has a profound effect on the metabolic processes of the body. On the other hand, blood glucose has been lowered by tDCS (Binkofski et al., 2011; Kistenmacher et al., 2017), which the authors explained as chiefly being caused by an increase in brain glucose uptake.

On the other hand, some studies have examined the effects of tDCS on central levels of glutamate and glutamine, with the compounds combined into a combination variable (Glx) due to technical limitations of magnetic resonance

spectroscopy (Hone-Blanchet et al., 2016; Hunter et al., 2015). While our study found no changes in the levels of circulating peripheral metabolites, this might be due to a few factors: either the changes in central levels are not reflected in the peripheral blood, or our study setting was not sensitive enough to detect such changes. Our power calculations indicated the latter to be the case for several of the measured metabolites.

6.3 STRENGTHS AND LIMITATIONS

The main strength of our studies is the reasonably large sample size, followed by the robust study protocols with randomized, sham-controlled study designs. In all of these studies, we provided detailed instructions for preparation to the participants in order to minimize confounding factors, such as the acute effects of alcohol or caffeine. In addition, in the tDCS sample, we instructed the subjects to fast before blood sampling to obtain fasting samples and minimize dietary effects on the investigated metabolites.

While our study settings do not allow us to differentiate between effects specific to a certain tES montage and effects that would be generated by *any* montage, the montages chosen are common and thus maximise the utility of our findings for any future research. Specifically, the gathered tDCS sample utilised the anode at F3 (according to the international 10-20 EEG system) and the cathode at F4, a commonly used montage for treating, for example, major depressive disorder. The tRNS montage we applied is also common, having been used, for example, to treat depression (Evans et al., 2018) and modify the reaction time (Brevet-Aeby et al., 2019). However, we consider most of the AEs (namely the skin reactions and sensations under the electrodes) local to the skin–electrode interface, and the specific montage should therefore have a minimal effect on them.

The tDCS sample has a few limitations. We had no information on whether, or how, changes in metabolite concentrations in the brain were reflected in the peripheral blood, and a concurrent MRS measurement would therefore have been illuminating, while not feasible due to economic issues. Another possible limitation is the fact that the participants were able to guess the group they were in with a frequency slightly deviating from randomness (please see substudy IV). Awareness of belonging to the tDCS group could have resulted in elevated levels of anxiety, which may have led to physiological changes. Although this could, in principle, have partly masked the changes in metabolites where the stimulation and the anxiety have opposing effects, the difference between the groups was so small that any

possible effect of this nature may be considered negligible. We also did not run models examining the relationship between stimulation awareness and AEs.

A third limitation is how we only had three time points for the metabolite analysis: day 1 pre-stimulation, day 1 post-stimulation and day 5 post-stimulation. This somewhat limited the analysis methods available. Having been able to obtain pre- and post-stimulation venous blood samples from all stimulation days would have provided ideal information from the perspective of any possible tDCS-induced, temporally changing metabolic patterns in peripheral circulation.

Both samples represented healthy volunteers. These individuals were predominately young and white, which might pose a problem for generalization of the results. In addition, having partially different types of measures available for lifestyle habits could have been ideal. For example, the AUDIT scale, while extremely widely used, is intended to measure problematic use of alcohol, not alcohol use in general. The measures used were chosen due to their active use in clinical settings.

Regarding the AEs, Brunoni et al. (2011) report that actively asking about different AEs increases their frequency in tES studies. While this might be considered to make our approach overly sensitive, overestimating the AEs, we consider this a strength: if we did not detect any effect on the AEs using this potentially more sensitive approach, we can safely extrapolate the obtained findings to the clinical use of tDCS without similar AE questionnaires.

6.3.1 Strengths and limitations of the statistical methods

Another strength of the conducted studies is the use of sophisticated statistical methods, tailored to both the data at hand and the questions I sought to answer. The design and execution of the analyses heavily involved professional statisticians, and the analyses allowed for the inclusion of confounding factors into the models, increasing the predictive power.

Our studies reported mostly negative findings. We decided to use the working hypothesis that stimulation does cause AEs, as while the statistical analysis of the matter in the literature is rather limited, the occurrence of AEs is rather commonly reported. As null hypothesis testing is not the ideal method for gathering evidence for the absence of an effect, this might not have been the best method in hindsight. While I, lacking a better explanation, conclude that our null hypotheses represent the most likely scenario given current evidence, experiments using other methods such as equivalence testing (Lakens et al., 2018) are needed.

In substudy four, we ran *post hoc* power analyses on the measured metabolites. This has been, and fairly so, criticized for resulting in poor estimates of required

sample sizes due to noisy observational data (Gelman & Carlin, 2014). Nevertheless, we decided to include the analyses in our paper to both help avoid replication issues and to rationalize our conclusion that tES probably has no effect on peripheral metabolites. In any given case, a negative result could mean that either the null hypothesis is correct and there is no effect, or that the findings are negative despite the null hypothesis being incorrect. The latter could be explained by either methodological issues within the study or a lack of statistical power to detect a real, but small, change. Despite being hotly debated, we maintain that our power calculations at least suggest that any possible effect would be small, leading us to suggest that the null hypothesis being correct is the more likely explanation.

I also believe that while the power calculations might be noisy, and using them on a per-metabolite basis to detect the necessary sample size for any given metabolite is ill-advised, they do offer a general guideline for any future studies on the effects of tES on metabolites, as no better data on the necessary sample sizes exist. In general, our calculations show that rather large sample sizes are necessary, so a false negative result based on our suggestions would be rather unlikely. Thus, they may serve to help avoid underpowered studies in the future, which have been a significant problem in health sciences. However, I continue to emphasize that due to the limitations of running *post hoc* calculations, our power analyses are likely to contain inaccuracies, and should only be used as general starting points, not as per-metabolite guides for necessary sample sizes.

6.4 IMPLICATIONS OF THE OBSERVED FINDINGS

6.4.1 Adverse effects and safety

While I investigated several factors that could potentially modulate the tES AEs (i.e., age, stimulation count and different lifestyle factors), none of the investigated potential modulators, except for age, appeared to modify tES AEs.

Even though data on lifestyle factors are commonly gathered and considered in clinical settings, it is helpful to know that they do not need to be taken into account while evaluating a patient's risk of tES AEs. However, as a higher age was associated with increased skin erythema, it should be considered when planning treatment, by warning the patient and by trying to find the minimal effective dose.

Given the current trend towards an increasing duration of treatment protocols under research, the fact that AEs – or skin redness as a proxy for AEs in general – do not appear to cumulate over consecutive treatment sessions is convenient. To the best of my knowledge, our study was the first time the progression of AEs over time has been statistically modelled.

6.4.2 Metabolomics

While we observed no metabolite changes in serum, it is not possible to exclude undetected metabolic changes in the brain tissue. The exploratory, multiple-comparisons nature of our study resulted in a poor sensitivity to smaller changes. Nevertheless, the lack of significant results does imply that tDCS, or at least the protocol we used, does not have a major effect on the circulating metabolites measured.

Transport over blood–brain barrier (BBB) is regulated, and not all changes in the intracerebral space might be reflected in the peripheral circulation. Metabolite profiles in the CSF could change, even if they do not do so in peripheral blood. Having an opportunity to also investigate cerebrospinal fluid levels of metabolites or to conduct MRS measurements would have enabled the testing of the above-stated hypothesis. However, performing spinal taps for a sample of healthy volunteers was not possible due to ethical reasons.

If other tDCS montages were to provide conflicting findings either in serum or other biofluids, these might be due to the altered function of the cortex modifying peripheral metabolic activity instead of altered local metabolism and/or local alteration in BBB permeability. The latter types of changes are what our study was most suited to finding, even if our power to detect such changes was quite low.

Cerebral blood flow changes following tDCS could also potentially affect the transfer of metabolites across the BBB, with increased blood flow facilitating increased transfer. However, Paquette et al. (2011) demonstrated that while tDCS affects the magnitude of the task-induced change in cerebral blood flow, tDCS had no effect on basal cerebral blood flow in the resting state. As the participants in our study received stimulation while resting, it is unlikely that changes in blood flow could have affected our observations. A study protocol where the participants would have been actively performing a mental task might have been more illuminating in this aspect.

The implications of the metabolomics substudy can be considered to be two-fold. First, the lack of peripheral effects makes evaluating the safety of tES among different patient populations an easier task. On the other hand, blood samples could have provided a convenient medium to measure some of the effects of tES, but our study suggested that this may not be feasible.

6.4.3 Blinding issues

While this work did not focus on blinding issues, substudy IV agrees with previous research suggesting that current blinding protocols are insufficient. In addition, skin redness has been suggested to be one of the AEs breaking blinding (Ezquerro et al., 2017). Thus, the fact that we found no proof that it worsens with consecutive stimulations is reassuring. However, our research does not provide new tools for better blinding protocols, nor can we answer whether other AEs worsen with consecutive stimulation sessions.

6.5 IMPLICATIONS FOR FUTURE RESEARCH

While the substudy regarding the longitudinal analysis of AEs in multi-day tDCS stimulation may be, to our knowledge, the first work of its kind, it has limitations: we could only appropriately investigate skin erythema due to the low prevalence of other AEs. Therefore, even larger samples are needed to properly investigate tES AEs other than skin erythema. While skin erythema could be a good proxy for other local effects arising from the skin–electrode interface, these results should not be generalised to other effects. Any effects arising from the actual stimulation of the brain tissue might behave differently over time – for example, by causing negative neuroplastic changes – and should be affected by the stimulation montage more than local effects. Furthermore, while our work implies that repeated stimulation sessions are safe, future research should be conducted with still longer stimulation protocols.

The power calculations conducted as part of the metabolomics substudy will hopefully prove helpful for future research. They provide a starting point for choosing a sample size for any future studies focusing on the effects of tES on peripheral metabolites. In addition, given that some of the predictions for the required sample size ran to tens of thousands, our study suggests that some of the metabolites might not have been affected by our stimulation, perhaps steering future research to other directions.

