Non-invasive Neuromodulation of Executive Functions

On the Effects of Transcranial Direct Current Stimulation and Cognitive Control Training

Dissertation

an der Mathematisch-Naturwissenschaftlichen Fakultät
der Eberhard Karls Universität Tübingen
zur Erlangung des Grades eines
Doktors der Naturwissenschaften
(Dr. rer. nat.)

vorgelegt von
Simone Weller, M.Sc.
aus Cuxhaven

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Erklärung

Ich erkläre hiermit, dass ich die zur Promotion eingereichte Arbeit mit dem Titel: *Non-invasive Neuromodulation of Executive Functions - On the Effects of Transcranial Direct Current Stimulation and Cognitive Control Training* selbständig verfasst, nur die angegebenen Quellen und Hilfsmittel benutzt und wörtlich oder inhaltlich übernommene Stellen als solche gekennzeichnet habe. Ich erkläre, dass die Richtlinien zur Sicherung guter wissenschaftlicher Praxis der Universität Tübingen (Beschluss des Senats vom 25.5.2000) beachtet wurden. Ich versichere an Eides statt, dass diese Angaben wahr sind und dass ich nichts verschwiegen habe. Mir ist bekannt, dass die falsche Abgabe einer Versicherung an Eides statt mit Freiheitsstrafe bis zu drei Jahren oder mit Geldstrafe bestraft wird.

Ort, Datum	Simone Weller

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I have learned a lot from working alongside all of you - and I still gladly do.

Contents

Zusammenfassung

Hintergrund

Kognitive Kontrollprozesse erlauben es uns, im alltäglichen Leben zielgerichtet und angepasst zu handeln. Ablenkende Reize, die Menschen zu ineffektiven oder gar unangemessenen Reaktionen verleiten würden, werden gefiltert und das Verhalten entsprechend angepasst. Defizite der kognitiven Kontrolle, wie sie oft in psychischen Erkrankungen wie zum Beispiel der Depression vorkommen, erschweren das Leben der Betroffenen und verursachen großen Leidensdruck. Kognitive Kontrolle zu stärken, zu verbessern oder wiederaufzubauen kann durch nichtinvasive Hirnstimulationsverfahren und spezielle Trainings erreicht werden. Diese Interventionen zielen oftmals auf den dorsolateralen Präfrontalkortex (*dorsolateral prefrontal cortex*, dlPFC) ab, welcher zu einem großen Teil für die Aufrechterhaltung kognitiver Kontrolle verantwortlich ist. Nach wie vor ist die Effektivität der Verfahren jedoch stark variabel und erfordert tiefergehende Untersuchungen.

Ziel

Ziel dieser Arbeit ist es, zu untersuchen, inwiefern kognitive Kontrollprozesse durch nichtinvasive Interventionen in gesunden sowie in depressiven Menschen unterstützt werden können. Zum Einen wurde strukturiert getestet, welche Parameterkombination bei transkranieller Gleichstromstimulation (*transcranial direct current stimulation*, tDCS) des dlPFC kognitive Prozesse in einem Training kognitiver Kontrolle (*paced auditory serial addition task*, PASAT) verbesserte und wie diese Effekte außerdem durch das biologische Geschlecht der Teilnehmenden beeinflusst wurden. Zum Anderen wurden zwei unterschiedliche Versionen des PASAT, welcher durch

seinen Aufbau insbesondere kognitive Kontrollfunktionen des dlPFC fordert, von depressiven Versuchspersonen angewandt, um eine mögliche Verbesserung depressiver Symptomatiken zu untersuchen.

Methodik

Zur systematischen Untersuchung der tDCS-Parameter durchliefen 162 gesunde Versuchspersonen ein zweiwöchiges PASAT-Training. Jede Versuchsperson wurde während dieses Trainings durch eine von neun möglichen tDCS-Konfigurationen - unterscheidbar durch Variation der Polarität, Intensität sowie Lateralität - stimuliert und der Trainingserfolg im PASAT gemessen. Um mögliche positive Auswirkungen des PASAT bei Vorliegen einer Depression zu untersuchen, wurden 32 Versuchspersonen in zwei Studiengruppen unterteilt und erhielten für einen Zeitraum von sechs Wochen entweder eine Reinform dieses kognitiven Trainings, oder aber eine mit gamification- und Psychoedukations-Elementen angereicherte Version. Das Training konnten sie in diesem Zeitraum nach eigenem Ermessen anwenden.

Ergebnisse

In der Untersuchung der Stimulationsparameter zeigte sich, dass anodale tDCS mit einer Intensität von 1 mA über dem linken dlPFC im Vergleich zur Scheinstimulation zu einem signifikanten Anstieg der Trainingsleistung im PASAT führte, während die anderen Stimulationsformen diese Überlegenheit nicht zeigten. Des Weiteren zeigte sich dieser Effekt in weiblichen, jedoch nicht männlichen, Teilnehmern. Diese Ergebnisse lassen darauf schließen, dass es sowohl einen nicht-linearen Zusammenhang zwischen Stimulationsintensität und Verbesserung der Trainingsleistung gibt, als auch, dass sich die Effekte der tDCS in Frauen und Männern unterscheiden. Ein herauszustellender Faktor ist hierbei vor allem die große Stichprobenzahl, durch die Zweifel an der Wirkung von tDCS auf kognitive Funktionen im Allgemeinen, und auf das Training kognitiver Kontrolle im Besonderen, ausgeräumt werden konnten. Die Anwendung des PASAT-Trainings durch depressive Patienten zeigte, dass die mit zusätzlichen gamification-Elementen angereicherte Version im Gegensatz zur Reinform zu einer anhaltenden signifikanten Reduktion depressiver Symptomatik führte. Diese Ergebnisse deuten auf eine potentielle positive Wirkung des angepassten kognitiven Trainings bei Vorliegen einer Depression hin.

Abstract

Background

Cognitive control (CC) processes allow us to act in a targeted and goal-oriented manner in everyday life. Distracting stimuli, which would otherwise lead to ineffective or even inappropriate actions, are filtered and the behaviour can subsequently be adequately adapted. Deficits in CC, often found in psychiatric disorders such as *major depressive disorder* (MDD), greatly impair the lives of those affected. Strengthening, improving, or rebuilding CC can be achieved through non-invasive brain stimulation techniques and specialised trainings. These interventions often target the *dorsolateral prefrontal cortex* (dlPFC), a cortical area that is largely responsible for maintaining CC. However, the effectiveness of these procedures is still highly variable and requires further thorough investigations.

Aim

The aim of this work is to investigate to what extent CC processes can be supported by non-invasive interventions in healthy study participants as well as depressed patients. In healthy participants, a structured parameter testing protocol was therefore implemented to determine which *transcranial direct current stimulation* (tDCS) configuration over the dlPFC improved cognitive processes during performance of a *cognitive control training* (CCT), the *paced auditory serial addition task* (PASAT). Additionally, the influence of biological sex on participants' performance was evaluated. Lastly, two different versions of the CCT were tested by patients with MDD to investigate a possible reduction of depressive symptoms, as this task specifically challenges CC functions of the dlPFC.

Methods

For the systematic investigation of tDCS parameters, 162 healthy participants underwent a two-week long PASAT training. Each participant received one of nine possible tDCS configurations during the CCT. The configurations were distinguishable by varying polarity, intensity, as well as localisation of the intervention. To investigate the effects of the PASAT in depression, 32 patients were divided into two study groups and received either a pure form of the PASAT or a version enriched with gamification and psychoeducational elements. They then could utilise their assigned training at their own discretion for the duration of six weeks.

Results

Examination of the stimulation parameters revealed that anodal tDCS with an intensity of 1 mA over the left dlPFC resulted in a significant increase in task performance compared to sham stimulation. Other tDCS combinations did not yield superior training effects. Furthermore, when comparing the study groups, this effect was evident in women but not men. These results suggest that there is a beneficial, albeit non-linear, relationship between stimulation intensity and improvement in task performance. Additionally, these performance-increasing effects differ between the sexes. One factor to be emphasised is the large sample size, which allowed to dispel doubts about the effect of tDCS on cognitive functions in general, and the training of CC in particular. The at-home implementation of the PASAT revealed that the version enriched with additional gamification elements led to a lasting significant reduction of patients' depressive symptoms over the pure form of the PASAT, showing the advantage of a specifically adapted CCT for the use in MDD.

Abbreviations

BDNF brain-derived neurotrophic factor

CACNA1C calcium voltage-gated channel subunit alpha1 C

CC cognitive control

CCT cognitive control training

CG control group

COMT catechol-O-methyltransferase

dlPFC dorsolateral prefrontal cortex

EF executive function

FPN frontoparietal network

IDS-SR Inventory of depressive symptomatology (self-report version)

IG intervention group

ISI interstimulus interval

LTP long-term potentiation

MADRS Montgomery-Åsberg depression rating scale

MDD major depressive disorder

NA negative affect

NIBS non-invasive brain stimulation

PA positive affect

PANAS positive and negative affect schedule

PASAT paced auditory serial addition task

PASST paced auditory serial subtraction task

PFC prefrontal cortex

PVSAT paced visual serial addition task

tACS transcranial alternating current stimulation

tDCS transcranial direct current stimulation

tES transcranial electric stimulation

TMS transcranial magnetic stimulation

tRNS transcranial random noise stimulation

WHO5 WHO-Five-Well-Being Index

List of Figures

List of Tables

List of Publications and Author's Contributions

Accepted Publications

Weller, S., Nitsche, M. A., & Plewnia, C. (2020a). Enhancing cognitive control training with transcranial direct current stimulation: A systematic parameter study. *Brain Stimulation*, *13*(5), 1358–1369. https://doi.org/10.1016/j.brs.2020.07.006

Simone Weller: methodology, software, validation, formal analysis, investigation, data curation, writing - original draft, writing - review and editing, visualisation, project administration.
Michael A. Nitsche: writing - review and editing.
Christian Plewnia: conceptualisation, methodology, formal analysis, resources, writing - original draft, writing - review and editing, supervision, project administration, funding acquisition.

Weller, S., Schroeder, P. A., & Plewnia, C. (2022). Gamification improves antidepressant effects of cognitive control training—A pilot trial. *Frontiers in Digital Health*, *4*. https://doi.org/10.3389/fdgth.2022.994484

Simone Weller: conceptualisation, methodology, software, validation, formal analysis, investigation, data curation, writing - original draft, writing - review and editing, visualisation, project administration.
Philipp A. Schröder: conceptualisation, writing - review and editing, funding acquisition.
Christian Plewnia: conceptualisation, methodology, formal analysis, resources, writing - original draft, writing - review and editing, supervision, funding acquisition.

Weller, S., Derntl, B., & Plewnia, C. (2023a). Sex matters for the enhancement of cognitive training with transcranial direct current stimulation (tDCS). *Biology of Sex Differences*, *14*(1), 1–13. https://doi.org/10.1186/s13293-023-00561-4

Simone Weller: conceptualisation, methodology, software, validation, formal analysis, investigation, data curation, writing - original draft, writing - review and editing, visualisation, project administration.
Birgit Derntl: writing - review and editing.
Christian Plewnia: conceptualisation, methodology, formal analysis, resources, writing - original draft, writing - review and editing, supervision, funding acquisition.

Supplementary Publication

Wiegand, A., Blickle, A., Brückmann, C., Weller, S., Nieratschker, V., & Plewnia, C. (2021a). Dynamic DNA Methylation Changes in the COMT Gene Promoter Region in Response to Mental Stress and Its Modulation by Transcranial Direct Current Stimulation. *Biomolecules*, *11*(11), 1726. https://doi.org/10.3390/biom11111726

Ariane Wiegand: conceptualisation, formal analysis, investigation, data curation, writing - original draft, visualisation, project administration.
Arne Blickle: investigation, writing - review and editing.
Christof Brückmann: investigation, writing - review and editing.
Simone Weller: formal analysis, writing - review and editing.
Vanessa Nieratschker: conceptualisation, writing - original draft preparation.
Christian Plewnia: conceptualisation, writing - original draft preparation, funding acquisition.

1 Introduction

1.1 Executive Functions in Human Cognition

Cognition can be defined as the mental process of acquiring knowledge and understanding through thought, experience, and senses. It is a complex concept that involves perception, attention, memory, problem-solving, and decision-making. In order to react adequately to ever changing environments, humans need to be able to adapt their behaviour to prevalent stimuli. *Executive function*s (EFs), a term commonly used in research to describe these top-down processes in humans, encompass a variety of traits that make flexible adaptation of cognitive functions possible (Diamond, 2013). They can be summarised as mechanisms that exert control over more elemental processes and regulate fundamental operations. By doing so, they allow for and facilitate adaptable goal-directed behaviour. This includes inhibition, working memory, cognitive flexibility, and hence is a central hallmark of higher cognition (Friedman & Robbins, 2022). Some of the basic processes that comprise EFs are attentional control, cognitive inhibition, inhibitory control, working memory, as well as cognitive and mental flexibility (Cristofori et al., 2019).

1.1.1 Introducing Cognitive Control

Another terminology commonly used in context with EFs is *cognitive control* (CC). While the two terms are often used interchangeably, several distinctions should be pointed out: while EFs describe the overarching *cognitive processes* that are necessary for CC, CC itself can be more aptly described as the dynamic *ability* to orchestrate and achieve the regulation of emotions, attention, actions, and task switching (Botvinick et al., 2001; Menon & D'Esposito, 2022; Miyake et al., 2000). By doing so, cognitive resources are focused towards situationally relevant stimuli

while prioritising the latter over distractions. This in turn is the basis for exercising and upholding goal-directed behaviour and as such, CC plays an essential role in almost all stages of information processing and behaviour in humans. Interestingly, the concept of CC has also spread to the development of artificial intelligences (Savage, 2019): through the use of abilities that can be classified as CC, agents learn from feedback, adapt to changing situations, and lastly solve problems of increasing complexity. This allows these artificial agents to mimic and surpass human performance in specific tasks (Robertazzi et al., 2022; Zhao et al., 2022). In humans, it has been proposed that these cognitive processes and abilities are modulated by multiple functional networks within the *prefrontal cortex* (PFC) (Menon & D'Esposito, 2022).

1.1.2 Neurobiological Foundations of Cognitive Control

EFs as well as the ability to exert CC are mostly associated with frontal lobe functioning (E. K. Miller & Cohen, 2001; E. K. Miller et al., 2002). Research has found that the PFC and its related networks and regions are essential cortical structures for this (Friedman & Robbins, 2022). Six functional networks have been proposed to be involved in the generation and maintenance of CC: the *frontoparietal network* (FPN) which engages the *dorsolateral prefrontal cortex* (dlPFC), posterior parietal cortex, and anterior inferior parietal lobule (Harding et al., 2015; Zanto & Gazzaley, 2013); the *salience* network, encompassing the anterior cingulate and ventral anterior insular cortices (Ham et al., 2013); the *cingulo-opercular* network, composed of operculum, dorsal anterior cingulate cortex, and thalamus (Wood & Nee, 2023); the *ventral attention* network which includes the temporoparietal junction and ventral frontal cortex (Shine et al., 2013); the *dorsal attention* network which incorporates intraparietal sulcus and frontal eye fields (Dosenbach et al., 2007); and lastly the *default mode* network which consists of the medial prefrontal cortex, posterior cingulate cortex, precuneus, and angular gyrus (Spreng, 2012). These networks are thought to operate as distinct functional units, exhibiting a high degree of symmetry that reflects the intrinsic functional connectivity, all while each supporting specific cognitive processes (Menon & D'Esposito, 2022). Research also suggests that dynamic cooperation as well as competition between these networks is essential for CC and that multidomain CC arises from these flexible patterns of interaction. Two interconnected meta-systems have been proposed: an

executive system that handles context-dependent processing of sensory, autonomic, and cognitive information for adaptive control as well as an *integration* system which allows for flexible integration of external and internal information (Cocchi et al., 2013). Under conditions when increased CC was required, two of these networks showed to become more integrated with each other: the FPN and the cingulo-opercular network. Furthermore, the increased integration of these two CC networks with a task-related but non-CC network was found to be associated with improved accuracy in cognitive tasks. This provides a basis for the theory that increased cognitive exertion leads functional networks towards adopting more efficient but less economical configurations by decreasing modularity (Cohen et al., 2014; Kitzbichler et al., 2011; Liang et al., 2016; Menon & D'Esposito, 2022).

On a more fine-grained structural level, another critical region of the brain's executive network, the dlPFC, plays a pivotal role in upholding CC (Gläscher et al., 2012). The dlPFC is a major component of the FPN (Marek & Dosenbach, 2018). It is implicated in a variety of cognitive processes and is believed to exert its influence through top-down modulation of task-relevant information processing (Cieslik et al., 2013). Changes in dlPFC activity are associated with shifts in CC capabilities (MacDonald et al., 2000), which will be described in more detail in the following subsection. Together with the previously introduced network dependencies, this highlights the importance of the dlPFC as an integral element to exert CC, modulate task-relevant information processing, and regulate impulsivity.

1.1.3 Impaired Cognitive Control

Executive dysfunctions are mostly represented by damage to the processes that encompass CC (Burgess & Alderman, 2003). Damage to regions involved in CC processes can result from traumatic injuries or neurological and psychiatric disorders (Larson et al., 2006; Olsen et al., 2015; Scheibel, 2017) and therefore negatively affect processes that are related to or rely on white matter connections (Kerchner et al., 2012), neurotransmitter systems (Barnes et al., 2011), and cortical activity (Brass et al., 2005; Wolkenstein & Plewnia, 2013). As CC (or lack thereof) is closely connected to the activity within the dlPFC, modification in dlPFC activity is often apparent in cases of impacted CC (Braver et al., 2009; Friedman & Robbins, 2022). Individuals that exhibit these dysfunctions can often be characterised by

difficulties to manage impulses and emotions (Amidfar et al., 2019; Kim & Lee, 2011; Salehinejad et al., 2017; Saunders et al., 2015); problems organising, planning, starting, or completing tasks (Alvarez & Emory, 2006); reduced attention, processing speed, and learning (Woodward et al., 2013); short-term memory issues (Funahashi, 2006); inability to multitask (Verghese et al., 2016); or suppression of socially inappropriate behaviour (Arnsten, 2009). Because of this, CC has been studied extensively in the context of various cognitive and psychiatric disorders that entail these symptoms. CC deficits have been linked to a decrease in dlPFC activity in schizophrenia (Yoon et al., 2008) as well as emotion dysregulation in *major depressive disorder* (MDD) (Joormann & Vanderlind, 2014; Villalobos et al., 2021). In MDD especially, the left dlPFC, amygdala, and hippocampus show functional and partly also structural changes (Palazidou, 2012; Zhang et al., 2020). Abnormal activity patterns within the cortices, which results in a dysbalance between the cerebral hemispheres, are often linked to deficits in attention, working memory, and action planning (Siddiqui et al., 2008). However, it should be noted that these neurobiological patterns are still not completely understood and therefore remain a topic of debate in research (Fitzgerald et al., 2006). Nevertheless, the change in activity leads to a change in emotional judgment towards negative aspects and furthermore promotes symptoms such as apathy and lack of motivation in depressed patients. Overall, it can be said that dysfunctional activity within the dlPFC is preferentially involved in disorders with prominent deficits in CC (Heilbronner & Chafee, 2019). Not surprisingly, the severity of MDD seems to be correlated with dlPFC activity, with more severe symptoms being associated with a steeper decline in activity (Grimm et al., 2008). Thus it can be summarised that impairment of CC is an integral component in the development and maintenance of psychiatric disorders and should therefore be prevented by employing proper interventions.

1.1.4 Supporting Cognitive Control

It stands to reason that aiding functions of the dlPFC in order to rebuild CC in patients, as well as enhancing CC in healthy participants to increase performance in specific tasks or everyday life, could prove to be a promising use for cognitive enhancement techniques (Taylor et al., 2022). CC develops well into adulthood and moreover is also a trainable ability. Hence, for more than 20 years, possible interventions that adequately target and support CC have been extensively sought

after and studied. There are various means by which CC can be influenced. Examples are forms of *non-invasive brain stimulation* (NIBS) (Hartwigsen & Silvanto, 2023), *cognitive control training*s (CCTs) (Koster et al., 2017), neurofeedback (Keizer et al., 2010), physical activity (Padilla et al., 2013), and structured educational programs (Diamond et al., 2007). Two of these methods are the focus of my dissertation and will be introduced in the following sections: *transcranial direct current stimulation* (tDCS) as a form of NIBS and a digital intervention in the form of an enhanced CCT which was enriched with elements that are hypothesised to improve its efficacy.

1.2 Non-invasive Neuromodulation

Non-invasive neuromodulation refers to techniques that can modulate brain activity without the need for surgery or implantation of electrodes. Various techniques fall under this category. Most of them apply *external* catalysts to modulate neuronal activity, where the neurons are actively targeted by magnetic or electric fields through medical devices. Commonly used forms of this kind of neuromodulation are: *transcranial magnetic stimulation* (TMS), vagus nerve stimulation, tDCS and its related forms *transcranial alternating current stimulation* (tACS) and *transcranial random noise stimulation* (tRNS). The use of NIBS has proven to be beneficial for healthy users as well as patients. TDCS especially has been used in the treatment of various neurological and psychiatric disorders, including MDD (Nitsche et al., 2009; Palm et al., 2016; Voineskos & Blumberger, 2023), anxiety disorders (Sagliano et al., 2019; Stein et al., 2020), and chronic pain (Wen et al., 2022). The second form of neuromodulation, CCT, is an umbrella term that summarises techniques that are meant to improve cognition by repeated engagement of cognitive processes (van Balkom et al., 2020), thereby strengthening the targeted networks. All in al, NIBS as well as CCTs have shown promise in improving symptoms of mental disorders as well as enhancing cognitive functions in healthy people. However, the field of non-invasive neuromodulation is still fairly young and has mostly been explored during the last two decades. Optimising parameters for stimulation or training, defining standardised protocols that can be used universally under various circumstances, have yet to be established. The following subsections will introduce these two techniques that were utilised for the studies that comprise my work.

1.2.1 Transcranial Direct Current Stimulation

TDCS is a form of *transcranial electric stimulation* (tES) that directs low electrical currents through the head in order to modulate brain functions (Nitsche et al., 2008). Electrodes are placed on the scalp, and sometimes extracephalic areas, to route the current through the target areas of the brain. The placement of two or more electrodes completes the circuit, allowing the current to flow from anode(s) to cathode(s). By doing so, neuronal membrane polarity and excitability are modulated. The threshold for action potential generation is thus changed, altering the likelihood of action potentials to occur and ultimately producing facilitatory or inhibitory effects on neuronal brain activity (Pelletier & Cicchetti, 2015; Thair et al., 2017). It should be noted that, unlike TMS, tDCS does not induce neuronal activity on its own. Instead, it changes the probability of action potentials occurring. *Anodal* tDCS describes a setup where the anode is placed over the target area, whereas *cathodal* tDCS describes a montage where the cathode is on top of the target area. In tDCS, the current flow causes a predominantly increase in excitability of neurons close to the anode (*depolarisation*). In case of cathodal tDCS, excitability is decreased (*hyperpolarisation*). Because of this, anodal tDCS is commonly referred to as *enhancing*, cathodal tDCS as *inhibitory* (Nitsche & Paulus, 2000), making especially the former form of stimulation a promising prospect to enhance cognitive functions by altering neuronal activity. Depending on the protocols used in tES, these changes in excitability can last from several minutes (Bastani & Jaberzadeh, 2013; Nitsche & Paulus, 2001) to hours or even days after the stimulation has ended (Nitsche & Paulus, 2001; Paulus, 2011b).

The prospect of enhancing neuronal activity within the PFC, particularly through tDCS, has been increasingly studied within recent years. Research has shown that stimulating the dlPFC can enhance CC (Wards et al., 2023; Wiegand et al., 2019), making it easier for individuals to stay focused on tasks, avoid distractions, and improve frustration tolerance (Plewnia et al., 2015). In addition to direct stimulation, tDCS can also indirectly enhance CC through the utilisation of network effects. As described before, functional networks within the brain are interconnected and stimulating one area/network is likely to have effects on other networks that are functionally connected to it. Therefore, tDCS can potentially enhance cognition by modulating not only the activity of neurons that are spatially close to the electrodes, but also of entire cortical networks (Li et al., 2019). Long-lasting plasticity effects

have also been observed, where sustained tDCS modulated *long-term potentiation* (LTP)- or *long-term depression*-like mechanisms in the brain (Frase et al., 2021; Jamil & Nitsche, 2017), though it remains debatable whether tDCS is acts as a modulator or inducer of synaptic plasticity (Kronberg et al., 2017).

Another approach how tDCS may enhance cognition is by reducing *cognitive interference*. Cognitive interference refers to the phenomenon where irrelevant information interferes with the processing of relevant information. In cases of efficiently applied CC, the interference will be adequately eliminated while relevant information will be prioritised (Friehs et al., 2020). Furthermore, tDCS may also increase the speed and flexibility of cognitive processes. This could involve changing functional activity patterns (Polanía et al., 2011) or switching between different brain states more efficiently (Li et al., 2019). Such enhancements could lead to improvements in various cognitive tasks, from problem-solving to multitasking (Filmer et al., 2013; Metuki et al., 2012). Finally, the stimulation may enhance CC by exploiting what is known as *cognitive reserve* (Barnett et al., 2006; Stern, 2009). It refers to the brain's resilience or ability to cope with damage or disease. By stimulating the brain, tDCS may help individuals tap into this reserve, potentially leading to improvements in cognitive function. It has to be noted though, that despite tDCS showing promise for successful interventions, there is currently still a lack of consensus between studies and it remains unclear what type of tDCS stimulation is most effective for a specific use case.

Lastly, many factors influence the outcome of a tDCS intervention: placement as well as shape and size of electrodes, stimulation intensity, direction of current flow, duration and number of stimulation sessions (Brunoni et al., 2012; Weller et al., 2020a). Additional to these stimulations-specific parameters are human-specific traits such as head morphology (Parazzini et al., 2011), brain state (Kurtin et al., 2021), medication (McLaren et al., 2018), (epi-)genetic factors (Wiegand et al., 2021a), and biological sex (Cahill, 2006). Because of this variability in possible setups and study groups, systematically evaluating and developing protocols, such as the titration and variation of stimulation parameters, is paramount to advance the application of tDCS (Berryhill & Martin, 2018).

1.2.2 Cognitive Control Training

CCTs are a widespread field of digital or computerised interventions, mostly in the forms of exercises that a participant or subject solves. Through regular use, the exercises are meant to recruit and activate prefrontal networks. Their goal is to strengthen CC functions. These tools often involve tasks that challenge and improve working memory, attention, inhibitory control, or emotion regulation (Hoorelbeke et al., 2016; Maraver et al., 2016; Peckham & Johnson, 2018; Vanderhasselt et al., 2021). It has been shown that maladaptive emotion regulation and depressive symptomatology can be reduced in patients who perform CCTs (Hoorelbeke et al., 2016) and that cognitive trainings can increase network activity within and connectivity between certain brain areas (Lockwood et al., 2004; van Balkom et al., 2020).

The Paced Auditory Serial Addition Task

The task used in all main studies presented in this dissertation is the *paced auditory serial addition task* (PASAT). It is a neuropsychological test that measures information processing and attention as well as how neurological pathology affects cognition (Tombaugh, 2006). Originally developed more than 40 years ago to evaluate patients with head injuries (Gronwall, 1977), it has since been extensively used in other areas related to the research of psychiatric disorders (Dujardin et al., 2007; Nikravesh et al., 2017; Polizzi et al., 2022; Rosti et al., 2007). In the PASAT, participants are asked to process single digits according to a specific arithmetic pattern. Most versions of this task require addition of the digits, though versions that utilise subtraction exist as well (Pope & Miall, 2012). The latter are referred to as *paced auditory serial subtraction task*s (PASSTs). Regardless of the arithmetic being used, the digits are presented randomly with an interval of a few seconds between presentations. Usually, participants are instructed to add or subtract each new number to the the one that immediately preceded it. Digit presentation can be either auditory, e.g. with the use of headphones (in case of the PASAT) or visually by flashing the respective digit on a computer screen (in that case the *paced visual serial addition task* (PVSAT)). Participants give the answer of their calculation either verbally or by pressing keys on keyboards. The speed with which the digits are presented is usually decreased either by a fixed amount after each

trial or adapted according to participants' performance where several consecutively correct or incorrect answers cause a change in digit presentation speed. During the task additional continuous performance feedback is given (Correia, 2011), usually in the form of a coloured flash on the screen: green for correct calculations, red for wrong calculations. This increases cognitive demands and task difficulty over time. The tasks are known to be associated with the increase of frustration and stress (Tombaugh, 2006). Interestingly, both the PASAT and PVSAT activate frontoparietal brain areas, which the dlPFC belongs to (Audoin et al., 2005; Bonzano et al., 2009; Lazeron et al., 2003; Staffen et al., 2002). Furthermore, it is therefore not only a tool to measure cognitive capabilities: as the activation of FPNs by the task has been found both in healthy participants and patients with psychiatric disorders (Mainero et al., 2004), this leads to the assumption that regular use of the task can strengthen the respective networks and hence CC functions - a core trait that constitutes any CCT. Indeed, the PASAT specifically has been used in MDD research (Segrave et al., 2014; Siegle et al., 2007) and shown promise to even decrease depressive symptoms (Calkins et al., 2015; Hoorelbeke et al., 2015).

1.2.3 Gamification as an Improvement to Cognitive Trainings

Gamification is described as the process of incorporating elements of gameplay into non-gaming environments such as cognitive tasks or tests. It can involve adding points systems, rewards, competition through leader boards, and achievement badges to an activity or task (Saleem et al., 2022). One major goal of adding gamification elements is to create an engaging and enjoyable experience, which can help increase participation and commitment towards the activity even though the activity itself is not necessarily enjoyable (Boot et al., 2016). This is thought to boost users' motivation and encourage a change towards desired behavioural patterns. Gamification can prove useful when added to CCTs, which are often seen as effortful, frustrating, and repetitive (Lumsden et al., 2016). By adding certain enriching elements, gamification can contribute to a more enjoyable experience that leaves users with a sense of accomplishment and keeps them engaged (Koivisto & Hamari, 2019). Meta-analyses have shown that gamified training tasks were more motivating and engaging but also more demanding and difficult than less or non-gamified tasks (Vermeir et al., 2020). However, it is of note that the type of gamification used is highly dependent on the task and target audience and should be chosen accordingly

(Lumsden et al., 2016; Vermeir et al., 2020). It is also important to point out that game elements could have adverse effects on the task they are applied to, such as causing distractions, which can outweigh their potential motivational benefits. That is why the rise of task complexity should be closely monitored (Lopez & Tucker, 2017). Furthermore, heterogeneous study designs are still quite common, making comparisons between the various gamification elements more challenging (Khaleghi et al., 2021; Vermeir et al., 2020).

1.2.4 Synergistic Effects of Combining Transcranial Direct Current Stimulation with Cognitive Control Training

While tDCS as well as CCTs have been independently used to enhance cognitive performance, multiple studies show that a combination of both neuroenhancement techniques can improve outcomes even further and amplify beneficial effects (Brunoni, Boggio et al., 2014; Koster et al., 2017; Segrave et al., 2014). Combining cognitive interventions can have synergistic effects not only on on cognition (Burton et al., 2023), but also upper limb function, gait, mobility, and posture (Beretta et al., 2020). One possible reason for this can be found in one major property that influences both CCTs and tDCS: *brain state* - i.e. recurring activity patterns across the brain which emerge from physiological or cognitive processes and which have functional relevance (Greene et al., 2023). A combination of tDCS and concurrent CCT that evokes and makes use of specific brain states can therefore: increase and extend training gains in tasks (Jones et al., 2015; Park et al., 2014; Stephens & Berryhill, 2016), alter excitatory neurotransmitter concentration in frontal cortices (Alvarez-Alvarado et al., 2021), and improve memory functions in early stages of cognitive impairment (Rodella et al., 2022). Some research suggests that tES combined with cognitive training enhances performance on CT tasks across a range of cognitive functions, though effects on transfer tasks remain mixed (Elmasry et al., 2015).

To summarise, the implications for the use of these interventions are promising, especially when the proper combination between NIBS and CC is found. Because of the intricate connections not only within but also between interventions, which ultimately result from the numerous intrinsic and extrinsic factors that were mentioned before, final and conclusive statements on *if* and *how* tDCS and CC should be combined have yet to be established. However, as each single intervention as well

as their combined application has shown encouraging results, this will be the topic for further research; some of which was done in the projects that are presented in the following chapters.

2 Objectives

Aim of my work is to examine mechanisms, application, and efficacy of two neuro-enhancement approaches that ultimately have the goal to support and enhance cognitive functions in humans: tDCS as a form of tES that can induce short- and long-term effects in the alteration of CC, and gamification elements in a CCT that was specifically adapted for and aimed at depressed patients. A healthy study group without any diagnosed psychiatric disorders as well as a group comprised of patients with MDD take part in the experiments. The dlPFC is chosen as a target area as it is involved in various CC processes. Although both approaches have been used increasingly in research and clinical settings, the study landscape remains diverse. The lack of systematic comparisons makes purposeful development difficult, as optimal parameters that make the techniques universally usable have yet to be established.

In the first study, multiple tDCS configurations are systematically examined in healthy participants. The stimulation is coupled with the PASAT as a measure of CC. This data provides a basis for individualised tDCS application and allows to determine which configuration is most beneficial to support cognitive processes in the provided setting (Section 3.1).

The objective of the second study is to take a more detailed look into one major human trait that can influence tDCS: biological sex. The gathered data is therefore analysed in more detail and with focus specifically on how sex affects the tDCS intervention (Section 3.2).

For the third study, potential antidepressant effects of an adapted version of the PASAT in patients diagnosed with MDD are investigated. Two versions of the CCT are compared against each other: the basic version as used in the first study and a gamified version specifically customised to be suitable for at-home use by patients. In this novel approach, patients are able to perform the training at their own

discretion, hence giving insight into usage patterns, tolerability, and the magnitude of reduction in depressive symptoms that can be expected (Section 3.3).

The combined results of these studies aim at answering questions on the mechanisms and potential of tDCS-enhanced training of CC and whether additional gamification elements added to CCT can prove valuable for patients with MDD. Overall, the viability of these neuroenhancement techniques is addressed with the goal to resolve some of the high variability that the current study landscape demonstrates and that impedes adoption into clinical practice.

3 Empirical Work

The subsequent sections give an abridged synopsis of the main studies that embody my dissertation and summarise the central findings. Detailed study protocols with full information, statistical analyses, and results are enclosed in the respective manuscripts (Appendix A). It is of note that *participants* will refer to healthy subjects without any diagnosed psychiatric disorders whereas *patients* refers to subjects with acute light to moderate MDD at the start of their involvement in the study. Participants and patients were recruited through flyers, websites, online forums, and the mailing list of the University of Tübingen. All individuals were selected at random and, before being enrolled in the study, gave informed written consent. In case of psychological ratings and interviews, raters were blind to patients' interventions. All studies were performed in accordance with the declaration of Helsinki and approved by the University of Tübingen ethics committee.

3.1 Systematic Testing of Transcranial Direct Current Stimulation Parameters

This study examined the effects of tDCS on a two-week long CCT in healthy humans (see Weller et al. (2020a), Appendix A.1.1; Weller et al. (2020b), Appendix A.1.2 for supplementary materials). It was hypothesised that active (more specifically anodal) tDCS would increase cognitive performance compared to sham stimulation.

3.1.1 Methods

In total, 192 right-handed participants without any history of psychiatric illnesses enrolled in the study and started the intervention. Ultimately, 163 participants

completed the paradigm and 162 were included in the final analyses (127 women, 35 men; aged between 18-39; mean age = 23.20 ±3.98 years); one subject had to be excluded as their performance deviated significantly from all other participants. Other reasons for exclusion were failure to partake in any of the nine study visits or not being able to attend on the day the study visit was scheduled as per the study protocol. Participants were instructed to perform the PASAT during each session. Before and after each PASAT session, participants rated the 20 items provided in the *positive and negative affect schedule* (PANAS) (Watson et al., 1988). Before and after the PASAT training phase, the *Eriksen flanker task* (Eriksen & Eriksen, 1974) was applied subsequently to the PASAT as a transfer task. An overview of the study visits is shown in Table 3.1.1.

Table 3.1.1: Overview of the study visits which constitute the parameter testing study.

	Session	Day	PASAT[a]	tDCS[b]	PANAS[c]	Flanker[d]
pre-training	1	1	x		x	x
training	2-7	4-15	x	x	x	
post-training	8	18	x		x	x
follow-up	9	102	x		x	x

Notes: [a]paced auditory serial addition task, [b]positive and negative affect schedule, [c]Eriksen flanker task

The PASAT settings in this study were as follows. The task was comprised of three blocks (5 min) divided by short breaks (30 s). With an initial *interstimulus interval* (ISI) of 3 s, random single digits (1-9) were presented via headphones during each block. To increase mental load for healthy participants and prevent ceiling effects, the PASAT was adjusted and modified from versions that are commonly used in research: for the first two studies (Section 3.1, Section 3.2), participants were asked to add the last digit to the digit that preceded it by two (n^{th} + n^{th-2}) instead of performing the more common addition of the last two digits (n^{th} + n^{th-1}), which was used in the third study that enrolled depressed patients (Section 3.3). The ISI was decreased by 0.1 s when four consecutively right answers were given. If four consecutively wrong answers were given, the ISI was increased by 0.1 s. The number of correct answers per session was used as a final score, as each training session was not limited by number of trials (calculations) but time to complete the task (15 min in total, excluding breaks). The ISI would be carried over from one block to the next block but would be reset for each new training session. With each

following number presentation, participants would get feedback on their last given answer. Feedback was presented in the form of a red or green flash of the screen (Figure 3.1.1). This resulted in a deliberately distracting delay in feedback. The PASAT was administered through a desktop computer and answers were given on a specifically prepared keyboard.