Regarding blinding, future research should examine whether AEs other than skin redness worsen with consecutive stimulations. In addition, deeper inspection of how reported AEs and the breaking of blinding correlate would be of value in any future studies, given that current blinding protocols appear insufficient.

6.6 DATA AVAILABLILITY STATEMENT

In general, free availability of the data is of considerable benefit for science. It allows independent re-analyses as well as meta-analyses of the data. However, our participant consents only allow sharing of the data with researchers based in countries with data privacy laws consistent with EU regulations. Therefore, our data are available to researchers in countries with EU-compatible data privacy laws, based on a reasonable request, including a scientifically well-justified research plan.

7 CONCLUSIONS

In this research, we determined that AEs of tES are largely unaffected by either the number of stimulation sessions or lifestyle effects (in this case, alcohol use, exercise, smoking and sleep), but the age of the individual receiving tDCS may modify the likelihood of skin erythema. These studies are, to the best of my knowledge, the first investigating the above issues. In addition, while we did not find any effect of tDCS on peripheral metabolites in our study sample, we did determine the sample sizes necessary for future, sufficiently powered studies. Finally, in our case study, we provided the first description of a delayed, tDCS-related lesion distant from the actual electrode, which we postulate was caused by saline leakage.

While the statistical analysis methods chosen revealed the limitations of the data, we were able to successfully conduct longitudinal analysis, introduce confounding factors and run sophisticated power analyses. This research strongly supports the view of tES as a safe treatment, even when utilising consecutive sessions. The simulations used for power calculations allowed us to offer recommendations for sample sizes. Based on our knowledge, this is the first work providing such figures.

Based on these results, future studies should look into the progression of other AEs as well. This would necessitate more robust datasets, and to achieve this, researchers in the field should consider systematically gathering AEs and either collaborating or publishing the data for others to use.

Even though we were unable to show any changes in peripheral blood, previous research has shown changes in both cortisol (Antal et al., 2014; Brunoni et al., 2013b; Sarkar et al., 2014) and blood glucose (Binkofski et al., 2011; Kistenmacher et al., 2017), suggesting that the concept of brain stimulation affecting the circulating metabolic compounds can be considered valid. Future research into the subject matter is needed, and this thesis offers further guidance for such research

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APPENDICES

Appendix 1: Adverse effect collection form for study participants. A translated version of the original, Finnish AE collection form. The form was the same in both studies, except for the question about skin redness being removed from the form for the tRNS study. This was done in order to improve the success of blinding.

Appendix 2: Adverse effect collection form for investigators. A translated version of the original, Finnish AE collection form.

APPENDIX 1. ADVERSE EFFECT COLLECTION FORM FOR STUDY PARTICIPANTS

Optimizing transcranial electrical stimulation for clinical applications: Systemic effects in healthy volunteers (sOptES)

QUESTIONNAIRE FOR THE PARTICIPANT **ID: _____**

IMMEDIATE ADVERSE EFFECTS

Date ____/____201___
Name _____

Stimulation group you estimate belonging to:
***Guide**: write down your estimation as a percentage so that the sum of the two options is 100%*
***Example**: active stimulation 40%, sham stimulation 60%*

Active stimulation _____% Sham stimulation _____%

TRANSCRANIAL ELECTRICAL STIMULATION ADVERSE EFFECTS SURVEY
Did you notice any of the following adverse effects during the stimulation or immediately after it? (0 = not at all; 100 = very intense):
***Guide**: If you did not have an adverse effect, leave the whole row blank. Remember to write down the intensity. Do not write more than one number on a row (e.g. separated by slash). When asked, describe the adverse effect with one, best-fitting word. For "Something else", you can be more specific.*

	During or immediately after stimulation	Is related to stimulation	Intensity on a scale of 1–100
Skin redness under the electrodes	☐	☐	_____
Sensation under the electrodes, what? _____	☐	☐	_____
Sensation elsewhere on the head, what? _____	☐	☐	_____
Change in alertness, what? _____	☐	☐	_____
Change in mood, what? _____	☐	☐	_____
Headache	☐	☐	_____
Something else, what? _____	☐	☐	_____
Something else, what? _____	☐	☐	_____
Something else, what? _____	☐	☐	_____

APPENDIX 2. ADVERSE EFFECT COLLECTION FORM FOR INVESTIGATORS

Optimizing transcranial electrical stimulation for clinical applications: Systemic effects in healthy volunteers (sOptES)

QUESTIONNAIRE FOR THE INVESTIGATOR **ID: _____**

IMMEDIATE ADVERSE EFFECTS

Date ____/____201___
Name _____

Stimulation group you estimate belonging to:
***Guide**: write down your estimation as a percentage so that the sum of the two options is 100%*
***Example**: active stimulation 40%, sham stimulation 60%*

Active stimulation _____% Sham stimulation _____%

TRANSCRANIAL ELECTRICAL STIMULATION ADVERSE EFFECTS SURVEY
Did you notice any of the following adverse effects during the stimulation or immediately after it? (0 = not at all; 100 = very intense):
***Guide**: If you did not have an adverse effect, leave the whole row blank. Remember to write down the intensity. Do not write more than one number on a row (e.g. separated by slash). When asked, describe the adverse effect with one, best-fitting word. For "Something else", you can be more specific.*

	During or immediately after stimulation	Is related to stimulation	Intensity on a scale of 1–100
Skin redness under the electrodes	☐	☐	_____
Change in alertness, what? _____	☐	☐	_____
Change in mood, what? _____	☐	☐	_____
Something else, what? _____	☐	☐	_____
Something else, what? _____	☐	☐	_____
Something else, what? _____	☐	☐	_____
Something else, what? _____	☐	☐	_____
Something else, what? _____	☐	☐	_____

ORIGINAL PUBLICATIONS (I – IV)

IV

Anodal tDCS over the left prefrontal cortex does not cause clinically significant changes in circulating metabolites.

Kortteenniemi A, Ortega-Alonso A, Javadi AH, Tolmunen T, Ali-Sisto T, Kotilainen T, Wikgren J, Karhunen L, Velagapudi V, Lehto SM.

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Anodal tDCS Over the Left Prefrontal Cortex Does Not Cause Clinically Significant Changes in Circulating Metabolites

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Background: Transcranial direct current stimulation (tDCS), a putative treatment for depression, has been proposed to affect peripheral metabolism. Metabolic products from brain tissue may also cross the blood–brain barrier, reflecting the conditions in the brain. However, there are no previous data regarding the effect of tDCS on circulating metabolites.

Objective: To determine whether five daily sessions of tDCS modulate peripheral metabolites in healthy adult men.

Methods: This double-blind, randomized controlled trial involved 79 healthy males (aged 20–40 years) divided into two groups, one receiving tDCS (2 mA) and the other sham stimulated. The anode was placed over the left dorsolateral prefrontal cortex and the cathode over the corresponding contralateral area. Venous blood samples were obtained before and after the first stimulation session, and after the fifth stimulation session. Serum levels of 102 metabolites were determined by mass spectrometry. The results were analysed with generalised estimating equations corrected for the family-wise error rate. In addition, we performed power calculations estimating sample sizes necessary for future research.

Results: TDCS-related variation in serum metabolite levels was extremely small and statistically non-significant. Power calculations indicated that for the observed variation to be deemed significant, samples sizes of up to 11,000 subjects per group would be required, depending on the metabolite of interest.

Conclusion: Our study found that five sessions of tDCS induced no major effects on peripheral metabolites among healthy men. These observations support the view of tDCS as a safe treatment that does not induce significant changes in the measured peripheral metabolites in healthy male subjects.

Keywords: brain stimulation, transcranial direct current stimulation, tES, metabolism, mass spectrometry

INTRODUCTION

Transcranial direct current stimulation (tDCS) is a non-invasive method for modulating neuronal activity by introducing a small electric current into the brain via electrodes placed on the scalp. It has attracted increasing interest among both clinicians and researchers during the past decade. It has been speculated to be a promising candidate in the treatment of major depressive disorder (1), bipolar depression (2), dependence and craving (3), as well as neuropathic and idiopathic pain (3). The low cost, safety and simplicity of use make tDCS an attractive option for clinical applications, even though more research is needed to determine the optimal treatment protocols and patient characteristics.

At the cellular level, tDCS is considered to alter neuronal resting membrane potentials (4, 5). For example, anodal stimulation has been observed to lead to increased neuronal activity in the motor cortex, while cathodal stimulation of the same area has been considered to inhibit neuronal firing (5, 6). In addition to local cortical effects in neuronal excitability, recent research has demonstrated that tDCS also appears to exert changes in neuronal activity in deeper brain structures, such as the midbrain nuclei (7).

The brain acts as a control unit that regulates the functions of the entire organism, and is in turn modified by peripheral physiology. Nevertheless, only limited knowledge is available on possible peripheral changes induced by brain stimulation using tDCS. Previous research has indicated that tDCS induces changes in cerebral blood flow (8), brain neurotransmitter levels (9) and central and peripheral metabolic activity (10). Furthermore, anodal tDCS in the area corresponding to the left dorsolateral prefrontal cortex (DLPFC) has been postulated to lead to changes in autonomic nervous system activity, measured by high- and low-frequency heart rate variability and the secretion of cortisol under normal conditions (11), as well as in stressful situations (12, 13). Thus, current evidence indicates that tDCS may induce very specific physiological responses in both the central nervous system and the periphery, although our understanding of possible systemic metabolic adaptations is very limited.

Electrical fields created by tDCS exert force on polar and/or charged molecules, and many proteins and amino acids, which often serve as neurotransmitters of the brain, are charged. This could lead to alterations in the concentrations of polar molecules and trigger larger metabolic cascades. These consequences may explain some of the observed physiological effects of tDCS in both the central nervous system and the periphery.

With regards to the peripheral effects of tDCS, a previous study conducted by Binkofski et al. (10) indicated that anodal tDCS led to a transient drop in high-energy phosphorus compounds (adenosine triphosphate (ATP), phosphocreatinine) both under the electrode and in the contralateral hemisphere, suggesting that there had been a widespread increase in neuronal glutaminergic activity. By using a hyperinsulinemic–euglycemic clamp, they also observed a simultaneous increase in glucose uptake in the peripheral circulation. The extent of the increase clearly correlated with the levels of the previously mentioned energetic compounds in the brain tissue, suggestive of elevated glucose uptake across the BBB.

As many physiological processes in the central nervous system result in observable changes in metabolite profiles in the periphery, metabolomic analysis of possible tDCS effects could provide a valuable tool for investigating the safety profile and mechanisms of action of tDCS. As many circulating metabolites are biologically active compounds, alterations in the levels of such compounds could result in safety concerns in healthy or clinical populations. We investigated the acute effects of tDCS on peripheral metabolites in a sample of 79 healthy men. The working hypotheses were that the metabolic state of the participants would change as a result of the stimulation and that differences between the study groups could be reflected in altered metabolite profiles.

MATERIALS AND METHODS

Study Population

This investigation formed part of the larger Optimizing Transcranial Electrical Stimulation for Clinical Applications (OptES) Study. The OptES Study was designed to generate novel information on the mechanisms of action of transcranial electrical stimulation, and to use this information to develop new, better clinical applications of transcranial electrical stimulation. The study protocol was approved by the Ethics Committee of the North Savo Hospital District (permit number 41/2015). All participants provided written informed consent after a full explanation of the study. The study conformed with the Declaration of Helsinki.

In order to decrease random variance in our findings, we chose to include only male participants, since our metabolomics platform also included some compounds known to be affected by sex hormones either centrally (14) or in the periphery (15, 16). A total of 80 male volunteers were recruited from the North Savo region of Finland. The participants received either tDCS or sham stimulation in a double-blind setting. The inclusion criteria were male gender (to avoid possible confounding metabolic effects of the menstrual cycle), aged between 18 and 40 years at the time of recruitment, right-handedness (i.e., belonging to the 1st to 10th right decile according to the Edinburgh Handedness Questionnaire (17)), and not having previously received tDCS.

The exclusion criteria were metal implants inside the skull or eye, severe skin lesions in the electrode placement areas, a pacemaker, a history of epilepsy or previous seizures, a history of intracerebral bleeding during the previous six months, a self-reported history of substance dependence/abuse during the past six months and a history of any endocrinological condition [i.e., any physician-defined E00-E32 diagnosis according to the International Statistical Classification of Diseases and Related Health Problems version 10 (IDC-10) (18)]. We were unable to obtain the post-stimulation venous blood sample from one participant, and this individual was therefore excluded from all analyses. Thus, the final sample size was 40 in the tDCS group (mean age 28.3 years, mean BMI 26.0 kg/m^2) and 39 in the sham group (mean age 27.7 years, mean BMI 25.4 kg/m^2).

Experimental Procedure

The participants were randomly assigned to either 1) the active stimulation or 2) the sham stimulation group in a 1:1 fashion, utilizing a computer-generated scheme. Basic sociodemographic information, including age, height and weight used to calculate the body mass index (BMI, kg/m^2), was collected from questionnaires that the participants completed prior to the first stimulation. Three venous blood samples were collected via venipuncture by a trained nurse.

Each participant received either tDCS or sham stimulation once per day for five consecutive days (**Figure 1**). Sociodemographic questionnaires were completed before the stimulation on day one. The baseline blood sample was collected immediately before the first stimulation session, with the second sample being drawn immediately after the first stimulation session (in order to investigate the acute effects of stimulation); the third sample was drawn immediately after the fifth stimulation session. A maximum of 5 min was allowed between stimulation and the collection of the blood samples.

Before the experiment, the participants were instructed to abstain from alcohol for 12 h and to have consumed no more than two doses during the preceding 24 h, to abstain from products containing caffeine for 3 h, and to abstain from smoking and heavy exercise for 1 h prior to the experiment. In addition, all experiments were conducted in the morning, and the participants were instructed to fast for 10 h to allow fasting blood samples to be obtained. This procedure enabled minimization of the confounding metabolic effects of the consumption of dietary products. Before stimulation, participant compliance with the instructions was checked, and those not conforming to the instructions were re-scheduled to another testing day.

Each participant received a 20-minute stimulation session with a current of 2 mA using a neuroConn DC-Stimulator (neuroConn GmbH, Ilmenau, Germany). The experimenter was unaware of the form of stimulation delivered (i.e., active stimulation vs. sham stimulation), and the participants were randomly divided between the study groups by a computer. The electrodes were made of conductive rubber placed inside sponge pads soaked with 12 ml of saline and held down with elastic straps. The electrode area was 25 cm^2, resulting in a current density of 0.8 A/m^2. The anode was placed at site F3 and the cathode at site F4 according to the international 10–20 electroencephalography system. In order to standardize the external stimuli, during the stimulation the participants were asked to refrain from talking and to watch a neutral landscape video with headphones on. The sham group received 15 seconds of ramping up and ramping down at the beginning, after which stimulation was discontinued.

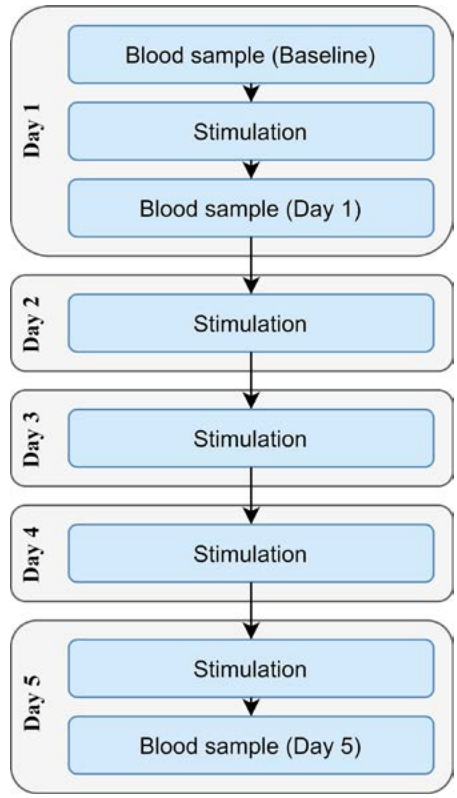

FIGURE 1 | Timeline of the study.

After the stimulation, both the participant and the experimenter filled in a form in which they were asked to provide their estimate (percentage) of the likelihood of the participant belonging to the sham group.

Blood Sample Analysis

Venous blood samples were collected into Vacuette 454078 4-ml serum gel tubes (Greiner Bio-One GmbH, Rainbach im Mühlkreis, Austria). They were left at room temperature for 30 min, followed by 10 min of centrifugation at 2,400×*g*, +20°C, to separate serum. The serum samples were frozen at −80°C until analysed. The metabolites were extracted from the serum samples using acetonitrile:formic acid (99:1 v/v) as a solvent (1:4, sample: solvent) and analysed using an ACQUITY UPLC-MS/MS system (Waters Corporation, Milford, MA, USA). A detailed protocol and instrument conditions have been published elsewhere (19).

Statistical Methods

Preliminary inspections of the metabolomic data allowed the detection of a total of 2,124 missing observations (8.786% of the values in the dataset). Because an excessive number of missing values for some of the measured compounds could potentially introduce unanticipated biases, metabolites with ≥50% of missing values within either of the experimental groups (n = 9) were excluded from any further statistical testing (**Supplementary Table 1**, **Supplementary Figure 1**), and the remaining metabolites (n = 93) were included in the further analyses.

After appropriate transformation and baseline standardization, each metabolite was subsequently entered into a generalized estimating equations model to investigate differences between groups in their respective values at baseline and after tDCS or sham stimulations were applied. The p-value threshold for statistical significance was set at p ≤5.376e−04, as the complexity of the analyses developed required accounting for both the family-wise error rate (Bonferroni method) and the intrinsic correlated nature of the metabolite data. Further details of the models implemented can be found in the **Supplementary Methods** and **Supplementary Figure 2**

These main statistical analyses were subsequently supplemented with a set of power-related computations utilizing statistical simulations within a sophisticated computer cluster environment. Firstly, we evaluated the sensitivity obtained with the current sample in its ability to detect significant longitudinal differences between groups with ≥80% power (for 0.05 and 5.376e−04 type-I error rates). This would allow an estimation of the minimum level of detection with the current experimental set-up. Subsequently, for each metabolite, we evaluated the sample size that would be required to detect the variation in metabolites caused by tDCS in this study as statistically significant with ≥80% power (type-I error rates 0.05 and 5.376e−04). This was done in order to make an estimate of the sample size needed in future experimental settings aiming to detect similar changes in metabolites resulting from tDCS (assuming that the detected values in this experiment would apply to other healthy populations with similar characteristics). Further details of these computations are offered in the **Supplementary Methods**.

Lastly, the success of the blinding procedure was investigated by running a set of Mann–Whitney U-tests for both the participants and the experimenters on days one and five. They were asked how likely they thought it was, as a percentage, that they were part of the sham group, and the answers were used in the analysis.

RESULTS

The current double-blind, randomized controlled trial failed to detect any metabolic changes due to tDCS after five treatment sessions; none of the models implemented for any of the investigated metabolites displayed statistically significant coefficients. **Figure 2** presents the model coefficients obtained for each metabolite, while the specific values provided by the statistical tests can be found in **Table 1**.

The computations related to statistical power made it possible to draw relevant conclusions concerning the usability of the current data. Firstly, the conducted analyses suggested that our study sample conferred enough power (80% level) to detect relatively small differences between groups due to tDCS (an absolute value for the "Time x Group" model coefficient of ≥0.1493 would be detectable with a type-I error rate of $5.376 \cdot 10^{-4}$). However, the majority of the tDCS effects observed here were substantially smaller than that threshold, and only a single metabolite, 2-aminoisobutyric acid, surpassed it. However, this metabolite was not ultimately considered as significant, because the model estimates displayed high uncertainties (for instance, see the extremely large standard error for the corresponding coefficients in **Table 1**).