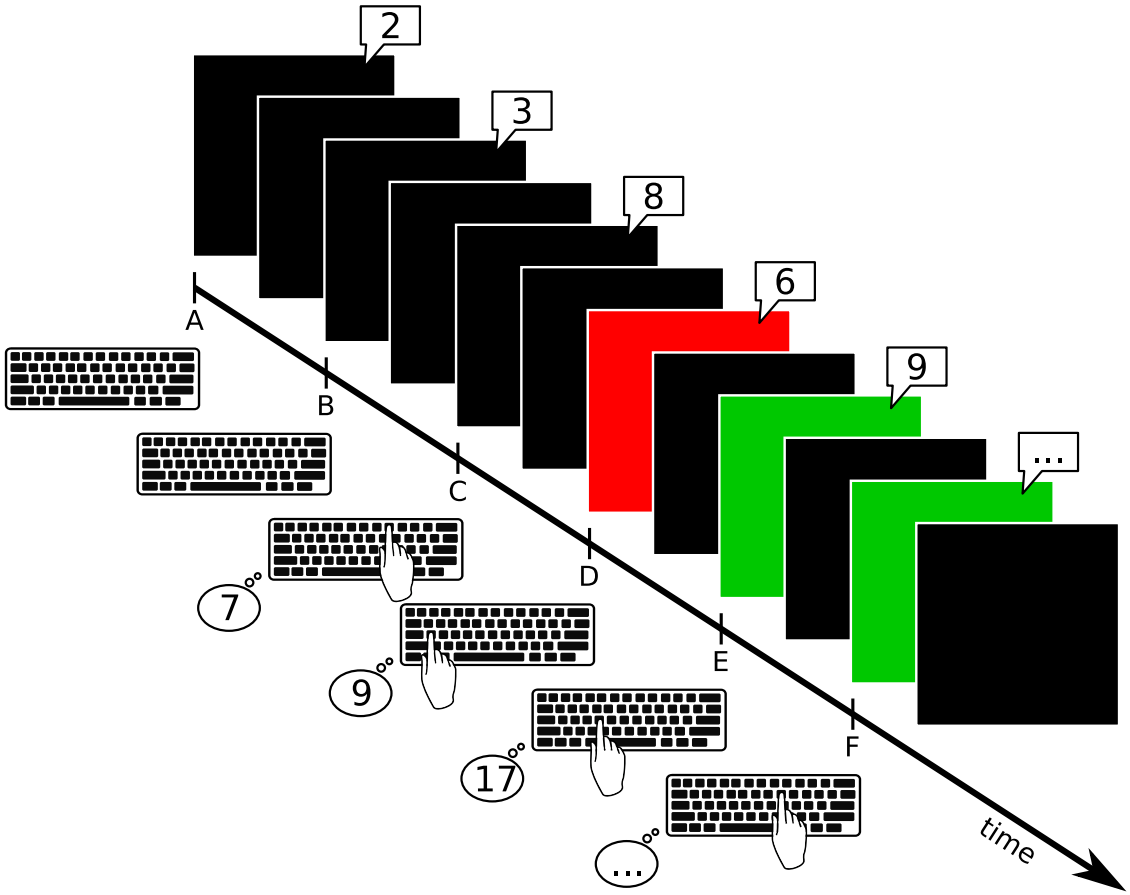

Figure 3.1.1: Overview of the PASAT used in this study. Participants were presented single digit numbers over headphones and were asked to add the digit that was presented last to the digit that was presented two before the last ($n^{th} + n^{th\text{-}2}$); e. g. A + C, B + D, C + E. Feedback was given in each following trial. Answers were to be given on a specially prepared keyboard. The initial ISI between each digit presentation was 3 s and would be adjusted after four consecutively right or wrong trials (reduction or increase of the ISI by 0.1 s respectively). The PASAT was divided into three blocks, each lasting 5 min with a 30 s pause between the blocks. *Note.* From 'Enhancing Cognitive Control Training with Transcranial Direct Current Stimulation' by Weller et al. (2020a). CC BY-NC-ND.

The final score of the PASAT session was used as a performance marker, as better performing participants were able to give more correct answers during a session. Due to the adaptive nature of the task, the PASAT cannot be solved perfectly and thus all participants were forced to make mistakes during each session, increasing

stress and frustration. During the six sessions that included tDCS (*training phase*), each participant received the intervention according to the study group they were assigned to. Stimulation was applied concurrently to the PASAT and was modified between participants by: *polarity* (anodal or cathodal), *intensity* (1 mA or 2 mA), and *laterality* (left or right dlPFC). This resulted in nine possible parameter combinations that were evenly distributed throughout the study sample (Figure 3.1.2).

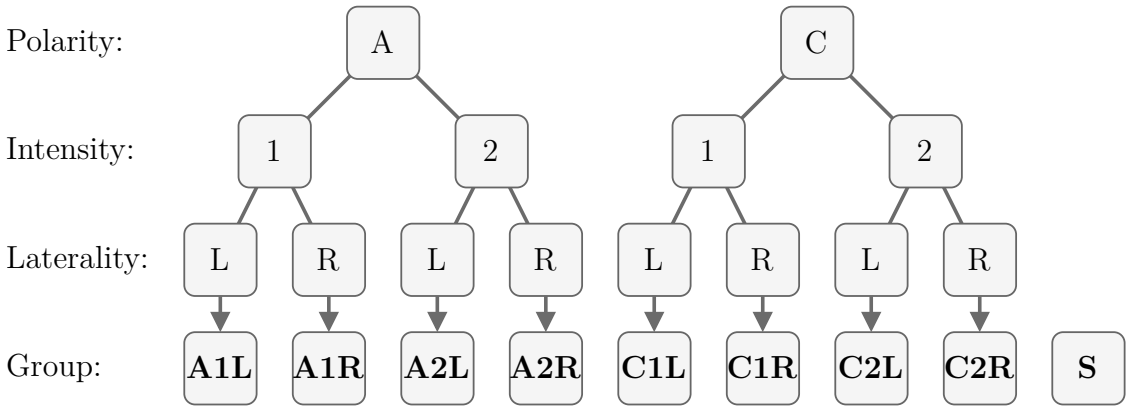

Figure 3.1.2: Overview of the possible parameter combinations resulting in eight active groups and one additional sham group. Number of participants for the respective level is reported in parentheses. A = anodal ($n = 60$); C = cathodal ($n = 59$); 1 = 1 mA ($n = 30$); 2 = 2 mA ($n = 30$); L = left dlPFC ($n = 15$); R = right dlPFC ($n = 15$); S = sham ($n = 43$). One participant in the C2R group had to be excluded from analyses as their performance deviated more than two standard deviations from all other participants.

3.1.2 Results

The tDCS conditions were analysed hierarchically, starting by grouping participants by stimulation polarity, then intensity (as a subgroup of polarity), and lastly laterality (as a subgroup of intensity). In case a significant effect was found in a superordinate group, the subordinate attribute would then be analysed.

These planned comparisons revealed that, compared to sham stimulation, anodal tDCS caused an increase in task performance ($p = 0.0235$). The study group that received cathodal tDCS did not exhibit any significant performance deviations from the sham group (Figure 3.1.3).

Splitting the anodal group further by intensity, it became evident that 1 mA was superior to sham stimulation ($p = 0.0069$), while the 2 mA condition did not cause diverging performance (Figure 3.1.4).

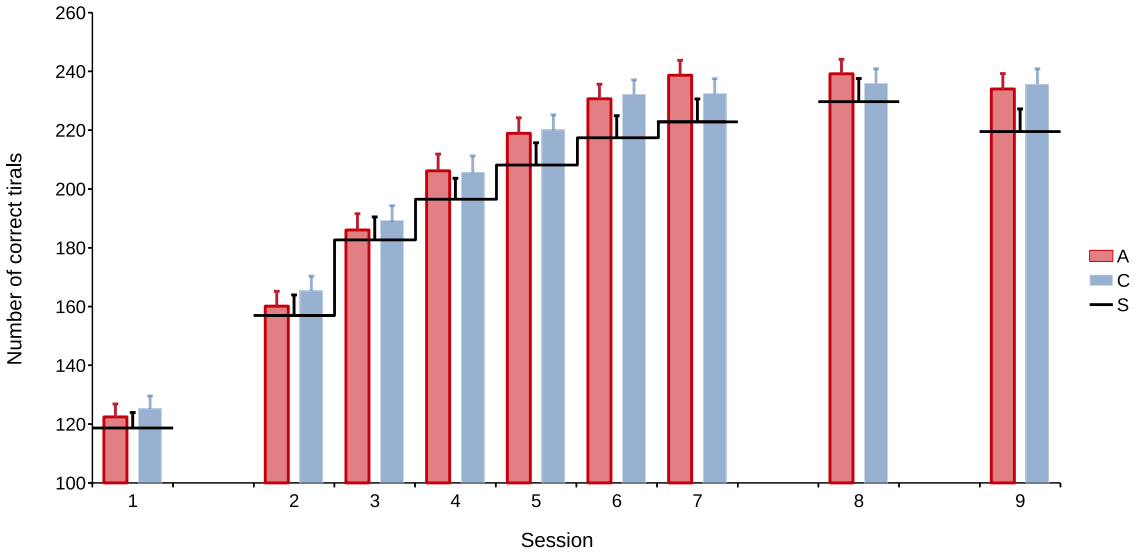

Figure 3.1.3: Effects of tDCS polarity. While all participants increased their performance over the course of the study, only the group that received anodal tDCS (A) showed a significant performance increase over sham stimulation (S). Cathodal tDCS (C) did not yield significant performance increases over sham tDCS. $N = 162$. *Note.* From 'Enhancing Cognitive Control Training with Transcranial Direct Current Stimulation' by Weller et al. (2020a). CC BY-NC-ND.

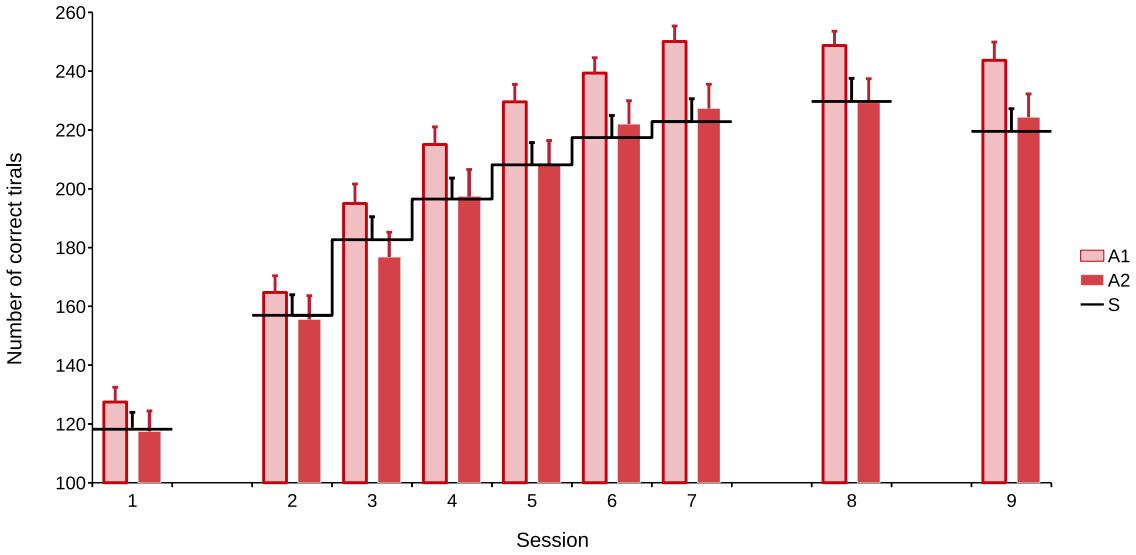

Figure 3.1.4: Effects of tDCS intensity. Here, the hierarchical analysis showed that anodal tDCS with an intensity of 1 maA (A1) was superior over sham stimulation (S), whereas the participants who received anodal tDCS with an intensity of 2 mA (A2) did not significantly improve their performance over sham stimulation. $N = 103$. *Note.* From 'Enhancing Cognitive Control Training with Transcranial Direct Current Stimulation' by Weller et al. (2020a). CC BY-NC-ND.

As a last step, the anodal/1 mA group was further split by laterality, revealing that only stimulation of the left dlPFC with this specific parameter combination resulted in increased task performance over sham stimulation ($p = 0.0117$; Figure 3.1.5).

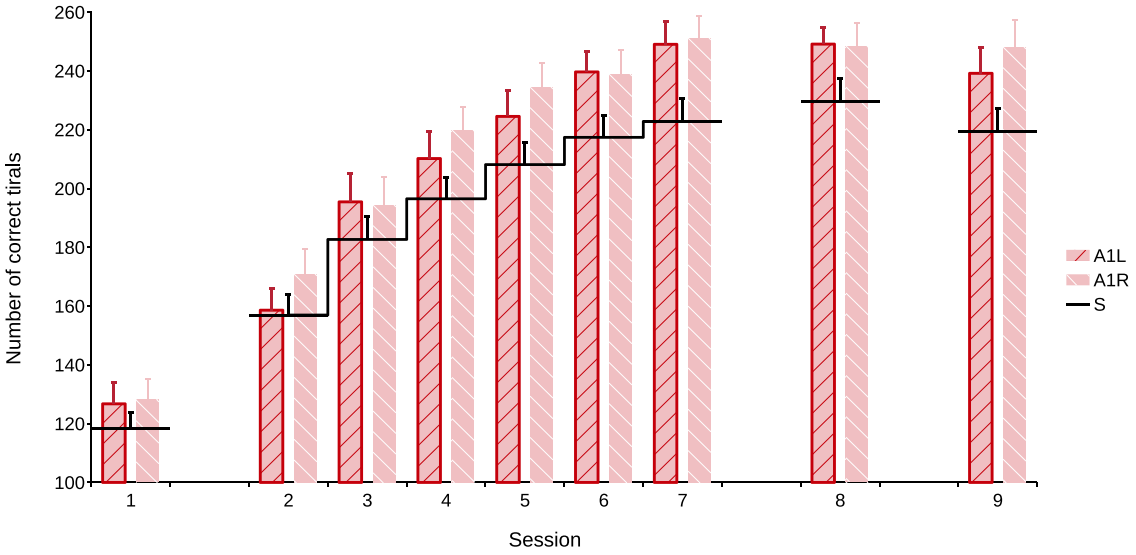

Figure 3.1.5: Effects of tDCS laterality. In this comparison, significant performance increases compared to sham stimulation (S) were only present when tDCS was applied to the left dlPFC (A1L). Stimulation over the right dlPFC (A1R) did not significantly differ from sham stimulation. $N = 73$. *Note.* From 'Enhancing Cognitive Control Training with Transcranial Direct Current Stimulation' by Weller et al. (2020a). CC BY-NC-ND.

Additionally to the hierarchical analysis, all eight groups at the most fine-grained level were also compared to sham condition within a single statistical model ($N = 162$). This confirmed the initial results showing that only anodal stimulation with an intensity of 1 mA over the left dlPFC increased CCT performance significantly ($p = 0.0111$).

Within each session, an increase in *negative affect* (NA), as assessed by the PANAS, from before to after completion of the PASAT was measurable ($p < 0.001$). Over the course of the training, the magnitude of this observation decreased. *Positive affect* (PA) decreased over the training period ($p < 0.001$). No influence of stimulation was found on either NA or PA.

While significant effects of time were found in the Eriksen flanker task, indicating that participants improved in this task over time, no tDCS-specific transfer effects became evident.

3.2 The Effects of Biological Sex on Transcranial Direct Current Stimulation

The findings presented in this study (Weller et al. (2023a), Appendix A.1.4; Weller et al. (2023b), Appendix A.1.5 for supplementary materials) resulted from a sex-specific analysis of the data gathered in the parameter testing study, this time to answer the question whether biological sex showed an influence on stimulation outcome.

3.2.1 Methods

The setup for this study is identical to the setup presented in Section 3.1. Therefore, as before, the study sample consisted of healthy participants that took part in a two-week CCT. The PASAT (Figure 3.1.1) was administered during each session (Table 3.1.1). Data from 162 participants were analysed (127 women, mean age = 22.73 ±3.67 years; 35 men, mean age = 24.89 ±4.64 years). All participants identified as either male or female according to their assigned sex at birth.

3.2.2 Results

For this investigation, the participants were first split into groups according to their self-reported birth sex and regardless of stimulation condition. This revealed higher performance gains in women compared to men ($p = 0.0038$, Figure 3.2.1).

In a next step, the groups were split by tDCS polarity to allow the comparison of performance gains for each stimulation condition. Here, anodal tDCS proved to improve women's performance significantly to men's ($p = 0.0070$, Figure 3.2.2). No such differences between the sexes were found for cathodal tDCS (Figure 3.2.3) and sham stimulation (Figure 3.2.4).

When comparing stimulation conditions within each sex, effects were found within the women's group where only anodal tDCS proved to be superior over sham stimulation ($p = 0.0354$, Figure 3.2.5). In men, neither anodal nor cathodal tDCS proved to be superior over sham stimulation (Figure 3.2.6).

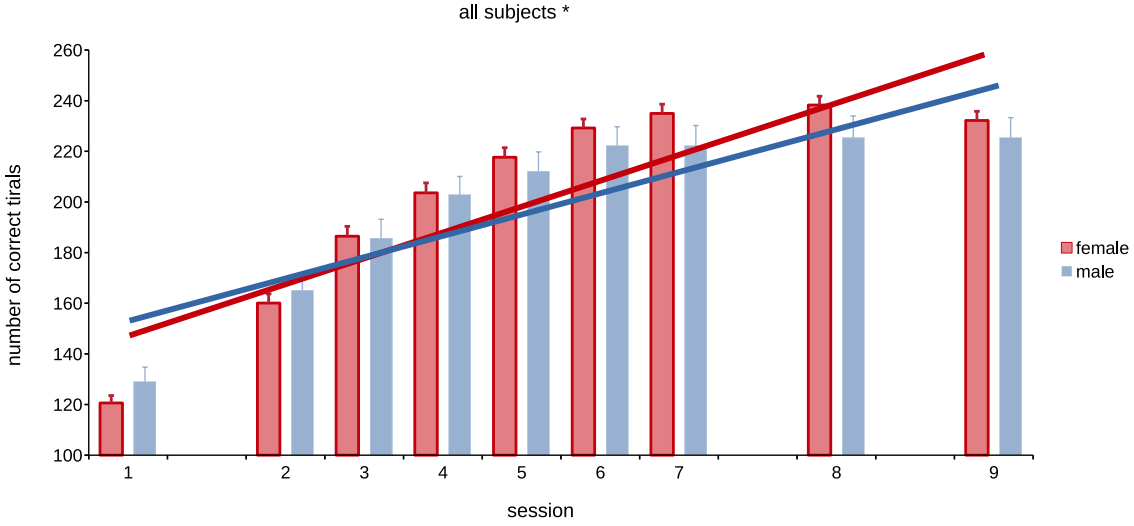

Figure 3.2.1: Grouping all participants by sex, regardless of their received stimulation condition, revealed a significant performance increase within the women's group but not men's (steeper trend line for women). $N = 162$. *Note.* From 'Sex Matters for the Enhancement of Cognitive Training with Transcranial Direct Current Stimulation (tDCS)' by Weller et al. (2023a). CC BY.

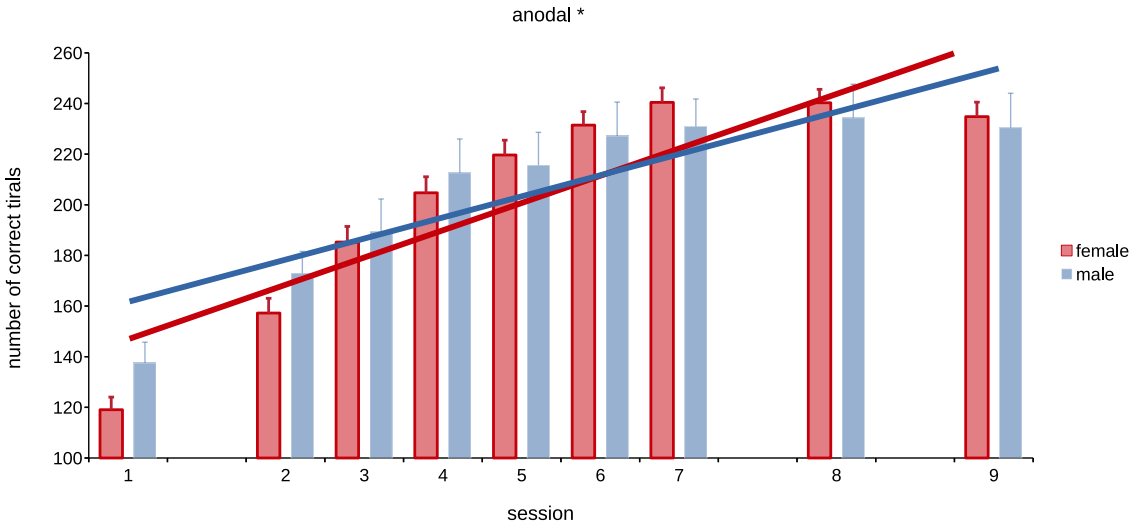

Figure 3.2.2: Split up by stimulation polarity, it became apparent that women's performance increased significantly compared to men's when anodal tDCS was applied concurrently to the CCT. $N = 60$. *Note.* From 'Sex Matters for the Enhancement of Cognitive Training with Transcranial Direct Current Stimulation (tDCS)' by Weller et al. (2023a). CC BY.

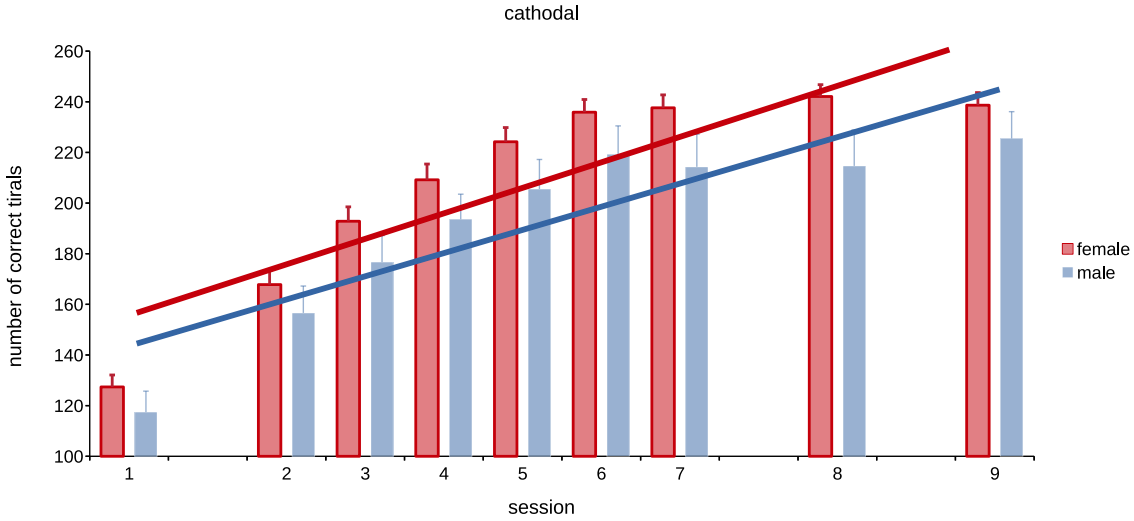

Figure 3.2.3: Split up by stimulation polarity, no significant performance deviations were found between men and women when cathodal tDCS was applied. $N = 59$. *Note.* From 'Sex Matters for the Enhancement of Cognitive Training with Transcranial Direct Current Stimulation (tDCS)' by Weller et al. (2023a). CC BY.

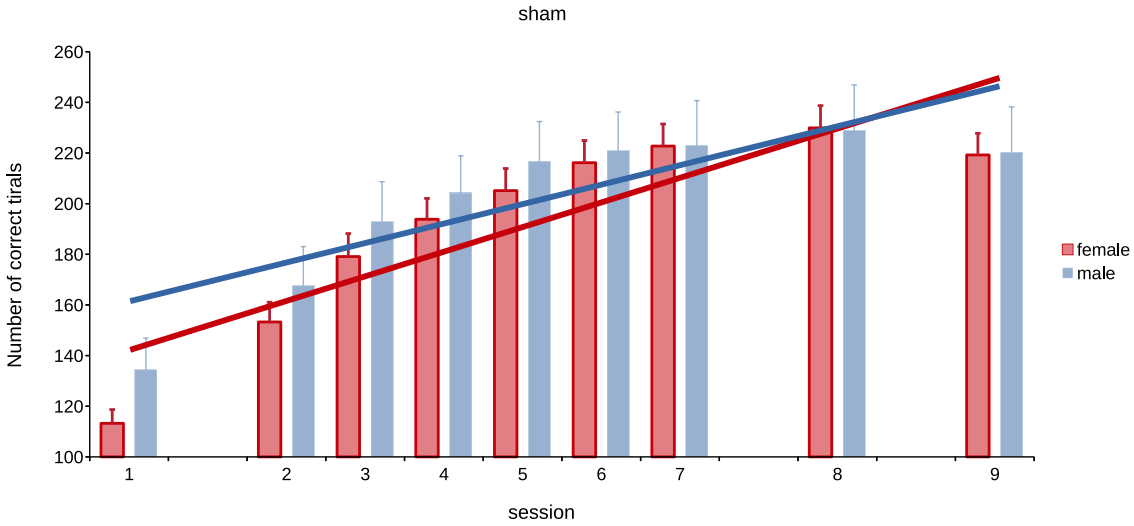

Figure 3.2.4: Men and women who received sham tDCS performed similar to each other over the course of the training and no significance performance differences were found. $N = 43$. *Note.* From 'Sex Matters for the Enhancement of Cognitive Training with Transcranial Direct Current Stimulation (tDCS)' by Weller et al. (2023a). CC BY.

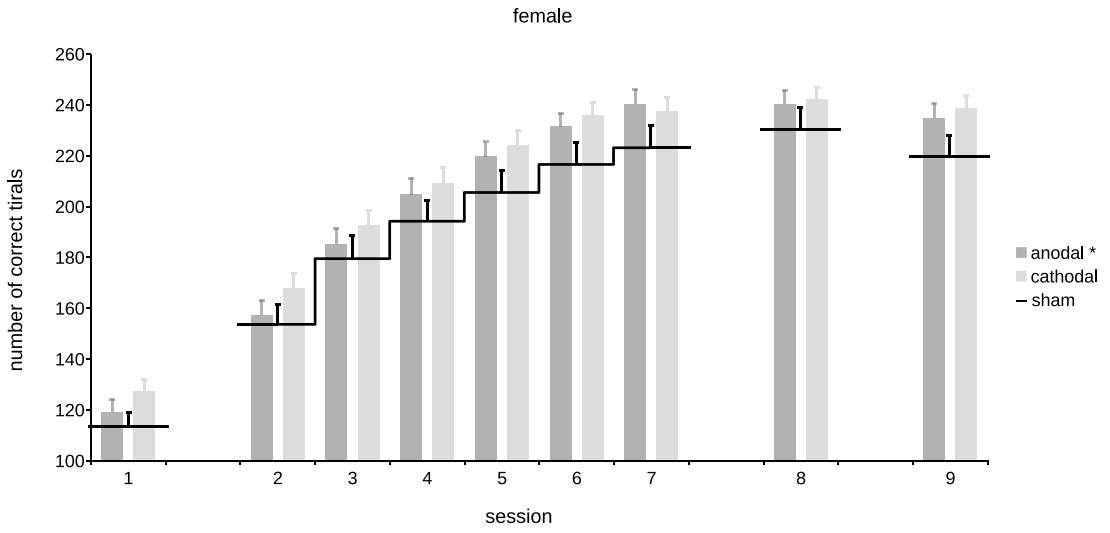

Figure 3.2.5: In women, anodal tDCS caused significant performance increases over sham tDCS. No such effects were found for cathodal stimulation. $N = 127$. *Note.* From 'Sex Matters for the Enhancement of Cognitive Training with Transcranial Direct Current Stimulation (tDCS)' by Weller et al. (2023a). CC BY.

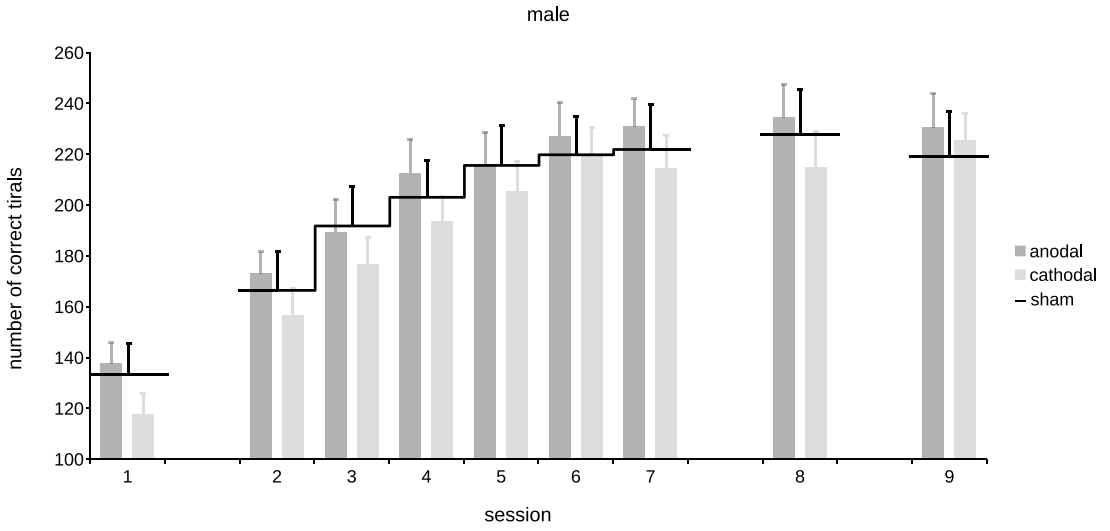

Figure 3.2.6: In men, neither anodal nor cathodal tDCS elicited superior effects over sham and all three groups improved at a similar rate. $N = 35$. *Note.* From 'Sex Matters for the Enhancement of Cognitive Training with Transcranial Direct Current Stimulation (tDCS)' by Weller et al. (2023a). CC BY.

3.3 Enhancing Cognitive Control Training through Gamification

In this study, possible antidepressant effects as well as the influence of gamification and psychoeducational elements added to a CCT in form of the PASAT were evaluated through patients with diagnosed MDD (Weller et al. (2022), Appendix A.1.3).

3.3.1 Methods

In total, 32 adult patients (19 women, 13 men) diagnosed with acute or chronic recurrent MDD were enrolled in the study. Originally, 55 applicants were screened, however 23 did not meet the inclusion criteria due to mostly *Montgomery-Åsberg depression rating scale* (MADRS) scores being either too high or low. Patients were aged between 18-76 (mean age in the *control group* (CG) = 30.00 ±13.33 years; mean age in the *intervention group* (IG) = 40.19 ±16.63 years). For these patients, the PASAT (Figure 3.3.1) was extensively overhauled, redesigned, and ported to an Android app. The CCT was distributed to patients on tablet computers. Patients were free to use the CCT at their own discretion for the duration of six weeks.

In total, patients attended five to six sessions during which psychological interviews in regards to depressive symptomatology were conducted. Additionally, questionnaires on well-being and user feedback were handed out. From study inclusion to the last follow-up, each patient was monitored for 16-18 weeks Table 3.3.1.

Table 3.3.1: Overview of the study visits in the CCT study.

	Visit	Day	CCT	Interviews/Questionnaires[a]
pre-baseline	0[b]	-14		x
baseline	1	1	x	x
training	2	14	x	x
training	3	42	x	x
follow-up 1	4	70		x
follow-up 2	5	126		x

Notes: [a]The following interviews and questionnaires were conducted: Montgomery-Åsberg depression rating scale, *Inventory of depressive symptomatology (self-report version)*, *WHO-Five-Well-Being Index*, usability and user feedback. [b]Only half of the participants were asked to attend visit 0.

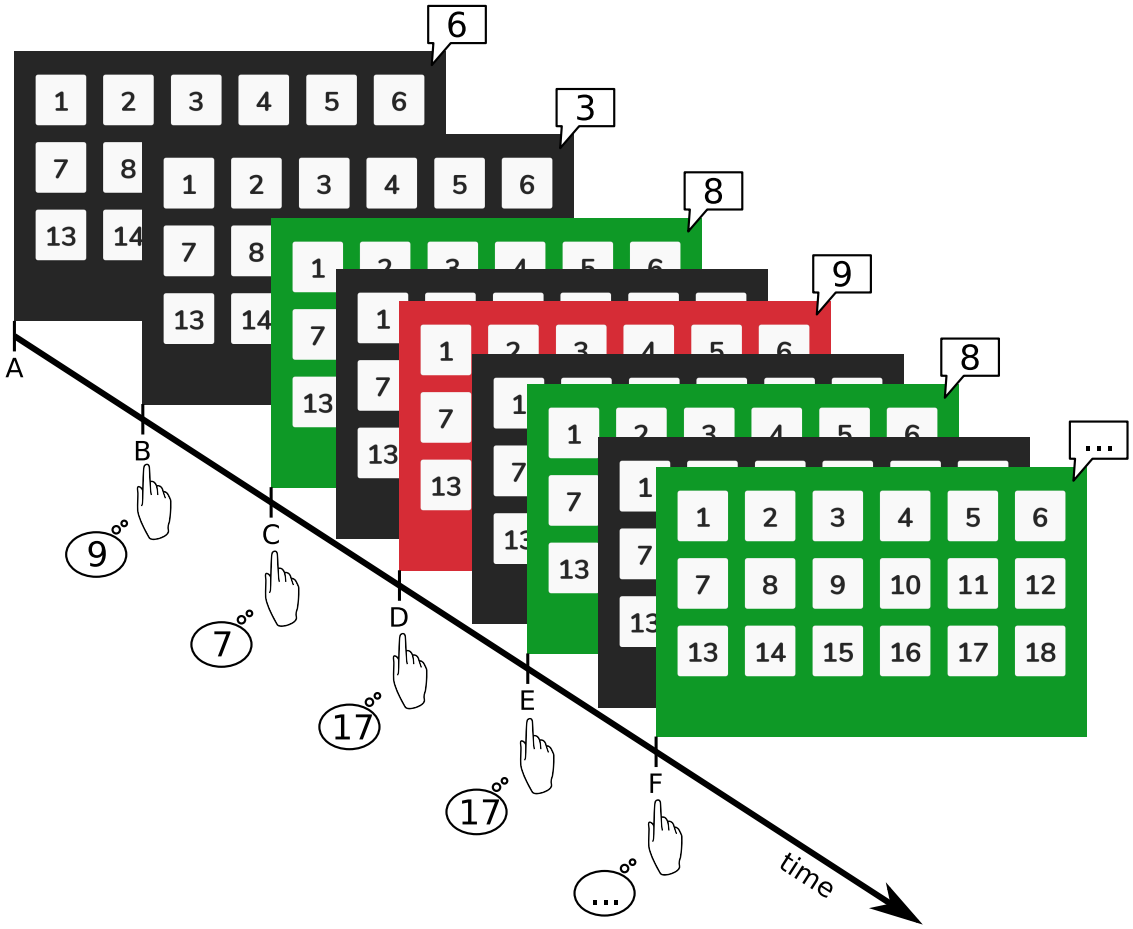

Figure 3.3.1: Overview of the PASAT used in the CCT study. Methodically, the PASAT used in this study was similar to the version described in Section 3.1. Differences lay within the following two major domains of the task: patients were instructed to add the digit that was presented last to the digit that was presented directly before it $n^{th} + n^{th\text{-}1}$; e. g. A + B, B + C, C + D. Also, in case of the IG, the gamification elements depicted in Table 3.3.2 were added. *Note.* From 'Gamification Improves Antidepressant Effects of Cognitive Control Training—A Pilot Trial' by Weller et al. (2022). CC BY.

All patients received the CCT at baseline, however half of them were scheduled for an additional study visit two weeks before this appointment (*pre-baseline*), to evaluate how symptoms might change prior to any influence of the intervention (assessed via the MADRS, *Inventory of depressive symptomatology (self-report version)* (IDS-SR), *WHO-Five-Well-Being Index* (WHO5)). After this, half of the group received a minimalistic CCT version that included only the training and corresponding instructions (active CG), while the other half of the study group were given a version of the PASAT that was enriched with additional information about the training as well as gamification elements to enhance engagement (IG; Table 3.3.2).

Table 3.3.2: Abridged description on the gamification elements used to enhance the PASAT. For an extensive overview please refer to Appendix A.1.3.

Component	CG	IG
Setting	PASAT instructions	Narrative that surrounds PASAT in a setting
Meaning and purpose	No additional info	Elaborated theme surrounding the PASAT
Progression	No feedback on progression	Animated graphs that show training performance
Levelling	None	Unlockable difficulty levels
Immediate feedback	Red/green flash after each trial	Red/green flash after each trial
Long-term feedback	None	Animated graphs, achievements, avatar interactions
Achievements/Rewards	None	Unlockable psychoeducational information
Avatar	None	Animated training companion

3.3.2 Results

As a primary endpoint changes in MADRS scores from start of training to first follow-up were used. Secondary endpoints were scores in the IDS-SR and WHO5. Analyses showed that whereas both, the CG as well as the IG, showed a decrease in depressive symptomatology over the course of the study, only the IG exhibited significantly alleviated symptoms up until follow-up 1 when compared to the CG

when the MADRS was assessed ($p = 0.019$). While the scores remained lowered even at follow-up 2, the significant differences between the two groups did not persist until this time point (Figure 3.3.2).

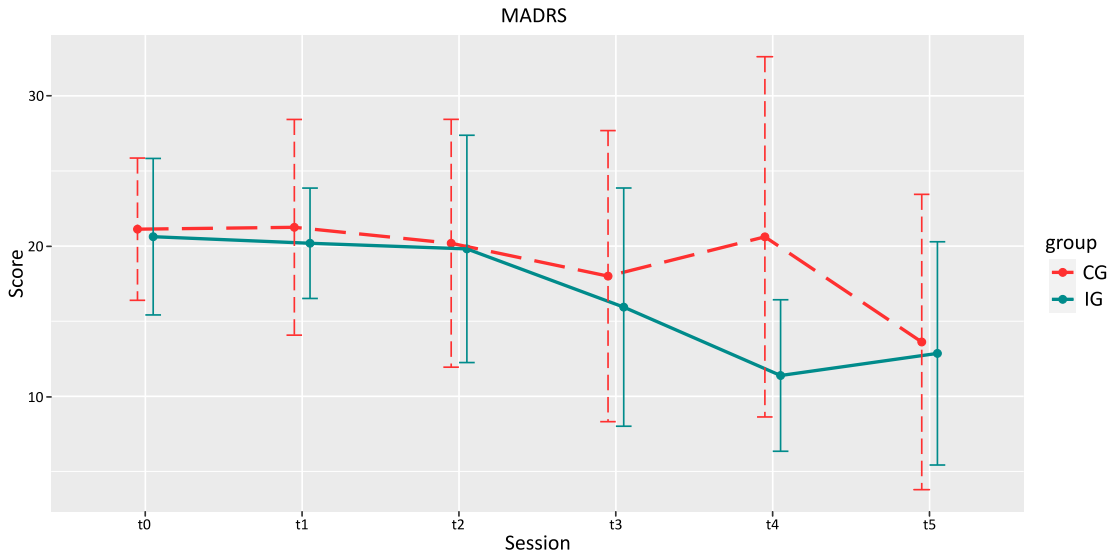

Figure 3.3.2: Development of Montgomery-Åsberg depression rating scale scores over the course of the study, shown are means for each session. The six-week long training session took place between time point t1 and t3. While both groups showed significantly decreased scores, indicating an improvement of depressive symptoms, the lasting effect until one month after training (t4, primary endpoint) was only visible in the IG. $N = 32$. *Note.* From 'Gamification Improves Antidepressant Effects of Cognitive Control Training—A Pilot Trial' by Weller et al. (2022). CC BY.

In case of the IDS-SR, both groups decreased greatly in depressive symptoms ($p = 0.051$) up until follow-up 1. However, there were no differences in reduction rate between the two groups (Figure 3.3.3). No significant increase in overall well-being as assessed by the WHO5 was reported (Figure 3.3.4) until follow-up 1.

At follow-up 2, scores of all scales were significantly reduced compared to baseline session, indicating that depression severity had successfully been decreased (MADRS: $p < 0.001$; IDS-SR: $p < 0.001$) whereas overall well-being increased significantly (WHO5: $p < 0.001$).

Patients' feedback on the training and usability of the app were positive, no adverse effects were reported.

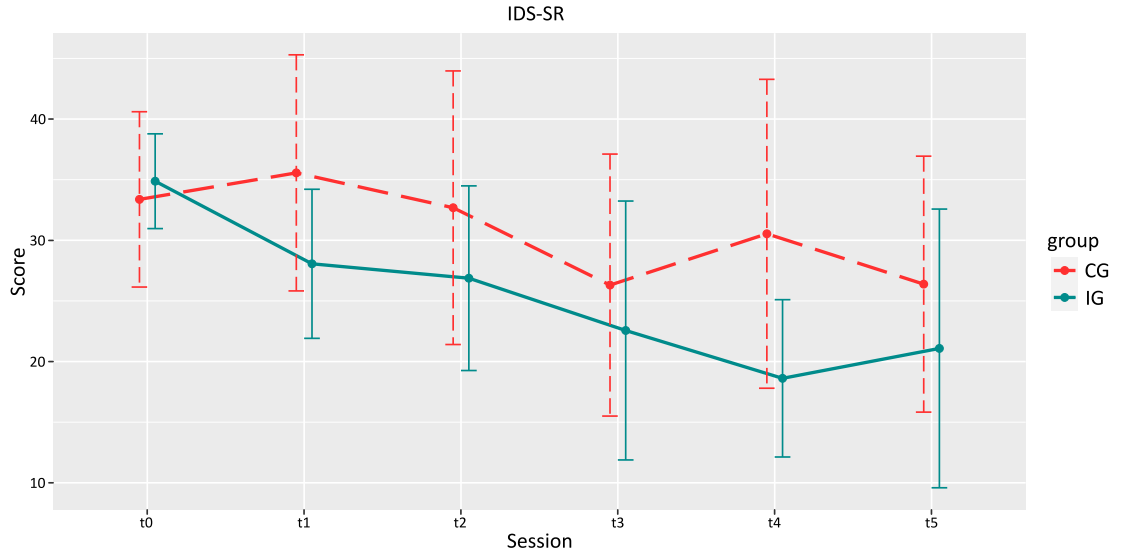

Figure 3.3.3: Development of Inventory of depressive symptomatology (self-report version) scores over the course of the study, shown are means for each session. The six-week long training session took place between time point t1 and t3. Both groups showed a significant decrease in depressive symptoms, however no difference between the CG and the IG was evident. $N = 32$. *Note.* From 'Gamification Improves Antidepressant Effects of Cognitive Control Training—A Pilot Trial' by Weller et al. (2022). CC BY.

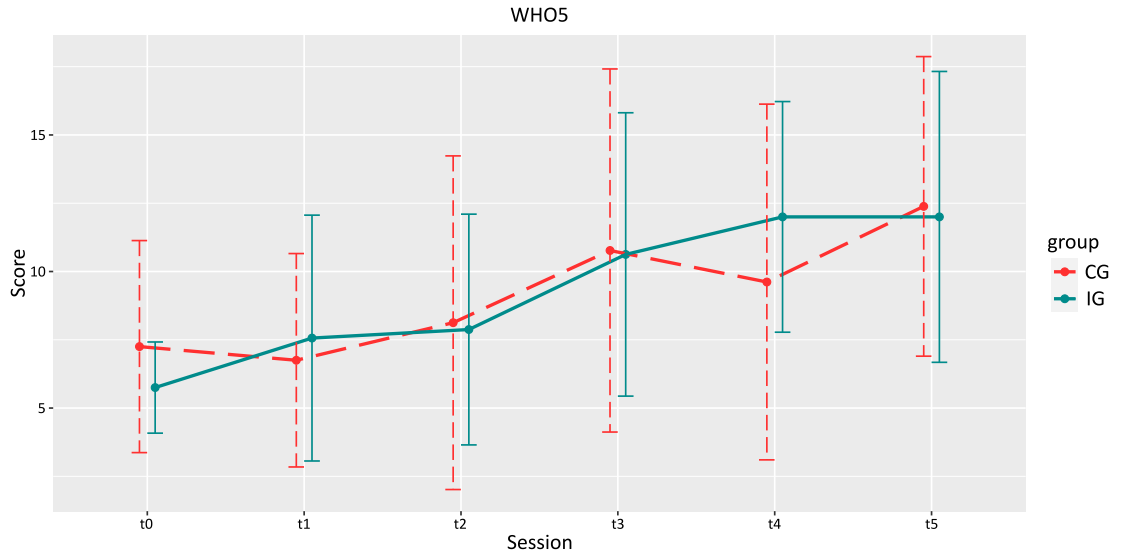

Figure 3.3.4: Development of WHO-Five-Well-Being Index scores over the course of the study, shown are means for each session. The six-week long training session took place between time point t1 and t3. Both groups reported increased well-being, however these increases were not statistically different between the groups. $N = 32$. *Note.* From 'Gamification Improves Antidepressant Effects of Cognitive Control Training—A Pilot Trial' by Weller et al. (2022). CC BY.

4 Discussion

In the investigations presented in my dissertation, the effectiveness and utilisation of two neuroenhancement approaches were examined. Both approaches focus on improving CC, a fundamental aspect of human cognition that plays a crucial role in regulating thoughts, emotions, and actions. CC encompasses attentional control, cognitive inhibition, inhibitory control, working memory, and cognitive flexibility. As these processes allow for the maintenance of focused attention on relevant information, the suppression of automatic responses, the control of impulsive behaviours, and the manipulation of information, they play a pivotal role in flexible coordination of sensory, emotional, and motor processes and are therefore ultimately vital for effective and goal-oriented actions in humans. Deficits in CC have been linked to reduced cognitive performance and various cognitive and emotional disorders. Therefore, my work focused on two subject groups that allow for targeted research on how CC can be improved: first, healthy participants without any diagnosed psychiatric disorders where CC was assumed to be properly functional. This allowed allowed for the systematic analysis of several major components in tDCS, a form of NIBS that shows to have enhancing effects on cognitive processes. The second group was composed of patients with MDD, where CC was assumed to be inhibited due to the illness. Including patients with MDD made it possible to determine if and how a CCT, that in itself can aid CC processes, can be improved further and in a purposeful manner to amplify its antidepressant effects.

Both methods, tDCS and CCT, can be used independently from each other but also in combined training-stimulation paradigms, which make them promising tools for future use in clinical settings. However, this flexibility comes with a price. CCT and tDCS can be modified in many ways and the person who administers the intervention is confronted with taking into consideration many aspects surrounding tES and CCT application, such as:

- shape/size/number of electrode(s),
- cortical area to choose for electrode montage,
- stimulation polarity,
- current intensity,
- appropriate choice of task or CCT,
- duration of stimulations per session/task duration,
- number and frequency of stimulation sessions/number and frequency of task applications,
- online or offline stimulation, i.e. task application concurrently or before/after stimulation,
- brain state during stimulation/task performance
- morphological traits of the recipient (e.g. head size, skull thickness),
- biological sex, hormonal states and their changes,
- concurrent intake of medication,
- underlying disorders and/or lesions,
- other factors that alter neuronal responses…

This non-exhaustive selection of topics illustrates the complexity that surrounds neuromodulation. When looking at targeted and personalised interventions, the interplay between CCT, tDCS, and their users becomes even more challenging, resulting in an inevitable diversity of possible choices. Even to date, literature on this topic still remains fairly divided (Medina & Cason, 2017; Moffa et al., 2020; Razza et al., 2020; Zhang et al., 2021) and systematic approaches that compare various interventions by modifying single aspects of the treatment are needed (Koo et al., 2023). My thesis aims to provide insight into some of these topics and unravel some of the uncertainty that still surrounds neuroenhancement.

4.1 Observed Benefits of Neuroenhancement

Among other neuromodulatory interventions, tDCS and CCT stand out. They are comparatively cheap to establish and, once a proper protocol is found, mobile and easy to administer. Both have a low possibility of inducing adverse effects and no gradual increase when starting or gradual decrease when discontinuing is necessary. This causes the barrier of entry for these methods to be low, especially when compared to other therapeutic tools such as psychotherapy or medication. As both interventions can target similar cortical structures, they can also be combined to increase their respective enhancing traits (Gill et al., 2015; Martin et al., 2013). Their complementing working mechanisms make versatile use cases and personalised approaches possible (Brunoni, Boggio et al., 2014; Martin et al., 2014; Segrave et al., 2014).

4.1.1 On Transcranial Direct Current Stimulation

While the hypothesis that anodal tDCS would prove beneficial over cathodal or sham tDCS was met in the parameter testing study, there is also research that indicates similar effects for both polarities (Brückner & Kammer, 2017) or superiority of cathodal over anodal tDCS (Pope & Miall, 2012). TDCS may also fail to produce effects altogether (Horvath et al., 2015a, 2015b; Jacobson et al., 2012). Possible reasons for this are multifarious. One of the critical factors that may contribute to the failure of tDCS to elicit effects is the variability in individual brain anatomy and physiology (Jamil & Nitsche, 2017). Furthermore, the effects of tDCS are highly dependent on the specific brain networks and structures that are targeted by the stimulation. These can vary significantly between individuals, making it challenging to achieve consistent results across different participants or patients. There are examples in literature where tDCS failed to manipulate motor behaviour in reaction time tasks, even though similar research came to different conclusions (Turkakin et al., 2018). Another such example are electrophysiological effects of tDCS over the motor cortex, which can change the magnitude of *motor evoked potentials* recorded from muscles. Even though this approach reduces other external factors such as specific tasks that influence brain state, there are cases in which the stimulation fails to elicit the expected effects. The influence of the multiple

intrinsic and extrinsic factors that interact with the stimulation become apparent. This includes the state of the individual's nervous system at the time of stimulation, the specific parameters of the tDCS protocol, and electrode setup (Wiltshire & Watkins, 2020). Nevertheless, the findings of the parameter study (Section 3.1) go in line with various previous studies that succeeded in showing how cognitive abilities throughout various domains can be supported by tDCS (Chase et al., 2019; Figeys et al., 2021; Hanley et al., 2020; Vall et al., 2021) and buttress the importance of structured research further. Especially big enough sample sizes need to be chosen to minimise variability and noise in data.

With the results presented here, further approaches can modify the intervention in more fine-grained steps to adapt the stimulation to its user base. This is why, based on the data gathered in this first study, a follow-up study was conducted in order to transfer the findings from healthy participants to a patient group diagnosed with MDD (Sommer & Plewnia, 2021). Here, two intensities (1 mA and 2 mA) of anodal tDCS were applied to the left dlPFC while patients performed the PASAT. While this study showed no effect of stimulation on task performance, a substantial improvement in depressive symptoms was uncovered, leading to the assumption that the CCT effects alone were responsible for the betterment. This is yet more evidence for the need for individualised neuroenhancement, rather than evidence for the inefficacy of tDCS. And while these results, for one, leave open the question why this *specific* parameter combination did not improve the depressed patients' performance, it strengthens another assumption: that CCT can have a positive impact on cognitive functions and depressive symptoms.

4.1.2 On Cognitive Control Training

The PASAT, as a well-established neuropsychological instrument, has demonstrated to be a viable choice as a CCT. It requires focused attention (Tombaugh, 2006) and the adequate processing of PA and inhibition of NA (Feldner et al., 2006; Iordan et al., 2013; Sommer et al., 2021). Success in the task is closely related to the inhibition of frustration and the ability to direct (and redirect) attention towards the task - even in situations where frustration about one's own performance might take over. It can thus be used to measure cognitive performance and is sensitive to cognitive deficits, while at the same time serving as a training that

boosts cognitive abilities if applied regularly (Siegle et al., 2014). It can reliably assess cognitive functions while providing a standardised method that was easily adaptable in case of the participant and patient groups presented in this dissertation. A significant advantage of utilising the PASAT is its inherent flexibility, permitting various modifications in the task while preserving the fundamental structure. An example from this work is the adaptation of difficulty for participants and patients: a simple change from *1-back* PASAT to *2-back* PASAT (i.e. the addition of the $n^{th} + n^{th\text{-}1}$ digit or $n^{th} + n^{th\text{-}2}$ digit respectively) allowed the task to be used by two different study groups. It can be assumed that the *1-back* PASAT would have been to easy for healthy participants, while the *2-back* PASAT would have likely been too hard and frustrating for depressed patients. As research has shown, the PASAT can reduce susceptibility to distractions by strengthening CC. As CC is not only about the control of thoughts and actions, but equally about controlling emotion, being confronted with one's own 'failure' in the task poses a great challenge to CC - one more reason why task difficulty was adjusted for the study groups.

Engaging in the task on a regular basis could cause habituation - 'ignoring the familiar, predictable, and inconsequential' (McDiarmid et al., 2017) which in turn then promotes beneficial behavioural and neurophysiological changes. Indeed, the participants of the parameter testing study reported an increase of NA within each session, likely due to the frustrating nature of the task), but the amount of reported NA decreased over the duration of the training, corroborating the aforementioned notion. With a patient sample in mind, getting used to failure and stress can prove beneficial by building resilience towards these sensations. Coupled with the activation of the appropriate networks related to the dlPFC, the assumption can be made the antidepressant effects of the training stem from both: behavioural and neuronal changes. The added gamification elements enhanced these outcomes both in magnitude and duration. Another huge advantage of the PASAT in the CCT study over the stationary version used in the parameter testing study was its increased usability. Patients were able to take the mobile device home with them and use it whenever their everyday permitted. The personal profile and avatar increased engagement with the task and gave patients an idea of their own progression, which was often perceived as a positive aspect of the training. Even performing the task itself was seen as an accomplishment, a form of self-efficacy that gave patients a sense of their own capabilities. In the end, even though the PASAT is a reliable tool with legitimate use, the application in clinical contexts is still restricted (Tombaugh,

2006), which is why in my work the translation of the task geared at a patient group was added.

As an interesting side note, while the Covid-19 pandemic did put constraints on people and the realisation of research (see Section 4.3), it also underlined the advantages and need of a digitised and mobile CCTs for location- and time-independent use. This allowed patients to engage in a new form of training at their own discretion - a circumstance that came with its own benefits and challenges. Patients were required to adhere to the training schedule on their own and without the guidance of research staff. The lack of external motivators proved difficult for some patients. This comes at no surprise, as MDD can massively impact motivation and energy (Fervaha et al., 2016; Otte et al., 2016; Treadway et al., 2012). Even more, cognitive deficits as a result of MDD can more specifically be related to impaired motivation during assessment or training (Scheurich et al., 2008). Apart from persistent sadness and low mood, people suffering from MDD often report a loss of interest, increase in irritability and intolerance, difficulties in concentrating on tasks, and feelings of worthlessness (Fried et al., 2016; Yorbik et al., 2004). The PASAT purposefully challenges all of these aspects. For a mobile training one should make sure that patients do not feel discouraged or alone while performing the CCT. Overcoming their own adverse feelings towards the task, or their own perceived inadequacy in performance, was a major hurdle to take for patients and participants alike. Encouragingly, most patients were able to overcome this hurdle despite lacking the commitment of regular and closely-monitored study visits throughout the training phase. This could be seen both in the overall positive feedback on the app and usage frequency, which was close to the recommended frequency. All in all, these findings do strengthen the notion that further systematic research is worthwhile.

4.2 The Necessity for and Role of the Individualisation of Neuromodulation

The necessity for individualised neuromodulation stems from the inherent variability in human neuroanatomy and neurophysiology. Each individual's brain exhibits unique structural and functional characteristics, leading to differential responses

to neuromodulatory interventions. By customising neuromodulation parameters to the individual, therapeutic efficacy can potentially be enhanced while adverse effects are kept at a minimum. Adapted to a specific group of users, the effects of neuromodulatory interventions might not only be bolstered, they might be made possible in the first place (Evans et al., 2023; Vergallito et al., 2022). Moreover, individualised neuromodulation allows for the incorporation of specific patient characteristics and clinical circumstances, thereby facilitating personalised care. This approach also provides the flexibility to modify treatment strategies in response to changes in a patient's condition over time. Many NIBS techniques already utilise some forms of individualisation. As an example, TMS is commonly applied after the motor threshold of the patient has been determined (Schwippel et al., 2019), and can even be coupled with *electroencephalography* signals (Chung et al., 2019). Neuronal activity depends on the type of task a person executes (Asaad et al., 2000), therefore making it all the more important to establish the proper brain state when applying any form of external stimulation (Bradley et al., 2022; Silvanto et al., 2008). While this task-dependency adds a layer of complexity to the search for proper intervention protocols, it also opens up the possibility to further enhance existing paradigms, where the combination of the right training increases NIBS effects or vice versa. As time goes on and more research is devoted to NIBS and CCTs, better task-stimulation combinations might arise. While there seems to be a general consensus on many aspects of tDCS (e.g. whether a certain polarity enhances or inhibits cognitive functions), the overall study landscape is far more ambiguous. As an example, cathodal tDCS has been found to modify executive functions during aerobic exercise while anodal tDCS failed to produce any effects (Thomas et al., 2021). Similarly, higher intensities do not necessarily surpass efficacy of lower stimulation intensities (Chew et al., 2015; Esmaeilpour et al., 2018). This illustrates once more how the results are not simply transferable between study populations, but require more intricate and systematic approaches.

For the longest time, studies were heavily biased towards men as other sexes were often simply excluded from participation (Beery & Zucker, 2011). In recent years, this has changed and the inclusion of all sexes in studies is now encouraged. Sexual traits present themselves in various ways, ranging from purely morphological up to psychological differences - all of which impact both tES, CCT, and other neuropsychological interventions (Bell et al., 2006; D. I. Miller & Halpern, 2014) during their application; sex-specific variability exists and needs to be properly

accounted for (Bhattacharjee et al., 2022; Rudroff et al., 2020). One step towards effective neuroenhancement will now be to acknowledge what exactly these diverging factors are and how to navigate them when applying any form of NIBS or CCT. The findings in the sex-specific analysis of the tDCS intervention (Section 3.2) buttress this need further, as a strong effect of biological sex on tDCS was apparent. It should be noted that the point of view from which this topic is addressed from is important. As an example: larger skull size, which influences electrical current flow and hence affects tDCS, can be viewed in two ways; it can be seen as a *general* trait that differs between women and men as groups. Alternatively, it can be considered an *individual* trait, as single individuals from one sex might show closer resemblance in this specific trait to individuals from the other group. This can be applied to any morphological but also cognitive trait or function. Especially in cognitive functioning these heterogeneities and task-specific sex differences gain importance (Gaillard et al., 2021) and only by addressing them, will effective modulation of cognition be viable.

Furthermore, several (epi-)genetic factors modulate and are modulated by tDCS (please see supplementary publication by Wiegand et al. (2021a), Appendix A.2.1). Three of the most focused on factors are: *brain-derived neurotrophic factor* (BDNF), *calcium voltage-gated channel subunit alpha1 C* (CACNA1C), and *catechol-O-methyltransferase* (COMT), as they are heavily involved in synaptic plasticity (Cocco et al., 2018; Paulus, 2011a). For this reason, another analysis will be done with most of the participants form the parameter testing study. Blood samples were taken at the end of the study to test for certain polymorphisms. First, BDNF, a neurotrophin that plays a crucial role in the growth, maintenance, and maturation of neurons (Hyman et al., 1991). It can be affected by antidepressants (Björkholm & Monteggia, 2016; Brunoni, Machado-Vieira et al., 2014) and tDCS, where the stimulation modulates LTP via regulating BDNF regulatory sequences that increase gene expression (Podda et al., 2016). So far, it has been found that the interaction between the BDNF Val66Met polymorphism and brain stimulation seems critical but heterogeneous. Second, CACNA1C, a protein that encodes an alpha-1 subunit of voltage-dependent calcium channels which in turn mediate calcium influx into cells. Membrane polarisation is therefore modulated which can possibly predict antidepressant tDCS response (Pereira Junior et al., 2015). Third, the COMT gene which plays an important role in regulating dopamine levels also in the dlPFC and that might prove to modulate behavioural effects of tDCS (Nieratschker et al., 2015;

Plewnia et al., 2013). The findings will be published separately. To summarise, it is possible that certain polymorphisms can act as predictors and modulators for tDCS effects and that this genetic diversity is one cause for the variability in tDCS research (Li et al., 2015). Future research on these factors can unveil their roles in neuroenhancement, allowing for more targeted NIBS approaches.

The aforementioned points can also be extended to other NIBS interventions, especially tACS and tRNS, which are closely related to tDCS and are similar to apply. Adding these additional methods to the equation opens up even more possibilities for enhanced neurostimulation. However, the number of studies is low and as tDCS is at the forefront in number of studies conducted, tACS and tRNS will be harder to evaluate still and need to be discussed in their own publications. However, it is likely that different individuals will profit from different forms of NIBS.

4.3 Limitations and Implications for Further Research

The parameter testing study included only young, well educated, and (psychologically) healthy participants. This is a very specific study group that is already capable of high cognitive performance - even in a version of the CCT that was harder to solve than the CCT commonly used in research. As such, significant improvements in performance are harder to obtain and may therefore be overlooked due to ceiling effects; especially as tDCS and CCT effects are often small. One solution could be an even tougher version of the task, e.g. the PASST, which increases difficulty without deviating from the original CCT too much. For this, however, one needs to ensure that still the same cortical target networks are activated by the novel task. Finally, for a healthy user group the ethical question arises: should *healthy* cognition be subject to artificial modification? Even though tES (and CCT) side effects are rare, there is no guarantee for their absence. Whilst accepting the occurrence of adverse effects might be tolerable in patient groups where the ultimate outcome is an improvement or even cure of certain impairments, the perspective changes when willingly accepting adverse effects by interfering with a healthy mind. This question has to be kept in mind by researchers, operators, and users alike, especially until the underlying mechanisms have been more thoroughly understood (Riggall et al., 2015).

Due to the outbreak of the Covid-19 pandemic, the CCT study had to be stopped after 10 patients had started the intervention. After lockdowns were lifted, the study was open for participation again and the full sample was recruited. However, recruitment remained difficult and ideally a higher number of patients should be included to add to the robustness of the results. Furthermore, possible impact of the new situation surrounding the pandemic on mental health and MDD symptom prevalence (Ettman et al., 2020), interactions between patients and staff, and ability to travel to and from the study site need to be taken into account (Sohrabi et al., 2021; Vindegaard & Benros, 2020).

The lack of a pure waiting group in the CCT study needs to be addressed in future trials. In many cases, episodes of MDD can subside on their own with time (Mekonen et al., 2022; Whiteford et al., 2013) and remission without treatment might have occurred during in this study as well. Nevertheless, symptom improvement was clearly facilitated by use of an improved version we of the CCT, indicating that the addition of such elements is beneficial to the training outcome. To tackle the drawback of the missing waiting group, a confirmatory study is currently in progress. Here, more than 100 patients with diagnosed MDD will be enrolled for a six week long intervention. All patients will attend three study visits and are either immediately given the gamified CCT as presented in this work, or they will receive the training after all study visits have been completed, therefore serving as a waiting group. The study will be conducted at multiple university hospitals and will allow to assess the effects of the training as an add-on to treatment as usual.

Transfer of results from one study to another (e.g. healthy participants to depressed patients) should be done with caution. It should not be tacitly implied that the findings are transferable without further considerations, but rather that the results need to be interpreted with the respective study sample in mind. Consequently, the future trajectory of neuromodulation is likely to be characterised by personalised methods that accommodate the unique neurobiological attributes of each individual.

4.4 Conclusion

EF play a pivotal role in day-to-day life, allowing humans to exert cognitive flexibility and manage behaviours in effective ways - they are important and vital cognitive functions that should be supported or, in case of dysfunction, be rebuilt and strengthened. The impairment of such functions is associated with numerous psychiatric disorders and places a significant burden on patients suffering from these conditions. Although the enhancement of cognitive functions could offer advantages beyond just treating disorders, potentially boosting cognition in healthy individuals as well, systematic parameter tests and training evaluations remain scarce. The work presented here shows that tDCS proves to be a viable option to support cognitive processes in healthy humans. Furthermore, it underscores the substantial level of variability and non-linearity that surrounds this intervention, indicating that further research is required to establish universal protocols, which ultimately lead to individualised and more effective tES. The potential benefits of a digitalised CCT in MDD patients illustrate another approach of how cognition and mental well-being can be improved. Future combination of tDCS and CCT holds promise as a viable means of neuroenhancement and therapy and these prospects open up promising perspectives for medicine, psychology, and neuroscience.

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A Appendix

A.1 Accepted Publications

A.1.1 'Enhancing Cognitive Control Training with Transcranial Direct Current Stimulation: A Systematic Parameter Study'

Note. Reprinted with permission (CC BY-NC-ND): Weller, S., Nitsche, M. A., & Plewnia, C. (2020a). Enhancing cognitive control training with transcranial direct current stimulation: A systematic parameter study. *Brain Stimulation*, *13*(5), 1358–1369. https://doi.org/10.1016/j.brs.2020.07.006

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Enhancing cognitive control training with transcranial direct current stimulation: a systematic parameter study

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ABSTRACT

Background: *Cognitive control* (CC) is an important prerequisite for goal-directed behaviour and efficient information processing. Impaired CC is associated with reduced prefrontal cortex activity and various mental disorders, but may be effectively tackled by *transcranial direct current stimulation* (tDCS)-enhanced training. However, study data are inconsistent as efficacy depends on stimulation parameters whose implementations vary widely between studies.
Objective: We systematically tested various tDCS parameter effects (anodal/cathodal polarity, 1/2 mA stimulation intensity, left/right prefrontal cortex hemisphere) on a six-session CC training combined with tDCS.
Methods: Nine groups of healthy humans (male/female) received either anodal/cathodal tDCS of 1/2 mA over the left/right PFC or sham stimulation, simultaneously with a CC training (modified adaptive *Paced Auditory Serial Addition Task* [PASAT]). Subjects trained thrice per week (19 min each) for two weeks. We assessed performance progress in the PASAT before, during, and after training. Using a hierarchical approach, we incrementally narrowed down on optimal stimulation parameters supporting CC. Long-term CC effects as well as transfer effects in a flanker task were assessed after the training period as well as three months later.
Results: Compared to sham stimulation, anodal but not cathodal tDCS improved performance gains. This was only valid for 1 mA stimulation intensity and particularly detected when applied to the left PFC.
Conclusions: Our results confirm beneficial, non-linear effects of anodal tDCS on cognitive training in a large sample of healthy subjects. The data consolidate the basis for further development of functionally targeted tDCS, supporting cognitive control training in mental disorders and guiding further development of clinical interventions.

Introduction

Continuously changing environments require dynamic adaptation by means of filtering and evaluating internal and external stimuli to orchestrate goal-directed behaviour. This is especially important for situations in which distractions might influence efficient responses. Important information is maintained, while non-relevant stimuli must be suppressed or ignored. Dysfunctions of *cognitive control* (CC) processes are at the core of many psychopathological conditions [1,2], comprise the intentional selection of thoughts, emotions, and behaviours based on current task demands [3] involving functions of attention, memory, and emotional control [4], and are associated with altered patterns of brain activation [5,6]. The *prefrontal cortex* (PFC), particularly the *dorsolateral prefrontal cortex* (dlPFC), is known to be highly involved in CC processes [7] by means of processes related to working memory [8], encoding of task relevant rules and responses [9], and emotion regulation [10].

Transcranial direct current stimulation (tDCS) has been put forward as a means to influence these processes by modulating the likelihood of neuronal firing in response to a stimulus [11]. At the

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macroscopic level, within the common and safe range of stimulation parameters (1–2 mA, up to 30 min of stimulation [12]), it is supposed that anodal tDCS predominantly enhances, while cathodal tDCS mainly reduces the excitability and spontaneous activity of the targeted and connected areas [13]. This polarity-dependent modulation of brain activity by tDCS has a remarkable potential to influence corresponding cognition and behaviour [14–16]. However, tDCS does not induce cortical activity per se. It develops its effects particularly in interaction with spontaneous neuronal activity [17,18]. This activity-dependent influence on brain networks allows for a 'functional targeting' of stimulation when tDCS is directly coupled with the respective cognitive or behavioural process [19], where the target regions are activated (i.e. by a task) and further specifically modulated by the stimulation [20]. Correspondingly, tDCS effects have been found especially in neuronal correlates of task features that were active during stimulation [21]. Therefore, the combination of tDCS with task training is suggested to have a synergistic 'neuroenhancing' effect that is currently subject of extensive research [22–25]. However, available data are still inconsistent as efficacy depends on stimulation parameters that vary widely between studies. For a meaningful clinical application, a sustainable enhancement of adaptive plasticity would be most desirable [26]. Based on this notion, a specific activation of the CC network and concomitant tDCS holds promise to provide new treatment strategies for cognitive and behavioural disorders [27–29]. In a plethora of studies, stimulation has already shown to enhance CC by changing emotion regulation processes [30], improving frustration tolerance [31], modulating emotional vulnerability [32], dissolving attentional biases [33], augmenting working memory training [16], and increasing multitasking capacity [34]. However, reliability of results and the plausibility of approaches leaves room for improvement, not at least because studies often yield varying results even for similar tasks [35–38]. Therefore, reliable knowledge about the efficacy of parameter settings is mandatory for further advancements [39].

To this aim, we systematically tested different standard stimulation parameters (anodal/cathodal tDCS with 1/2 mA to the left/right dlPFC) in 162 healthy subjects, combining repeated CC training (6 sessions within 2 weeks) with tDCS, and additionally analysed pre- and post-training assessments. We applied a modified adaptive *paced auditory serial addition task* (PASAT) to challenge and train CC [40]. This task requires continuous updating of working memory with parallel distracting performance feedback; it is known to activate CC [31], critically involves resources within the PFC [41], and adapts task difficulty to individual performance [42]. We hypothesized that adding anodal but not cathodal tDCS to PASAT-induced neuronal activity of the dlPFC [43,44] can enhance cognitive training effects [45,46], improve performance of the PASAT or similar, even more challenging tasks [16,31,46–50], and that higher stimulation intensity does not increase efficacy [51]. Furthermore, we wanted to test if the laterality of stimulation matters. Therefore, PASAT performance under eight different tDCS conditions (combined $N = 119$) was compared to a sham intervention group ($N = 43$). Analyses were conducted hierarchically, allowing us to narrow down the responsible factors for the most efficient combination of CC training and tDCS.

Materials and methods

The study was performed in accordance with the Declaration of Helsinki, approved by the University of Tübingen local ethics committee, and conducted in the University Hospital Tübingen, Department of Psychiatry and Psychotherapy. Subjects were recruited through flyers, online forums, and the e-mail distribution list of the University of Tübingen. Before inclusion, each subject gave informed written consent.

Experimental design

Subjects

Out of 192 eligible subjects, 163 right-handed subjects finished all experimental sessions and 162 were eventually included in the data analysis of this single-blind between-subject study (127 females, 35 males; ages between 18 and 39; mean age = 23.20 years, standard deviation = 3.98 years). Dropouts were caused by inability to adhere to the strictly timed training schedule, as shifting appointments was not allowed and resulted in termination of participation. Minimum group size was set to 15 subjects per group in accordance with previous studies with similar sample sizes, interventions, and significant outcomes, since standard procedures for calculation of sample size are not available for linear mixed effect models which were fitted to analyse our data [9,52]. Exclusion criteria were diagnosed neurological or psychiatric disorders, achromatopsia, metallic implants or tattoos near electrode sites, consumption of tobacco to an equivalent of ten or more cigarettes per day, German language skills lower than CEFR level B, and simultaneous brain stimulation from other sources during attendance of this study. Subjects chose between monetary compensation or course credits. To create an incentive for increased effort in solving the task, participants were informed that the top twelve performers additionally received a bonus pay-out at the end of the study.

TDCS procedure and experimental groups

Stimulation was delivered by a CE-certified direct current stimulator (DC-Stimulator MC, NeuroConn GmbH, Ilmenau, Germany), version 1.3.8, and two rectangular rubber electrodes (5 × 7 cm). Experimental groups were specified by stimulation polarity, current intensity, and electrode laterality. Polarity-dependent effects were examined by placing either the anode or cathode over the PFC. Stimulation intensity was varied by applying a current of either 1 mA or 2 mA, resulting in densities of 0.03 or 0.06 mA/cm^2 respectively. Impedances were kept below 8 kΩ. Laterality was defined by positioning the electrode centre either over F3 or F4 according to the international 10–20 system, with the foreside oriented towards the nasion, the backside with the attached cable oriented towards the inion. The extracephalic electrode was mounted over the opposite lateral deltoid muscle to avoid confounding effects of opposing stimulation polarities on brain physiology which can occur with bipolar cephalic tDCS [53]. Consequently, these parameter combinations resulted in nine groups (referred to as *conditions*) that each subject was randomly assigned to: sham (S), anodal/1 mA/left PFC (A1L), anodal/1 mA/right PFC (A1R), anodal/2 mA/left PFC (A2L), anodal/2 mA/right PFC (A2R), cathodal/1 mA/left PFC (C1L), cathodal/1 mA/right PFC (C1R), cathodal/2 mA/left PFC (C2L), cathodal/2 mA/right PFC (C2R).

Electrode surfaces were coated with conductive electrode paste (Ten 20 conductive Neurodiagnostic Electrode Paste, Weaver and Company, Aurora, Colorado), placed on skin areas previously prepared with 70 % ethanol and mildly abrasive peeling gel (Nuprep Skin Prep Gel, Weaver and Company, Aurora, Colorado), and secured by a fabric cap and tape.

For verum stimulation, the current was ramped up within 5 s to start, and down within 5 s to end the stimulation. Stimulation at target intensity comprised 19:10 min. For sham tDCS, a short stimulation block before and after the training was applied. For the first block, the current was ramped up within 5 s, kept at target intensity (1 or 2 mA) for 40 s, then ramped down within 5 s. After 18:30 min the second block was initiated and the current was

A Appendix

ramped up slowly within 39 s, kept at target intensity for 10 s and ramped down within 1 s. This was done to distinctly mark the end of the second sham phase so that subjects of the sham procedure would feel similar sensations as the subjects treated with continuous tDCS. All subjects started the task 1 min after the stimulation was initiated, ensuring that verum stimulation spanned the entire time subjects worked on the task, while sham stimulation blocks were only active before and after completion of the task, which is too short to induce after-effects [11,17].

CC training: PASAT

Our version of the *Paced Auditory Serial Addition Task* (PASAT) was implemented in PsychoPy 1.82.01 [54]. Subjects were seated in front of a computer screen while hearing random single digit numbers (1–9) over headphones. They were instructed to sum up the current digit and the digit that preceded it by two trials ($n^{th} + n^{th-2}$; 'two-back'), hence deviating our task from the 'one-back' ($n^{th} + n^{th-1}$) version. This was done to increase mental load as well as to prevent subjects from reaching their peak performance too early and therefore having no room for further improvement. Answers were given immediately after each stimulus presentation on a keyboard marked with all possible results (2–18). Only the usage of one finger of the right hand was permitted, preventing subjects from tagging numbers. The added benefit of the keyboard setup compared to the more conventional use of a mouse cursor was to reduce the intricate element of dexterity, therefore linking the reaction times closer to cognitive abilities.

At the beginning of each session, the interval between digit presentations was 3 s. After four consecutive correct answers, the interval was lowered by 0.1 s, while after four consecutive wrong answers, it was increased by 0.1 s. Feedback on each calculation was given together with the succeeding digit by means of a green (correct) or red (incorrect/missed) computer screen (Fig. 1A). Sessions were divided into three blocks of 5 min of continuous digit presentations, separated by 30 s rest. The total number of trials within each session was not limited and intervals between digit presentations were carried over from preceding blocks within, but not between, sessions. This adaptive presentation speed at fixed time windows qualifies the total number of correct answers in each session as the most sensible performance parameter.

Subjects were asked to answer correctly as fast as possible and resume quickly after mistakes or failure to respond in time. Each session began with 11 supervised practice trials which were excluded from analyses.

Transfer: flanker task

The flanker task [55] was used to measure possible far transfer effects to CC processes, as successful task performance requires the subject to effectively suppress automated responses in favour of a closely but quickly evaluated response, and hence requires appropriate CC capabilities and conscious processing. It was implemented in PsychoPy 1.82.01. Subjects were seated in front of a computer screen and were instructed to respond to a target, an arrowhead pointing either to the left (<) or right (>), by indicating its direction via button press with the left or right index finger respectively. Each target was surrounded by three distractors on each side, defining the type of the stimulus. This stimulus type could either be congruent (distractors pointing in the same direction as the target: <<<<<<< or >>>>>>>) or incongruent (distractors pointing in the opposite direction to the target: <<<><<< or >>><>>>). In addition to these two conditions, the task included a neutral condition (===<=== or ===>===), during which the direction of the target was also to be indicated, and a pure no-go-condition (XXX<XXX or XXX>XXX) during which no button had to be pressed (Fig. 1B). An experimental session involved two blocks consisting of 88 trials each. Each of the aforementioned conditions was randomly displayed 11 times during the task. Before each stimulus presentation, a white dot appeared for 0.3 s to facilitate eye fixation at the target's position and minimise response time. Stimuli were displayed for maximally 2 s, but proceeded as soon as subjects gave their response. For incorrect answers, an error message was displayed for 0.6 s, for right answers the screen remained black for 0.6 s. Before each session, subjects went through 15 supervised practice trials which were excluded from analyses.