Secondly, future replication studies aiming to detect significant group differences due to tDCS will need to increase their sample size considerably, as the treatment effects detected in our data were extremely small. For example, increasing the sample size to n = 150 participants per group (i.e., n = 300 in total) would have made it possible to identify six metabolites as displaying statistically significant differences between the study groups: 2-aminoisobutyric acid and propionylcarnitine being upregulated and hippuric acid, L-glutamine, xanthine and gamma-aminobutyric acid being downregulated in the tDCS group. **Figures 3** and **4** provide more details on this sample size estimation. Detailed results from the calculations are available in **Supplementary Table 2**.

Finally, it should be acknowledged here that the blinding protocol was not totally successful, as a larger number of participants and experimenters on day 1 compared to day 5 successfully guessed that they belonged to the sham group (p = 0.041 for participants, 0.012 for experimenters).

DISCUSSION

This investigation revealed that five daily sessions of tDCS applied to healthy male adults exerted no significant impact on serum metabolite levels when compared to sham stimulation. Although our study was adequately powered to detect relatively small effect sizes (more specifically, ≥0.1493), tDCS produced either no or extremely small effects for the majority of the metabolites evaluated. This study further demonstrated that in order to detect the observed minuscule effects as statistically significant, the sample sizes would need to be extremely large, making such a hypothetical study unfeasible.

In principle, tDCS could affect peripheral metabolite levels via two mechanisms: either indirectly through changes in the central nervous system that would modify peripheral neural responses and consequently peripheral metabolism, or directly through central metabolites passing through the BBB. While tDCS does not appear to lead to long-lasting changes in BBB permeability (20), short-lasting functional changes in its permeability could evoke changes in metabolite levels in the peripheral circulation. Our findings did not support a clinically significant presence of such changes, and the above hypotheses remain to be further tested in animal or human models.

Peripheral metabolic effects and serum effects caused by tDCS have previously been investigated in a limited number of

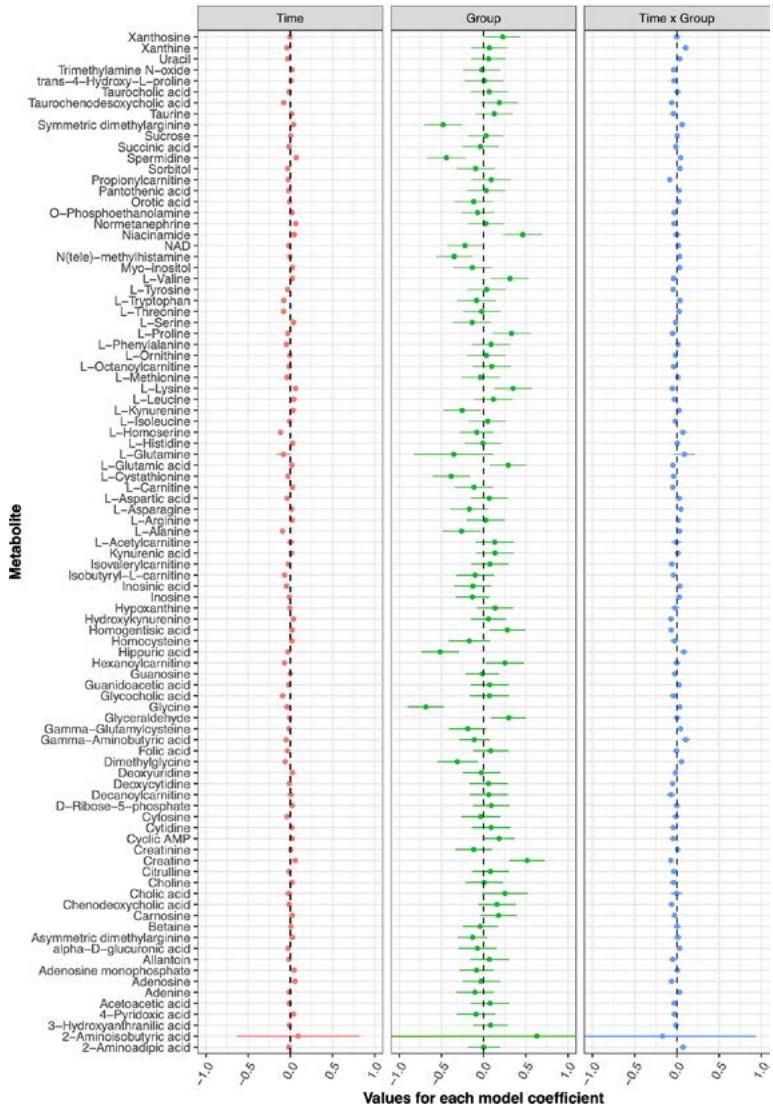

FIGURE 2 | Coefficient values with 95% confidence intervals from each metabolite model analysis.

studies.(10–13, 21–23) In these studies, one session of tDCS was found to cause changes in glucose metabolism (10, 21) and cortisol secretion (11–13, 23) when investigating healthy individuals. The sample sizes of the glucose studies were 15 (sham-controlled cross-over design), and 9 (within-subjects design, no sham, measurements before and after stimulation), respectively, while the sample sizes of the cortisol studies ranged from 20 to 60. Interestingly, one of the studies indicated that the hormone-related effect of tDCS varies based on pre-existing conditions. Subjects with high math anxiety experienced reduced serum cortisol with tDCS compared to sham stimulation, while receiving tDCS was associated with no change in serum cortisol in subjects with low math anxiety (13).

In addition, Khedr et al. (22), in a study with 40 participants, observed that 10 consecutive sessions of tDCS lowered pain, a finding that correlated with an increase in serum endorphin levels

TABLE 1 | Descriptive statistics from the pre-processed data, with main results from the models evaluated for each metabolite.

Metabolite Name	Baseline		Post session 1		Post session 5		Coefficient for "Time" **	Coefficient for "Group" ** ^	Coefficient for "Time × Group" **
	tDCS Group *	Sham Group *	tDCS Group *	Sham Group *	tDCS Group *	Sham Group *			
2-Aminoadipic acid	39; 0.012 (0.166)	39; −0.012 (0.156)	40; 0.092 (0.153)	39; 0.062 (0.177)	39; 0.228 (0.136)	39; −0.106 (0.151)	−0.018 (0.027); 0.434; 0.51	0.001 (0.193); 0; 0.994	0.073 (0.041); 3.113; 0.078
2-Aminoisobutyric acid	40; 0.127 (0.129)	39; −0.13 (0.185)	40; 0.137 (0.131)	39; −0.065 (0.18)	40; 0.283 (0.141)	39; 0.217 (0.156)	0.092 (0.726); 0.016; 0.899	0.629 (3.6); 0.031; 0.861	−0.172 (1.137); 0.023; 0.88
3-Hydroxyanthranilic acid	40; 0.055 (0.151)	39; −0.056 (0.169)	40; −0.051 (0.128)	39; −0.139 (0.168)	40; −0.082 (0.153)	39; −0.092 (0.143)	−0.011 (0.025); 0.206; 0.65	0.082 (0.206); 0.158; 0.691	−0.012 (0.036); 0.11; 0.74
4-Pyridoxic acid	40; −0.044 (0.121)	39; 0.045 (0.192)	40; -0.067 (0.118)	39; 0.034 (0.186)	40; −0.05 (0.16)	39; 0.214 (0.19)	0.037 (0.019); 3.726; 0.054	−0.091 (0.229); 0.158; 0.691	−0.03 (0.029); 1.032; 0.31
Acetoacetic acid	40; 0.005 (0.154)	39; −0.005 (0.167)	40; −0.044 (0.158)	39; −0.193 (0.174)	40; −0.223 (0.18)	39; −0.148 (0.163)	−0.012 (0.018); 0.413; 0.52	0.075 (0.228); 0.107; 0.744	−0.031 (0.028); 1.207; 0.272
Adenine	40; −0.021 (0.176)	39; 0.022 (0.142)	40; −0.174 (0.132)	39; 0.007 (0.195)	40; 0.04 (0.135)	39; −0.044 (0.134)	−0.012 (0.027); 0.205; 0.651	−0.103 (0.223); 0.215; 0.643	0.029 (0.042); 0.46; 0.498
Adenosine	40; −0.066 (0.164)	39; 0.067 (0.155)	40; 0.252 (0.163)	39; 0.225 (0.177)	40; 0.057 (0.186)	39; 0.378 (0.167)	0.054 (0.024); 4.931; 0.026	−0.03 (0.223); 0.018; 0.893	−0.061 (0.044); 1.963; 0.161
Adenosine monophosphate	40; 0.04 (0.142)	39; −0.041 (0.177)	40; 0.101 (0.138)	39; 0.349 (0.145)	40; 0.251 (0.123)	39; 0.211 (0.14)	0.044 (0.027); 2.721; 0.099	−0.082 (0.205); 0.161; 0.688	0.005 (0.038); 0.017; 0.895
Allantoin	40; 0.038 (0.173)	39; −0.039 (0.144)	40; −0.125 (0.174)	39; −0.103 (0.154)	40; −0.312 (0.177)	39; −0.177 (0.154)	−0.017 (0.025); 0.439; 0.507	0.067 (0.228); 0.086; 0.769	−0.049 (0.042); 1.348; 0.246
alpha-D-glucuronic acid	40; −0.016 (0.165)	39; 0.016 (0.155)	40; −0.176 (0.158)	39; −0.071 (0.172)	40; −0.054 (0.15)	39; −0.132 (0.142)	-0.028 (0.021); 1.87; 0.172	−0.073 (0.226); 0.103; 0.748	0.032 (0.034); 0.879; 0.348
Asymmetric dimethylarginine	40; −0.145 (0.16)	39; 0.149 (0.157)	40; 0.171 (0.135)	39; 0.062 (0.134)	40; 0.034 (0.132)	39; 0.236 (0.154)	0.022 (0.041); 0.282; 0.595	−0.13 (0.176); 0.547; 0.459	0.012 (0.051); 0.055; 0.815
Betaine	40; 0.008 (0.16)	39; −0.008 (0.16)	40; −0.083 (0.153)	39; 0.048 (0.133)	40; 0.01 (0.176)	39; 0.003 (0.119)	0.005 (0.027); 0.036; 0.85	−0.04 (0.212); 0.036; 0.849	0.003 (0.044); 0.004; 0.95
Carnosine	40; 0.114 (0.153)	39; −0.116 (0.165)	40; 0.05 (0.143)	39; 0.061 (0.134)	40; 0.064 (0.129)	39; −0.004 (0.115)	0.021 (0.025); 0.752; 0.386	0.177 (0.217); 0.662; 0.416	−0.028 (0.033); 0.711; 0.399
Chenodeoxycholic acid	40; 0.046 (0.143)	39; −0.047 (0.176)	40; 0.026 (0.138)	39; −0.14 (0.167)	40; −0.286 (0.123)	39; −0.133 (0.186)	−0.013 (0.023); 0.321; 0.571	0.159 (0.224); 0.501; 0.479	−0.061 (0.034); 3.145; 0.076
Cholic acid	30; 0.08 (0.186)	28; −0.086 (0.188)	26; −0.159 (0.209)	25; −0.175 (0.161)	27; 0.002 (0.194)	23; −0.387 (0.202)	−0.018 (0.047); 0.151; 0.697	0.252 (0.27); 0.867; 0.352	−0.004 (0.072); 0.003; 0.955
Choline	40; 0.03 (0.148)	39; −0.031 (0.171)	40; −0.024 (0.175)	39; −0.044 (0.157)	40; −0.121 (0.158)	39; 0.05 (0.197)	0.019 (0.036); 0.287; 0.592	0.006 (0.223); 0.001; 0.979	−0.038 (0.05); 0.578; 0.447
Citrulline	40; 0.035 (0.166)	39; −0.036 (0.154)	40; −0.127 (0.152)	39; −0.163 (0.151)	40; −0.229 (0.182)	39; −0.164 (0.18)	−0.014 (0.026); 0.284; 0.594	0.081 (0.217); 0.138; 0.71	−0.036 (0.034); 1.085; 0.298
Creatine	40; 0.302 (0.151)	39; −0.309 (0.154)	40; 0.308 (0.146)	39; −0.074 (0.133)	40; 0.229 (0.141)	39; −0.051 (0.137)	0.057 (0.019); 8.719; 0.003	0.516 (0.21); 6.014; 0.014	−0.072 (0.028); 6.578; 0.01
Creatinine	40; −0.087 (0.154)	39; 0.089 (0.165)	40; −0.098 (0.149)	39; −0.024 (0.157)	40; −0.026 (0.164)	39; 0.076 (0.176)	0.005 (0.015); 0.098; 0.754	−0.118 (0.218); 0.293; 0.588	0.008 (0.022); 0.129; 0.72
Cyclic AMP	40; 0.129 (0.174)	39; −0.133 (0.141)	40; 0.086 (0.141)	39; −0.054 (0.14)	40; 0.043 (0.135)	39; 0.021 (0.141)	0.02 (0.023); 0.77; 0.38	0.184 (0.188); 0.956; 0.328	−0.044 (0.045); 0.967; 0.326
Cytidine	40; 0.038 (0.14)	39; −0.039 (0.179)	40; −0.013 (0.133)	39; −0.054 (0.177)	40; −0.045 (0.167)	39; 0.066 (0.195)	0.016 (0.035); 0.207; 0.649	0.089 (0.228); 0.153; 0.696	−0.04 (0.046); 0.762; 0.383
Cytosine	40; −0.013 (0.18)	39; 0.013 (0.137)	40; −0.079 (0.16)	39; 0.015 (0.139)	40; −0.304 (0.16)	39; −0.232 (0.14)	−0.04 (0.026); 2.348; 0.125	−0.033 (0.232); 0.021; 0.886	−0.012 (0.042); 0.084; 0.772
D-Ribose-5-phosphate	40; 0.053 (0.162)	39; −0.054 (0.157)	40; 0.038 (0.147)	39; 0.02 (0.144)	40; 0.129 (0.128)	39; −0.023 (0.14)	0.02 (0.027); 0.555; 0.456	0.09 (0.22); 0.17; 0.68	−0.002 (0.042); 0.002; 0.969