Study timeline

Subjects attended nine sessions: eight sessions were conducted within four weeks while the last session took place three months later (Fig. 1C). The first session (*pre-training*, week 1) included the initial collection of the demographic data as well as the first PASAT run without tDCS, a 5-min pause, and eventually the flanker task. The second to seventh sessions (*training*, week 2 and 3) included the PASAT as well as simultaneous tDCS. The eighth (*post-training,* week 4) and ninth (*follow-up*, three months after post-training) sessions followed the same general procedure as the first session, without the initial assessment questionnaires. A day of training combined with tDCS always alternated with a day without any stimulation or training. Between weeks, there were at least two, at most three intervention-free days [56].

During pre- and post-training, we also recorded EEG of groups who underwent treatment of the right PFC (A1R, A2R, C1R, and C2R). Blood samples were collected from all subjects after the follow-up session to assess a possible impact of genetic factors. These data will be published separately.

Questionnaires

Right-handedness was ascertained by the ten-item *Edinburgh-Handedness-Inventory* (EHI) [57]. Only subjects scoring laterality quotients equal or higher 60 were included in the study [58]. Other sample characteristics, such as age, or educational level were gathered in a custom questionnaire (see Table 1). To account for initial interest and perceived challenge of the task, the *Questionnaire on Current motivation* (QCM) [59] was applied. It assesses four factors which are defined as follows: anxiety (assumptions of failure attributable to the pressure created by the task), probability of success (successfulness in the task), interest (appreciation of the task), and challenge (how demanding the task is perceived). To evaluate changes of affective states, subjects rated the twenty adjectives provided in the *Positive and Negative Affect Schedule* (PANAS) [60] both, before and after the PASAT. Possible adverse effects were reported on a custom five-point Likert-type scale. At the end of post-training, blinding was assessed and subjects stated whether they thought they had received 'sham' or 'verum' stimulation during their training. Questionnaires were provided in their respective German versions.

Statistical analyses

Threshold for type I error was set to 5 % for all analyses. In case of necessary correction of these values, we provide the adjusted thresholds in the respective results section. Reported values refer to two-tailed tests.

CC training: PASAT

All statistical analyses were done with IBM SPSS Statistics Software version 24 [61], R version 3.5.1 [62], and packages nlme [63] together with reghelper [64] in particular. Type I error was corrected by the Bonferroni-Holm method for each step of analysis, the respective corrected levels are presented in the results section.

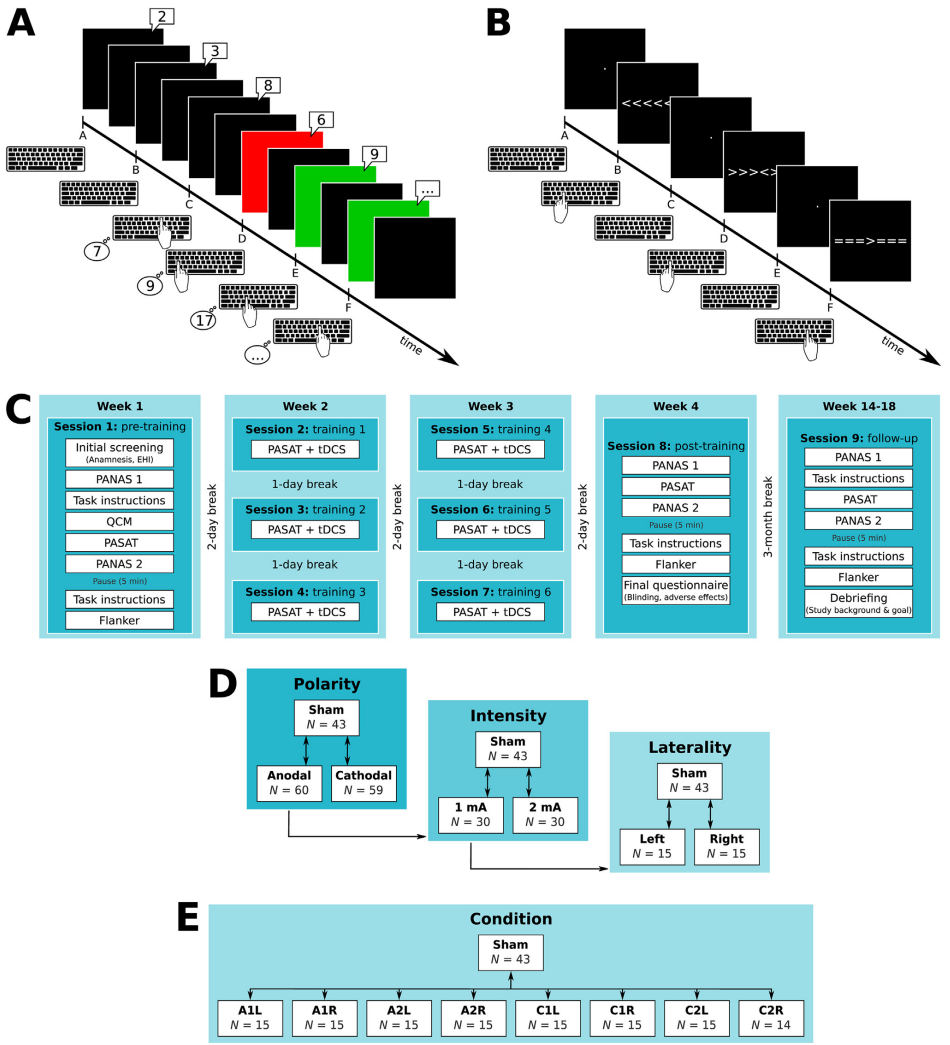

Fig. 1. **A** During the PASAT, subjects heard single digit numbers over headphones and were asked to sum up the current digit (n^{th}) and the second-to-last digit that preceded it (n^{th-2}): e. g. digits at timepoints C and A, D and B, E and C, and so on. Answers were given on a keyboard using one hand only, subsequent feedback appeared simultaneously with the following digit presentation. Consecutive correct answers shortened the interval between digit presentations, wrong answers prolonged it. **B** During the flanker task, subjects were asked to indicate the direction of a centre stimulus surrounded by distractors. The three go-conditions, consisting of congruent (e. g. <<<<<<<), incongruent (e. g. >>><>>>), and neutral distractors (e. g.===>===), were accompanied by one no-go-condition (XXX < XXX). **C** The study was divided into nine sessions. Sessions were spread in a way that training days alternated with days without any intervention. Between weeks, there were two resting days. EHI = Edinburgh Handedness Inventory; PANAS = Positive and Negative Affect Schedule; QCM = Questionnaire on Current Motivation; PASAT = Paced Auditory Serial Addition Task; Flanker = Flanker task. **D** Analysis of the PASAT data was divided by stimulation parameters, starting with broad classifications that were further split into specified intervention parameter sub-categories. This hierarchical analysis was only continued out for groups where significant findings were obtained. **E** Analyses were additionally made for all stimulation parameter combinations separately in one single model to assess whether effects would still be present in this analysis. All groups were compared to sham, not between each other.

Table 1
Demographic group characteristics. If applicable, means and standard deviations (M(SD)) are listed, otherwise the number of subjects belonging to each parameter are shown. One Subject was removed from analyses (group C2R) as performance deviated more than 2 SD from all other subjects. a: Fisher's Exact test; b: Kruskal-Wallis H test; c: Welch ANOVA; d: one-factorial ANOVA.

Level	Label									Test statistic
Polarity	Sham	Anodal	Anodal	Anodal	Anodal	Cathodal	Cathodal	Cathodal	Cathodal	
Intensity	Sham	1 mA	1 mA	2 mA	2 mA	1 mA	1 mA	2 mA	2 mA	
Laterality	Sham	Left	Right	Left	Right	Left	Right	Left	Right	
Group	S	A1L	A1R	A2L	A2R	C1L	C1R	C2L	C2R	
Subjects (N)	43	15	15	15	15	15	15	15	14	
Sex (m/f)[a]	11/32	2/13	4/11	2/13	3/12	5/10	4/11	2/13	2/12	$p = 0.863$
Age[b]	22.767	22.800	24.733	23.533	22.267	25.600	23.133	23.533	21.071	χ^2 (8, $N = 162$) = 15.211,
(M(SD))	(3.611)	(4.092)	(5.147)	(3.270)	(3.283)	(3.924)	(4.389)	(4.853)	(2.336)	$p = 0.055$
EHI-Score[c]	0.872	0.900	0.927	0.886	0.960	0.900	0.960	0.893	0.986	F(8, 151) = 1.961, $p = 0.055$
(M(SD))	(0.153)	(0.107)	(0.116)	(0.146)	(0.074)	(0.146)	(0.063)	(0.127)	(0.036)	
Academic Degree[a] (high school/ middle school)	42/1	15/0	15/0	15/0	15/0	15/0	15/0	15/0	14/0	$p = 1.000$
QCM: anxiety[d]	3.516	3.293	4.027	3.640	3.107	3.720	3.627	3.200	4.071	F(8, 153) = 1.174, $p = 0.318$,
	(1.141)	(1.331)	(1.071)	(0.882)	(1.195)	(1.121)	(1.331)	(1.694)	(1.016)	$\eta^2 = 0.058$)
QCM: probability of success[d]	4.192	4.050	4.133	4.567	3.989	3.933	4.267	4.143	3.875	F(8, 152) = 0.381, $p = 0.929$,
	(1.266)	(1.477)	(1.109)	(1.155)	(1.441)	(1.513)	(0.810)	(1.709)	(1.108)	$\eta^2 = 0.020$
QCM: interest[d]	4.065	3.907	4.440	4.333	3.737	3.840	4.053	3.867	4.157	F(8, 153) = 0.566, $p = 0.805$,
	(1.282)	(0.959)	(1.127)	(1.024)	(1.383)	(1.157)	(0.987)	(1.608)	(1.017)	$\eta^2 = 0.029$
QCM: challenge[d]	5.064	5.100	5.583	5.450	5.533	5.433	5.250	5.017	5.125	F(8, 153) = 1.006, $p = 0.434$,
	(1.047)	(0.687)	(0.497)	(0.902)	(0.640)	(0.848)	(0.675)	(1.571)	(0.663)	$\eta^2 = 0.050$
Menstruating during experiment[a] (yes/no)	17/14	5/8	8/3	7/5	9/3	6/4	7/4	7/5	10/2	$p = 0.786$
Hormonal contraceptive (women only)[a] (yes/no)	16/16	8/5	5/6	7/5	4/8	4/6	6/5	7/5	7/5	$p = 0.978$
Smoker[a] (yes/no)	10/33	4/11	1/14	2/12	2/13	3/12	0/15	0/14	1/13	$p = 0.182$

We used the number of correct trials as measure of performance (n_{corr}). In the adaptive PASAT, correct responses are followed by faster digit presentation and thus a higher number of correct trials. Comparing the number of correct trials within a fixed task duration allowed to measure performance during constant stimulation periods throughout all sessions.

To test for differences in pre-training performance (session one), a one-factorial ANOVA with *condition* as between-subjects factor and $n_{corr(pre)}$ as within-subject factor was used.

To analyse tDCS effects on performance gain during training, sessions including tDCS (training sessions) and sessions without tDCS (pre-training, post-training, and follow-up) were investigated separately. The experimental groups were compared to the sham group by hierarchical analysis. First, the two polarity groups (anodal [A; $N = 60$], cathodal [C; $N = 59$]) were each compared to sham (S; $N = 43$). If significantly different, the respective group was then further split by intensity (1 mA [1; $N = 30$], 2 mA [2; $N = 30$]) and then, again if significant, by stimulation laterality (left PFC [L; $N = 15$], right PFC [R; $N = 15$]). For each of these planned comparisons, sham was used as comparator (Fig. 1D). Additionally, we analysed a full model with groups split by all three parameters within a single step and compared these eight groups to sham (Fig. 1E).

A linear mixed model was applied to each hierarchical level since it allows to analyse performance gain over time while accounting for variability explained by the variables as well as accounting for variation not explained by these variables. In the model, n_{corr} was used as the dependent variable. For planned contrasts, performance of the sham group was used as the reference as we were interested in changes compared to a non-stimulated sample. Fixed effects in each model were the experimental *groups* at their respective hierarchical level (*polarity*: S, A, C; *intensity*: S, 1, 2; *laterality*: S, L, R; and lastly *condition* for the full model at the lowest level: S, A1L, A1R, A2L, A2R, C1L, C1R, C2L, C2R), session during which the measurement was taken (*time*: session two to seven), the interaction *condition* x *time*, and lastly *pre-training performance* as a regression coefficient ($n_{corr(pre)}$: number of correct trials during pre-training), resulting in the following model: n_{corr} ~ *group* x *time* + $n_{corr(pre)}$. Random effects were measurement timepoint and individual subject: *~1 + time | subject*. To compare strength of effects, non-standardised (B) and standardised beta coefficients (β) were computed for each model. These models allow for the comparison of training gains, forming a slope over the course of all sessions, corresponding to how much participants improved in their performance over time.

To investigate the stability of effects at post-training and follow-up, data of conditions that were found to be significantly different from sham during the training phase were additionally compared to sham by t-tests. Here, Cohen's d was calculated to assess for effect sizes.

Transfer: flanker task

For analysis of the flanker task we used neutral, congruent and incongruent trials. Outliers (±3 SD deviation from overall reaction time mean), incorrect trials as well as the respective subsequent trial (which could be subject to inflated reaction times due to potential post error slowing) were removed from the data. Grouping factors of the flanker trials were chosen according to congruency, resulting in three *trial type* groups: *congruent*, *incongruent*, and *neutral* trial conditions. The repeated measures ANOVA included the dependent variable *mean reaction time* pooled by *trial type*, and the main effects *trial type*, *condition* (S, A1L, A1R, A2L, A2R, C1L, C1R, C2L, C2R), *time* (session in which measurement was taken), as well as the interactions *time* x *condition* and *time* x *condition* x *trial type*.

Questionnaires

Comparisons regarding data gathered from the anamnesis and questionnaires preceding the experimental procedure to ensure group homogeneity were performed on the nine *conditions* (S, A1L, A1R, A2L, A2R, C1L, C1R, C2L, C2R) as independent factors and the

respective questionnaire outcome as dependent variable. Fisher's Exact Test was used to analyse the distribution across groups of the following variables: gender, educational degree, stage of menstrual cycle for women, application of hormonal contraception, and smoking behaviour. For the distribution of age across groups, the Kruskal-Wallis H test was used. Due to non-homogenous variances, handedness was analysed by Welch ANOVA. QCM items were analysed by one-factorial ANOVAs. Effects of PASAT and tDCS on PANAS scores were analysed by repeated measures ANOVAs, with PANAS scores in the nine *conditions* before and after PASAT completion (*session*) as well as before and after the course of training (*training*). Positive affect (PA) and negative affect (NA) were analysed separately. Adverse effects were analysed item-wise by one-factorial ANOVA. Blinding was evaluated through χ^2-test.

Results

Sample characteristics

Composition of the nine groups did not differ regarding demographic data. See Table 1 for a detailed overview of group compositions and the respective test statistics. No differences were found between groups for pre-training performance in the PASAT (conditions: $F(8, 153) = 0.939$, $p = 0.486$, $\eta^2 = 0.047$).

Effects of tDCS on cognitive control training

Fig. 2 shows the performance (n_{corr}) for each level of analysis (polarity [Fig. 2A], intensity [Fig. 2B], laterality [Fig. 2C]). Main effect *time* was significant ($p < 0.001$), illustrating that subjects' performance continually improved in all intervention conditions. Pre-training performance turned out to be a predictor for overall performance increase, $n_{corr(pre)}$ was significant in all analyses ($p < 0.001$): subjects performing better during pre-training also showed higher performance gains. Please see Supplementary Table 1 for the numeric values.

TDCS-enhanced training: hierarchical analysis

I) First level: polarity (Supplementary Table 2)

We found an effect for *anodal polarity* ($t(806) = 2.27$, $p = 0.0235$; $B = 2.66$, SE = 1.17; $\beta = 0.04$, SE = 0.02), indicating that *anodal tDCS* applied during PASAT training enhances performance gains compared to sham stimulation. *Cathodal polarity* did not yield an effect ($t(806) = 0.82$, $p = 0.4129$; $B = 0.96$, SE = 1.17; $\beta = 0.02$, SE = 0.02). The Bonferroni-Holm corrected threshold for level of significance for this level of analysis is 0.025.

II) Second level: intensity (Supplementary Table 3)

Subsequently narrowing down the effects of anodal stimulation, intensities of 1 and 2 mA were compared to sham tDCS. Here, *1 mA* ($t(511) = 2.71$, $p = 0.0069$; $B = 3.69$, SE = 1.36; $\beta = 0.06$, SE = 0.02) showed a highly significant increase in performance gain compared to sham, but *2 mA* did not support training ($t(511) = 1.19$, $p = 0.2345$; $B = 1.62$, SE = 1.36; $\beta = 0.02$, SE = 0.02). The Bonferroni-Holm corrected threshold for level of significance for this level of analysis is 0.01.

III) Third level: laterality (Supplementary Table 4)

Finally, testing the effect of stimulation laterality (left, right) in the subsample of 1 mA anodal tDCS, we found a significant effect of the *left* ($t(362) = 2.53$, $p = 0.0117$; $B = 4.40$, SE = 1.74; $\beta = 0.06$, SE = 0.02), but not the *right PFC* ($t(362) = 1.72$, $p = 0.0869$; $B = 2.98$, SE = 1.74; $\beta = 0.04$, SE = 0.02). The Bonferroni-Holm corrected threshold for level of significance for this level of analysis is 0.0167.

TDCS-enhanced training: single group analysis (Supplementary Table 5)

Complementary, testing each of the applied tDCS conditions against sham tDCS within a single model indicated that *1 mA anodal tDCS to the left PFC* significantly increased PASAT performance gains with training ($t(800) = 2.55$, $p = 0.0111$; $B = 4.40$, SE = 1.73; $\beta = 0.04$, SE = 0.02). The Bonferroni-Holm corrected threshold for level of significance for this level of analysis is 0.0125.

TDCS-enhanced training: sensitivity analysis (Supplementary Table 6)

To control for possible placebo effects, a sensitivity analysis was performed, including only subjects who assumed to have received verum tDCS. The result coincides with the overall analysis, as the *A1L* group still shows significantly improved performance gains over sham ($t(670) = 1.98$, $p = 0.0485$; $B = 3.91$, SE = 1.98; $\beta = 0.04$, SE = 0.02). The Bonferroni-Holm corrected threshold for level of significance for this level of analysis is 0.05.

Pre-, post-training, and follow-up

To investigate the stability of tDCS effects, post-training and follow-up performance were compared for each condition to sham on the different levels of analysis. No persistent effects were found for the overall group of anodal polarity. After training with 1 mA anodal tDCS, superior effects in comparison with sham stimulation were measurable at post-training ($p = 0.043$) and follow-up ($p = 0.017$). At the single group level (1 mA anodal tDCS to the left PFC), enhanced performance was observed at the post- ($p = 0.049$) but not the follow-up session. See Table 2 for the statistics of the conducted t-tests; for the sake of completeness, we show calculations for all sessions.

Changes of affective state (PANAS)

Analyses of PA revealed that the interaction of *session* and *training* was significant, indicating a slight decrease over the course of the experiment ($F(1, 146) = 12.048$, $p < 0.001$, $\eta^2 = 0.076$). For NA, *session* was significant ($F(1, 148) = 28.634$, $p < 0.001$, $\eta^2 = 0.162$), with increase in NA immediately after completion of the PASAT within the session. NA decreased between sessions as reflected by significance of *training* ($F(1, 148) = 13.567$, $p < 0.001$, $\eta^2 = 0.084$). The interaction of *session* and *training* was also significant, pointing towards a reduction of PASAT-induced NA with training ($F(1, 148) = 17.272$, $p < 0.001$, $\eta^2 = 0.105$). See Table 3 for the reported mean scores.

Effects of tDCS and CC training on the flanker transfer task

In all analyses and under all conditions, we found highly significant effects of *time*, *trial type*, and the interaction *time* x *trial type*, indicating that subjects improved over time and that the *trial type* consistently affected subjects' reaction times with congruent trials eliciting faster reaction times than incongruent trials. No effects of tDCS on flanker task performance were found. For an overview of the flanker scores please refer to Supplementary Table 7, for test statistics to Supplementary Table 8.

Adverse effects

There were statistically significant differences between groups for itching sensations under the electrode surface ($F(8, 153) = 2.081$, $p = 0.041$, $\eta^2 = 0.098$). Least significant post hoc analysis showed that the sensations were significantly stronger in A2L, A2R, and C2R compared to S. Significant differences were also

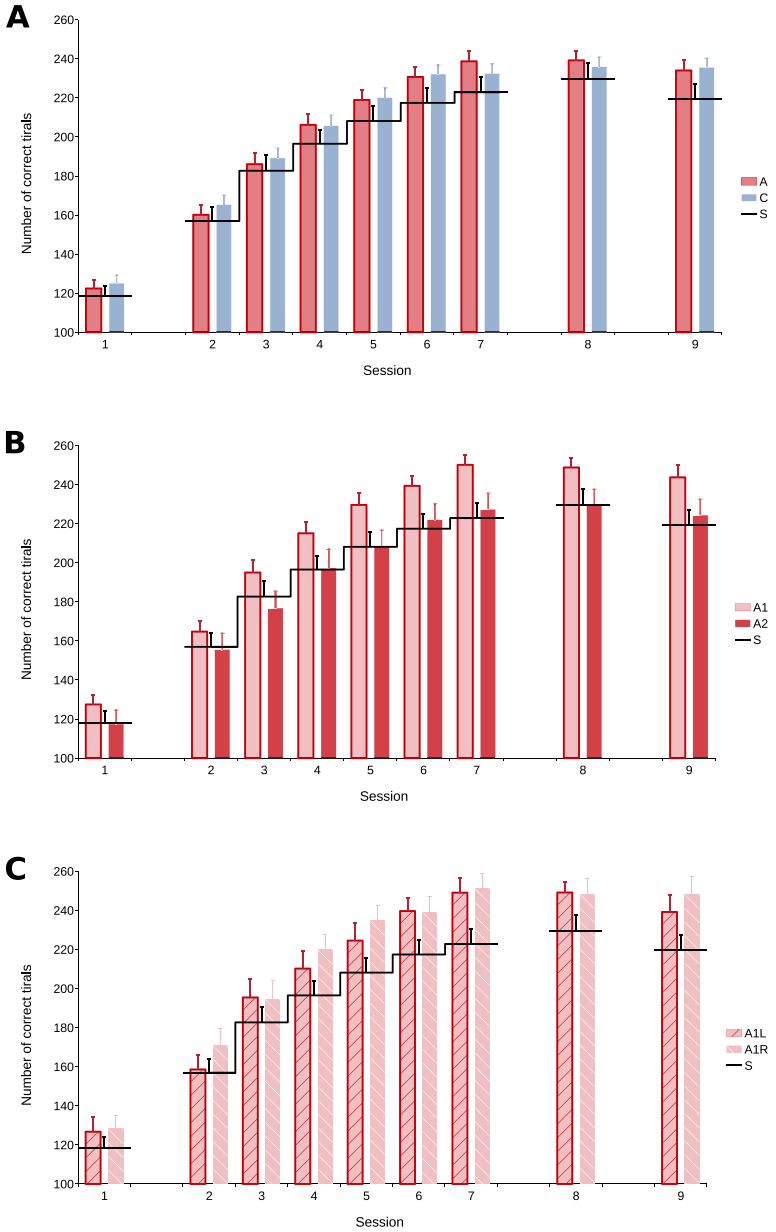

Fig. 2. Shown are the number of correct trials (sum of the respective session) for each analysis level (**A**: polarity; **B**: intensity; **C**: laterality). Performance of the sham group is shown as a line plot to facilitate comparison. All experimental groups improved significantly over time. In each planned comparison, sham was used as the reference and no comparisons were performed between stimulation groups. **A** For the first analysis level, the anodal group proved to benefit significantly over sham, therefore this group was further divided by their respective intensity levels (**B**), for which 1 mA showed significant effects compared to sham. After then additionally dividing this group by laterality (**C**), we were able to deduce that the left side of this subgroup provided significant performance gain over sham while the right side showed a trend in comparison to sham. Groups that benefited from tDCS and therefore exhibited increased performance are outlined in bold. Error bars show standard error of the mean.

Table 2
t-test statistics showing stability of tDCS effects. Depicted are mean number of correct trials at the respective level (n_{corr} (level)), differences in number of correct trials compared to the sham group (Δ_n_{corr}), standard deviations (SD), standard errors of the mean (SEM), and test statistics.*p < 0.05; **p < 0.01; ***p < 0.001.

Stability of tDCS effects over time											
Polarity level: S vs. A (N = 43 vs. 60)											
Session	n_{corr} (S)	n_{corr} (A)	Δ_n_{corr}	SD (S)	SD (A)	SEM (S)	SEM (A)	t	df	p	Cohen's d
1	118.67	122.45	−3.776	34.503	34.126	5.262	4.406	−0.551	101	0.583	0.110
2	156.95	160.13	−3.180	45.810	38.967	6.986	5.031	−0.379	101	0.705	0.075
3	182.67	186.05	−3.376	51.251	42.381	7.816	5.518	−0.364	100	0.717	0.072
4	196.51	206.18	−9.672	46.769	44.026	7.132	5.684	−1.071	101	0.287	0.213
5	208.14	218.93	−10.794	49.656	40.911	7.572	5.282	−1.207	101	0.230	0.237
6	217.42	230.68	−13.265	49.217	38.293	7.505	4.944	−1.538	101	0.127	0.301
7	222.84	238.70	−15.863	51.060	39.288	7.787	5.072	−1.707	75.524	0.092	0.348
8	229.70	239.19	−9.489	51.610	37.812	7.870	4.923	−1.022	73.180	0.310	0.210
9	219.53	234.02	−14.482	50.533	40.590	7.706	5.240	−1.611	101	0.110	0.316
Intensity level: S vs. A1 (N = 43 vs. 30)											
Session	n_{corr} (S)	n_{corr} (A1)	Δ_n_{corr}	SD (S)	SD (A1)	SEM (S)	SEM (A1)	t	df	p	Cohen's d
1	118.67	127.47	−8.792	34.503	27.430	5.262	5.008	−1.162	71	0.249	0.282
2	156.95	164.73	−7.780	45.810	31.058	6.986	5.670	−0.865	70.963	0.390	0.199
3	182.67	195.00	−12.326	51.251	36.286	7.816	6.625	−1.133	71	0.261	0.278
4	196.51	215.07	−18.555	46.769	32.768	7.132	5.983	−1.993	70.994	0.050*	0.460
5	208.14	229.57	−21.427	49.656	32.640	7.572	5.959	−2.224	70.802	0.029*	0.510
6	217.42	239.37	−21.948	49.217	28.499	7.505	5.203	−2.403	68.992	0.019*	0.546
7	222.84	250.10	−27.263	51.060	28.853	7.787	5.268	−2.900	68.471	0.005**	0.657
8	229.70	248.73	−19.036	51.610	26.589	7.870	4.854	−2.059	66.166	0.043*	0.464
9	219.53	243.70	−24.165	50.533	34.003	7.706	6.208	−2.442	70.935	0.017*	0.561
Laterality level: S vs. A1L (N = 43 vs. 15)											
Session	n_{corr} (S)	n_{corr} (A1L)	Δ_n_{corr}	SD (S)	SD (A1L)	SEM (S)	SEM (A1L)	t	df	p	Cohen's d
1	118.67	126.73	−8.059	34.503	28.542	5.262	7.369	−0.812	56	0.420	0.255
2	156.95	158.60	−1.647	45.810	27.954	6.986	7.218	−0.164	40.632	0.871	0.043
3	182.67	195.47	−12.792	51.251	37.428	7.816	9.664	−0.886	56	0.380	0.285
4	196.51	210.20	−13.688	46.769	35.184	7.132	9.084	−1.034	56	0.306	0.331
5	208.14	224.53	−16.394	49.656	34.234	7.572	8.839	−1.181	56	0.243	0.384
6	217.42	239.67	−22.248	49.217	26.757	7.505	6.909	−2.181	45.446	0.034**	0.562
7	222.84	249.07	−26.229	51.060	29.572	7.787	7.635	−2.405	42.823	0.021**	0.629
8	229.70	249.13	−19.436	51.610	21.620	7.870	5.582	−2.014	53.937	0.049*	0.491
9	219.53	239.20	−19.665	50.533	33.593	7.706	8.674	−1.399	56	0.167	0.458

found for overall itching sensations (F(8, 153) = 2.910, p = 0.005, η^2 = 0.132), with post hoc analysis revealing that sensations were stronger in A2L, A2R, and C2R compared to S. Overall, these results show that higher intensities generally caused more noticeable sensations.

No differences were found for tingling sensations on the head area (F(8, 153) = 1.364, p = 0.217, η^2 = 0.067), fatigue (F(8, 153) = 1.456, p = 0.178, η^2 = 0.071), headache (F(8, 153) = 0.987, p = 0.448, η^2 = 0.049), nausea (F(8, 153) = 0.667, p = 0.720, η^2 = 0.034), and other miscellaneous effects (F(8, 153) = 1.488, p = 0.166, η^2 = 0.072). See Supplementary Table 9 for the reported values regarding the magnitude of the perceived sensations.

Blinding

Of the sham group (N = 43), 29 subjects (67.44 %) guessed that they received verum stimulation. Out of the 119 subjects that actually received verum tDCS, 107 (89.92 %) guessed correctly. Of note, in the verum group, ratios did not differ regarding stimulation intensity (Fig. 3): 53/60 subjects (1 mA), 54/59 subjects (2 mA), χ^2 (1, N = 119) = 0.33, p = 0.563, Cramer's V = 0.053.

Table 3
PANAS sum scores (standard deviations in parentheses). Missing Ns resulted from subjects not reporting a score for at least one adjective of the PANAS, hence the overall score was not calculated.

Timepoint within session	Before PASAT	After PASAT	Before PASAT	After PASAT	Before PASAT	After PASAT	Before PASAT	After PASAT
Session	Positive affect				Negative affect			
	Pre-training		Post-training		Pre-training		Post-training	
S (N = 41)	30.049 (6.797)	30 (7.029)	26.439 (7.991)	26.073 (8.563)	12.286 (2.075)	14.952 (4.288)	12.595 (4.591)	12.714 (3.293)
A1L (N = 15)	30.533 (5.705)	31.6 (6.905)	27.067 (7.216)	24.667 (8.516)	14.467 (6.457)	13.467 (5.167)	13.667 (6.651)	13.667 (5.778)
A1R (N = 14)	28.5 (4.848)	29.643 (7.792)	26.857 (7.655)	26.714 (9.042)	11.571 (1.651)	15.286 (3.931)	11.429 (1.604)	12.071 (3.025)
A2L (N = 14)	31.643 (7.045)	30.786 (7.557)	29.071 (7.322)	29.5 (9.59)	11.8 (2.731)	14.667 (4.865)	12.067 (4.284)	12.4 (3.996)
A2R (N = 15)	26.867 (5.54)	32.2 (7.002)	25.733 (8.336)	27.933 (7.166)	12.071 (3.339)	13.929 (5.595)	11.714 (1.939)	12.143 (2.248)
C1L (N = 15)	31.667 (6.102)	29.133 (5.397)	31.733 (6.819)	27.267 (6.017)	12.267 (2.463)	15.4 (5.889)	11.4 (1.502)	13.467 (5.963)
C1R (N = 12)	23.333 (4.774)	27.75 (7.06)	25.5 (4.89)	25.333 (6.499)	12.2 (2.624)	16.133 (5.475)	12.133 (2.696)	13.133 (3.796)
C2L (N = 15)	31.467 (5.693)	34.2 (6.951)	29.467 (8.236)	28.533 (8.175)	12 (2.353)	13.143 (4.4)	12.071 (3.339)	12.143 (2.958)
C2R (N = 14)	26.714 (6.031)	31.643 (6.476)	24.571 (4.274)	27.286 (4.697)	11.538 (2.367)	13.385 (3.948)	11.231 (2.204)	12.538 (5.06)

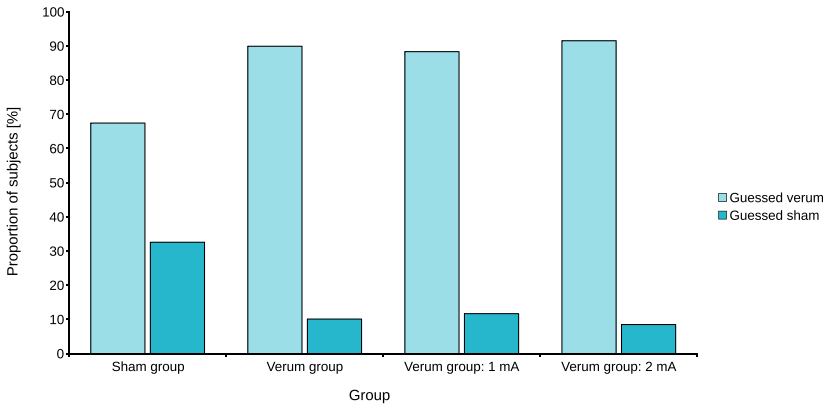

Fig. 3. Number of subjects and their respective guesses on blinding. Regardless of stimulation intensity, subjects were able to discern whether they received verum tDCS or not. The majority of subjects in the sham group also assumed to have received verum stimulation.

Discussion

To provide comprehensive evidence for the beneficial effects of tDCS on CC training, and the critical dependency of these effects on stimulation parameters, we systematically tested multiple tDCS parameter combinations applied concurrently to a challenging modified two-back adaptive PASAT training in a large sample of healthy subjects ($N = 162$). By means of hierarchical analysis we found that: *i)* CC training gains were enhanced by anodal - but not cathodal - tDCS and superior to sham stimulation, confirming the polarity-dependence of tDCS; *ii)* stimulation intensity of 1 mA was superior to sham tDCS, supporting previous findings that the influence of stimulation intensity on task-related cognitive processing and plasticity is not linear [51]; *iii)* this effect was particularly salient when applying 1 mA anodal tDCS to the left PFC, suggesting spatial specificity as right-sided stimulation missed the significance level; and *iv)* the effects were observable until three months after training.

These results are particularly valuable to identify reliable stimulation protocols for further development of translational applications [65,66]. Current lack of conclusive evidence might be primarily due to the multitude of studies with small sample sizes, ill-defined mechanistic models, varying parameter settings, and experimental designs [66]. With this study, we addressed these requirements by investigating a large sample in a standardised intervention, identifying reliably effective stimulation conditions. We tested the effects on adaptive plasticity measured by means of training effects in contrast to single session interventions. Although there is no doubt about the malleability of cognitive functions with transcranial brain stimulation [67,68], beneficial neuroplastic effects of tDCS as reflected in lasting improvements or amelioration of goal-directed behaviour are still under debate [69]. Therefore, the primary endpoint of our study was performance-gain during a training paradigm. Harnessing state-dependency of tDCS effects [14,48,70,71], functional targeting [18] of the cognitive control networks was implemented by stimulating strictly concurrent to PASAT training, in accordance with previous studies which showed a boosting effect of tDCS on task-relevant networks [22].

Regarding stimulation polarity, the dichotomy of anodal (activity-enhancing) and cathodal (activity-decreasing) effects has often been challenged in research [72]. Here, we provide new evidence for polarity-dependence of tDCS effects on CC, showing that only anodal stimulation over the PFC boosted training gains. Additionally, our data support the notion that tDCS causes non-linear effects, e. g. higher intensities do not necessarily elicit more prominent outcomes in a cognitive task, which was so far shown mainly for physiological tDCS effects [51,73]. None of the higher-intensity groups analysed in our study showed significantly improved performance gains compared to sham intervention. Particularly relevant for the clinical context, these findings imply that a simple increase of stimulation intensity does not necessarily enhance efficacy but, by contrast, might compromise efficacy of the respective intervention. Nevertheless, clinical populations may require higher stimulation intensities due to pathology- or medication-dependent impairments of neuroplasticity [74,75]. Finally, laterality of tDCS modulating potentially lateralised cognitive functions might be critical for yielding effects, as shown by our present and previous studies [16]. However, conclusive evidence for clear hypotheses on CC functions is scarce [76,77]. Consistently, although we observed superior effects of 1 mA anodal tDCS to the left PFC, the trendwise effect of 1 mA anodal tDCS to the right PFC does not allow drawing definite conclusions regarding the prefrontal lateralization of CC processes.

Since the PASAT was used to target CC of emotionally relevant information, affective states were assessed regularly. The PASAT was indeed frustrating as reflected by an increased NA after completion of the task within a session. Interestingly, the magnitude of NA changes decreased with training, which might be due to habituation, but could also indicate improved CC which might support keeping focus on the task at hand and not the distracting, often negative, feedback, hence lowering frustration elicited by the task. Nevertheless, our data do not reveal an effect of tDCS on the affective responses measured with the PANAS as indicated by our previous trials [31,49]. Apparently, the effect of tDCS on acute mood states in healthy subjects is less consistently measurable than on cognitive performance. Additionally, not limited to tDCS but also observable in rTMS trials, emotional states in healthy subjects are quite resistant against modulatory interventions. This might be due to a low precision of assessment tools and high variability of affective reactions to the PASAT interacting with stimulation.

Although in our study effects of tDCS on CC training gains were unambiguous and partially lasting for at least three months, transfer effects as tested with the flanker task were absent. However, the flanker task is rather far from the trained and improved two-back PASAT and transferability of cognitive training gains to other cognitive domains is generally under debate [78,79]; our study sample of healthy subjects at the prime of their cognitive abilities poses additional challenges for improvements. Yet, the lack of transfer effects argues for the specificity of tDCS for trained tasks in accordance with the notion of a synergistic effect of training and stimulation-activated networks [18,27].