Decanoylcarnitine	40; 0.04 (0.156)	39; −0.041 (0.165)	40; −0.105 (0.18)	39; −0.401 (0.18)	40; −0.36 (0.173)	39; −0.287 (0.112)	0.005 (0.039); 0.015; 0.902	0.061 (0.226); 0.072; 0.788	−0.069 (0.051); 1.878; 0.171
Deoxycytidine	40; 0.018 (0.128)	39; −0.018 (0.188)	40; −0.036 (0.123)	39; −0.127 (0.184)	40; −0.267 (0.136)	39; −0.044 (0.177)	−0.01 (0.014); 0.528; 0.468	0.057 (0.227); 0.064; 0.8	−0.051 (0.025); 4.151; 0.042
Deoxyuridine	40; −0.02 (0.151)	39; 0.02 (0.169)	40; 0.058 (0.153)	39; −0.025 (0.17)	40; 0.031 (0.139)	39; 0.121 (0.189)	0.026 (0.02); 1.6; 0.206	−0.026 (0.225); 0.013; 0.908	−0.018 (0.029); 0.372; 0.542
Dimethylglycine	40; −0.106 (0.145)	39; 0.109 (0.173)	40; −0.129 (0.145)	39; -0.007 (0.162)	40; −0.089 (0.13)	39; 0.112 (0.169)	−0.061 (0.031); 3.848; 0.05	−0.311 (0.24); 1.673; 0.196	0.053 (0.037); 2.086; 0.149
Folic acid	40; 0.063 (0.175)	39; −0.064 (0.142)	40; 0.181 (0.16)	39; 0.016 (0.134)	40; −0.053 (0.184)	39; −0.169 (0.148)	−0.034 (0.026); 1.706; 0.191	0.083 (0.21); 0.156; 0.693	−0.006 (0.037); 0.025; 0.875
Gamma-Aminobutyric acid	40; −0.116 (0.163)	39; 0.119 (0.155)	40; 0.004 (0.155)	39; −0.102 (0.149)	40; 0.184 (0.148)	39; −0.18 (0.129)	−0.05 (0.032); 2.479; 0.115	−0.112 (0.183); 0.372; 0.542	0.105 (0.051); 4.207; 0.04
Gamma-Glutamylcysteine	38; −0.05 (0.169)	34; 0.055 (0.165)	37; −0.205 (0.133)	38; 0.035 (0.175)	38; 0.014 (0.159)	34; −0.005 (0.145)	−0.014 (0.019); 0.505; 0.477	−0.188 (0.218); 0.743; 0.389	0.042 (0.029); 2.127; 0.145
Glyceraldehyde	40; 0.167 (0.143)	39; −0.171 (0.172)	40; −0.089 (0.135)	39; −0.271 (0.163)	40; 0.089 (0.139)	39; −0.243 (0.154)	−0.007 (0.029); 0.064; 0.8	0.298 (0.209); 2.026; 0.155	0.001 (0.045); 0; 0.991
Glycine	40; −0.296 (0.169)	39; 0.304 (0.133)	40; −0.319 (0.177)	39; 0.36 (0.143)	40; −0.304 (0.183)	39; 0.094 (0.125)	−0.039 (0.018); 4.72; 0.03	−0.685 (0.216); 10.095; 0.001	0.033 (0.029); 1.291; 0.256
Glycocholic acid	40; 0.011 (0.165)	39; −0.012 (0.155)	40; −0.31 (0.172)	39; −0.465 (0.164)	40; −0.61 (0.163)	39; −0.467 (0.153)	−0.092 (0.036); 6.521; 0.011	0.067 (0.233); 0.082; 0.775	−0.039 (0.048); 0.681; 0.409
Guanidoacetic acid	40; 0.028 (0.149)	39; −0.029 (0.171)	40; −0.159 (0.144)	39; −0.209 (0.169)	40; 0.005 (0.2)	39; −0.216 (0.187)	−0.021 (0.023); 0.838; 0.36	0.071 (0.223); 0.102; 0.749	0.025 (0.037); 0.439; 0.508
Guanosine	40; −0.068 (0.159)	39; 0.07 (0.16)	40; 0.179 (0.133)	39; 0.023 (0.161)	40; −0.064 (0.137)	39; 0.06 (0.161)	0 (0.022); 0; 0.994	−0.014 (0.198); 0.005; 0.943	−0.026 (0.031); 0.681; 0.409
Hexanoylcarnitine	40; 0.133 (0.162)	39; −0.136 (0.155)	40; −0.165 (0.161)	39; −0.496 (0.165)	40; −0.251 (0.177)	39; −0.45 (0.127)	−0.067 (0.031); 4.866; 0.027	0.253 (0.227); 1.235; 0.266	−0.002 (0.044); 0.002; 0.961
Hippuric acid	40; −0.254 (0.145)	39; 0.261 (0.164)	40; −0.451 (0.135)	39; 0.039 (0.159)	40; 0.003 (0.192)	39; 0.116 (0.167)	−0.029 (0.034); 0.718; 0.397	−0.515 (0.224); 5.288; 0.021	0.083 (0.045); 3.437; 0.064
Homocysteine	40; -0.08 (0.15)	39; 0.082 (0.169)	40; −0.094 (0.156)	39; 0.088 (0.168)	40; −0.118 (0.146)	39; 0.125 (0.158)	0.017 (0.035); 0.235; 0.628	−0.171 (0.25); 0.468; 0.494	−0.029 (0.047); 0.387; 0.534
Homogentisic acid	40; 0.144 (0.165)	39; −0.147 (0.151)	40; −0.06 (0.135)	38; −0.261 (0.183)	40; −0.131 (0.131)	39; −0.102 (0.137)	0.02 (0.022); 0.865; 0.352	0.281 (0.215); 1.705; 0.192	−0.066 (0.034); 3.668; 0.055
Hydroxykynurenine	40; 0.053 (0.158)	39; −0.055 (0.161)	40; −0.004 (0.128)	39; −0.043 (0.171)	40; −0.092 (0.154)	39; 0.143 (0.156)	0.038 (0.025); 2.291; 0.13	0.058 (0.213); 0.074; 0.785	−0.069 (0.037); 3.59; 0.058
Hypoxanthine	40; 0.135 (0.155)	39; −0.138 (0.163)	40; −0.129 (0.117)	39; −0.02 (0.141)	40; 0.041 (0.131)	39; −0.181 (0.119)	−0.005 (0.033); 0.022; 0.883	0.136 (0.214); 0.404; 0.525	−0.02 (0.046); 0.19; 0.663
Inosine	40; −0.109 (0.159)	39; 0.112 (0.159)	40; 0.154 (0.12)	39; 0.083 (0.155)	40; 0.062 (0.148)	39; 0.055 (0.16)	−0.011 (0.028); 0.155; 0.694	−0.133 (0.198); 0.451; 0.502	0.028 (0.039); 0.526; 0.468
Inosinic acid	40; 0.008 (0.17)	39; −0.008 (0.149)	40; −0.085 (0.171)	39; 0.136 (0.161)	40; −0.113 (0.174)	39; −0.159 (0.159)	−0.046 (0.022); 4.373; 0.037	−0.128 (0.218); 0.344; 0.558	0.032 (0.04); 0.641; 0.423
Isobutyryl-L-carnitine	40; −0.09 (0.145)	39; 0.092 (0.174)	40; −0.285 (0.134)	39; −0.131 (0.165)	40; −0.555 (0.139)	39; −0.192 (0.175)	−0.067 (0.022); 9.072; 0.003	−0.102 (0.225); 0.206; 0.65	−0.042 (0.031); 1.906; 0.167
Isovalerylcarnitine	40; 0.035 (0.134)	39; −0.035 (0.183)	40; −0.001 (0.128)	39; −0.214 (0.176)	40; −0.385 (0.145)	39; −0.147 (0.154)	−0.025 (0.03); 0.698; 0.403	0.073 (0.219); 0.112; 0.738	−0.059 (0.041); 2.097; 0.148
Kynurenic acid	40; 0.057 (0.137)	39; −0.059 (0.181)	40; −0.065 (0.133)	39; −0.23 (0.167)	40; 0.118 (0.155)	39; −0.066 (0.175)	0.013 (0.027); 0.236; 0.627	0.133 (0.225); 0.351; 0.554	0.011 (0.038); 0.079; 0.779
L-Acetylcarnitine	40; 0.049 (0.154)	39; −0.05 (0.166)	40; −0.027 (0.171)	39; −0.111 (0.168)	40; 0.044 (0.2)	39; −0.009 (0.16)	0.004 (0.044); 0.007; 0.931	0.129 (0.227); 0.322; 0.57	−0.009 (0.058); 0.023; 0.88