A relevant limitation of this study is effective blinding: verum tDCS was detected by most participants, independent from stimulation intensity. In the sham group, most but nonetheless fewer subjects assumed having received verum stimulation, following results of studies highlighting challenges of proper tDCS blinding [80,81]. Recent findings show that blinding aided by topical anaesthetics was successful up to 3 mA [82]. Nevertheless, as established by sensitivity analysis, our results remain basically the same when individuals that judged their stimulation as 'not real' were removed. Furthermore, to warrant a safe and valid stimulation of three subjects in parallel, this study was conducted in a single-blind fashion. To avoid experimenter bias, randomisation was performed prior to pre-training measures, instructions were strictly read from scripts, and other interactions with the subjects were limited to a minimum.

In conclusion, our study confirms polarity-dependent, non-linear, beneficial effects of optimised tDCS on CC training. Based on systematic testing of parameters in a large group of healthy subjects, these data provide a solid basis for further developments in the neuroscientific and clinical use of electrical brain stimulation. Linking tDCS with cognitive tasks may allow for targeted enhanced brain network retraining, opening new perspectives for cognitive enhancement and efficient treatment of symptoms in various psychiatric disorders.

Author contributions

CP designed the study. SW performed the experiments. SW and CP analysed the data, interpreted the results, and wrote the manuscript; SW, MAN, and CP participated in the result interpretation and finalised the paper. All authors read and approved the final manuscript.

CRediT authorship contribution statement

Simone Weller: Methodology, Formal analysis, Investigation, Software, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Michael A. Nitsche:** Writing - review & editing. **Christian Plewnia:** Conceptualization, Methodology, Formal analysis, Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

SW and CP 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. MAN is scientific advisor for Neuroelectrics, and NeuroDevice.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2020.07.006.

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A Appendix

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A.1.2 'Supplementary Material to: Enhancing Cognitive Control Training with Transcranial Direct Current Stimulation: A Systematic Parameter Study'

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1 **Supplementary Tables**

Supplementary Table 1. Numeric values for the training effects as shown in **Figure 2A-C**. Shown are the number of correct trials and the respective standard deviations in parentheses.

	Session								
Group	1	2	3	4	5	6	7	8	9
S	118.67 (34.50)	156.95 (45.81)	182.67 (51.25)	196.51 (46.77)	208.14 (49.66)	217.42 (49.22)	222.84 (51.10)	229.70 (51.61)	219.53 (50.53)
A	122.45 (34.13)	160.13 (38.97)	186.05 (42.38)	206.18 (44.03)	218.93 (40.91)	230.68 (38.29)	238.70 (39.29)	239.19 (37.81)	234.02 (40.59)
C	125.20 (31.49)	165.34 (38.41)	189.27 (38.63)	205.76 (41.07)	220.10 (39.42)	232.22 (35.87)	232.49 (38.32)	235.95 (37.96)	235.67 (34.34)
A1	127.47 (27.43)	164.73 (31.06)	195.00 (31.06)	215.07 (32.77)	229.57 (32.64)	239.37 (28.50)	250.10 (28.85)	248.73 (26.59)	243.70 (34.00)
A2	117.43 (39.56)	155.53 (45.62)	176.79 (56.72)	197.30 (52.02)	208.30 (45.89)	222.00 (44.89)	227.30 (45.16)	229.31 (45.06)	224.33 (44.74)

A1L	126.73	158.60	195.47	210.20	224.53	239.67	249.07	249.13	239.20
	(28.54)	(27.95)	(37.43)	(35.18)	(34.23)	(26.76)	(29.57)	(21.61)	(33.50)
A1R	128.20	170.87	194.53	219.93	234.60	239.07	251.13	248.33	248.20
	(27.25)	(33.71)	(36.42)	(30.59)	(31.31)	(31.08)	(29.11)	(31.57)	(34.97)
A2L	124.80	162.67	180.57	201.87	209.80	220.13	227.00	230.64	223.80
	(50.27)	(55.23)	(55.75)	(59.67)	(54.73)	(54.52)	(52.73)	(57.58)	(52.13)
A2R	110.07	148.40	173.27	192.73	206.80	223.87	227.60	228.07	224.87
	(24.46)	(33.92)	(38.09)	(44.73)	(36.90)	(34.55)	(37.98)	(31.30)	(37.79)
C1L	138.53	180.27	202.60	218.93	228.00	237.33	232.40	241.53	242.20
	(42.34)	(51.10)	(45.09)	(42.08)	(44.17)	(34.59)	(43.47)	(44.94)	(35.07)
C1R	116.73	159.60	182.47	201.67	216.07	229.07	229.20	229.47	228.80
	(30.94)	(37.47)	(43.75)	(45.52)	(38.87)	(41.42)	(42.05)	(42.12)	(41.77)
C2L	124.93	157.73	185.40	202.00	218.87	235.60	234.80	237.50	238.20
	(26.69)	(28.36)	(31.88)	(40.74)	(42.95)	(32.95)	(38.37)	(33.69)	(31.08)

C2R	120.29	163.64	186.43	200.07	217.29	226.50	233.64	235.36	232.36
	(19.55)	(32.07)	(32.07)	(36.47)	(33.44)	(36.67)	(31.73)	(31.78)	(31.44)

4

Supplementary Table 2. Results from the linear mixed model at the polarity level. All calculations use the sham group as a reference. Number of subjects: $N = 162$. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Factor	Δ n_{corr}	SEM	*df*	*t*	*p*	β
Time	12.722	0.893	806	14.245	< 0.001***	0.491
Pre-training	1.104	0.048	158	23.075	< 0.001***	0.747
Anodal	-6.696	4.990	158	-1.342	0.182	0.052
Cathodal	-1.430	5.012	158f	-0.285	0.776	0.029
Anodal:time	2.656	1.170	806	2.270	0.024*	0.045
Cathodal:time	0.962	1.174	806	0.819	0.413	0.016

Supplementary Table 3. Results from the linear mixed model at the intensity level (subgroup of polarity). All calculations use the sham group for comparison. Number of subjects: $N = 103$. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Factor	Δ n_{corr}	SEM	*df*	*t*	*p*	β
Time	12.722	0.873	511	14.579	< 0.001***	0.480
Pre-training	1.135	0.062	99	18.432	< 0.001***	0.759
1 mA	-8.367	5.733	99	-1.460	0.148	0.074
2 mA	-5.246	5.714	99	-0.918	0.361	0.018
1 mA:time	3.690	1.361	511	2.711	0.007**	0.056
2 mA:time	1.621	1.362	511	1.190	0.235	0.025

Supplementary Table 4. Results from the linear mixed model at the laterality level (subgroup of polarity and intensity). All calculations use the sham group as a reference. Number of subjects: $N = 73$. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Factor	Δ n_{corr}	SEM	*df*	*t*	*p*	β
Time	12.722	0.883	362	14.410	< 0.001***	0.486
Pre-training	1.173	0.080	69	14.634	< 0.001***	0.743
Left	-13.750	7.210	69	-1.907	0.061	0.049
Right	-3.652	7.222	69	-0.506	0.615	0.079
Left:time	4.400	1.736	362	2.534	0.012*	0.061
Right:time	2.981	1.736	362	1.717	0.087	0.041

Supplementary Table 5. Results from the linear mixed model at the single group level including all subjects. All calculations use the sham group for comparison. Number of subjects: $N = 162$. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Factor	Δ n_{corr}	SEM	*df*	*t*	*p*	β
Time	12.722	0.878	800	14.483	< 0.001***	0.491
Pre-training	1.108	0.049	152	22.474	< 0.001***	0.749
A1L	-13.230	7.480	152	-1.769	0.079	0.039
A1R	-3.037	7.484	152	-0.406	0.686	0.061
A2L	-3.114	7.495	152	-0.416	0.678	0.021
A2R	-7.428	7.481	152	-0.993	0.322	0.045
C1L	6.330	7.533	152	0.840	0.402	-0.017
C1R	0.422	7.470	152	0.056	0.955	0.046
C2L	-12.450	7.475	152	-1.665	0.098	0.008
C2R	-0.032	7.665	152	-0.004	0.997	0.031
A1L:time	4.400	1.727	800	2.547	0.011*	0.044
A1R:time	2.981	1.727	800	1.726	0.085	0.030

A2L:time	-0.092	1.729	800	-0.053	0.958	0.000
A2R:time	3.331	1.727	800	1.928	0.054	0.034
C1L:time	-2.038	1.727	800	-1.180	0.238	-0.021
C1R:time	1.626	1.727	800	0.942	0.347	0.016
C2L:time	3.072	1.727	800	1.778	0.076	0.031
C2R:time	1.204	1.773	800	0.679	0.497	0.012

Supplementary Table 6. Results from the linear mixed model at the single group level as a sensitivity analysis. Hereby we included only subjects who thought to have received verum stimulation. All calculations use the sham group for comparison. Number of subjects: $N = 136$. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Factor	Δ n_{corr}	SEM	*df*	*t*	*p*	β
Time	12.965	1.071	670	12.101	< 0.001***	0.498
Pre-training	1.096	0.052	126	21.154	< 0.001***	0.747
A1L	-5.594	8.464	126	-0.661	0.510	0.070
A1R	-0.807	8.240	126	-0.098	0.922	0.074
A2L	0.301	8.044	126	0.037	0.970	-0.005
A2R	-3.531	7.850	126	-0.450	0.654	0.067
C1L	12.276	8.100	126	1.516	0.132	-0.004
C1R	5.709	8.021	126	0.712	0.478	0.057
C2L	-8.377	7.847	126	-1.068	0.288	0.028
C2R	4.662	9.039	126	0.516	0.607	0.070
A1L:time	3.914	1.980	670	1.977	0.049*	0.039

A1R:time	2.890	1.926	670	1.501	0.134	0.030
A2L:time	-0.245	1.880	670	-0.130	0.897	-0.002
A2R:time	3.089	1.835	670	1.683	0.093	0.034
C1L:time	-2.869	1.878	670	-1.528	0.127	-0.031
C1R:time	0.762	1.878	670	0.406	0.685	0.008
C2L:time	2.830	1.835	670	1.542	0.124	0.031
C2R:time	1.864	2.116	670	0.881	0.379	0.017

11

Supplementary Table 7. Flanker task mean reaction times in seconds (standard deviations in parentheses).

	Pre-training (*N* = 161)			Post-training (*N* = 161)			Follow-up (*N* = 160)		
Condition	Congruent	Incongruent	Neutral	Congruent	Incongruent	Neutral	Congruent	Incongruent	Neutral
S	0.524 (0.077)	0.620 (0.087)	0.547 (0.082)	0.482 (0.056)	0.563 (0.062)	0.503 (0.063)	0.460 (0.055)	0.540 (0.054)	0.489 (0.057)
A1L	0.500 (0.060)	0.611 (0.095)	0.519 (0.056)	0.461 (0.031)	0.561 (0.048)	0.487 (0.027)	0.466 (0.034)	0.558 (0.057)	0.487 (0.032)
A1R	0.521 (0.042)	0.613 (0.070)	0.550 (0.064)	0.477 (0.036)	0.569 (0.067)	0.509 (0.046)	0.442 (0.048)	0.526 (0.053)	0.467 (0.045)
A2L	0.505 (0.073)	0.618 (0.125)	0.531 (0.080)	0.476 (0.076)	0.571 (0.095)	0.510 (0.104)	0.445 (0.040)	0.529 (0.047)	0.465 (0.047)
A2R	0.521 (0.064)	0.609 (0.078)	0.547 (0.069)	0.474 (0.036)	0.550 (0.048)	0.493 (0.046)	0.431 (0.043)	0.509 (0.040)	0.464 (0.055)
C1L	0.511 (0.065)	0.618 (0.098)	0.530 (0.071)	0.476 (0.038)	0.553 (0.057)	0.486 (0.039)	0.468 (0.033)	0.549 (0.046)	0.486 (0.040)

C1R	0.526	0.641	0.558	0.476	0.559	0.499	0.432	0.519	0.463
	(0.086)	(0.119)	(0.099)	(0.048)	(0.054)	(0.050)	(0.058)	(0.067)	(0.073)
C2L	0.493	0.574	0.523	0.476	0.547	0.489	0.443	0.518	0.466
	(0.059)	(0.062)	(0.067)	(0.059)	(0.060)	(0.061)	(0.058)	(0.053)	(0.062)
C2R	0.498	0.575	0.517	0.450	0.527	0.476	0.422	0.506	0.451
	(0.040)	(0.055)	(0.040)	(0.034)	(0.044)	(0.032)	(0.034)	(0.037)	(0.034)

12

13

Supplementary Table 8. Test statistics for the results of the flanker task from pre-training to post-training and pre-training to follow-up at the single group level. Analysed were reaction times from pre- to the respective following session (either post-training or follow-up) via repeated measures ANOVA. In cases where assumptions of sphericity were violated, Greenhouse-Geisser (GG) corrections were applied. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Factor	Pre-training to post-training	Pre-training to follow-up
Time	$F(1, 152) = 121.076$, $p < 0.001$***, $\eta^2 = 0.443$)	$F(1, 151) = 281.302$, $p < 0.001$***, $\eta^2 = 0.651$)
Time*condition (GG)	$F(8, 152) = 0.797$, $p = 0.606$, $\eta^2 = 0.040$)	$F(8, 151) = 2.469$, $p < 0.015$**, $\eta^2 = 0.116$)
Trial type (GG)	$F(1.481, 225.124) = 507.963$, $p < 0.001$***, $\eta^2 = 0.770$)	$F(1.462, 220.719) = 512.745$, $p < 0.001$***, $\eta^2 = 0.773$)
Trial type*condition (GG)	$F(11.849, 225.124) = 1.002$, $p = 0.447$, $\eta^2 = 0.050$)	$F(11.694, 220.719) = 0.900$, $p = 0.545$, $\eta^2 = 0.046$)
Time*trial type (GG)	$F(1.756, 266.904) = 11.115$, $p < 0.001$***, $\eta^2 = 0.068$)	$F(1.646, 248.614) = 13.028$, $p < 0.001$***, $\eta^2 = 0.079$)
Time*trial type*condition (GG)	$F(14.048, 266.904) = 1.065$, $p = 0.390$, $\eta^2 = 0.053$)	$F(13.172, 248.614) = 0.939$, $p = 0.514$, $\eta^2 = 0.047$)
Condition	$F(1, 8) = 0.669$, $p = 0.718$, $\eta^2 = 0.034$)	$F(1, 8) = 0.813$, $p = 0.592$, $\eta^2 = 0.041$)

Supplementary Table 9. Mean intensities of adverse effects (standard deviations in parentheses), ranging from 1 (not at all) to 5 (extremely).

Miscellaneous = individually reported occurrences such as vertigo, metallic taste in mouth, sensation of warmth. *N* = 162.

Condition	Tingling (head)	Tingling (electrode)	Fatigue	Itching	Headache	Nausea	Miscellaneous
S	1.279 (0.630)	2.837 (1.271)	1.326 (0.522)	1.860 (1.125)	1.395 (0.791)	1.116 (0.448)	1.419 (1.052)
A1L	1.200 (0.561)	2.933 (1.100)	1.667 (1.047)	2.133 (1.187)	1.200 (0.561)	1.000 (0.000)	1.067 (0.258)
A1R	1.200 (0.561)	3.000 (1.134)	1.133 (0.352)	1.800 (0.941)	1.067 (0.258)	1.067 (0.258)	1.267 (0.704)
A2L	1.333 (0.816)	3.600 (1.183)	1.000 (0.000)	3.067 (1.223)	1.133 (0.352)	1.000 (0.000)	1.733 (1.223)
A2R	1.267 (0.799)	3.733 (1.223)	1.400 (0.632)	2.533 (1.246)	1.200 (0.561)	1.000 (0.000)	2.067 (1.486)
C1L	1.267 (0.458)	2.667 (0.900)	1.267 (0.594)	1.867 (0.834)	1.267 (0.594)	1.067 (0.258)	1.600 (1.056)
C1R	1.133 (0.352)	3.000 (1.309)	1.533 (0.834)	1.800 (0.862)	1.000 (0.000)	1.000 (0.000)	1.267 (0.799)

C2L	1.067 (0.258)	3.267 (1.163)	1.400 (0.910)	1.733 (1.387)	1.267 (0.594)	1.000 (0.000)	1.933 (1.163)
C2R	1.786 (1.188)	3.786 (1.051)	1.214 (0.579)	2.714 (1.326)	1.214 (0.426)	1.071 (0.267)	1.571 (1.284)

15

A.1.3 'Gamification Improves Antidepressant Effects of Cognitive Control Training—A Pilot Trial'

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Gamification improves antidepressant effects of cognitive control training—A pilot trial

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Objective: Computerised cognitive trainings have been put forward to improve control over negatively biased information processing and associated depressive symptomatology. Yet, disease-related impairments of motivation and endurance, as well as insufficient accessibility hinder use of this promising therapeutic opportunity. Here, we developed an app (*de:)press*©) that utilizes a cognitive control training (paced auditory serial addition task) enriched with gamification and information elements. We compared a six-week training with *de:)press*© to a non-gamified version (active control group).
Methods: Thirty-two depressed participants were included. Each received either *de:)press*© or the non-gamified version and was instructed to train three times per week for two weeks. Afterwards (four weeks) they were free to train at their own discretion. Depression severity was assessed during training and two follow-up sessions. Primary endpoint was defined as difference between groups [change of Montgomery-Åsberg Depression Rating Scale (MADRS)] four weeks after end of training.
Results: Depression severity decreased in both groups. At primary endpoint, MADRS scores were significantly lower in the *de:)press*©-group compared to the control group. No differences were observed at three months' follow-up. Intervention usability was consistently rated positively. Participants who had trained with *de:)press*© maintained the recommended training frequency without further prompting. Besides transient fatigue or frustration, no adverse effects were observed.
Conclusion: This pilot demonstrates that gamification and information elements can substantially increase cognitive control training efficacy in alleviating depressive symptoms. Moreover, it provides first evidence for the feasibility and efficacy of *de:)press*© as an add-on intervention to treat depression.
Clinical trial registration: The study is registered under ClinicalTrials.gov, identifier: NCT04400162.

KEYWORDS

cognitive control, gamification, depression, cognitive control training, digital intervention, APP

Introduction

Major depressive disorder (MDD) is a very common cause of morbidity and mortality that presents with low mood, loss of joy, hopelessness, lack of motivation, brooding, and other symptoms (1). Standard and mostly effective treatment approaches for MDD encompass psychotherapy, medication, and brain stimulation. Nevertheless, insufficient symptom relief remains a significant therapeutic challenge. This clinically relevant proportion of therapy-resistant symptomatology suggests that the available standard treatment does not sufficiently consider the pathophysiological variability (2), is not yet targeted enough or is underutilised due to lack of tolerance, high treatment costs, limited mobility, long waiting lists, lack of motivation, and concerns regarding stigma and privacy (3, 4). An expansion of therapeutic options would therefore be highly desirable.

Recent comprehensive evidence demonstrates that depression is linked with a wide range of cognitive deficits which for instance are indicated by dysfunctions in executive control, working memory, and processing speed (5). These impairments substantially affect quality of life (6) and represent a critical mediator of the association between depression and impaired psychosocial functioning (7). Even more importantly, this attenuated cognitive control (CC) is a critical factor in the development and maintenance of depression by means of a more salient experience and also preferential processing of negative information (*negativity bias*) (8–11). Consistent with Beck's cognitive model of depression (12, 13) attentional resources are withdrawn from external environment and predominantly allocated to negative internal experiences resulting in symptoms of depression (e.g., sadness, rumination, loss of motivation, hopelessness) (14). Moreover, the negative interpretation and negatively biased attention constitutes a feedback loop with mutually reinforcing subjective and behavioural symptoms. Therefore it can be derived that negatively biased cognition is not only a symptom of the acute depressive state but also a key pathophysiological factor. Consistently, studies with pharmacological (15), psychological (16), and neuromodulatory (17) interventions indicate that most of the effective treatments are linked with the normalisation of these biases (18) and an improvement of emotion regulation capacities (19), suggesting that improving CC—and in turn balancing out negativity biases—can be a viable addition to treatment. From the neurophysiological perspective, impaired cognitive control in depression is linked with a decreased prefrontal top-down regulatory influence on bottom-up activity (e.g., amygdala, hippocampus, and cingulate cortex) (8).

Based on this notion, cognitive control trainings (CCT) have been put forward as new ways to improve CC on negatively biased information processing and the associated depressive symptomatology (10, 20–22). Additionally, correcting the processing of such information may prove an effective tool for secondary prevention (23–26). In sum, CCT can be considered as a promising new tool for a multi-dimensional individualised treatment of MDD.

Training of cognitive or behavioural skills harnesses neuroplasticity to achieve clinical gains. It is assumed, that *via* constant and targeted exercise, critically weakened brain circuits will be strengthened and the associated control mechanisms will be restored. Therefore, to support clinically relevant and meaningful adaptive neuroplasticity, systems neuroscience-based circuit-specific trainings should be especially promising (27).

Yet, the number of clinical trials is small and hampers the drawing of conclusions on clinical utility of CCT (26). Clinical evidence for the efficacy of CCT predominantly comes from smaller laboratory studies, often with analogue mild depressed samples showing mixed results—full-scale controlled clinical trials with MDD patients are scarce. Nevertheless, recent meta-analyses indicate a small to medium effect size of CCT on mood and cognitive symptoms in MDD (28, 29). Naturally, methodological concerns must also be considered: nonspecific factors including patients' expectancy, engagement, novelty, and motivation (20) regarding the presented intervention may support efficacy of CCT. While these elusive factors cast doubt regarding the concrete mechanism of action, it has to be considered that, among others, environmental enrichment (30), reward (31), novelty (32) and background network activity (33) represent critical elements of the complex conditional structure within which adaptive neuroplasticity exists. Depending on the research question, these factors should be thoroughly assessed in future studies. For example, lack of motivation and decreased frustration tolerance can inhibit successful implementation and thus lower the effectiveness of CCT for the treatment of depression. Supporting and strengthening user engagement as well as training adherence may substantially improve efficacy (34, 35).

To address these challenges, we utilised gamification principles such as integrating psychoeducative elements, unlockable levels and progression tracking to enrich a digital and individually adaptive training paradigm: the *Paced Auditory Serial Addition Task* (PASAT) (36). This task has shown to have beneficial potential in supporting the treatment of depressive symptomology (37–40). Originally used for neurological testing (36), it was later applied in depressed patients as they exhibit decreased function of CC networks and re-activation of these networks can enhance cognitive functioning (40). The PASAT requires continuous attention, challenges the brain's processing speed by presenting stimuli with the individually determined minimal inter-stimulus interval, and trains the participant's ability to overcome distractions from negative feedback. The PASAT has shown to be quite demanding, monotonous, and sometimes frustrating (41, 42). It transiently induces negative affect (43, 44) as well as mental stress, indicated by increased cortisol levels (45).

TABLE 1 Comparison between the apps that CG and IG received.

Topic	Control group (CG)	Intervention group (IG)
Setting	Apart from PASAT instructions, there was no further explanation on reasoning or mechanisms of the task within the app. However, similarly to the IG group, the CG group was briefly given information on why the PASAT was chosen for this study.	A narrative that encapsulated the PASAT and its working mechanisms in a meaningful setting. Participants were taught about the biological and psychological background of depression aetiology (and possible supportive treatment options). This included artwork and other design options of the app.
Meaning and purpose	No additional information was given.	This provided participants with a theme that elaborated on the training's purpose: both in helping to improve their quality of life as well as giving them the opportunity for expressing feedback on game development and steering it in a useful, user-oriented direction. Participants were encouraged to browse through the different areas within the app and thus explore more of the background information on their own.
Progression	No feedback on progression was given.	The group was able to see their training progression over time *via* animated graphs. This was done to create an incentive to keep up with the training schedule and foster interest in continuing. Participants were made aware that drops in performance should be expected and to not be discouraged by them.
Levelling	No levels to unlock (ascending keyboard layout only).	If keeping up with the training schedule, participants could unlock further difficulty levels (ascending, descending, randomised keyboard layout) while the task itself remained the same. We included them to prevent ceiling effects in task performance, reset muscle memory, and increase cognitive load. Participants were allowed to switch freely between the unlocked levels during each training session.
Immediate feedback	Red or green screen after each trial, indicating whether the last response was wrong or right.	Identical to CG.
Long-term feedback	None.	Animated graphs on performance and training count, unlocking of achievements, interactions with avatars (see following lines).
Achievements/ rewards	None.	When keeping up with the training schedule, participants unlocked up to 10 achievements (e.g., psychoeducation and information on the brain, the task, MDD, etc.). These achievements were also used to strengthen the "setting" and "meaning and purpose" aspect of the app.
Avatar	None.	An animated avatar acted as a "training companion" by guiding participants through the app, appearing in crucial screens, and visualise key components in the respective screens.

For best effects, this CCT needs to be performed on a regular basis over the course of at least several weeks. It can be assumed that the integration of gamification elements into the PASAT training (35) will likely improve the clinical feasibility and efficacy of CCT in the treatment of MDD. Various forms of gamification, i.e., the use of gaming elements in non-game contexts (46), were introduced to the PASAT to aid motivation and adherence to the task (47), resulting an easy to use mobile app (*de:)press*©). Each element we used (for a list see **Table 1**) can be categorised into one of five main dimensions of gamification: purpose, feedback, ownership, challenge, reward (46, 48–50). Providing the user with an elaborative context on the working mechanisms of a task has shown to not only add purpose to the training, but also allowing participants to set their own goals on what they want to achieve, which finally in itself is meant to increase motivation (51). The addition of feedback on performance creates a form of reinforcement, further fostering adherence to stick to the training paradigm (47).

To test feasibility and efficacy of *de:)press*© training in addition to standard treatment of MDD, we compared the gamified training to the same PASAT training paradigm without any gamification elements in a randomized controlled pilot trial. We expected sustainable reduction of MDD symptoms 4 weeks after a 6-week intervention phase.

Materials and methods

Ethics

The study was conducted in accordance with the declaration of Helsinki on the ethical principles of medical research involving human subjects. The ethics committee of the University Hospital Tübingen gave their positive vote on the protocol for this study.

Participants and study groups

In a previous study that used the PASAT (40), large reductions in depressive symptoms and rumination ($d = 1.26$ /

$d = 1.28$) were observed. To reproduce these effects with high power ($\alpha = 0.05$; $1—\beta = 0.95$), a total of $n = 11$ participants per group would be needed. This relatively small sample size would be sufficient to provide first effect size estimates of the gamified training and to allow for larger high-quality follow-up trials. To ensure more robust results, we increased that number and did enrol 16 participants per group.

Out of 55 persons 32 adult applicants (female and male) met the inclusion criteria. They were diagnosed with acute or chronic recurrent major depressive disorder (MDD) and were recruited through the University of Tübingen mailing lists, posters, and flyers displayed around campus. Participants were randomly assigned to either the *control group* (CG) or the *intervention group* (IG; $n = 16$ respectively). While the CG received the "bare" CCT without any gamification elements, the IG received the same CCT enriched with several motivational and educational elements (see section *Gamified Cognitive Control Training: de:)press*©).

Inclusion criteria: at least 18 years old, ability to give consent, appropriate knowledge of German (at least CEFR level B), current MDD (F32, F33) as diagnosed by the Mini-International Neuropsychiatric Interview, light to moderate manifestation of the MDD as defined by a MADRS score between 10 and 34 at the time of the first study visit, either no or stable antidepressant/psychoactive medication (since at least 6 weeks before inclusion in the study).

Exclusion criteria: psychotic symptoms or schizophrenia, dementia, a history of epilepsy, other mental disorders (current or in the past—an exception to this rule were anxiety phobic or panic disorders as they often occur concurrently with MDD), suicidality.

Gamified cognitive control training: *de:)press*©

As a CCT we chose the PASAT. This task has proven to be a frustrating challenge regardless of cognitive state as it adapts to a participant's performance and provides a continuous cognitive challenge (36, 42). Participants were each given a tablet computer which had the PASAT installed in a "kiosk mode", allowing only interaction with the task and no other tablet functions. Hence, for this study, participants were not required to use their own devices or go through the install process themselves. No updates were deployed over the course of the study, each participant received the same final version. The app is a native Android app and registered under the name *de:)press*©. It requires at least Android 8.1.0 and 2 GB of RAM and was developed with Android Studio. All data collected and processed by the app remained on the device until exported to perform statistics, no internet connection was needed.

Participants were presented with a continuous auditory stream of single digit numbers with an initial *inter-stimulus interval* (ISI) between digit presentations of 3 s. Participants were then instructed to add the last two digits they heard to each other: the current digit and the digit that was presented directly before it. Answers were given by pressing the respective answer button shown on the screen. See **Figure 1** for a visual representation of the task and **Figure 2** for screenshots of the app. In general, to successfully perform in this CCT, participants must stay focused and not let themselves be distracted by errors, the provided feedback of their recent addition (green screen for correct responses, red screen for wrong, late, or non-responses), or negative thoughts. Four consecutively correct answers shortened the ISI by 0.1 s, four consecutively wrong answers lengthened the interval by the same amount. Consequently, the PASAT adapted to individual performance and provided a continuous challenge. The task was divided into three blocks, each block lasting five minutes. Blocks were intercepted by short breaks (30 s) and an initial countdown of 30 s, amounting to a training duration of 16 min 30 s per session. The ISI was carried over from block to block, however it was reset for each new training session.

Gamification elements of the cognitive training

To specifically test the additional effects of gamification elements and mediation of purpose-driven motivation, the app was kept as minimal as possible for the CG, while the IG received the enriched training. **Table 1** highlights the main differences between the two versions. Please refer to **Figure 2** for screenshots of the app.

Study timeline

Before taking part in the study, all participants gave written informed consent. They were to attend 5 sessions (t1–t5, see **Figure 3**), each during which they answered questionnaires and took part in psychological interviews. Half the participants of each group attended an additional session 2 weeks before start of the training (t0). This was done to evaluate possible changes in depressive symptomatology prior to our intervention. In t1, all participants were given the tablet with the CCT installed on it. In the 2 weeks between t1–t2 they were instructed to train at least every second day (equal to 3 times per week). From t2–t3 (4 weeks) they were asked to train as often as they saw fit. In t3 they returned the tablets. Four weeks after end of training (t4) main outcomes were assessed, final follow-up was 12 weeks after end of training (t5).

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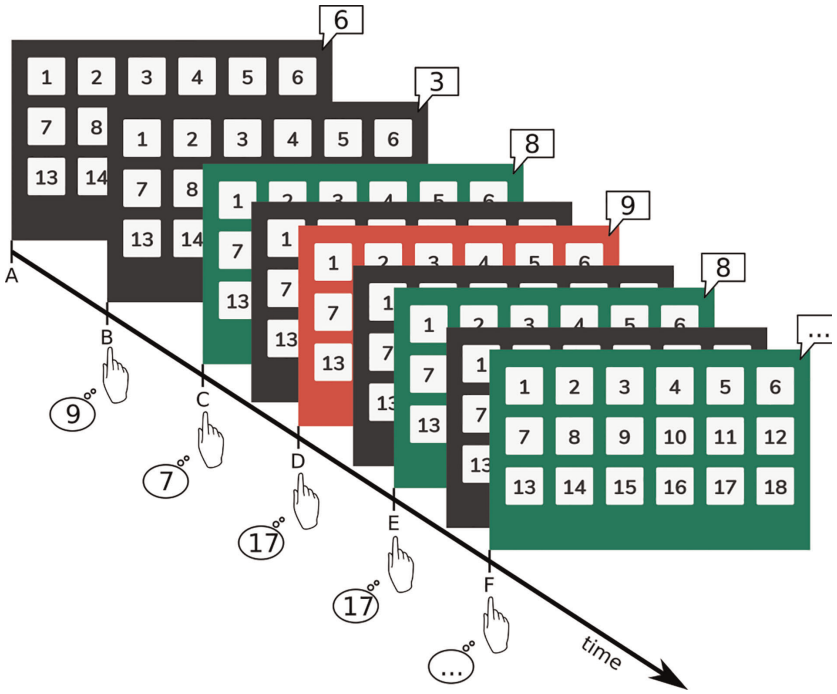

FIGURE 1
Visual representation of the PASAT. Participants heard single digit numbers (here shown in speech bubbles) from the tablet's speakers and were asked to add the last digit to the second-to-last digit (e.g., digits at timepoints A + B, B + C, C + D, and so forth). Numbers were presented with an initial interval of 3 s. Answers were then given on the keyboard. For correct answers the screen briefly flashed green, for wrong answers the screen flashed red and then immediately return to a dark background. This feedback was given *concurrently* to the following digit presentation (e.g., green feedback at E refers to the correct result given for the addition of C + D).

Questionnaires

Montgomery-Åsberg depression rating scale (MADRS)

The MADRS (52) is a semi-structured interview to assess MDD severity. The assessment period is the previous week and consists of 10 items, each of which is rated on a 7-point scale from 0 to 6 by a trained psychologist. The psychologists who performed the ratings were blind to the intervention that each participant received. The MADRS is considered the gold standard for measuring the severity of depressive symptoms (53), especially because its high sensitivity to changes.

Inventory of depressive symptomatology, self-report version (IDS-SR)

The Inventory of Depressive Symptoms (IDS-SR) is a 28-item self-report depression scale utilised to determine the severity of depressive symptoms (54, 55).

WHO-Five-Well-Being Index (WHO5)

The WHO5 is a short (5 items) self-report questionnaire designed to assess overall well-being. It has been recommended by the World Health Organization as a screening questionnaire for depression and is suitable as an outcome for clinical trials (56).

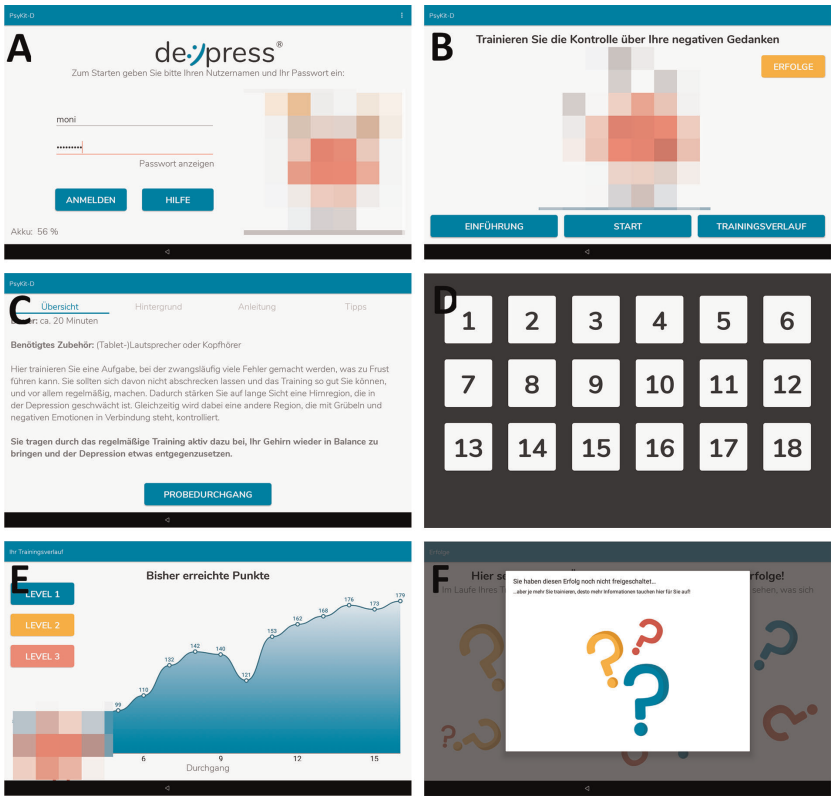

FIGURE 2
Screenshots from the IG app. (**A**) Login screen. (**B**) Training hub, from which participants were able to chose which area of the app they wanted to explore. (**C**) Introduction and instructions for the training. Furthermore, psychoeducative information was provided. (**D**) Keyboard layout during training. This layout shows the first level with an ascending button layout. (**E**) Progression graph that shows the number of correct responses within each training session ("highscore"). (**F**) Achievements that can be unlocked during training. In this screenshot no achievements have been unlocked yet. The CG app consisted of screens A (without avatars), (**C**,**D**).

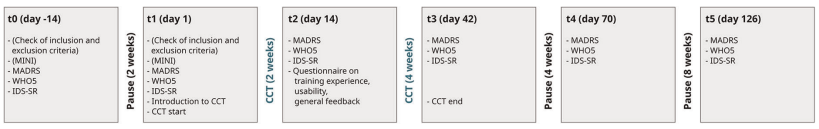

FIGURE 3
Study timeline showing the content of each session.

TABLE 2 Demographic and general data.

Measure	Control Group (CG)	Intervention Group (IG)
Age range (min-max)	18–63	21–76
Mean age (±SD)	30.00 ± 13.33	40.19 ± 16.63
Sex (f/m)	9/7	10/6
Psychotherapy (y/n)	9/7	6/10
Medication (y/n)	9/7	5/11
Mean number of trainings (t1–t2)	5.69 ± 4.11	6.47 ± 1.92
Mean number of trainings (t2–t3)	8.81 ± 8.39	12.80 ± 10.61
Mean number of trainings (total)	14.50 ± 11.39	19.27 ± 10.92

Usability and general user feedback

Participants answered 18 custom questions regarding the usability, stability, and design of the software, as well as the training paradigm itself. Additionally, there were 7 free-text questions for participants to give feedback and recommendations on the training and software.

Statistical analyses

All statistical analyses were done with IBM SPSS version 27 (57) and R version 4.0.4 (58). The factors used in the statistical models are defined as such: *group* (CG or IG) and *time* (session during which the measurement was taken, t0–t5).

We used t-tests to analyse differences between study samples (measured at t1) and usage frequency (measured at t3). Distributions within groups (sex, current pharmacotherapy, current psychotherapy) were measured *via* fisher's exact tests. Possible changes during the pre-training phase (t0–t1) were measured *via* t-tests. A linear mixed model (LMM with restricted maximum likelihood estimation) was used to analyse the development of depression symptoms from start of training to primary endpoint (t1–t4) as this method is most robust against single missing sessions (see following sections for drop-out rates). Fixed effects were scores of the respective questionnaire, group, session during which the measurement was taken, the interactions between scores and session, and baseline scores of the questionnaire. Random effects were measurement timepoint and individual subject. Post-hoc analyses of t4 (primary end point) and t5 (follow-up) were done *via* t-tests.

Results

See **Table 2** for an overview on demographic data and **Table 3** for the scores of each interview and questionnaire. See **Figures 4–6** for a visual representation of primary and secondary outcomes.

Overview of the study sample and pre-training phase (t0−t1)

Study sample (t1)

There were no differences between groups in age ($t(30) = -1.912$, $p = 0.065$, $d = -0.676$). The distribution of sexes ($p = 1$), participants taking any form of psychiatric medication ($p = 0.285$), and participants undergoing psychotherapy ($p = 0.479$) was equal in both groups.

Pre-training phase (t0−t1) and baseline (t1)

In the waiting groups there were no significant changes from t0 to t1 in either MADRS or WHO5. However, IDS-SR scores in the IG lowered significantly during this period. See **Table 4** for an overview of the statistics.

Scores at start of training (t1) were not significantly different between CG and IG for MADRS [$t(30) = 0.527$, $p = 0.603$, $d = 0.186$] and WHO5 [$t(30) = -0.545$, $p = 0.590$, $d = -0.193$]. Yet, the IDS-SR was significantly higher for IG [$t(30) = 2.605$, $p = 0.014$, $d = 0.921$], denoting a higher perceived depressive symptomatology within this group.

TABLE 3 Scores for questionnaires and interviews per session. Shown are mean and standard deviations (±SD).

Timepoint	CG			IG		
	MADRS	IDS-SR	WHO5	MADRS	IDS-SR	WHO5
t0	21.13 ± 4.73	33.36 ± 7.23	7.25 ± 3.88	20.62 ± 5.21	35.86 ± 3.91	5.75 ± 1.70
t1	21.25 ± 7.19	35.56 ± 9.74	6.75 ± 3.91	20.19 ± 3.67	28.06 ± 6.15	7.56 ± 4.50
t2	20.19 ± 8.25	32.69 ± 11.28	8.13 ± 6.12	19.81 ± 7.56	26.88 ± 7.61	7.88 ± 4.22
t3	18.00 ± 9.69	26.31 ± 10.80	10.77 ± 6.65	15.94 ± 7.93	22.56 ± 10.68	10.63 ± 5.19
t4	20.62 ± 11.99	30.54 ± 12.74	9.62 ± 6.51	11.38 ± 5.04	18.62 ± 6.49	12.00 ± 4.22
t5	13.62 ± 9.83	26.38 ± 10.56	12.38 ± 5.49	12.86 ± 7.43	21.08 ± 11.49	12.00 ± 5.33

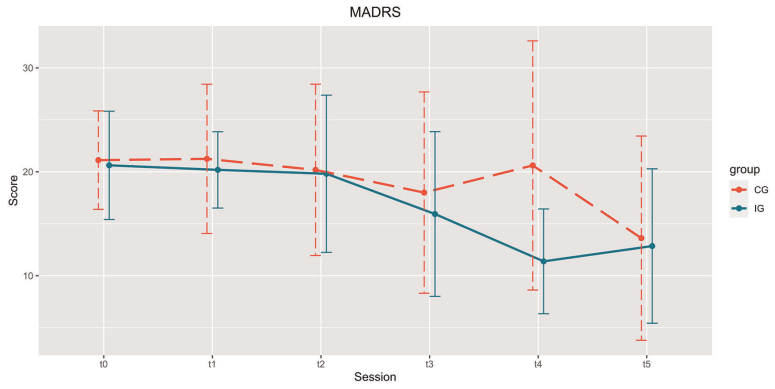

FIGURE 4
Development of the MARDS scores over the course of the study. Depicted are means and standard deviations (SD). Participants of the IG showed significantly higher improvement in the reduction of depression scores up until 4 weeks after the end of the training (t4).

Training and primary endpoint (t1–t4)

Primary endpoint: MADRS

Compared to the CG, gamified training led to a significantly stronger alleviation of depressive symptoms during the intervention and the following 4 weeks [*group* x *time*: $t(85) = -2.395$, $p = 0.019$, $B = -2.652$, $\beta = 1.107$]. The main effects *group* [$t(85) = 1.881$, $p = 0.063$, $B = 4.258$, $\beta = 2.264$] and *time* [$t(85) = -0.101$, $p = 0.920$, $B = -0.080$, $\beta = 0.794$] were not significant. Post-hoc comparison between IG and CG at t4 (CG: 20.62 ± 11.990, IG: 11.38 ± 5.042) showed a significant superiority of the gamified training [$t(16.116) = 2.559$, $p = 0.021$] with large effect size ($d = 1.004$). The comparison between MADRS scores in t1 and t4 for the IG shows a significant improvement [$t(12) = 5.503$, $p < 0.001$, $d = 1.526$], which is not found in the comparison between t1 and t4 in the CG.

IDS-SR

There was a significant reduction of the total score for all patients [main effect *time*, $t(84) = -1.984$, $p = 0.051$, $B = -1.870$, $\beta = 0.942$], but no significant interaction between *group* and *time* [$t(84) = -0.843$, $p = 0.401$, $B = -1.088$, $\beta = 1.290$], and no main effect of *group* [$t(84) = 0.962$, $p = 0.339$, $B = 2.311$, $\beta = 2.403$].

WHO5

There was no significant increase in WHO5 scores in either group for main effect *time*, $t(84) = 1.480$, $p = 0.143$, $B = 0.815$, $\beta = 0.551$), between *group* x *time* [$t(84) = 1.022$, $p = 0.310$, $B = 0.756$, $\beta = 0.739$] or main effect *group* [$t(84) = -1.143$, $p = 0.256$, $B = -1.434$, $\beta = 1.255$].

Follow-up (t5)

MADRS

Twelve weeks after the end of the intervention, the total sample showed a significant reduction in MADRS score compared to baseline [*t*1: 20.67 ± 5.428, t5: 13.22 ± 8.505; $t(26) = 4.281$, $p < 0.001$, $d = 0.824$].

IDS-SR

There was a significant decrease in self-reported depressive symptomatology for the whole group [t1: 32.56 ± 9.106, t5: 23.84 ± 11.116; $t(24) = 4.256$, $p < 0.001$, $d = 0.851$].

WHO5

There was a significant increase in overall well-being for the total sample [t1:7.04 ± 4.449, t5: 12.20 ± 5.299; $t(24) = -4.151$, $p < 0.001$, $d = -0.830$].

Usage

Usage frequency

From t1–t2, during which the participants were instructed to train at least 3 times per week, IG trained 6.46 ± 1.92 times on average, CG 5.69 ± 4.11 times on average. During the following four weeks (t2–t3), participants were asked to

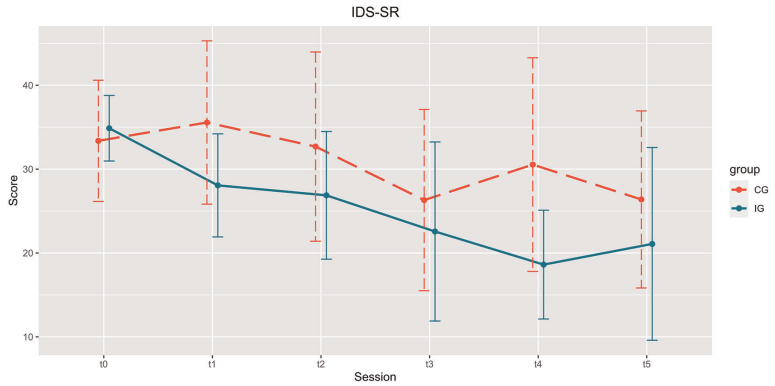

FIGURE 5
Development of the IDS-SR scores over the course of the study. Depicted are means and standard deviations (SD).

exercise as often as they found helpful. In the IG, this was on average 12.80 ± 10.61 times, in the CG amount of training sessions was 8.81 ± 8.39 times. Hence, we can conclude that the IG maintained the recommended training frequency of 3 times per week without further prompting. However, the difference in the number of total training session between *de:)press*© and the non-gamified PASAT was not statistically significant in this sample (19.267 ± 10.924 vs. 14.500 ± 11.390; $t = -1.188$; $p = 0.245$, $d = -0.427$).

Usability

Regardless of intervention type, the training was perceived positively: reliability and overall feedback of the software reached 82.81% (where +100% corresponds to a maximally positive evaluation, 0% to a neutral evaluation, and −100% to a maximally negative evaluation). Design and usability reached 74.06%. The training itself scored 48.05%. In a questionnaire on the intuitive use of the system no differences were found between the groups.

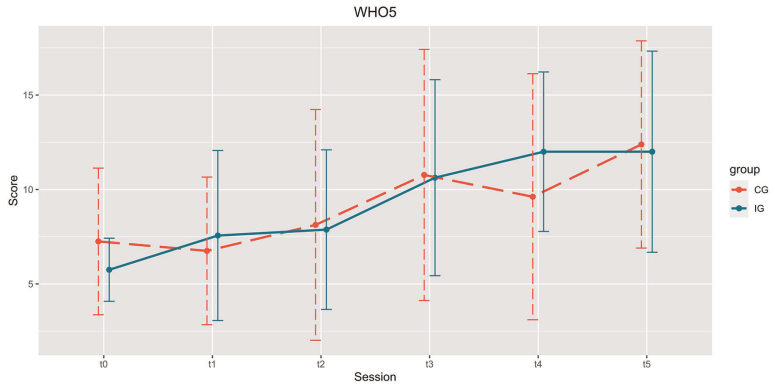

FIGURE 6
Development of the WHO5 scores over the course of the study. Depicted are means and standard deviations (SD).

TABLE 4 Statistical analysis of the pre-training phase (t0t1).

	CG	IG
MADRS	t (7) = 0.751, p = 0.477, d = 0.265	t (7) = 0.632, p = 0.547, d = 0.224
IDS-SR	t (7) = 0.081, p = 0.938, d = 0.028	t (7) = 2.799, p = 0.027, d = 0.990
WHO5	t (7) = 0.000, p = 1.000, d = 0.000	t (7) = −1.910, p = 0.098, d = −0.675

There were no significant changes in either group during this phase except for IDS-SR scores in the IG, which lowered significantly.

Side effects

No severe side effects were reported. Fatigue was occasionally reported as occurring directly after the training, which is an expected outcome of a demanding cognitive training. Participants also reported frustration during the task, however this subsided the longer the training was continued.

Drop-out rates and aborted training sessions

Some subjects were not able to attend certain sessions but might have been available at later sessions again. During the training phase no subject dropped out in the IG but 2 in the CG (missing values at t3). At the 4 weeks follow-up assessment (t4) 3 subjects were not available in either group. At the 12-weeks follow-up (t5) data from 2 subjects in the IG and 3 in the CG were missing. No training sessions were terminated prematurely.

Discussion

With this study, we tested the feasibility of an app-based, gamified PASAT training (*de:)press*©) and its effect on depression severity. To focus on the relevance of gamification and to allow for a meaningful effect-size estimation of *de:)press*©, a non-gamified PASAT was used as active control condition. With *de:)press*©, we found a greater decrease in depressive severity (MADRS) during and up to four weeks after the intervention. Additionally, both depression severity and usage frequency were more stable in the enriched compared to the control version of the CCT. These findings indicate that *de:)press*© has the potential for an adjunctive treatment of depression and that the antidepressive effect of this gamified digital health intervention may even surpass the PASAT training without the added motivating, playful, and informative elements. However, at follow-up, 12 weeks after the end of the training phase, a substantial reduction of depressive symptoms was visible in both groups. The usability of the intervention was consistently rated positively by its users. Except for slight occasional fatigue and transient frustration, no adverse events or side effects were observed.

Improvement of depression with PASAT-training

The superiority of *de:)press*© compared to the active control condition (reduction by 9.2 MADRS points, 45%) as well as to baseline (reduction by 8.8 MADRS points, 44%) in a real-world sample of patients with depression is clinically meaningful (59) and is maintained for up to 3 months after intervention. This beneficial effect is in line with previous findings in studies that applied the PASAT to alleviate symptoms of depression (37–40). However, with this it is not shown that other that other forms of CCT cannot also be effective. Nevertheless, by simultaneously challenging cognitive core features of depression (60) such as: deficits in working-memory (61), attention (62), processing speed (63), cognitive effort (64), and the control of negative feedback (25) at the individual performance maximum, *de:)press*© allows for a retraining of brain networks that are critical for the development and maintenance of MDD. Given the goal of maximizing the clinical effectiveness, simultaneous activation of the various processes seems most promising. However, the specific contribution of each of these processes to antidepressant efficacy, remains to be elucidated.

Facilitative effects of gamification on training

Our data show that depression adapted gamification as well as the comprehensive and patient-oriented information about the purpose of the training can substantially enhance anti-depressive features of the CCT. So far, gamification was not systematically used to enhance the efficacy of PASAT training in the treatment of MDD, and evidence regarding the facilitatory effect of gamification in mental health apps is mixed (65–67). Improving motivation and frequency of use through engaging and motivational elements could support those patients who have deficits specifically in this area. Notably, depression adapted gamification goes beyond the mere inclusion of game elements but encompasses meaning, psychoeducation, and broader support (see **Table 1**) derived from clinical experience and patient feedback. While the training proved to be a challenge for participants, the vast majority kept up with the training schedule, and the few dropouts were caused by external factors such as sudden family issues or non-related illnesses. It can therefore be assumed that gamification makes cognitive training programs more acceptable and increases the motivation to get it done. However, neuroplasticity-enhancing factors of gamification should also be considered. Beneficial effects of reward (31), motivation and attention (27, 68), and environmental enrichment (69) may additionally support adaptive

reorganization and recovery. In *de:)press*©, a pragmatic, user- and usability-oriented mixture of these factors is used, as the app tries to utilise these gamified elements without overwhelming the user with too many options. It can be assumed that these factors also, by facilitating adaptive reorganization, contributed to the antidepressant effect and its sustainability. Accordingly, with added gamification elements more patients may benefit from the intervention, while also benefitting more from training. Unfortunately, this question cannot be answered based on the present sample.

Need for long-term training and follow-up

Of note, the specific efficacy of *de:)press*© in the reduction of depressive symptoms is particularly visible 4 weeks after the end of its use. Considering the assumed mechanisms of action, this is not surprising. On the one hand, similar to physical exercise, it takes a while before cognitive training produces benefits that are recognizable for the trainee; on the other hand the PASAT-training as used in *de:)press*© aims to improve control of negative and stressful information (40, 43)—a process that may take time to induce a clinically tangible impact (70). The need for a sufficiently long training and observation period is illustrated by a recently published study indicating a lack of antidepressant effect of PASAT training compared to a sham-training control condition. Here, a non-gamified PASAT intervention comprising 10 training session within 2 weeks in the context of an inpatient treatment did not yield superior effects on depression severity. However, an exploratory analysis revealed significantly higher levels of subjective well-being in the active compared to the sham group at 1-year follow-up (71). This is consistent with prior studies showing significant between-group differences in depression symptomatology only at 3 months follow up after PASAT training (70). It indicates that training effects on depressive symptoms do not become visible immediately after the end of training but after a longer period of time. Regarding the amount and the spacing of training, available studies on CCT point to an optimum of 10–15 h of training spanned over several weeks (20). However, in the case of depression, a limited endurance of the patients must be considered. Consistently, most interventions elicit positive effects if a long enough training period is chosen (35, 38, 72). In this context, our training schedule comprising a 6 week intervention with three trainings per week proved to be adequate.

Limitations

Several limitations should be considered, most of which will be addressed in the follow-up study. First, an increased number of participants would have been beneficial for the stability of the findings. However, the Corona pandemic hindered recruitment.

While we saw beneficial effects of the training, due to the multimodality of the task itself and the surrounding gamification elements, it remains to be seen which factors contributed most (and in which way) to recovery. This could be targeted by strategically comparing versions of the app that differ in their number of gamified elements and how they are implemented.

Within this study, we compare two active groups against each other. While this allows us to draw conclusions on how either of the app versions worked, we have no comparison to treatment as usual. In an ongoing follow-up study, we will address these points.

Conclusions

This pilot study shows the feasibility and usability of *de:)press*© as an adjunctive treatment option of MDD by demonstrating that participants adhere to the training paradigm and show a lasting decrease in depressive symptoms. Based on the notion that good mental health is an active process (18), *de:)press*© empowers, enables and encourages patients to regain cognitive control and thus effectively participate in a key aspect of overcoming their depression. By inclusion of depression adapted gamification elements and mediation of purpose-driven motivation the beneficial effects of CCT can substantially be enhanced.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics committee of the University Hospital Tübingen. The patients/participants provided their written informed consent to participate in this study.

Author contributions

SW: Conceptualization, data curation, formal analysis, investigation, project administration, visualisation, writing (original draft preparation, review & editing). PAS: Conceptualization, funding acquisition, writing (review & editing). CP: Conceptualization, formal analysis, funding acquisition, resources, supervision, writing (original draft

preparation, review & editing). All authors contributed to the article and approved the submitted version.

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Conflict of interest

SW and CP are founders, associates, and chief executive officer (CP) of PsyKit GmbH. The company was founded after data collection of the study had finished and to allow certification of *de:)press*© as a medical device. PAS does not have any conflicts of interest to declare.

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A.1.4 'Sex Matters for the Enhancement of Cognitive Training with Transcranial Direct Current Stimulation (tDCS)'

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Biology of Sex Differences

RESEARCH

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Sex matters for the enhancement of cognitive training with transcranial direct current stimulation (tDCS)

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Abstract

Background Transcranial direct current stimulation (tDCS) can influence brain network activity and associated cognitive and behavioural functions. In addition to the extensive variety in stimulation parameters, numerous biological factors drive these effects, however these are yet poorly understood. Here, we investigate one of the major biological factors by focusing on sex-dependent effects of tDCS on a challenging cognitive control task (*adaptive paced auditory serial addition task* [PASAT]) in healthy humans.

Methods This sex-specific re-analysis was performed on data of 163 subjects who underwent a 2-week cognitive control training (6 sessions in total). Subjects received either verum (anodal/cathodal) or sham tDCS. Electrodes were placed over the left or right dorsolateral prefrontal cortex and the respective contralateral deltoid muscle. Cognitive control was measured as performance in the PASAT and was analysed in respect to stimulation conditions (sham, anodal, cathodal) and sex.

Results Regardless of stimulation condition, performance gains between the sexes were higher in females compared to males ($p = 0.0038$). Female's performance during anodal tDCS exceeded male's ($p = 0.0070$), yet no effects were found for cathodal or sham tDCS. Moreover, in females we found a superior effect for anodal tDCS over sham stimulation (f_{anodal}: $p = 0.0354$; $f_{cathodal}$: $p = 0.6181$), but no such effect in males (m_{anodal}: $p = 0.6882$; $m_{cathodal}$: $p = 0.4822$).

Conclusions This study highlights the relevance of biological sex for the effects of tDCS on cognitive training. Thus, an increased attention to biological sex is advisable in future brain stimulation research to highlight and in consequence better understand potentially underlying sex-specific mechanisms. Considering biological sex will further advance customisation and individualisation of tDCS interventions.

Trial registration ClinicalTrials.gov, NCT04108663.

Highlights

- This study provides evidence that tDCS affects females and males differently: females, compared to males, show higher performance gains in a demanding cognitive control task when tDCS is applied concurrently to the task.

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- The performance altering effects of tDCS in females were observable for anodal, yet not cathodal stimulation. In males, we did not detect any differences in performance, suggesting that tDCS affects females and males differently and that sex-specific customisation can prove to enhance stimulation efficacy even further.
- Our study highlights that biological sex needs to be taken into account in order to further personalise and optimise the application of tDCS in humans.

Keywords Brain stimulation, Cognitive control, Cognitive enhancement, Sex differences, Biological sex, Prefrontal cortex, Transcranial direct current stimulation, Neuropsychiatry

Plain language summary

In previous studies, brain stimulation techniques like transcranial direct current stimulation (tDCS) have been shown to support cognitive trainings. However, these effects are rather small and vary between people. A key factor of variability is the biological sex. Hence, in this study we were interested in whether the effects of tDCS differ between females and males. To answer this research question, we analysed the data of 163 human subjects who underwent a 2-week cognitive control training program, which incorporates a challenging cognitive task (the *adaptive paced auditory serial addition task* [PASAT]). During the PASAT, subjects have to solve a stressful calculation exercise. Concurrently to solving this task, the subjects received either real (further divided into anodal [= enhancing] and cathodal [= inhibiting]) or placebo tDCS. We found that females had greater performance gains in the task than males, regardless of the type of tDCS they received. Furthermore, females performed particularly well when they received anodal tDCS, but there were no significant effects for cathodal or placebo tDCS. For males, we did not find any significant benefits of tDCS. These findings highlight the importance of considering biological sex in future brain stimulation research and suggest that biological sex is an important component to consider when studying the effects of tDCS. By paying more attention to this factor, researchers can better understand how tDCS works and develop more effective and personalised interventions.

Background

Transcranial brain stimulation techniques, particularly *transcranial direct current stimulation* (tDCS), have proven to modulate cognitive processes both in healthy as well as patients diagnosed with mental disorders [1–5]. To modify cortical activity via tDCS, electrodes are placed on the scalp with low electrical currents being routed through them. Without evoking action potentials itself, the stimulation is capable to shift the resting membrane potential which in turn affects the resulting neuronal response, i.e. the likelihood of action potentials to occur [6]. In conventional stimulation protocols the anode enhances the neuronal response of the target area, while the cathode reduces cortical excitability at the macroscale level. This property of tDCS has been used to elicit changes in cortical excitability that can last several hours, to modulate cognitive performance in a number of ways, and even to reduce the symptoms of neurological or mental disorders [7].

In a recent study, we systematically analysed the most common stimulation parameters and were able to show that anodal tDCS with an intensity of 1 mA to the left dorsolateral prefrontal cortex (dlPFC) supports cognitive control (CC) processes in healthy humans, while other tDCS configurations did not yield similar results [8]. These cognitive control processes are needed to uphold effective and goal-directed behaviour [9–12], which is required to perform well in the challenging task. Effectiveness of tDCS relies on a plethora of factors. This includes electrode setup (size, shape, orientation) and stimulation polarity as well as brain and head morphology, brain state, pre-existing disorders, usage of psychotropic drugs, hormonal states, age, and sex. The variability in the factors that influence tDCS still limits a systematic use in clinical settings. Not alone the multitude of parameters but also the complex interaction between individual psychological, anatomical, and physiological characteristics with the current flow shape the direction and magnitude of effects. Therefore, it is not surprising that biological sex has already been discussed as a critical factor that contributes to the individual variability of the effects, yet the significance of biological sex for the scientific and clinical use of tDCS remains unclear and an increasing amount of empirical studies reporting sex to be an important variable reinforce this notion [13].

Sex differences include morphological and structural variations [14–16] such as overall head size, larger brain volumes (up to 10%) for males in cerebrum, cerebellum, cerebrospinal fluid, intracranial volume, and deviating tissue density across various brain regions [17]. Additionally, larger volumes of white matter for several brain regions, most notably the frontal cortex,

yet no significant difference for global white matter volume [18], and diverging distributions of cancellous bone in the skull [19]. A recent study in a large sample of 240 subjects has shown the extent to which anatomical parameters of the cortex affect the electrical current distribution caused by tDCS [13]. Notably, current densities at the regions of interest varied considerably between females and males, and the distribution of cerebrospinal fluid and grey matter allowed the prediction of current intensities at the target sites. These findings suggest that the ratio between male and female subjects in a study sample influences the outcome. Consistently, a recent meta-analysis on 61 studies supports the notion that, particularly in healthy females, higher current density and/or charge can enhance response accuracy, and that the higher the percentage of females included in the study, the stronger the effect sizes [20].

In addition to these morphological traits, hormone receptors, neurochemicals and -transmitters, which impact neuronal pathways, brain architecture and behaviour [21–24], are expressed at different rates in distinct brain areas between sexes [25], but also between individuals of the same sex [26]. In females, cyclic fluctuations of sex hormones such as endogenous oestradiol [27] should be taken into account [28–31]. As ovarian hormones are known to influence neurotransmission and neuronal excitability [32, 33], they can thereby affect female's performance in verbal, spatial, and cognitive tasks across the menstrual cycle [30, 31]. Interestingly, the use of hormonal contraception has been found to further influence brain activity, with some activation patterns rather resembling brain activity in males [34].

In terms of sex differences in regard to tDCS, previous studies have shown different outcomes for males and females in specific brain regions such as the visual cortex [35], motor cortex [36], and in different tasks that focus on, e.g. decision-making [37] or theory of mind [38, 39]. Evidently, biological sex affects tDCS efficacy, thereby contributing to the high inter-subject and inter-study variability [40, 41]. To circumvent this, many studies excluded females and were carried out in study samples only including males, thus heavily biasing previous insight towards a male population.

Hence, within this study we focus on this fundamental characteristic of human biology. We re-analysed the sample of 162 healthy subjects form our previously published data [8] with regard to sex differences. The training gains in a challenging cognitive control task over two weeks were compared between females and males receiving either concurrent anodal, cathodal or sham tDCS.

Methods

This re-analysis is based on previously published data, therefore, we report the materials and methods in brief. A comprehensive description of the experiments is provided in Weller et al. [8]. The study was approved by the University of Tübingen local ethics committee and executed in accordance with the Declaration of Helsinki.

Experimental design

Subjects

In total, 162 subjects were included in the study (127 females, 35 males). Subjects were aged 18 to 39 years (mean $age_f = 22.73$ years, SD = 3.67 years; mean age_m: 24.89 years, SD = 4.64 years). We acknowledge biological sex not being binary. We distinguish it from gender identity and are aware that sex and gender need not necessarily align.

Before participation, all subjects gave written informed consent. Potential subjects were only included if they reported no diagnosed mental or neurological disorders in the past, no achromatopsia (colour blindness), no metallic implants or tattoos near electrode sites, consumed less than 10 cigarettes per day, sufficient German skills (minimum CEFR level B), and did not take part in any brain stimulation studies while enrolling in this study. Subjects were discharged from our study, and hence their data not used, if they missed a study visit. As compensation, money or course credits were provided with an additional bonus for the best 12 performers.

TDCS procedure

Verum stimulation was applied for 19:10 min, therefore starting and ending shortly before and after the PASAT, respectively. Sham stimulation was applied in two blocks, one before and one after the PASAT, limited to a total of 50 s. The current was applied through a CE-certified direct current stimulator (DC-Stimulator MC, NeuroConn GmbH, Ilmenau, Germany; version 1.3.8) and two rectangular rubber electrodes (5×7 cm). The stimulation was applied as either sham stimulation (S) or verum stimulation. For verum stimulation, the following configurations were applied: anodal or cathodal polarity (A/C) with an intensity of either 1 mA or 2 mA, applied to either the left or right dlPFC. The position for the first electrode was determined by the international 10–20 system (F3 for left dlPFC, F4 for right dlPFC), the second electrode was placed over the opposing deltoid muscle. The subject's skin was prepared with mild abrasive gel (Nuprep Skin Prep Gel, Weaver and Company, Aurora, Colorado) and 70% alcohol, electrode surfaces were coated with conductive electrode paste (Ten20

conductive Neurodiagnostic Electrode Paste, Weaver and Company, Aurora, Colorado) and subsequently attached to the skin with adhesive tape.

Experimental groups

The two groups (female and male) were split according to tDCS polarity (A/C) to allow the comparison with sham tDCS. To conserve statistical power and group sizes, we did not split the groups further by intensity and laterality as we did in our previous publication. For an overview on the demographical data, see Table 1.

Cognitive control training: PASAT (Fig. 1A)

We used a modified adaptive version of the PASAT. Subjects were seated in front of a computer screen. Over headphones, they heard single digit numbers in random order and were instructed to add the current digit to the digit that preceded it by 2 ($n^{th}+n^{th-2}$). Responses were given on a keyboard with all possible results printed on it (i.e. the numbers 2 to 18). Subjects were instructed to answer as quickly and correctly as possible. If subjects answered correctly/incorrectly four times in a row, the interval with which the digits were presented was decreased/increased by 0.1 s, resulting in performance-dependent task speed. At the beginning of each session, the interval between digits was 3 s and then adjusted according to performance. Each training session was divided into 3 blocks, 5 min each, with the achieved interval being carried over from block to block. Between each block a 30 s pause was implemented. Subjects were only allowed to give answers with their right index finger.

This form of the *2-back* PASAT [42] was chosen over the standard *1-back* PASAT, where the last digit must be added to the digit directly before it. From our experience, the 1-back PASAT would likely have been too easy for our healthy group and would have culminated in ceiling effects.

Table 1 Demographic group characteristics

Sex	f	m	Test statistic
N subjects	127	35	Not applicable
Age[b]	22.73 (3.67)	24.89 (4.64)	$t(160) = 2.898$, $p = 0.004$*
EHI-Score[b]	0.904 (0.1318)	0.940 (0.0914)	$t(160) = 1.520$, $p = 0.131$
Last math grade[b]	2.29 (1.078)	2.26 (0.954)	$t(160) = -0.113$, $p = 0.910$
QCM (anxiety)[b]	3.5795 (1.2094)	3.5086 (1.2313)	$t(160) = -0.306$, $p = 0.760$
QCM (success)[b]	4.0735 (1.2791)	4.3897 (1.2839)	$t(159) = 1.279$, $p = 0.203$
QCM (interest)[b]	3.9925 (1.1751)	4.2457 (1.2603)	$t(160) = 1.111$, $p = 0.268$
QCM (challenge)[b]	5.2165 (0.9206)	5.3571 (0.9301)	$t(160) = 0.798$, $p = 0.426$
RSES[b]	39.09 (5.314)	40.06 (5.263)	$t(158) = 0.956$, $p = 0.341$
Hormonal contraceptive (yes/no)[c]	64/63	Not applicable	$\chi^2(1) = 0.008$, $p = 0.929$
Smoking (yes/no)[a]	15/112	8/27	$p = 0.087$

Means and standard deviations (M(SD)) are shown; if not applicable, the number of subjects belonging to each trait are shown. One female subject in the cathodal group was removed from our analyses, as her performance deviated more than 2 SD from all other subjects, resulting in a total of 162 instead of 163 subjects

EHI Edinburgh Handedness Inventory, *QCM* Questionnaire on Current Motivation, *RSES* Rosenberg Self-esteem Scale

[a] Fisher's exact test

[b] *t*-test

[c] Chi^2

*$p < 0.05$

Study timeline (Fig. 1B)

In total, each subject attended nine sessions. Session one to eight happened within one months' time (pre-training in week 1, six training sessions in week 2 and 3, post-training in week 4). The last session (follow-up) was conducted three months later. During each session subjects carried out the PASAT, however tDCS was applied only during training sessions. Training sessions alternated with one training-free day.

Questionnaires

To assess for right-handedness, only subjects scoring higher than 60 in the Edinburgh Handedness Inventory (EHI) could participate in this study [43]. This was done to minimise possible variability in tDCS response caused by subjects' handedness [44]. Through the Questionnaire on Current Motivation (QCM), we a priori accounted for overall interest and perceived challenge in the task as this might have subsequently influenced performance [45]. Other anamnestic data such as age and formal education were inquired about in a custom questionnaire. We measured subjects' self-esteem through a modified Rosenberg Self Esteem Scale (RSES) which allowed to measure self-esteem scores between 10 and 50 [46]. For a summary of the assessed items please refer to Table 1. It is of note, that this RSES utilises a 5-point Likert scale incorporating a middle category of agreement ("neither agree nor disagree"), unlike the original version of the questionnaire which only offers 4 points. This might increase variability of responses or reduce acquiescence bias, and analyses regarding varying numbers of Likert scale points show no difference in external validity [47].

Statistical analyses

Unless stated differently, threshold for type I error was set to 5% and all tests refer to two-tailed tests. R version

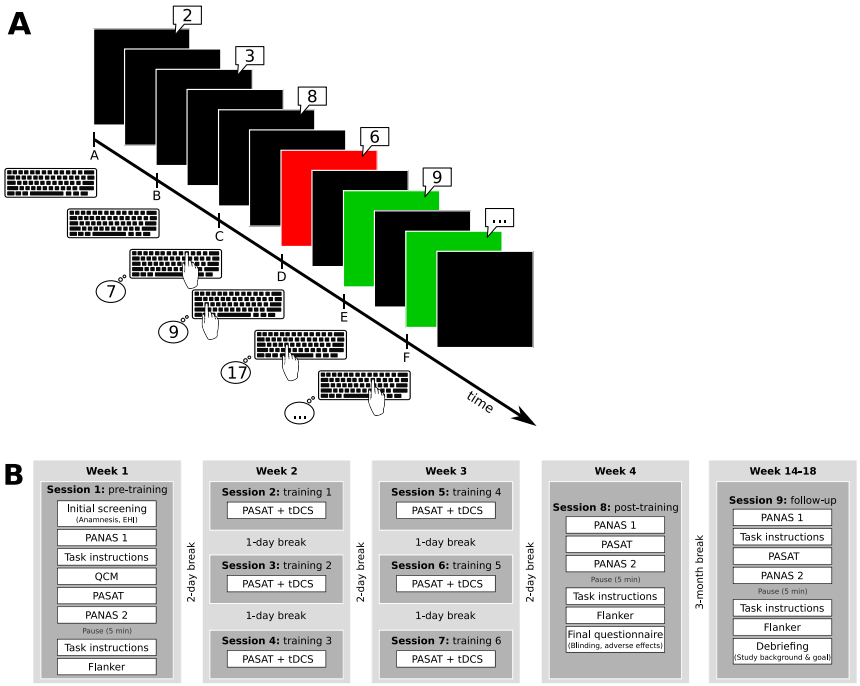

Fig. 1 During the PASAT, single digit numbers were presented to each subject. They were asked to sum the current (*n*th) and second-to-last digit (n^{th-2}): e.g. digits at timepoints C and A, D and B, E and C, and so on. Several correct answers in a row shortened the interval between digit presentations while long answers prolonged it (**A**). Subfigure **B** shows the timeline of the experiment. Figure adapted from Weller et al. [8], reprinted with permission

3.5.1 to 4.0.2 [48] with packages nlme [49] as well as rcompanion [50] and IBM SPSS Statistics Software version 24 [51] were used for all analyses.

Cognitive control training: effects of tDCS between sexes

To assess performance, the number of correct trials within each training session (n_{corr}) was calculated. This was done as the PASAT was limited by time (15 min raw PASAT), hence subjects were able to solve as many calculations during a session as their abilities allowed. As faster digit presentations were a result of better performance (i.e. higher count of n_{corr}), this variable was chosen as a comparator between the study groups.

Since pre-training performance ($n_{corr(pre)}$) might prove to be an indicator for overall performance, this value was compared separately between the female and male group. For this, we used a mixed-effects ANOVA with the within-subject factor $n_{corr(pre)}$ and between-subjects factor sex.

Next, the training sessions (session two to seven) were analysed. For each of the following steps, performance gain measured in one sex was compared to performance gain of the other sex. All this was done in a linear mixed-effects model: sex, time (i.e. session number), and the interaction sex x time were used as fixed effects. Performance (n_{corr}) was used as the dependent variable, and $n_{corr(pre)}$ was included as a regression coefficient. Random effects were measurement timepoint and individual subject ($\sim 1 + time \mid subject$). Firstly, subjects were grouped by sex only, regardless of the tDCS intervention (males,

pooled: m_p; females, pooled: f_p). Secondly, we split the groups by tDCS *polarity* to test for possible polarity-dependent deviations: performance gain of all males from the sham group (m_S) was compared to the performance gain of all females from the sham group (f_S). Analogous, the performance gain of the anodal (m_A and f_A) and cathodal (m_C and f_C) groups were compared.

Cognitive control training: effects of tDCS within each sex

While the afore-described steps allow for the analysis of effects between the two sexes, they do not answer the question whether any tDCS condition caused effects within each sex. Therefore, we ran a linear mixed-effects model for each sex independently. For this, we compared performance of subjects who had received either anodal or cathodal tDCS to subjects of the same sex who had received sham tDCS (m_A and m_C compared to m_S; f_A and f_C compared to f_S). Again, n_{corr} was used as the dependent variable and $n_{corr(pre)}$ was included as a coefficient. Fixed effects were defined as the condition (S/A/C), time (corresponding to session), and the interaction between condition x time. As random effects, measurement timepoint and individual subject (*~ 1 + time | subject*) were used.

We refrained from computing non-standardised (B) and standardised beta coefficients (β), for why measuring effect strength is still a topic of discussion where an optimal roadmap has yet to be developed. This goes in accordance with the reasoning given when the *beta()* function included in R's reghelper package [52–55] was deprecated. The between-sex analyses were corrected via the Bonferroni–Holm method, as the polarity sample is a subgroup of the pooled sample. Lastly, to look at possible long-term effects, performance gains of the groups were tested against each other via t-tests.

Questionnaires

The questionnaires were implemented to ensure similar group compositions, comparative analyses between male and female groups were performed using Fisher's Exact test, t-test, Chi^2 test with each questionnaire's *outcomes* as dependent variables (Table 1).

Results

Sample characteristics

No disparities were found for pre-training performance between females and males, showing that subjects started the study at similar performance levels: $F(1, 160) = 1.809$, $p = 0.180$, $\eta^2 = 0.011$. The distribution of females menstruating during the training phase did not differ between the groups split by tDCS polarity (S/A/C; Fisher's exact $p = 0.659$). While there was a significant age difference (males being older by an average of 2 years), there were no differences for any of the other descriptive factors; please refer to Table 1 for a comprehensive overview.

Cognitive control training: between sex effects

Figure 2 shows subjects' performance gains over time. Additional file 1: Table S1 provides the raw n_{corr} for all possible groups. The exhaustive statistics for all analyses are provided in Additional file 1: Table S2–S7. Main effects of time and $n_{corr(pre)}$ were highly significant in all cases ($p < 0.001$), showing that subjects improved their performance over the course of the training and that pre-training performance was a predictor for further performance gains, with higher pre-training performance correlating with increased performance gains during the subsequent training period. The significant differences in training effects between the two sexes, that we were able to find during the training period, did not persist for post-training or follow-up (post-training: $t(45.568) = -1.391$, $p = 0.171$; follow-up: $t(158) = 0.841$, $p = 0.402$). No difference in baseline performance was found for either analysis (pooled group: $p = 0.180$; anodal group: $p = 0.105$; cathodal group: $p = 0.320$; sham group: $p = 0.078$. Bonferroni–Holm corrected threshold: $p = 0.025$).

Analysis of all subjects combined regardless of tDCS parameters (m_p/f_p)

Between the two pooled groups, we found an effect of sex, indicating that females exhibited higher training gains compared to males ($p = 0.0038$). The Bonferroni–Holm corrected threshold for significance is 0.0125. Based on this significant general sex difference, we split groups according to the applied polarity. See Additional file 1: Table S2.