(Continued)

TABLE 1 | Continued

Metabolite Name	Baseline		Post session 1		Post session 5		Coefficient for "Time" **	Coefficient for "Group" ** ^	Coefficient for "Time × Group" **
	tDCS Group *	Sham Group *	tDCS Group *	Sham Group *	tDCS Group *	Sham Group *			
L-Alanine	40; −0.119 (0.166)	39; 0.122 (0.151)	40; −0.152 (0.184)	39; 0.02 (0.142)	40; −0.485 (0.168)	39; −0.373 (0.149)	−0.093 (0.028); 10.741; 0.001	−0.262 (0.23); 1.298; 0.255	0.029 (0.039); 0.569; 0.451
L-Arginine	40; 0.023 (0.153)	39; −0.024 (0.167)	40; 0.054 (0.149)	39; −0.008 (0.151)	40; 0.225 (0.148)	39; 0.097 (0.172)	0.022 (0.019); 1.408; 0.235	0.025 (0.226); 0.012; 0.912	0.016 (0.03); 0.277; 0.599
L-Asparagine	40; −0.08 (0.166)	39; 0.082 (0.152)	40; −0.129 (0.181)	39; 0.023 (0.155)	40; 0.13 (0.167)	39; 0.092 (0.161)	0.013 (0.025); 0.261; 0.61	−0.17 (0.226); 0.564; 0.453	0.046 (0.032); 2.072; 0.15
L-Aspartic acid	40; 0.107 (0.165)	39; −0.109 (0.153)	40; −0.375 (0.143)	39; −0.205 (0.163)	40; −0.091 (0.175)	39; −0.333 (0.157)	−0.038 (0.031); 1.457; 0.227	0.065 (0.219); 0.087; 0.767	0.025 (0.051); 0.241; 0.624
L-Carnitine	40; −0.027 (0.16)	39; 0.028 (0.16)	40; −0.065 (0.163)	39; 0.059 (0.154)	40; −0.128 (0.17)	39; 0.159 (0.154)	0.026 (0.014); 3.701; 0.054	−0.113 (0.227); 0.248; 0.618	−0.049 (0.02); 5.801; 0.016
L-Cystathionine	40; -0.184 (0.13)	39; 0.189 (0.181)	40; −0.378 (0.122)	39; 0.058 (0.187)	40; −0.457 (0.117)	39; 0.09 (0.175)	−0.027 (0.023); 1.368; 0.242	−0.384 (0.22); 3.037; 0.081	−0.039 (0.035); 1.229; 0.268
L-Glutamic acid	40; 0.159 (0.171)	39; −0.163 (0.143)	40; 0.062 (0.136)	39; −0.033 (0.152)	40; 0.053 (0.126)	39; −0.198 (0.131)	0.017 (0.022); 0.564; 0.453	0.292 (0.218); 1.788; 0.181	−0.047 (0.036); 1.776; 0.183
L-Glutamine	40; −0.089 (0.15)	39; 0.091 (0.169)	40; −0.04 (0.148)	39; 0.136 (0.17)	40; 0.064 (0.136)	39; 0.095 (0.153)	−0.083 (0.088); 0.896; 0.344	−0.353 (0.473); 0.558; 0.455	0.088 (0.121); 0.53; 0.467
L-Histidine	40; 0.006 (0.161)	39; −0.006 (0.159)	40; 0.013 (0.144)	39; −0.036 (0.16)	40; 0.155 (0.164)	39; 0.104 (0.176)	0.026 (0.025); 1.094; 0.296	−0.009 (0.221); 0.002; 0.968	0.004 (0.035); 0.015; 0.903
L-Homoserine	40; −0.017 (0.167)	39; 0.017 (0.152)	40; −0.243 (0.156)	39; −0.11 (0.145)	40; −0.319 (0.155)	39; −0.604 (0.148)	−0.116 (0.028); 17.574; 0	−0.081 (0.201); 0.161; 0.688	0.072 (0.047); 2.341; 0.126
L-Isoleucine	40; 0.043 (0.161)	39; −0.044 (0.158)	40; −0.162 (0.133)	39; −0.154 (0.171)	40; −0.13 (0.144)	39; −0.122 (0.155)	−0.008 (0.014); 0.29; 0.59	0.048 (0.223); 0.047; 0.828	−0.02 (0.027); 0.516; 0.473
L-Kynurenine	40; −0.082 (0.148)	39; 0.084 (0.171)	40; −0.128 (0.138)	39; 0.101 (0.163)	40; 0.159 (0.159)	39; 0.252 (0.191)	0.035 (0.032); 1.198; 0.274	−0.255 (0.221); 1.33; 0.249	0.024 (0.043); 0.307; 0.58
L-Leucine	40; 0.051 (0.151)	39; −0.052 (0.169)	40; −0.102 (0.167)	39; −0.24 (0.161)	40; 0.05 (0.169)	39; 0.049 (0.188)	0.042 (0.016); 6.525; 0.011	0.117 (0.226); 0.266; 0.606	−0.03 (0.028); 1.136; 0.286
L-Lysine	40; 0.184 (0.152)	39; −0.188 (0.163)	40; 0.266 (0.162)	39; −0.151 (0.159)	40; 0.236 (0.159)	39; 0.061 (0.202)	0.062 (0.029); 4.611; 0.032	0.349 (0.226); 2.375; 0.123	−0.053 (0.042); 1.572; 0.21
L-Methionine	40; 0.015 (0.154)	39; −0.016 (0.166)	40; −0.182 (0.151)	39; −0.086 (0.169)	40; −0.103 (0.16)	39; −0.224 (0.148)	−0.039 (0.022); 3.154; 0.076	−0.037 (0.227); 0.027; 0.87	0.012 (0.035); 0.118; 0.731
L-Octanoylcarnitine	40; 0.049 (0.162)	39; −0.05 (0.158)	40; −0.032 (0.187)	39; −0.292 (0.163)	40; −0.236 (0.179)	39; −0.237 (0.115)	−0.012 (0.033); 0.131; 0.717	0.095 (0.226); 0.176; 0.675	−0.037 (0.043); 0.768; 0.381
L-Ornithine	40; 0.051 (0.161)	39; −0.052 (0.158)	40; −0.152 (0.167)	39; −0.094 (0.168)	40; −0.161 (0.169)	39; −0.086 (0.155)	−0.005 (0.019); 0.059; 0.809	0.032 (0.23); 0.019; 0.889	−0.019 (0.031); 0.373; 0.541
L-Phenylalanine	40; 0.061 (0.167)	39; −0.062 (0.151)	40; −0.129 (0.145)	39; −0.225 (0.145)	40; 0.011 (0.153)	39; −0.201 (0.14)	−0.046 (0.014); 10.562; 0.001	0.088 (0.228); 0.15; 0.698	0.013 (0.03); 0.194; 0.66
L-Proline	40; 0.162 (0.171)	39; −0.166 (0.143)	40; 0.06 (0.169)	39; −0.163 (0.132)	40; −0.212 (0.186)	39; −0.355 (0.153)	−0.03 (0.031); 0.936; 0.333	0.33 (0.228); 2.091; 0.148	−0.05 (0.041); 1.504; 0.22
L-Serine	40; −0.006 (0.129)	39; 0.006 (0.188)	40; −0.056 (0.127)	39; 0.168 (0.194)	40; 0.093 (0.145)	39; 0.233 (0.18)	0.037 (0.026); 2.04; 0.153	−0.136 (0.226); 0.363; 0.547	−0.016 (0.033); 0.23; 0.632
L-Threonine	40; 0.004 (0.149)	39; −0.004 (0.171)	40; −0.098 (0.143)	39; −0.047 (0.169)	40; −0.241 (0.189)	39; −0.443 (0.137)	−0.081 (0.026); 10.062; 0.002	−0.022 (0.221); 0.01; 0.922	0.026 (0.042); 0.375; 0.54
L-Tryptophan	40; −0.009 (0.138)	39; 0.009 (0.18)	40; −0.272 (0.145)	39; −0.262 (0.174)	40; −0.179 (0.154)	39; −0.298 (0.151)	−0.078 (0.029); 7.173; 0.007	−0.083 (0.228); 0.134; 0.714	0.036 (0.038); 0.871; 0.351
L-Tyrosine	40; 0.035 (0.153)	39; −0.036 (0.167)	40; −0.225 (0.16)	39; −0.226 (0.162)	40; −0.323 (0.155)	39; −0.216 (0.187)	−0.032 (0.018); 3.203; 0.073	0.035 (0.23); 0.023; 0.879	−0.045 (0.028); 2.544; 0.111
L-Valine	40; 0.148 (0.138)	39; −0.152 (0.177)	40; −0.008 (0.145)	39; −0.282 (0.164)	40; 0.025 (0.168)	39; −0.096 (0.169)	0.022 (0.025); 0.745; 0.388	0.312 (0.225); 1.926; 0.165	−0.04 (0.037); 1.185; 0.276
Myo-inositol	40; −0.009 (0.156)	39; 0.01 (0.164)	40; −0.171 (0.142)	39; 0.024 (0.196)	40; 0.181 (0.16)	39; 0.09 (0.163)	0.024 (0.027); 0.833; 0.361	−0.133 (0.227); 0.345; 0.557	0.031 (0.043); 0.517; 0.472
N(tele)-methylhistamine	40; −0.108 (0.158)	39; 0.111 (0.161)	40; −0.047 (0.148)	39; 0.175 (0.15)	40; 0.016 (0.152)	39; 0.094 (0.162)	−0.004 (0.013); 0.072; 0.788	−0.349 (0.214); 2.66; 0.103	0.032 (0.023); 1.863; 0.172
NAD	40; −0.08 (0.168)	39; 0.082 (0.15)	40; −0.056 (0.135)	39; 0.135 (0.177)	40; −0.08 (0.133)	39; 0.022 (0.156)	−0.019 (0.025); 0.593; 0.441	−0.222 (0.205); 1.178; 0.278	0.015 (0.042); 0.134; 0.714
Niacinamide	40; 0.23 (0.143)	39; −0.236 (0.168)	40; 0.929 (0.189)	39; 0.394 (0.147)	40; 0.377 (0.162)	39; −0.08 (0.175)	0.045 (0.037); 1.425; 0.233	0.463 (0.226); 4.194; 0.041	−0.003 (0.052); 0.003; 0.958
Normetanephrine	40; 0.053 (0.176)	39; −0.054 (0.142)	40; 0.287 (0.145)	39; 0.376 (0.177)	40; 0.22 (0.144)	39; 0.301 (0.148)	0.065 (0.023); 7.89; 0.005	0.026 (0.221); 0.013; 0.908	−0.032 (0.039); 0.672; 0.412
O-Phosphoethanolamine	40; 0.002 (0.136)	39; −0.002 (0.182)	40; −0.053 (0.138)	39; 0.079 (0.122)	40; −0.049 (0.143)	39; 0.075 (0.15)	0.018 (0.036); 0.242; 0.623	−0.072 (0.198); 0.133; 0.716	−0.029 (0.041); 0.508; 0.476
Orotic acid	40; 0.087 (0.155)	39; −0.089 (0.164)	40; −0.139 (0.187)	39; 0.256 (0.219)	40; 0.026 (0.158)	39; 0.045 (0.161)	−0.007 (0.029); 0.058; 0.809	−0.117 (0.228); 0.264; 0.607	0.019 (0.039); 0.237; 0.626
Pantothenic acid	40; 0.058 (0.153)	39; −0.059 (0.166)	40; −0.074 (0.151)	39; −0.054 (0.191)	40; 0.011 (0.157)	39; −0.123 (0.193)	−0.018 (0.023); 0.652; 0.419	0.029 (0.227); 0.016; 0.899	0.023 (0.033); 0.499; 0.48
Propionylcarnitine	40; 0.051 (0.138)	39; −0.052 (0.18)	40; −0.168 (0.141)	39; −0.235 (0.174)	40; −0.404 (0.142)	39; −0.103 (0.18)	−0.027 (0.017); 2.461; 0.117	0.09 (0.231); 0.153; 0.696	−0.085 (0.026); 10.381; 0.001
Sorbitol	40; −0.02 (0.184)	39; 0.02 (0.13)	40; −0.199 (0.187)	39; −0.012 (0.133)	40; 0.028 (0.188)	39; −0.174 (0.142)	−0.032 (0.017); 3.476; 0.062	−0.095 (0.228); 0.173; 0.677	0.036 (0.037); 0.962; 0.327
Spermidine	40; −0.156 (0.173)	39; 0.16 (0.14)	40; −0.033 (0.171)	39; 0.47 (0.181)	40; 0.378 (0.191)	39; 0.562 (0.162)	0.069 (0.024); 8.464; 0.004	−0.441 (0.229); 3.722; 0.054	0.045 (0.035); 1.636; 0.201
Succinic acid	40; −0.057 (0.157)	39; 0.058 (0.163)	40; −0.282 (0.158)	39; −0.253 (0.152)	40; −0.27 (0.141)	39; −0.121 (0.171)	−0.015 (0.022); 0.47; 0.493	−0.038 (0.218); 0.03; 0.863	−0.014 (0.032); 0.198; 0.656
Sucrose	40; 0.054 (0.156)	39; −0.056 (0.164)	40; −0.049 (0.16)	39; 0.088 (0.134)	40; 0.068 (0.147)	39; −0.039 (0.144)	0.003 (0.02); 0.022; 0.882	0.029 (0.206); 0.02; 0.889	0.004 (0.03); 0.021; 0.885
Symmetric dimethylarginine	40; −0.224 (0.161)	39; 0.23 (0.15)	40; −0.169 (0.154)	39; 0.113 (0.148)	40; 0.201 (0.154)	39; 0.378 (0.134)	0.037 (0.026); 2.134; 0.144	−0.481 (0.22); 4.752; 0.029	0.06 (0.04); 2.222; 0.136
Taurine	40; 0.102 (0.158)	39; −0.104 (0.16)	40; −0.285 (0.138)	39; −0.114 (0.155)	40; −0.057 (0.161)	39; −0.079 (0.159)	0.014 (0.04); 0.129; 0.72	0.126 (0.218); 0.334; 0.563	−0.039 (0.052); 0.575; 0.448
Taurochenodesoxycholic acid	40; 0.057 (0.171)	39; −0.059 (0.147)	40; −0.222 (0.158)	39; −0.401 (0.129)	40; −0.487 (0.131)	39; −0.332 (0.145)	−0.078 (0.03); 6.61; 0.01	0.187 (0.223); 0.705; 0.401	−0.059 (0.042); 1.97; 0.16
Taurocholic acid	40; 0.02 (0.138)	39; −0.021 (0.18)	40; −0.064 (0.138)	39; −0.152 (0.17)	40; 0.011 (0.16)	39; −0.047 (0.186)	−0.014 (0.035); 0.155; 0.694	0.064 (0.224); 0.083; 0.773	0.005 (0.047); 0.01; 0.919
trans-4-Hydroxy-L-proline	40; 0.012 (0.165)	39; −0.012 (0.154)	40; 0.01 (0.163)	39; 0.007 (0.152)	40; −0.301 (0.159)	39; −0.166 (0.165)	0.008 (0.033); 0.062; 0.803	0.006 (0.233); 0.001; 0.979	−0.032 (0.043); 0.555; 0.456
Trimethylamine N-oxide	40; −0.046 (0.144)	39; 0.047 (0.175)	40; −0.058 (0.139)	39; 0.099 (0.181)	40; −0.131 (0.147)	39; 0.043 (0.178)	0.018 (0.03); 0.347; 0.556	−0.019 (0.223); 0.007; 0.931	−0.037 (0.038); 0.934; 0.334
Uracil	40; 0.038 (0.177)	39; −0.039 (0.141)	40; −0.165 (0.176)	39; −0.158 (0.154)	40; −0.011 (0.134)	39; −0.235 (0.155)	−0.031 (0.031); 0.986; 0.321	0.058 (0.202); 0.083; 0.773	0.03 (0.044); 0.482; 0.488
Xanthine	40; 0.107 (0.155)	39; −0.11 (0.163)	40; −0.092 (0.145)	39; −0.092 (0.169)	40; 0.278 (0.158)	39; −0.296 (0.135)	−0.039 (0.03); 1.726; 0.189	0.065 (0.212); 0.095; 0.758	0.103 (0.042); 5.941; 0.015
Xanthosine	40; 0.138 (0.153)	39; −0.141 (0.164)	40; 0.086 (0.166)	39; 0.023 (0.144)	40; 0.064 (0.177)	39; −0.155 (0.149)	−0.003 (0.024); 0.018; 0.893	0.227 (0.209); 1.183; 0.277	−0.001 (0.044); 0; 0.989

**values represent: "N; Mean (SE)"; **values represent: "Estimate (SE); Wald statistic; P-value"; ^"Group" coefficient specified in the model as variation in the tDCS group respect to the Sham.*