Analysis of subjects according to stimulation polarity (m_S/f_S; m_A/f_A; m_C/f_C)

Here, we found a significant effect for anodal polarity. As above, females showed higher performance gains than males when anodal tDCS was applied ($p = 0.0070$ with Bonferroni–Holm corrected threshold of 0.0167), however no such sex effect emerged for the sham ($p = 0.2063$ with Bonferroni–Holm corrected threshold of 0.025) or cathodal condition ($p = 0.3258$ with Bonferroni–Holm corrected threshold of 0.05, respectively). See also Additional file 1: Table S3–S5.

Cognitive control training: within-sex effects

While the hierarchical analysis above answers the question whether females and males varied in their performance gains, it does not allow to draw conclusions about performance changes based on tDCS polarity within each sex. Hence, we analysed the two sexes independently thereby exploring possible tDCS effects that are

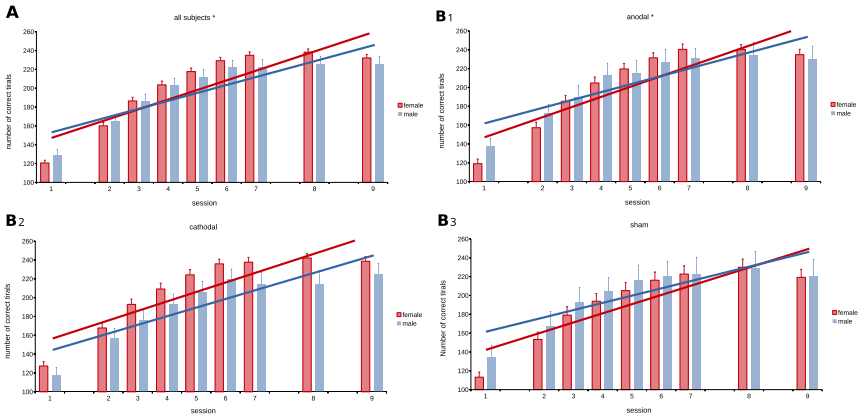

Fig. 2 Performance development between the two sexes. Measurement of performance is the sum of correct trials per session. Shown here is the performance for **A**: all subjects and **B1**–**B3**: separated by tDCS polarity. For every subfigure, females' performance gains are compared to males'. Trendlines indicate performance gains over time, with steeper inclines corresponding to higher performance gains. We found a significant effect of sex when analysing all conditions in a single group (**A**), with female's performance increase surpassing male's ($p = 0.0038$). For polarity (**B1**–**B3**), we found that females improved significantly over males under anodal conditions (**B1**; $p = 0.0070$), whereas no difference was found under cathodal (**B2**; $p = 0.3258$) or sham condition (**B3**; $p = 0.2063$). *Groups where performance gains differ significantly ($p < 0.05$)

prominent in one sex but absent in the other. Figure 3 shows the number of correct trials per training session within each sex.

Male group: polarity ($m_S/m_A/m_C$)

We found no differences in performance gains for *polarity* between the male verum group compared to the male sham group (m_A: $p = 0.6882$; m_C: $p = 0.4822$). See also Additional file 1: Table S6.

Female group: polarity ($f_S/f_A/f_C$)

Assessing for polarity effects in females, we observed a significant effect with females receiving anodal tDCS performing better over the training sessions than the female sham group ($p = 0.0354$). This was not the case for the cathodal group compared to sham ($p = 0.6181$). The effect for the anodal group did not persist throughout post-training ($t(52.994) = -1.009$, $p = 0.318$) or follow-up ($t(79) = -1.578$, $p = 0.119$). See also Additional file 1: Table S7.

Discussion

In this re-analysis of previously published data, we tested the influence of biological sex on tDCS-supported enhancement of cognitive control training in healthy females and males who underwent a challenging 2-week training paradigm (PASAT). For the whole study group, including all stimulation conditions (anodal, cathodal, sham), we found a larger training benefit in females compared to males over the course of the training phase. More precisely, females had consistently higher training gains compared to males when anodal tDCS (but not cathodal or sham) was applied to the prefrontal cortex during training. Consistently, the comparison of stimulation conditions within sexes demonstrated a beneficial effect of anodal over sham tDCS in females, but no such effect in males. No effects were found in either sex for cathodal over sham tDCS. Thus, our analysis indicates that the enhancement of cognitive control training by anodal tDCS is critically modulated by biological sex, with females being more susceptible for beneficial effects than males. A similar level of performance at baseline and the lack of differential effects in the sham group underline the specificity of this effect: the absence of differences in educational levels and expectation towards the task as assessed via questionnaire indicate comparable performance prerequisites in both sexes, and both groups had similar math abilities and motivation to perform well in the task.

So far, the available data on the interaction between biological sex and tDCS are highly inconsistent with some studies reporting effects only in one sex while being absent, or even opposite, in the other [20, 56, 57]. For example, Meiron et al. showed beneficial effects of anodal

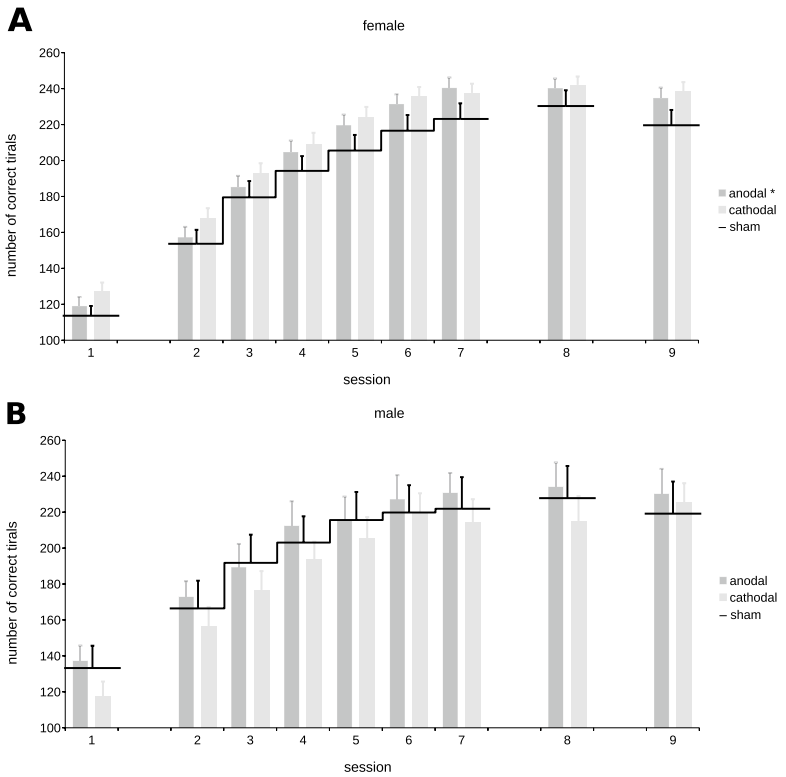

Fig. 3 Performance development within each sex. Measurement of performance is the sum of correct trials per session. Shown are the number of correct trials for all females in **A** and males in **B**, split each by tDCS polarity. The performance for each polarity condition (bars) was compared to sham stimulation (black line). We found significant performance gains for females in the anodal group compared to sham ($p = 0.0354$). This effect was not seen in the cathodal group, where the performance increase of cathodal and sham stimulation was of similar magnitude throughout the training phase ($p = 0.6181$). No significant effects were found for either polarity in the male group, as both polarities resulted in similar performance gains compared to sham stimulation (m_A: $p = 0.6882$; m_C: $p = 0.4822$). *Groups where performance gains differ significantly ($p < 0.05$)

tDCS during a verbal n-back task with stimulation of the left dlPFC in their male sample, whereas in females stimulation of the right dlPFC proved to achieve similar positive effects [58]. He et al. found, that while both sexes benefitted from anodal tDCS over the left dlPFC in the Iowa Gambling Task, in females the stimulation effect was more pronounced [59]. In our study, we were able to show that anodal tDCS facilitated training gains in females, but not males.

As diverse as the outcomes of anodal tDCS alone are, so are the results from studies focusing on cathodal tDCS. For example, some research has found excitatory effects of cathodal tDCS over the motor cortex. This effect however, was only prominent for certain current intensities. More strikingly, the magnitude, duration, and direction of these non-linear effects were dependent on stimulation intensity [60]. Another study found that cathodal tDCS increased performance in a cognitive task, instead of

degrading performance [61]. These examples alone highlight even more how complex the relationship between tDCS parameters and their potential effects is. While we were not able to observe any effects for cathodal tDCS in our study, it is possible that this resulted from a certain parameter combination we did not analyse, the study group that was included in the experiments, or the task that was done during the stimulation. Lastly, it is not yet clear if or how the magnitude of outcomes from anodal and cathodal tDCS are related.

It seems reasonable to assume that the sex-dependent variability in our study is at least partially explained by anatomical differences for instance in volume of cerebrospinal fluid, skull thickness, gyrus orientation, or the individual location of the dlPFC. It should be noted that the relevance of sex differences in brain architecture for cognitive functions and mental health are still under debate. Two recent analyses based on MRI data [62, 63] coincide that structural differences in brain morphology between males and females exist, but draw contrasting conclusions on their impact: ranging from the differences being trivial (e.g. derived from height and size of the subject) and the brain not being sexually dimorphic [63], to several regions still being significantly distinct even when accounting for overall body morphology [64]. In the end, current status can potentially agree on the "mosaic" hypothesis, indicating that no typical female or typical male brain exists without neglecting frequently reported differences in brain anatomy and function [65]. Based on this variability, it can be assumed that current flow that reaches the cortical area relevant for cognitive control processes differs between females and males [66]—and hence identical tDCS configurations not necessarily lead to comparable results in all sexes. However, some researchers suggests that this variability is more appropriately described by means of these anatomical features than in regard to biological sex differences [13, 67, 68]. Nevertheless, it has also to be considered that inter-individual variability possibly outweighs sex effects [69], though greater variance in brain structure was reported in males than females across the lifespan [16].

Besides anatomical variability, variations in task-specific activation of brain networks exist and reinforce the significance of the task being conducted during the application of tDCS. Sex-specific activation patterns have been found in various cognitive tasks as well as in the processing of emotional information [70–72]. For instance, under specific task conditions, females have been shown to more strongly involve higher-order frontal regions such as the prefrontal cortex which could be further enhanced by tDCS [69, 73]. In another study more pronounced effects of tDCS were visible when tDCS was applied on the hemisphere that was predominantly activated during a specific task [74]. With this evidence we can assume that the beneficial effects of tDCS in females were likely also influenced by the underlying specific activation patterns within the frontal brain regions.

More specifically, and in addition to the aforementioned differences, variability between the sexes in challenging cognitive tasks might be linked with the fact that females and males show different brain activity particularly in response to cognitive stress, which then in turn affects performance during those stressful tasks [75–77]. Therefore, it can be assumed that tDCS shows sex-specific differences of brain activity in a stressful task like the PASAT [78]. More precisely, females have been shown to be more sensitive to negative feedback [79], which is a major component in the PASAT, posing an additional challenge for the executive system. Both workload and cognitive state can influence the efficacy of tDCS. Li et al. showed that effects of anodal tDCS were more pronounced without a concurrent task while the opposite was true for cathodal tDCS, yet, results like these seem to highly depend in the task itself [80, 81]. However, as stress increases workload [82], a consequently higher activity of the dlPFC might be the basis for a higher response to tDCS in females specifically.

Within our sample we found an age difference of approximately 2 years between males and females. Previous studies have shown that age seems to be related to tDCS efficacy. Supposedly, this effect can be related to brain atrophy and that the aging brain, with its accompanying changes in morphology, requires different tDCS parameters to be effective [83]. This data suggests that the higher age difference between groups, the more closely parameters should be inspected and adapted—especially current dose. However, the study also notes that the brain ratio as a measure of brain atrophy, rather than chronological age, plays the larger role in the response to tDCS. As the age difference in our sample is very small and both groups would still fall within the same age cluster (i.e. young adults), we presume that biological sex is the main driving factor for the performance variations we found.

Conclusively, structural and functional anatomy of cognitive control training likely varies between males and females. Indeed, recent studies allow the assumption that individual components of cognitive control may be altered differently in the sexes (yet without systematic advantage) and that these effects depend on the modality of testing and respective parameters [58, 75]. It stands to reason that both sexes employ different strategies when presented with challenging tasks [76, 84, 85] such as the PASAT. The specific strategies and how they can be enhanced by concurrent tDCS, remains elusive so far, but increasing evidence for this theory has been found recently.

A limitation of this retrospective analysis is the unequal distribution of sexes in our sample. While the applied statistical models are robust enough to account for the numerical distribution, the smaller number of male subjects did not allow to analyse the influence of sex on stimulation intensity and laterality. Another challenge is that the hormonal states of individual subjects can influence tDCS and should therefore be monitored thoroughly: oestradiol is known to enhance cortical excitability and should be considered when excitability is modified via tDCS, while at the same time, progesterone can decrease excitability [86]. Higher levels in oestradiol (compared to lower levels) have shown greater neuroplastic responses when tDCS was applied to the frontal cortex, hence suggesting that oestradiol contributes to inter-individual variability in tDCS outcomes [27]. As our data allow to analyse the distribution between females menstruating/not menstruating during the experiment, we found that this distribution was equal. However, we did not collect more specific data on menstrual cycles to determine the exact cycle phase of each female. As oestradiol peaks before ovulation but rises again during the mid-luteal phase, simply comparing females who are menstruating (low levels) with not menstruating females (varying levels) is not enough. Additionally, half of our female sample were using hormonal contraception, thus further influencing sex hormone levels. This needs to be addressed in more detail in further prospective studies, focussing specifically on this research question.

Another aspect to consider is the question whether the observed effects are actually related to biological sex or whether they are mostly correlated to anatomical differences. However, as certain anatomical features and sex heavily correlate with each other, this is more a matter of perspective and phrasing and hence the critical interaction between sex and tDCS intervention outcomes remains.

Finally, in an adult human sample effects of sex can hardly be disentangled from effects of gender and gender roles. Self-concepts and personality traits, such as neuroticism and conscientiousness, that are more expressed in females [87], can influence behaviour and are thought to be influenced by experience, social desirability concerns, and societal norms. We did not assess gender identity, gender norms and gender expression in our participants which should be done in future studies to shed light on how gender and other diversity aspects influence reported results.

In sum, we can conclude that research is picking up on the importance of sex differences in the neuromodulation of the human cortex. With this study we shed further light on the variable impact of tDCS on performance in a cognitive task and whether this is influenced by biological sex. Most likely, sex-related diversities are not binary but lie on a complex spectrum composed of morphological, hormonal, and neurobiological factors. Researchers should harness the knowledge on sex differences to stratify and personalise brain stimulation interventions. Especially in the light of tDCS being a viable tool for the treatment of various illnesses, it is vital to further uncover the (biological) characteristics that have a bearing on tDCS efficacy and hence contribute to the high variability we currently see in the study landscape. By doing so, personalised interventions may prove to surpass standardised paradigms soon.

Perspective and significance

Non-invasive transcranial brain stimulation is a powerful tool to influence cognitive performance and training. The stimulation effects can be modulated by a multitude of factors one of them is sex/gender. While the results of this study suggest that tDCS works better in females when faced with a challenging cognitive task, we cannot conclude that there are no effects in males. This will require a more focused and sex-based approach. Understanding how sex interacts with tDCS is a critical step on the path to personalised and effective cognitive interventions and treatments.

Conclusions

Our results are the first to show that beneficial effects of anodal tDCS on cognitive control training are more prominent in females than in males. This supports the notion that biological sex is one of the critical sources of variability in tDCS responses on cognitive training in particular and most likely in neuromodulation in general. Notably, these sex effects are measurable under anodal tDCS, however not under cathodal or sham condition. When comparing tDCS polarities within the sexes, anodal tDCS proved to be beneficial over sham and cathodal tDCS for females, however that was not the case for males. Accordingly, our results clearly point towards a further individualisation of tDCS by recognising biological sex. Further research is required to elucidate the specific interrelations between biological, social and functional characteristics of individuals and stimulation techniques. Based on this, more refined tDCS interventions show a promising perspective to yield optimal results for research and therapy.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13293-023-00561-4.

Additional file 1: Table S1. Training performance as measured by the number of correct trials for all groups. Shown are mean of correct trials

and standard deviations in parentheses. **Table S2**. Results from the linear mixed model for all males and females pooled, regardless of tDCS setting. All calculations use the female group (f_p) as a reference. Number of subjects: $N = 162$. **Table S3.** Results from the linear mixed model for groups organised by tDCS polarity (sham). All calculations use the female group (f_s) as a reference. Number of subjects: $N = 43$. **Table S4.** Results from the linear mixed model for groups organised by tDCS polarity (anodal). All calculations use the female group (f_A) as a reference. Number of subjects: $N = 60$. **Table S5.** Results from the linear mixed model for groups organised by tDCS polarity (cathodal). All calculations use the female group (f_C) as a reference. Number of subjects: $N = 59$. **Table S6.** Results from the linear mixed model from the male group divided by tDCS polarity. All calculations use the sham group (m_S) as a reference. Number of subjects: $N = 35$. **Table S7.** Results from the linear mixed model from the female group divided by tDCS polarity. All calculations use the sham group (f_S) as a reference. Number of subjects: $N = 127$.

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Author contributions
CP designed the study. SW performed the human experiments. SW and CP analysed the data, interpreted the results, and wrote the manuscript; SW, BD and CP participated in the result interpretation and finalised the paper. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved by the University of Tübingen local ethics committee and executed in accordance with the Declaration of Helsinki. All subjects gave informed written consent before taking part in this study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Publisher's Note

A.1.5 'Supplementary Material to: Sex Matters for the Enhancement of Cognitive Training with Transcranial Direct Current Stimulation (tDCS)'

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Additional Tables

Table S1. Training performance as measured by the number of correct trials for all groups. Shown are mean of correct trials and standard deviations in parentheses.

Group	Session								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
m_P	129.11 (5.64)	165.23 (6.70)	185.8 (7.36)	202.94 (7.14)	212.25 (7.54)	222.29 (7.41)	222.29 (7.92)	225.37 (8.62)	225.46 (7.86)
f_P	120.61 (2.93)	160.07 (3.63)	186.48 (3.89)	203.61 (3.92)	217.66 (3.78)	229.22 (3.56)	234.97 (3.65)	238.29 (3.47)	232.18 (3.62)
m_S	134.46 (12.44)	167.64 (15.43)	193 (15.69)	204.273 (14.6)	216.82 (14,65)	221 (15.22)	223.09 (17.60)	229 (17.89)	220.36 (17.87)
f_S	113.25 (5.44)	153.28 (7.79)	179.13 (9.07)	193.84 (8.24)	205.156 (8.73)	216.19 (8.76)	222.75 (8.72)	229.94 (8.79)	219.25 (8.53)
m_A	137.55 (8.18)	173 (8.55)	189.36 (12.93)	212.64 (13.36)	215.64 (12.97)	227.18 (13.38)	230.91 (10.86)	234.36 (13.25)	230.46 (13.59)
f_A	119.06 (4.98)	157.25 (5.81)	185.29 (6.16)	204.74 (6.33)	219.67 (5.84)	231.47 (5.32)	240.45 (5.73)	240.45 (5.30)	234.82 (5.71)
m_C	117.46 (8.25)	156.62 (10.61)	176.69 (10.50)	193.62 (9.89)	205.54 (11.61)	219.23 (11.25)	214.31 (12.89)	214.69 (14.17)	225.54 (10.57)

f_C	127.39 (4.71)	167.80 (5.68)	192.83 (5.68)	209.20 (6.22)	224.22 (5.62)	235.89 (5.02)	237.63 (5.10)	242.09 (4.67)	238.66 (4.98)

Table S2. Results from the linear mixed model for all males and females pooled, regardless of tDCS setting. All calculations use the female group (f_P) as a reference. Number of subjects: N = 162.

Factor	Δ n_{corr}	SEM	*df*	*t*	*p*
Time	14.748	0.514	807	28.707	<0.0001*
Pre-training	1.108	0.047	159	23.406	<0.0001*
m_P	1.428	4.791	159	0.298	0.7660
m_P:time	-3.204	1.105	807	-2.900	0.0038*

Table S3. Results from the linear mixed model for groups organised by tDCS polarity (sham).

All calculations use the female group (f_S) as a reference. Number of subjects: *N* = 43.

Factor	Δ n_{corr}	SEM	*df*	*t*	*p*
Time	13.424	1.095	213	12.265	<0.0001*
Pre-training	1.286	0.101	40	12.750	<0.0001*
m_S	-5.676	8.932	40	-0.635	0.5288
m_S:time	-2.744	2.164	213	-1.268	0.2063

Table S4. Results from the linear mixed model for groups organised by tDCS polarity (anodal). All calculations use the female group (f_A) as a reference. Number of subjects: *N* = 60.

Factor	Δ n_{corr}	SEM	*df*	*t*	*p*
Time	16.228	0.730	297	22.227	<0.0001*
Pre-training	1.055	0.078	57	13.569	<0.0001*
m_A	2.876	7.899	57	0.364	0.7172
m_A:time	-4.628	1.704	297	-2.715	0.0070*

Table S5. Results from the linear mixed model for groups organised by tDCS polarity (cathodal). All calculations use the female group (f_C) as a reference. Number of subjects: *N* = 59.

Factor	Δ n_{corr}	SEM	*df*	*t*	*p*
Time	14.096	0.890	293	15.830	<0.0001*
Pre-training	1.036	0.074	56	13.982	<0.0001*
m_C	1.759	8.566	56	0.205	0.8381
m_C:time	-1.867	1.897	293	-0.984	0.3258

Table S6. Results from the linear mixed model from the male group divided by tDCS polarity.

All calculations use the sham group (m_S) as a reference. Number of subjects: N = 35.

Factor	Δ n_{corr}	SEM	*df*	*t*	*p*
Time	10.681	1.618	172	6.602	<0.0001*
Pre-training	1.134	0.096	31	11.782	<0.0001*
m_A	-3.823	10.170	31	-0.376	0.7095
m_C	2.327	9.903	31	0.235	0.8158
m_A:time	0.919	2.288	172	0.402	0.6882
m_C:time	1.548	2.198	172	0.704	0.4822

Table S7. Results from the linear mixed model from the female group divided by tDCS polarity. All calculations use the sham group (f_S) as a reference. Number of subjects: *N* = 127.

Factor	Δ n_{corr}	SEM	*df*	*t*	*p*
Time	13.424	1.034	631	12.982	<0.0001*
Pre-training	1.115	0.056	123	19.811	<0.0001*
f_A	-7.587	5.801	123	-1.308	0.1933
f_C	-2.583	5.917	123	-0.436	0.6632
f_A:time	2.803	1.330	631	2.108	0.0354*
f_C:time	0.672	1.347	631	0.499	0.618

A.2 Supplementary Manuscripts

A.2.1 'Dynamic DNA Methylation Changes in the COMT Gene Promoter Region in Response to Mental Stress and Its Modulation by Transcranial Direct Current Stimulation'

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Dynamic DNA Methylation Changes in the *COMT* Gene Promoter Region in Response to Mental Stress and Its Modulation by Transcranial Direct Current Stimulation

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Abstract: Changes in epigenetic modifications present a mechanism how environmental factors, such as the experience of stress, can alter gene regulation. While stress-related disorders have consistently been associated with differential DNA methylation, little is known about the time scale in which these alterations emerge. We investigated dynamic DNA methylation changes in whole blood of 42 healthy male individuals in response to a stressful cognitive task, its association with concentration changes in cortisol, and its modulation by transcranial direct current stimulation (tDCS). We observed a continuous increase in *COMT* promotor DNA methylation which correlated with higher saliva cortisol levels and was still detectable one week later. However, this lasting effect was suppressed by concurrent activity-enhancing anodal tDCS to the dorsolateral prefrontal cortex. Our findings support the significance of gene-specific DNA methylation in whole blood as potential biomarkers for stress-related effects. Moreover, they suggest alternative molecular mechanisms possibly involved in lasting behavioral effects of tDCS.

Keywords: transcranial direct current stimulation; epigenetics; DNA methylation; stress response; *COMT*

1. Introduction

Epigenetic patterns are known to be dynamic and associated with environmental factors. Without altering the DNA sequence, epigenetic modifications affect chromatin structure and gene expression. Currently, one of the best studied epigenetic modifications is DNA methylation (DNAm), which plays an important role in gene regulation [1]. One factor which has consistently been associated with differential DNAm is stress [2,3]. Many studies link early life stress to long lasting differences in DNAm [4,5] or correlate severe psychiatric symptoms caused by traumatic and stressful life events with differential DNAm profiles [6]. Hence, epigenetic alterations might be an underlying mechanism how exposure to stress increases the risk of developing psychiatric disorders. Nevertheless, only little is known about the short-term dynamics of methylation changes after stress exposure. Immediate changes in DNAm can be induced by chemical stressors, such as dimethyl sulfoxide (DMSO) [7–9], and can already occur within 20 min after T-cell activation [10]. Furthermore, an experimental psychological stressor, the Trier Social Stress Test, has shown

to be associated with dynamic DNAm alterations in a stress-associated gene within 90 min after exposure [11]. The dynamic malleability of methylation changes in genes involved in cognitive stress is, therefore, a potentially critical mechanism for the regulation of human behavior. However, a precise characterization of the degree and time course of these changes is required. Moreover, opportunities to influence this process would be useful and may open new perspectives for individualized therapeutic strategies.

To this aim, we use transcranial direct current stimulation (tDCS), a non-invasive brain stimulation technique, which has been shown to modulate neuroplasticity [12]. Many studies have demonstrated the impact of tDCS on cognitive processes and training [13,14]. Most importantly it has been discussed as a potential treatment approach for neuropsychiatric disorders which are often associated with aberrant brain activation patterns [15]. However, so far little is known about the underlying molecular mechanisms of stimulation effects and how they potentially manifest as long-lasting cognitive improvements and amelioration of psychiatric symptoms. Since epigenetic modifications present a mechanism of how environmental factors can influence physiological reactions and, moreover, seem to be involved in the pathophysiology of psychiatric disorders, they might also be important for the manifestation of tDCS effects.

There is accumulating evidence that genetic factors interact with stimulation effects and contribute to inter-individual variability in tDCS responses [16]. Particularly, the Val108/158Met polymorphism of the catechol-O-methyltransferase (*COMT*) gene that regulates the dopamine metabolism [17] is associated with differential tDCS effects on executive functions [16,18]. COMT is involved in the degradation of dopamine and, therefore, plays a critical role in cognitive processes and executive functioning [19,20]. A physiological concentration of dopamine in the prefrontal cortex is important for optimal cognitive functioning [21] and a dysregulation of the dopaminergic system is associated with the pathophysiology of neurological and psychiatric disorders, such as schizophrenia and depression [22]. Furthermore, it has been shown that acute stress leads to an activation of the dopaminergic system [23,24], and the *COMT* gene seems to be crucially involved in the stress response [25]. The *COMT* genotype, which influences the enzyme's stability and hence dopaminergic activity [17], is also associated with an altered cortisol response [26,27]. Therefore, the promotor region of this gene appears to be a promising candidate to exemplify the epigenetic signatures of mental stress and its malleability by tDCS.

Thus, the present study aims at (i) determining the effects of tDCS on task performance, negative affect, and the physiological stress response, (ii) testing the notion that DNAm of the *COMT* gene is subject to immediate modulation by mental stress, and (iii) providing initial evidence that tDCS can influence this process.

2. Materials and Methods

2.1. Participants

The study sample was recruited in two cohorts. As a pilot study, 22 healthy participants (mean age: 23.6 years, SD = 3.0; mean years of formal education: 16.9, SD = 3.3) took part in the experiment. The effects of tDCS on task performance and affect were described in Wiegand et al. (2019) in more detail [28]. See Supplementary Figures S1 and S2 for *COMT* DNAm and cortisol data from this pilot cohort. Since, to our knowledge, this is the first human study investigating dynamic DNAm in the context of cognitive stress and its modulation by tDCS, no previously reported effect sizes for a power and sample size estimation were available. To increase reliability of our findings, we replicated the same experiment with another 20 healthy participants (mean age: 23.3 years, SD = 3.5; mean years of formal education: 14.4, SD = 6.2). See Supplementary Figures S3 and S4 for *COMT* DNAm and cortisol data from this replication cohort. Since there were no prominent differences between the data from the two cohorts, results in the main manuscript are reported for the merged cohort including all 42 participants. The inclusion criteria, experimental procedure, and sample handling and storage were the same for both cohorts, and instructions were given by the same instructor using a detailed script. All participants

were recruited within two years. The two cohorts showed no significant differences with respect to age ($t(40) = -0.39$, $p = 0.97$) or years of education ($t(40) = 1.13$, $p = 0.26$). To reduce inter-individual DNAm variability, all participants were aged between 18–30 years, male, non-smoking, and of European descent. Furthermore, screening excluded participants with a history of mental or neurological illness, relevant somatic disorders (two participants were suffering from hypothyroidism), dyscalculia, metallic foreign particles around the head, a cardiac pacemaker, and the usage of psychotropic or other medication that may impact DNAm status (two participants took L-thyroxine). All participants were right-handed according to the Edinburgh Handedness Inventory (laterality index = 98.06, SD = 6.44) [29] and German native speakers. Prior to study inclusion, all participants gave written informed consent to the experimental procedure approved by the University of Tübingen local ethics committee. The study was conducted in accordance with the Declaration of Helsinki in its latest version.

2.2. Adaptive 2-Back Paced Auditory Serial Addition Task (PASAT)

Participants were exposed to an adaptive, 2-back version of the Paced Auditory Serial Addition Task (PASAT) [28]. Numbers ranging from 1 to 9 were continuously presented via headphones. Participants were asked to add the current number to the number presented before the previous one (2-back) and to type in their answer by pressing a correspondingly labeled keyboard button. Parallel to the next stimulus presentation, they received visual feedback, i.e., the screen flashed green for a correct answer and red for an incorrect, late or missed answer. The inter-stimulus interval between digit presentations adapted to participants' performance. Initially set to 3 s, it was decreased by 0.1 s after four consecutive correct answers and increased 0.1 s after four consecutive wrong answers. The PASAT consisted of 16 practice trials followed by three task blocks lasting for 5 min, which were separated by breaks of 30 s. Due to the adaptive design the error percentage remained similar, although the number of correct trials could vary between task blocks.

2.3. Positive and Negative Affect Schedule (PANAS)

To assess changes in negative affect during the experimental procedure, participants were administered the German version of the 'Positive and Negative Affect Schedule' (PANAS), a self-report to determine the participants' current affective states [30,31]. Ten positive and ten negative adjectives were rated on a five-point Likert scale ranging from 1 'not at all' (in German: 'gar nicht') to 5 'very much' (in German: 'äußerst'). Participants completed the PANAS three times throughout each session: before starting the PASAT (pre), immediately after they completed the PASAT (post), and 90 min after task completion (follow-up).

2.4. Transcranial Direct Current Stimulation (tDCS)

A direct current of 1 mA was generated by a portable, battery-driven stimulator (NeuroConn GmbH, Illmenau, Germany) and applied via a pair of 5 × 7 cm electrodes covered with conductive paste (Ten20®, Weaver and Company, Aurora, CO, USA). The anodal electrode was placed over the left dorsolateral prefrontal cortex at F3 according to the international 10–20 system of electrode placement [32], whereas the cathodal reference electrode was fixated on the right upper arm over the deltoid muscle to prevent any opposite polarization of other brain regions that were not the target of the stimulation protocol [33]. Two minutes before PASAT onset, the current was faded in for 5 s. During the anodal stimulation session, a continuous current of 1 mA was delivered for 20 min until task completion and then faded out for another 5 s. During sham stimulation, the current was only administered for 30 s before fading out. Impedance was controlled by the device and did not exceed 10 kΩ. To ensure blinding effectiveness, participants were asked for tDCS adverse effects at the end of each session (see Supplementary Table S1).

2.5. Experimental Procedure

The experimental design was identical to Wiegand et al. (2019), where the behavioral data and changes in affect of the pilot cohort are described in more detail [28].

The study followed a single-blind, sham-controlled cross-over design. Each participant took part in two sessions with an interval of 7 days in between. To reduce variability, each session started at 2 PM. To ensure that the inclusion criteria were met, a brief screening including the Symptom-Checklist-90-Revised (SCL-90-R) to detect psychiatric symptoms and distress was performed in the first session [34]. Apart from that, the two sessions only differed in the type of stimulation (anodal or sham) participants received. The order of stimulation was randomized and counterbalanced across participants.

Each session started with a saliva sampling. Then, a venous catheter was placed, and the tDCS electrodes were fixated. Affective states were assessed (PANAS pre), and the instructions for the PASAT were given. The first blood sampling was done just before the stimulation started, but at least 15 min after the venous access had been established. Afterwards, participants were exposed to the 2-back PASAT while receiving tDCS (anodal or sham). Immediately after task completion, the second blood sample was collected and the PANAS (post) was administered. After removal of the electrodes, participants were exposed to relaxing music (genre: ambient electronic) via headphones until the end of the experiment, which was 90 min after task completion. Four more blood samples were collected 20, 40, 60, and 90 min after task completion. In addition, a second saliva sample was collected 30 min after the beginning of the 2-back PASAT. Finally, participants were administered the PANAS for a third time (follow-up). Supplementary Figure S5 depicts the experimental procedure graphically.

2.6. DNAm Analysis

Blood samples were collected in EDTA tubes (2.7 mL Monovette®, Sarstedt AG & Co. KG, Nümbrecht, Germany) and stored at −80 °C. DNA was extracted with the QIAamp Blood Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions using 400 µL whole blood sample. To increase DNA yield, the final elution step was repeated using the 100 µL eluate of the first elution. DNA was quantified using the Qubit® 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). Samples were stored at −20 °C.

Five hundred nanograms of genomic DNA were bisulfite converted using the EpiTect Fast Bisulfite Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Bisulfite-converted DNA was eluted with 20 µL of the provided elution buffer. The purified bisulfite-converted DNA was stored at −20 °C.

A region-specific polymerase chain reaction (PCR) was performed for the *S-COMT* promoter region using the PyroMark PCR Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions with previously published primers (F: 5-GAGTAGGTTGTGGATGG GTTGTA-3, R: 5-Biotin-ACATTTCTAAACCTTACCCCTCTA-3) [35]. Successful amplification and specificity of the PCR products was verified and visualized via agarose gel electrophoresis.

DNAm was analyzed by pyrosequencing on a PyroMark Q24 system (Qiagen, Hilden, Germany) using 5 µL biotinylated PCR product of each sample and a previously published sequencing primer (S: 5-GTAATATAGTTGTTAATAGTAGA-3) [35]. As in previous studies using the same pyrosequencing assay, DNAm levels of two CpG sites (hg19 reference genome coordinates: chr22:19,950,055 and chr22:19,950,064) were quantified using the PyroMark Q24 Software 2.0 (Qiagen, Hilden, Germany). Each sample was analyzed twice, and the mean percentage was used for further analysis. Samples with a deviation $\geq$ 3% between duplicates were repeated. To detect disparate amplification of unmethylated DNA fragments, a titration assay using standardized bisulfite-converted control DNA samples (EpiTect Control DNA, Qiagen, Hilden, Germany) with established DNAm levels of 0%, 25%, 50%, 75%, and 100% DNAm was performed.

2.7. Saliva Cortisol Concentration

Saliva was sampled in Salivettes® (Sarstedt AG & Co. KG, Nümbrecht, Germany) and stored at −80 °C. For analysis of cortisol levels, Salivettes® were thawed and centrifuged for 2 min at 1000× *g* to collect saliva. Cortisol concentrations were determined using the Cortisol Saliva ELISA kit (IBL International, Hamburg, Germany) according to manufacturer's instructions. Cortisol concentrations were determined in duplicates, and the mean coefficient of variation was below 10%.

2.8. Statistical Analysis

All statistical calculations were performed using the software R (Version 3.5.1) [36] including the package nlme [37]. The two cohorts were pooled for data analysis. Mean numbers of correct trials for each of the three task blocks were extracted from the adaptive 2-back PASAT as measure of task performance. For the PANAS questionnaire, mean scores were calculated for the 10 items comprising negative affect. Since DNAm at the two analyzed CpG sites was highly correlated at all time points ($r > 0.88$, $p < 3.43 \times 10^{-14}$), DNAm of the *COMT* gene promoter region was expressed as the mean level of methylation of the two CpG sites.

Multilevel modeling was chosen over repeated-measures ANOVA to allow analyses of the effects of *stimulation* and *session* within the same statistical model. For all analyses (i.e., task performance, affect changes, DNAm changes and cortisol level changes), a multilevel model with the fixed effects *stimulation*, *session*, and *time* (or *task block* accordingly in task performance data analyses) was estimated using maximum likelihood. A random intercept for each participant and random slopes for the effects of *session* and *time* (or *task block*) were included to account for individual differences in the outcome variable, in the effect of *session*, and in the effect of *time* (or *task block*) within each session. The error term was modeled as a first order autoregressive process to account for serial autocorrelations due to the repeated measures design. The severity of multicollinearity was assessed by the variance inflation factor (VIF). To assure a VIF < 10, the interaction of *stimulation* and *session* and, hence, the three-way interaction of *stimulation*, *session*, and *time* (or *task block*) was eliminated from the models for task performance, DNAm and cortisol levels [38,39]. For changes in affect, a full model was estimated as VIF < 10 was given for all predictors. A linear model was fitted for the analyses of task performance, DNAm and cortisol levels, whereas a quadratic term for time was included in the model for affect resulting in a better fit for changes over time. Unstandardized (B) as well as standardized (β) parameter estimates were reported and statistical significance was assessed at $p < 0.05$. Additionally, an analysis of variance table for each model is given in the Supplementary Tables S2–S5 reporting the overall significance of all terms [40]. Post-hoc pairwise comparisons were performed after significant effects.

Similarly, a multilevel model was fitted with the fixed effects *stimulation in first session* and *session*, including only DNAm data of the first time point of each session, to examine whether DNAm changes induced in the first session were preserved until the second session with respect to the type of stimulation received during the first session. Random intercepts estimated for each participant and random slopes for the effect of session were included to account for individual variance.

Finally, Pearson's correlation was used to test for an interrelation between changes in DNAm levels (*COMT*-methylation$_{post90}$-*COMT*-methylation$_{pre}$) and cortisol concentration (cortisol$_{post}$-cortisol$_{pre}$) and between changes in negative affect (negative affect$_{post}$-negative affect$_{pre}$) and in cortisol concentration during the first session.

3. Results

3.1. Study Sample

Participants were randomly assigned to the order of stimulation (anodal/sham or sham/anodal) they received during the two experimental sessions. The two resulting groups showed no significant differences with respect to age ($t(40) = 1.52$, $p = 0.14$), years

of formal education ($t(40) = 1.06$, $p = 0.30$), math performance at school ($t(36) = -0.54$, $p = 0.59$), body mass index ($t(40) = -0.24$, $p = 0.81$), global severity index (SCL-90-R) ($t(40) = 0.59$, $p = 0.56$), or *COMT* Val108/158Met genotype ($x^2 = 0.15$, $p = 0.93$). A more detailed description of the sample characteristics including sociodemographic variables and information on the *COMT* Val108/158Met genotype can be found in Supplementary Table S6.

3.2. Task Performance

Task performance in the adapted version of the PASAT was evaluated by a linear mixed model with the predictors *stimulation* (anodal, sham), *session* (1, 2), and *task block* (1, 2, 3). *Task block* (B = 3.80, SE = 0.99, β = 0.24, $t(202) = 3.84$, $p < 0.001$) and *session* (B = 9.53, SE = 0.79, β = 0.60, $t(202) = 12.07$, $p < 0.001$) significantly predicted task performance due to an increasing number of correct trials over the course of the three task blocks within each session and from the first to the second session, respectively. Furthermore, the interaction of *session* and *task block* predicted task performance significantly (B = −2.36, SE = 1.12, β = 0.15, $t(202) = -2.11$, $p = 0.036$). Neither *stimulation* (B = −0.39, SE = 0.97, β = −0.02, $t(202) = -0.40$, $p = 0.69$) nor the interaction between *stimulation* and *task block* (B = 1.54, SE = 1.29, β = 0.10, $t(202) = 1.19$, $p = 0.23$) predicted task performance significantly.

Follow-up t-tests showed significant increases in the number of correct trials from task block 1 to task block 2 ($t(41) = -2.86$, $p = 0.007$, $|d| = 0.44$), from task block 2 to task block 3 ($t(41) = -3.20$, $p = 0.003$, $|d| = 0.49$) and from task block 1 to task block 3 ($t(41) = -5.94$, $p < 0.001$, $|d| = 0.92$) during session 1. During session 2, there was a significant increase from task block 1 to task block 3 ($t(41) = -2.63$ $p = 0.012$, $|d| = 0.41$) and from task block 2 to task block 3 ($t(41) = -2.98$ $p = 0.005$, $|d| = 0.46$), but not from task block 1 to task block 2 ($t(41) = -0.33$ $p = 0.74$). Figure 1 depicts the number of correct trials for each task block during each session with regard to stimulation condition.

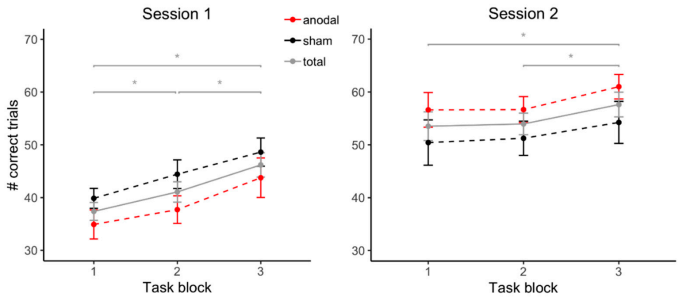

Figure 1. Task performance during each session with regard to stimulation condition. As the order of received stimulation ('anodal/sham' or 'sham/anodal') was a between-subject factor, participants receiving anodal stimulation during the first session ($n = 21$) received sham stimulation during their second session, and vice versa ($n = 21$). Error bars depict standard errors of the mean; asterisks mark $p < 0.05$.

3.3. Affective Changes

Changes in negative affect were investigated by a multilevel mixed model with the predictors *stimulation* (anodal, sham), *session* (1, 2), and *time* (pre, post, follow-up).

Session (B = −0.09, SE = 0.04, β = −0.38, $t(199) = -2.45$, $p = 0.015$) and *time* (B = −0.12, SE = 0.03, β = −0.53, $t(199) = -3.84$, $p < 0.001$) both significantly predicted changes in negative affect. While the interaction of *stimulation* and *time* did not predict negative affect significantly ((B = 0.07, SE = 0.04, β = 0.29, $t(199) = 1.59$, $p = 0.11$), the interaction of *session* and *time* predicted the outcome variable significantly (B = 0.14, SE = 0.05, β = 0.60,

$t(199) = 3.05$, $p = 0.003$). Furthermore, the three-way interaction of *stimulation*, *session*, and *time* predicted negative affect by trend (B = −0.12, SE = 0.07, β = −0.54, $t(199) = -1.85$, $p = 0.065$), indicating that the effect of tDCS on changes in negative affect might be different in the two sessions.

In the first session, participants receiving sham stimulation showed an increase in negative affect by trend ($t(20) = -1.95$, $p = 0.066$, $|d| = 0.42$), whereas the negative affect did not change in participants under anodal stimulation ($t(20) = -0.38$, $p = 0.71$). There were no changes in negative affect during the second session, neither for participants under anodal stimulation ($t(20) = -1.16$, $p = 0.26$), nor for participants in the sham condition ($t(20) = 0.13$, $p = 0.90$). Figure 2 depicts the changes in negative affect during each session with regard to stimulation condition.

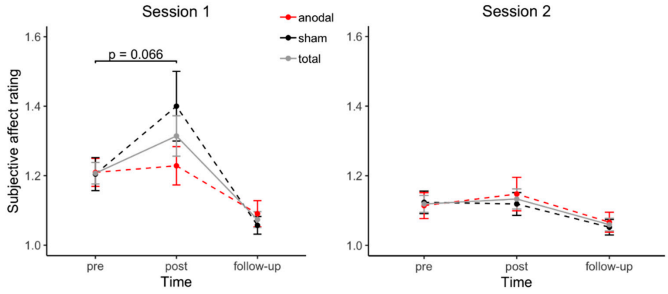

Figure 2. Changes in negative affect during each session with regard to stimulation condition. Subjective rating of negative affect is shown separately for each session in pre- and post-task and follow-up condition. As the order of received stimulation ('anodal/sham' or 'sham/anodal') was a between-subject factor, participants receiving anodal stimulation during the first session ($n = 21$) received sham stimulation during their second session, and vice versa ($n = 21$). Error bars depict standard errors of the mean.

3.4. DNAm Changes in COMT Gene Promoter Region

In a linear mixed model with the predictors *stimulation* (anodal, sham), *session* (1, 2) and *time* (hours), *stimulation* (B = 0.98, SE = 0.42, β = 0.13, $t(457) = 2.34$, $p = 0.020$) and *time* (B = 0.65, SE = 0.17, β = 0.06, $t(457) = 3.75$, $p < 0.001$) predicted DNAm levels significantly, implying an effect of tDCS on the DNAm and dynamic DNAm changes during the experimental procedure. While the interaction of *stimulation* and *time* (B = −0.09, SE = 0.23, β = −0.01, $t(457) = -0.37$, $p = 0.71$) did not predict the outcome variable significantly, the interaction of *session* and *time* significantly predicted DNAm levels (B = −0.64, SE = 0.17, β = −0.05, $t(457) = -3.77$, $p < 0.001$). This indicates that the effect of the PASAT performance on *COMT* DNAm over time differs between the first and the second session and that the tDCS effect occurs between and not within the interventions.

Follow-up t-tests showed a significant increase in DNAm during session 1 from time point pre (57.70% methylated) to post90 (59.33% methylated) disregarding the stimulation condition ($t(41) = -4.30$, $p < 0.001$, $|d| = 0.66$), driven by an almost continuous increase in DNAm levels during the experimental procedure. Further t-tests comparing 'pre' with all post time points during the first session, showed that this increase is significant from time point post20 (58.69% methylated) onwards ($t(41) < -2.48$, $p < 0.017$, $|d| > 0.38$). For session 2, no change in DNAm from time point pre to any post time point was observed ($|t(41)| < 1.8$, $p > 0.08$). Figure 3A depicts changes in DNAm over the experimental procedure separately for the two sessions.

Demonstrating that the increase in DNAm during session 1 was still present in session 2, i.e., that it was preserved over 1 week, a multilevel model was fitted with the predictors *stimulation in first session* (anodal, sham) and *session* (1, 2) including only DNAm data of

the first time point of each session. The interaction of *stimulation in first session* and *session* significantly predicted DNAm levels at the beginning of each session (B = 1.54, SE = 0.68, β = 0.21, $t(40) = 2.26$, $p = 0.030$). As depicted in Figure 3B, the increase in DNAm during the first session could still be detected in the beginning of session 2 in sham-treated participants ($t(20) = -2.95$, $p = 0.008$, $|d| = 0.64$), but not in anodal-treated participants ($t(20) = -0.13$, $p = 0.90$). Supplementary Figure S6 depicts individual DNAm data of the first time point of each session.

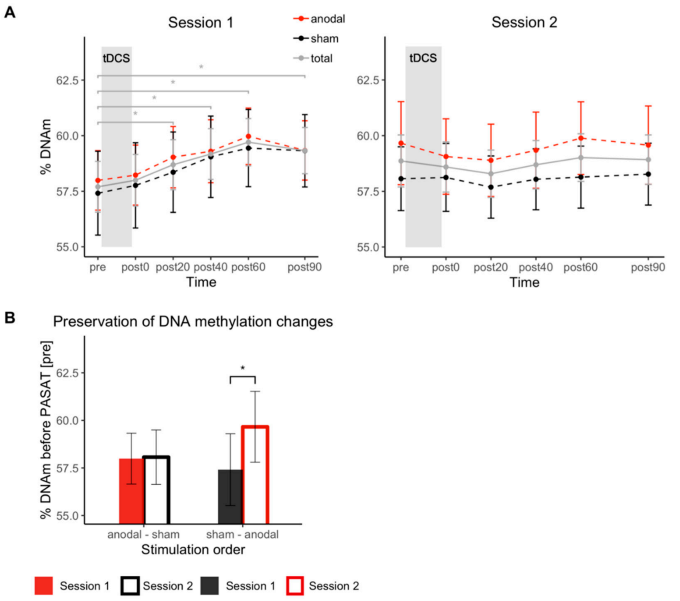

Figure 3. DNAm changes during each session with regard to stimulation condition and its preservation over one week. (**A**) % DNAm is shown separately for the six time points during each session. Each participant in the anodal stimulation group in session 1 ($n = 21$) was receiving sham stimulation in session 2, and vice versa ($n = 21$). (**B**) % DNAm for session 1 and 2 at time point 'pre' grouped by order of stimulation conditions ('anodal/sham' ($n = 21$) or 'sham/anodal' ($n = 21$)). The figure illustrates the comparison of % DNAm before ('pre') the first (session 1) and second (session 2) PASAT training within subjects who received tDCS ('anodal/sham') and subjects who did not receive effective tDCS in session 1 ('sham/anodal'). Error bars depict standard errors of the mean; asterisks mark $p < 0.05$.

3.5. Cortisol Changes

In a linear mixed model with the predictors *stimulation* (anodal, sham), *session* (1, 2) and *time* (pre, post), *session* (B = −0.08, SE = 0.02, β = −0.28, $t(121) = -3.40$, $p < 0.001$) predicted cortisol levels significantly, and *time* predicted cortisol levels by trend (B = 0.05, SE = 0.03, β = 0.18, $t(121) = 1.90$, $p = 0.060$). Neither *stimulation* (B = 0.02, SE = 0.03, β = 0.06, $t(121) = 0.76$, $p = 0.45$) nor the interaction of *stimulation* and *time* (B = −0.02, SE = 0.03, β = −0.06, $t(121) = -0.54$, $p = 0.59$) significantly predicted the outcome variable. However, the interaction of *session* and *time* predicted cortisol levels significantly (B = −0.07, SE = 0.03, β = −0.25, $t(121) = -2.60$, $p = 0.011$), indicating differences in cortisol changes in the two sessions.

Follow-up t-tests showed a significant increase in cortisol levels during session 1 ($t(41) = -2.50$, $p = 0.017$, $|d| = 0.39$), while no cortisol changes were detected in session 2 ($t(41) = -0.26$, $p = 0.80$). Figure 4 depicts changes in cortisol levels during each session.

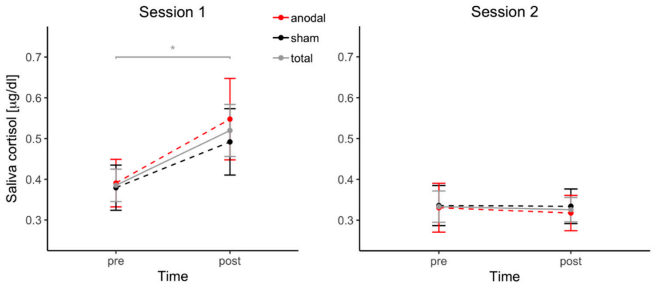

Figure 4. Cortisol concentration changes during each session with regard to stimulation condition. Saliva cortisol levels are shown separately for each session in pre- and post-task condition. As the order of received stimulation ('anodal/sham' or 'sham/anodal') was a between-subject factor, participants receiving anodal stimulation during the first session ($n = 21$) received sham stimulation during their second session, and vice versa ($n = 21$). Error bars depict standard errors of the mean; asterisk marks $p < 0.05$.

3.6. Correlation of DNAm Changes and Cortisol Changes

During the first session, there was a significant correlation between changes in cortisol concentration and changes in DNAm levels ($r = 0.359$, $p = 0.019$), as depicted in Figure 5. There was no correlation between changes in cortisol concentration and changes in negative affect ($r = -0.139$, $p = 0.38$).

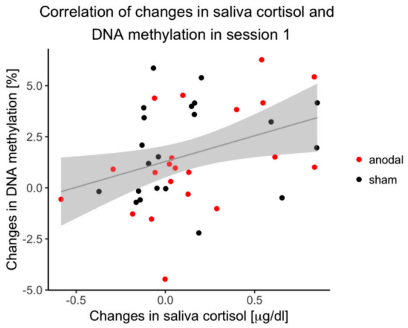

Figure 5. Correlation of DNAm changes and cortisol changes. Correlation of changes in DNAm during session 1 with changes in saliva cortisol concentration ($n = 42$). Regression line with 0.95 confidence interval.

4. Discussion

The key findings of the present study are (i) a continuous increase of *COMT* gene promotor methylation in blood after a challenging, stressful, and frustrating cognitive task which correlates with an increase in salivary cortisol, (ii) increased *COMT* DNAm detectable one week later, and (iii) a suppression of this lasting effect by concurrent activity-enhancing anodal tDCS to the dorsolateral prefrontal cortex. These data support the notion

of dynamic DNAm in response to mental stress that is associated with changes in cortisol levels and can be modulated by tDCS.

To date, few studies have reported dynamic changes in DNAm in response to a mental stress paradigm. In fact, DNAm levels have been regarded as rather stable, long-term epigenetic marks in somatic cells, which might even be maintained over numerous cell divisions [41]. However, previous studies have associated differential DNAm patterns with diseases, such as post-traumatic stress disorder, which can be triggered by the experience of a single stressful life event [42]. Yet, little is known about the time frame of the formation of these methylation changes and the amount of stress load required to induce these changes. An increase in *OXTR* DNAm was observed already 10 min after the exposure to the TSST [11] and changes in *FKBP5* gene expression within 70 min [43]. Congruent with these studies, our data add evidence to those immediate effects on DNAm and, moreover, the continuous increase in *COMT* promoter methylation over the course of five independent measurements within 90 min after stress exposure makes a random variation rather unlikely.

The correlation of changes in saliva cortisol and DNAm might indicate that the stress hormone cortisol links stress with DNAm changes detectable in peripheral blood. This is also in line with previous findings reporting DNAm differences after glucocorticoid exposure [44,45]. We specifically chose to investigate methylation dynamics of the *COMT* gene, as several studies indicate its role in stress reactivity on a genetic [46,47] and epigenetic level [48]. Furthermore, the *COMT* gene interacts with cognitive performance and tDCS effects, supporting its eligibility as candidate gene [16,49]. Due to the inaccessibility of living brain tissue, we investigated DNAm differences in whole blood. This leads to the question of what extent blood DNAm can serve as proxy marker for DNAm in neuronal tissue. Indeed, for *COMT* DNAm several studies show that methylation status in peripheral tissue can serve as surrogate for brain DNAm [48,50]. More importantly, our data show that changes in DNAm correlate with changes in cortisol concentration. Saliva cortisol correlates well with the concentration of free circulating cortisol [51]. Therefore, it is possible that the dynamic DNAm is mediated by alterations in plasma cortisol levels. Since cortisol can cross the blood brain barrier, it might elicit similar effects on DNAm in neuronal tissue. However, the observed correlation could also be due to differences in catecholamines, which are released in parallel with cortisol in the context of stress. Taken together, these data support the hypothesis that dynamic epigenetic modifications are invoked immediately by exposure to stress and associated with the released stress hormones.

The COMT enzyme is involved in the degradation of catecholamines, such as dopamine, and changes in its expression levels could consequently affect catecholamine concentrations. There are two isoforms of COMT regulated by two different promotors. The predominant form in peripheral tissue is the soluble isoform (S-COMT) [52]. The CpG sites investigated in our study are located within its promotor region and, hence, may be involved in the regulation of *S-COMT* expression. However, since the observed DNAm differences were relatively small, their functional relevance needs to be clarified in future studies including gene expression data. Furthermore, in the brain, the primarily expressed mRNA is encoding the membrane-bound isoform (MB-COMT); however, there is evidence that, to a lower extent, the S-COMT form is also expressed [52]. The investigated CpG sites fall within the gene body region of *MB-COMT*. Therefore, if *COMT* DNAm is affected to a similar extent in neuronal tissue, effects on *COMT* expression levels in the brain might be diverse [53]. Given this limitation, conclusions about a potential epigenetic feedback loop controlling prefrontal dopamine activity, as well as neuroplasticity and behavior, is beyond the scope of this study. The dynamics of stress-related epigenetic changes and its relation to the neurotransmitter metabolism in the brain are likely better suited to animal studies.

Considering that the PASAT only presents a relatively mild stressor, it is quite remarkable that it elicited significant changes in DNAm which persisted even over one week. Of note, the PASAT task is originally designed as a measure of information processing ability [54]. However, previous studies have also demonstrated that the PASAT induces

psychological stress [55]. In this study, a more challenging design (2-back task) was used, probably leading to an even more stressful experience while performing this not only cognitively but also emotionally challenging task. This is supported by the increase in cortisol levels after exposure to the first task. Interestingly, no changes in cortisol levels were observed during the second session. Probably, participants are adapting to the task, which is why a stress response is only elicited when the task is unfamiliar. Being already mentally prepared to encounter a difficult task that comes along with frequent negative feedback might attenuate the stress experience. Although this indicates the limited validity of the PASAT as a mere stress task in a cross-over design, it circumvents the necessity of a less stressful control task. It controls already for the possibility that any other parameter, such as, for example, the physical stress of the venous catheter placement, might have led to the observed changes. Nevertheless, venous catheter placement was done with a relatively short interval before the first blood sample was collected, which could have affected baseline measurements.

Similar to the observed changes in cortisol and DNAm levels, there was also an increase in negative affect by trend during the first, but not during the second experimental session. However, in contrast to the molecular markers, these changes in affect seemed to be suppressed by anodal tDCS. As this short-term effect of tDCS on the affective experience is not observed in DNAm and cortisol levels, a correlation between changes in affect and cortisol is missing. The at least trend-wise effect of tDCS on negative affect is in line with the hypothesis that activity enhancing stimulation applied over the left dorsolateral prefrontal cortex increases cognitive control over emotion and, thereby, leads to stabilization in affect [56,57].

Furthermore, the application of tDCS during the first session affected the preservation of DNAm changes. While, in sham-treated participants, an increase in DNAm was still detectable one week later, methylation levels returned to baseline when anodal stimulation had been applied. Although we were not able to detect a significant difference between the active and sham stimulated subjects in salivary cortisol levels 30 min after intervention, it is possible that these differed in the cumulated amount of cortisol excretion over the time of the experiment. This was not captured by our experimental design, but a modulation of the stress response by brain stimulation has been reported previously [58,59], and may have been involved in mediating the observed differences in the stability of stress-induced DNAm changes. It has been previously shown in a mouse model that activity enhancing tDCS can lead to alterations in histone modifications, chromatin remodeling and changes in gene expression [60]. Our data provide preliminary evidence for a lasting modulation of DNAm changes by tDCS, suggesting that tDCS induces system-wide effects detectable in peripheral tissues, such as whole blood, through intermediary pathways.

One confounding factor which might affect DNAm are alterations in blood cell composition. Since epigenetic patterns are cell type specific, DNAm measured in whole blood might be influenced by cell type composition. According to the iMETHYL database, which provides cell type specific DNAm patterns based on a Japanese population, the methylation level of the investigated CpG sites in the *COMT* gene promoter region is very similar between neutrophils and monocytes but substantially lower for CD4-positve T-lymphocytes [61–63]. Studies investigating stress-induced immunological reactions report changes in leukocyte counts after severe physical or acute psychological stress, which is often accompanied by increased glucocorticoid levels [64]. Whether these effects can already occur within a narrow time frame of two hours is disputed and potentially species specific [65]. For humans, there is evidence that cortisol induced effects on leukocyte composition occur with a time lag of two hours [66,67]. Therefore, we believe that it is rather unlikely that the increase in *COMT* promotor methylation already present 20 min after stress exposure reported here is merely a secondary effect of changes in cell type composition. Nevertheless, controlling for potential cell type composition effects in future studies will be reasonable.

The novel findings of the present study evoke several lines of research to validate and extend our results. Previous research suggests that activity-enhancing stimulation applied over the left dorsolateral prefrontal cortex increases cognitive control over emotion and, thereby, leads to stabilization in affect [56,57]. Since task-induced changes in negative affect missed significance in our study, future studies using well-established mental stress paradigms with greater power to induce changes in affect are needed to investigate the relation between tDCS effects on emotion regulation, cortisol concentration changes, and the persistence of DNAm changes. Furthermore, longitudinal studies with several sessions of tDCS application in the context of mental stress may help to investigate long-term effects of tDCS treatment on DNAm. Previous studies have shown that DNAm might not only change in association with stress exposure but also in response to different psychological treatments, such as cognitive-behavioral therapy (CBT) [68]. Given that our data suggest that tDCS reduces potentially maladaptive epigenetic effects in response to stressful experiences, it might be a valuable addition to complement conventional therapies, such as exposure-based CBT.

We specifically chose to investigate the *COMT* gene because of its role in stress response, cognitive performance, and tDCS effects. However, there are also other genes that have been associated with differences in neurostimulation efficacy. In particular, a polymorphism within *BDNF*, the gene encoding the brain-derived neurotrophic factor, has been shown to interact with the effects of tDCS [16]. Moreover, in a mouse model, previous research has observed differences in epigenetic patterns of the *BDNF* gene in response to tDCS [60]. In addition, there are further genes that are important in dopamine regulation, particularly in other tissues. Further studies are needed to clarify whether DNAm in *BDNF* and other dopamine-related genes also plays a role in tDCS effects in humans, and, in addition, genome-wide approaches may be important to identify new candidate genes.

Another limitation of our study concerns the sample size. For tDCS effects, we included an adequate number of participants as we can assume small to intermediate effect sizes [69]. However, since this is the first human study investigating the effects of tDCS on DNAm changes, it was an explorative approach to detect potential effects and provide a first estimation of effect sizes for future research. Randomized clinical trials using a parallel design are needed to replicate our findings and to investigate whether changes in DNAm might also be involved in long-lasting therapeutic effects of tDCS.

In the field of molecular psychiatry, the inaccessibility of living brain tissue is a major challenge. However, our findings support the notion of DNAm status in whole blood as a potential biomarker in the context of stress-related behavior, most likely via intermediary hormones. On that account, it is reasonable to study peripheral tissue in order to track epigenetic traces of a stressful cognitive effort in behaving human subjects, with due caution regarding the direct transferability to changes in the brain. Finally, by showing that prefrontal tDCS can affect the stability of stress-induced DNAm changes in a gene regulating neurotransmission, these data point towards possible alternative pathways that might be involved in the therapeutic effects of brain stimulation.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/biom11111726/s1, Figure S1: DNA methylation changes in pilot cohort during each session with regard to stimulation condition, Figure S2: Cortisol concentration changes in pilot cohort during each session with regard to stimulation condition, Figure S3: DNA methylation changes in replication cohort during each session with regard to stimulation condition, Figure S4: Cortisol concentration changes in replication cohort during each session with regard to stimulation condition, Figure S5: Experimental procedure, Figure S6: Preservation of DNAm changes over one week with regard to the order of stimulation condition. Table S1: TDCS adverse effects, Table S2 Analysis of Variance Table: Task performance, Table S3: Analysis of Variance Table: Affect changes, Table S4: Analysis of Variance Table: DNA methylation changes, Table S5: Analysis of Variance Table: Cortisol concentration changes, Table S6: Sample characteristics.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the local ethics committee of the University of Tübingen (protocol code 129/2016BO2 and approved on 12 April 2016).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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A.2.2 'Supplementary Material to: Dynamic DNA Methylation Changes in the COMT Gene Promoter Region in Response to Mental Stress and Its Modulation by Transcranial Direct Current Stimulation'

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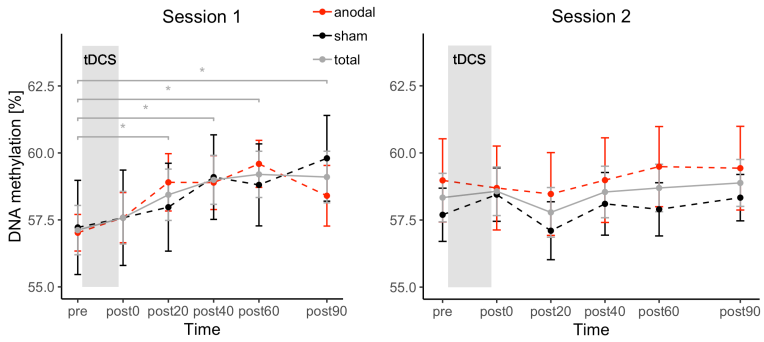
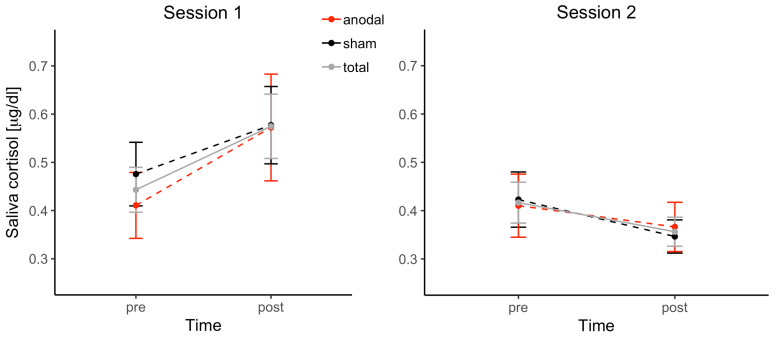
Supplementary Material

Data from pilot cohort only (n = 22)

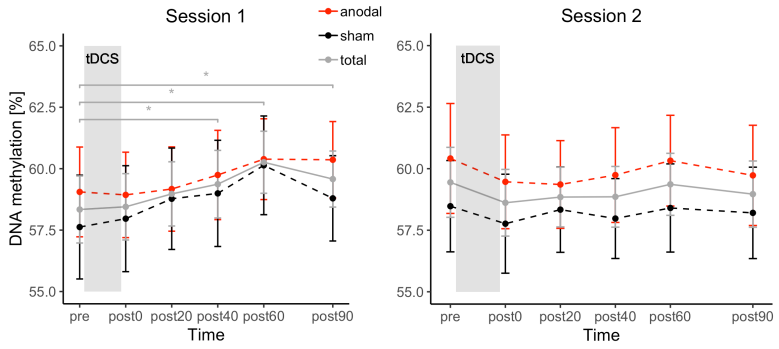


Supplementary Figure S1. DNA methylation changes in pilot cohort during each session with regard to stimulation condition. % DNA methylation is shown separately for the six time points during each session. Each participant in the anodal stimulation group in session 1 (n = 11) was receiving sham stimulation in session 2, and vice versa (n = 11). Error bars depict standard errors of the mean; asterisks mark $p < 0.05$.

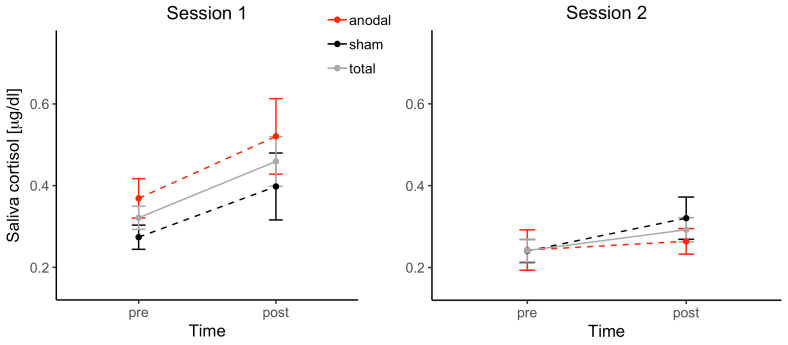


Supplementary Figure S2. Cortisol concentration changes in pilot cohort during each session with regard to stimulation condition. Saliva cortisol levels are shown separately for each session in pre- and post-task condition. As the order of received stimulation (anodal/sham or sham/anodal) was a between-subject factor, participants receiving anodal stimulation during the first session (n = 11) received sham stimulation during their second session, and vice versa (n = 11). Error bars depict standard errors of the mean.

Data from replication cohort only (n = 20)



Supplementary Figure S3. DNA methylation changes in replication cohort during each session with regard to stimulation condition. % DNA methylation is shown separately for the six time points during each session. Each participant in the anodal stimulation group in session 1 (n = 10) was receiving sham stimulation in session 2, and vice versa (n = 10). Error bars depict standard errors of the mean; asterisks mark $p < 0.05$.



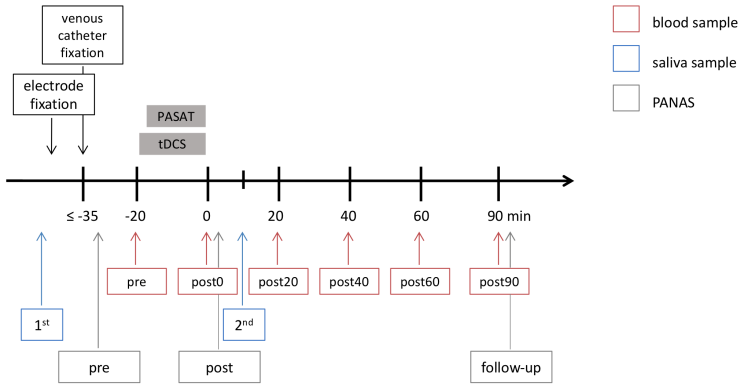
Supplementary Figure S4. Cortisol concentration changes in replication cohort during each session with regard to stimulation condition. Saliva cortisol levels are shown separately for each session in pre- and post-task condition. As the order of received stimulation (anodal/sham or sham/anodal) was a between-subject factor, participants receiving anodal stimulation during the first session (n = 10) received sham stimulation during their second session, and vice versa (n = 10). Error bars depict standard errors of the mean.

Supplementary Table S1. tDCS adverse effects. Adverse sensations were assessed on a 5-point Likert scale (n = 42). A nominal significant difference was observed for a tingling sensation at the site of the electrode. However, this difference did not remain significant when correcting for multiple testing.

Sensation	Sham tDCS Mean (SD)	Anodal tDCS Mean (SD)	*t*	*P*
Tingling at the site of the electrode	2.17 (1.20)	2.71 (1.35)	2.31	0.026
Tingling elsewhere in the area of the head	1.22 (0.52)	1.41 (0.92)	1.19	0.243
Exhaustion	1.07 (0.26)	1.20 (0.46)	1.40	0.168
Slight itching	1.68 (0.99)	2.05 (1.22)	1.78	0.083
Headache	1.05 (0.22)	1.05 (0.32)	0.00	1.000
Nausea	1.00 (0.00)	1.00 (0.00)	-	-

Furthermore, we asked participants at the end of both sessions for their subjective assessment of whether they received sham or verum stimulation. No significant differences in subjective assessments were found between sham and verum conditions (Fisher's exact test: p=0.758 (session 1), p=0.058 (session 2)).

Experimental Procedure



Supplementary Figure S5. Experimental procedure. Schematic representation of an experimental session.

Analysis of Variance Tables

Supplementary Table S2. Task performance

Term	*F*	*p*
Intercept	*F(1,202)* = 776.10	*p* < 0.001
Stimulation	*F(1,202)* = 0.17	*p* = 0.68
Session	*F(1,202)* = 142.71	*p* < 0.001
Task block	*F(2,202)* = 5.40	*p* = 0.005
Stimulation * task block	*F(2,202)* = 0.83	*p* = 0.44
Session * task block	*F(2,202)* = 2.33	*p* = 0.10

Supplementary Table S3. Affect changes

Term	*F*	*p*
Intercept	*F(1,199)* = 5366.20	*p* < 0.001
Stimulation	*F(1,199)* = 0.11	*p* = 0.75
Session	*F(1,199)* = 2.75	*p* = 0.09
Time	*F(2,202)* = 11.02	*p* < 0.001
Stimulation * session	*F(1,199)* = 0.28	*p* = 0.60
Stimulation * time	*F(2,202)* = 1.17	*p* = 0.31
Session * time	*F(2,199)* = 7.18	*p* = 0.001
Stimulation * session * time	*F(2,199)* = 1.72	*p* = 0.18

Supplementary Table S4. DNA methylation changes

Term	*F*	*p*
Intercept	*F(1,457)* = 3876.71	*p* < 0.001
Stimulation	*F(1,457)* = 5.89	*p* = 0.016
Session	*F(1,457)* = 0.41	*p* = 0.52
Time	*F(1,457)* = 17.09	*p* < 0.001
Stimulation * time	*F(1,457)* = 0.14	*p* = 0.71
Session * time	*F(1,457)* = 14.25	*p* < 0.001

Supplementary Table S5. Cortisol concentration changes

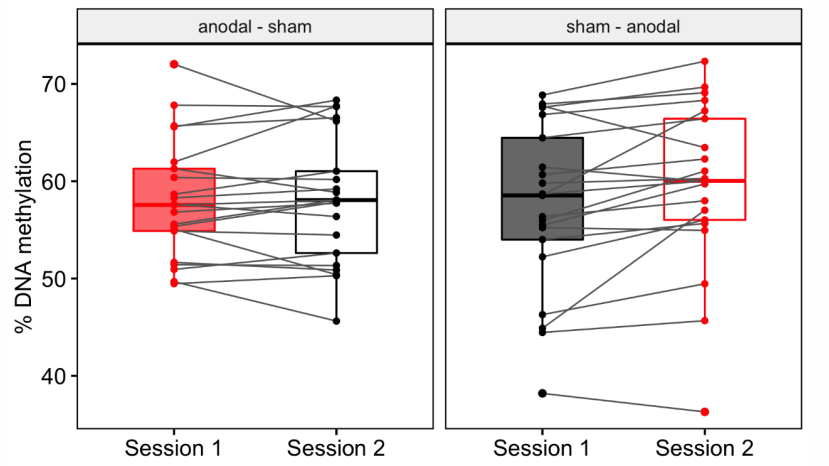
Term	*F*	*p*
Intercept	*F(1,121)* = 6.33	*p* = 0.013
Stimulation	*F(1,121)* = 2.51	*p* = 0.12
Session	*F(1,121)* = 6.12	*p* = 0.014
Time	*F(1,121)* = 0.38	*p* = 0.54
Stimulation * time	*F(1,121)* = 0.01	*p* = 0.93
Session * time	*F(1,121)* = 6.75	*p* = 0.011

Sample description

Supplementary Table S6. Sample characteristics

	Group sham – anodal (n=21)	**Group anodal – sham (n=21)**
Age [years]	22.71 (± 2.81; 18-28)	24.19 (± 3.44; 18-29)
Years of education [years]	16.38 (± 2.99; 12-22)	17.40 (± 3.29; 12-23)
Math performance at school*	10.76 (± 2.47; 5-15)	10.23 (± 2.93; 5-15)
Laterality index**	99.05 (± 4.37; 80-100)	97.08 (± 8.00; 70-100)
BMI [kg/m^2]	23.00 (± 2.10; 19.59-27.44)	22.86 (± 1.83; 19.94-25.98)
SCL-90-R		
Somatization	0.16 (± 0.18; 0.00-0.58)	0.21 (± 0.22; 0.00-0.75)
Obsessive-compulsive	0.41 (± 0.36; 0.00-1.20)	0.38 (± 0.29; 0.00-1.00)
Interpersonal sensitivity	0.18 (± 0.19; 0.00-0.67)	0.22 (± 0.28; 0.00-1.00)
Depression	0.19 (± 0.22; 0.00-0.69)	0.27 (± 0.27; 0.00-1.00)
Anxiety	0.18 (± 0.13; 0.00-0.40)	0.11 (± 0.13; 0.00-0.40)
Anger-hostility	0.19 (± 0.22; 0.00-0.83)	0.21 (± 0.26; 0.00-0.83)
Phobic anxiety	0.05 (± 0.12; 0.00-0.43)	0.01 (± 0.03; 0.00-0.14)
Paranoid ideation	0.12 (± 0.21; 0.00-0.83)	0.20 (± 0.33; 0.00-1.17)
Psychoticism	0.06 (± 0.09; 0.00-0.30)	0.15 (± 0.21; 0.00-0.70)
GSI	0.18 (± 0.12; 0.00-0.44)	0.21 (± 0.18; 0.00-0.69)
***COMT* genotype**		
Val/Val	7	7
Val/Met	8	9
Met/Met	6	5

Mean (± standard deviation, range), GSI: Global severity index, SCL-90-R: Symptom-Checklist-90-Revised
* According to the German academic grading system (15-point scale)
** Edinburgh Inventory[1]



Supplementary Figure S6. Preservation of DNAm changes over one week with regard to the order of stimulation condition. % DNAm for session 1 and 2 at time point 'pre' grouped by order of stimulation conditions ('anodal - sham' (n = 21) or 'sham - anodal' (n = 21)). The figure illustrates the comparison of % DNAm before ('pre') the first (session 1) and second (session 2) PASAT training within subjects who received tDCS ('anodal - sham') and subjects who did not receive effective tDCS in session 1 ('sham - anodal').

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