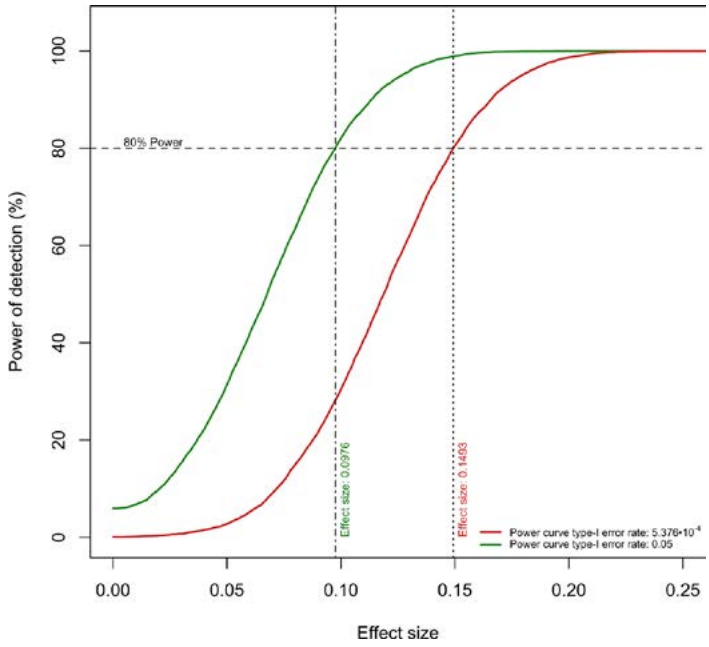

FIGURE 3 | Power curves for the detection of "Time × Group" coefficient values. The current sample size per experimental group confers ≥80% power to detect "*Time Group*" coefficients of ≥0.0976 (type-I error rate: 0.05) and ≥0.1493 (type-I error rate: $5.376 \cdot 10^{-4}$).

during the 10-session intervention. Brunoni et al. (24–26) investigated the effect of tDCS on circulating neurotrophins and their receptors (brain-derived neurotrophic factor (BDNF), neurotrophins 3 and 4 (NT-3 and NT-4), nerve growth factor, glial cell line derived neurotrophic factor (GDNF), and soluble tumour necrosis factor receptors 1 and 2), but found no difference between individuals receiving tDCS vs. sham stimulation. A few year later (27), the same group expanded their research to interleukins and tumour necrosis factor alpha, and again observed no differences observed between participants receiving tDCS vs. sham stimulation. In contrast, in a study by Hadoush et al. (28), belonging to the tDCS group vs. sham stimulation was associated with an increase in serum BDNF levels in Parkinson's disease patients undergoing a 10-session tDCS intervention. The effects of tDCS on the concentrations of soluble neuronal cell adhesion molecules in minimally conscious subjects have also been studied; the authors observed no significant tDCS-related changes (29). Unfortunately, our metabolomics panel did not include any of the aforementioned metabolites, which limits comparisons with these previous observations. However, these studies (with the exception of glucose studies) focused more on larger molecules in peripheral blood in contrast to the small metabolic products we measured.

Some earlier reports have suggested that tDCS may cause intracerebral changes in the concentrations of certain metabolites. For example, Dickler et al. (30) found an increase in dorsolateral prefrontal cortical GABA levels under the stimulated area in patients with a gambling disorder, while Hone-Blanchet et al. (31) recorded elevated striatal levels of N-acetylaspartate and Glx (a combined measurement of glutamate and glutamine), but no differences in the levels of GABA in healthy subjects. Although a previous study (10) did claim that tDCS-induced alterations in central metabolism are reflected in the peripheral circulation, we observed no tDCS-related alterations in the serum levels of N-acetylaspartate or GABA among healthy individuals. Nevertheless, the possibility of such alterations at the cerebral level cannot be ruled out; tDCS may have induced central nervous system alterations that are not reflected as meaningful alterations at the peripheral level.

The main strengths of our study are the relatively large sample size (previous studies focusing on tDCS-induced possible peripheral metabolic changes have utilised samples ranging from 14 to 60 individuals) (13, 32) and the implementation of a randomized, double-blind, controlled study design. Furthermore, in order to reduce potential confounding due to individual lifestyle factors, we provided the study participants with detailed instructions regarding lifestyle behaviours potentially modifying the effects of tDCS before each tDCS/sham stimulation session. All participants were instructed to fast before providing blood samples. Controlling for these factors most likely contributed to

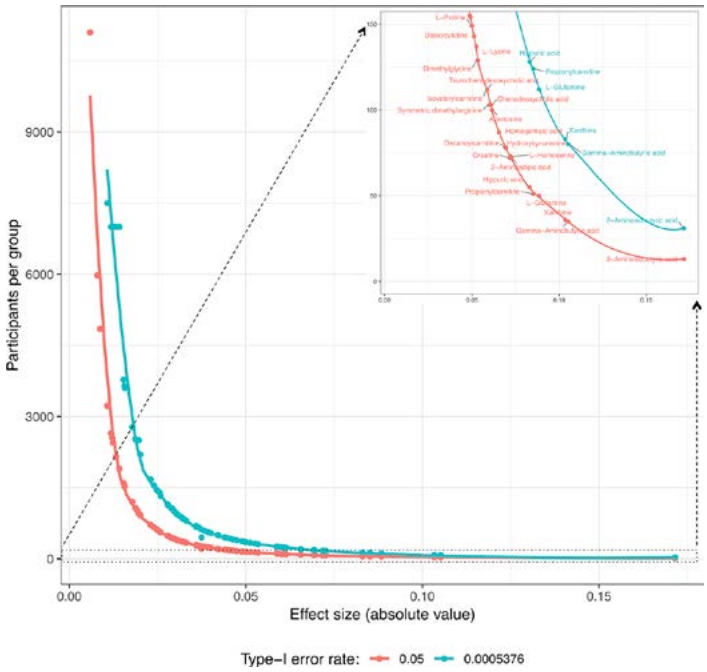

FIGURE 4 | Estimation of the number of participants per group required for the significant detection of "*Time* × *Group*" coefficients (type-I error rates: 0.05 and $5.576 \cdot 10^{-4}$; minimum detection power: 80%) for each metabolite according to the coefficient values obtained in these study models. In the upper right corner, there is a resized section of the overall curves clarifying the metabolites for which the estimated number of participants per group is ≤150.

the more homogeneous findings between the two study groups, whereas some previous studies have not reported whether they provided similar instructions to their participants.

Some issues need to be taken into consideration while interpreting the findings from our have received a similar tDCS regime (i.e., a 20-minute stimulation session with a current of 2 mA for five consecutive days). While the montage we used is common in both clinical and experimental tDCS studies, caution is advised when extrapolating our results to other montages. The tDCS group had a BMI of 26, compared to the BMI of 25.4 in the control group. While the difference between these group means is statistically non-significant, it is of note that a BMI of 26 is considered as overweight, and tDCS has been demonstrated to have different effects in overweight individuals compared to those of normal weight (decreased systemic glucose uptake in response to tDCS in obese individuals compared to normal weight individuals) (33). Thus we cannot completely rule out BMI affecting the results.

In addition, due to the extensive number of comparisons and the stringent alpha levels resulting from correction to the number of these comparisons, we cannot rule out some real effects erroneously appearing as non-significant. The study protocol allowed a maximum time interval of 5 min between the end of stimulation and the venipuncture, but unfortunately we did not use a timer to record this interval. In practice, the circa 20-metre transfer from the stimulation room to the blood sampling station, as well as preparations necessary for the blood draw, were estimated to take approximately 3 min, leaving approximately 2 min for individual variation in the interval. Therefore, we cannot exclude potential random variation that this time interval may have induced in our findings.

It is also important to note that any tDCS-related changes may have a ceiling effect in the healthy brain. For example, a greater improvement in cognitive task performance following tDCS was observed in neuropsychiatric patients than in healthy controls (34). Therefore, our observations may have been different among, for example, patients with depression. Nevertheless, we sought to provide a healthy male volunteer 'baseline' of findings that we hope both our group and others will in the future be able to use as a healthy individual reference when conducting further investigations among patients.

In the present investigation, both the participants and the experimenters were able to distinguish sham and active stimulation at the beginning of the protocol, which is in contrast to some previous studies (35). This might be a result of the different protocols applied here, with its higher currents

and greater current density as compared to earlier investigations. In addition, we also had larger study groups, resulting in a higher power to detect smaller failures in blinding. However, this issue should not have significantly impacted on our findings.

Our power calculations suggested that enormous sample sizes would be necessary to detect any changes in most of the studied compounds. However, while the power calculations indicated that some of the metabolites would require sample sizes at the level of thousands of individuals to be detected as statistically significant, some metabolites, such as xanthine and hippuric acid, could be detected as significant with group sizes of less than 100. These power calculations may be beneficial when planning future studies. Furthermore, GABA and glutamate, for which our observations suggest sufficient power for detection with sample sizes of 35 and 164 per group, respectively, are of special interest for clinical tDCS research. Both GABAergic and glutaminergic systems have been suggested to play a significant part in, for example, the pathophysiology of depression (36), and any changes in these markers may be of interest from the point of view of treatment mechanisms or the prediction of treatment efficacy.

CONCLUSIONS

We found that five daily sessions of tDCS, applied to healthy male adults, did not result in significant changes in peripheral blood metabolites. These results indicate that tDCS may be metabolically safe, at least for healthy participants, but more research is needed to determine whether the results would be the same in populations with metabolic disturbances. Further studies with larger samples as well as with female volunteers are also warranted. Our power calculations offer a useful, evidence-based baseline for designing such studies.

DATA AVAILABILITY STATEMENT

The informed consent received from the participants, as well as the ethics permission from the research ethics committee who reviewed the study protocol, only permits data transfer to countries with data privacy laws compatible with the respective laws in Finland. Therefore, the data cannot be published in an open repository. Nevertheless, the data will be made available on request and can be obtained by contacting the corresponding author (soili.lehto@helsinki.fi).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee, Hospital District of Northern Savo, Kuopio University Hospital. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Data acquisition—LK, VV. Study design—TK, SL, JW. Data analysis and interpretation—AK, AO-A, A-HJ, TT, TA-S. Project supervision—SL, JW. Wrote the manuscript—AK. Commented on the manuscript—AO-A, A-HJ, TT, TA-S, TK, JW, LK, JN, VV, SL.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2020.00403/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Transcranial electrical stimulation is a novel neuromodulation method, showing promise in the treatment of, for example, depression and substance dependence. This thesis examined the tolerability of the stimulation during consecutive treatment days, and how lifestyle factors affect the adverse effects. Effects of stimulation on peripheral metabolomics was also investigated.

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