Aus der Klinik für Psychiatrie und Psychotherapie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Modifying Emotional Memory in Healthy Human Subjects Through Transcranial Direct Current Stimulation of the Prefrontal Cortex

zur Erlangung des akademischen Grades
Medical Doctor – Doctor of Philosophy in Medical Neurosciences
(MD/PhD in Medical Neurosciences)

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CONTENTS:

1. SUMMARY

a) ABSTRACT (ENGLISH)

Background:
Various pharmacological and non-pharmacological interventions have been used to modify emotional memory. Influencing emotional memory could have important therapeutic implications in the treatment of psychiatric disorders. Many interventions that have shown promising results in animal studies cannot be used in humans because of their invasive nature. Therefore, it is critical to investigate non-invasive interventions that can safely modify emotional memory in humans with minimal side effects.

Methods:
We used a non-invasive form of brain stimulation, more specifically transcranial direct current stimulation (tDCS) to target different emotional memory processes in healthy human subjects. TDCS influences cortical excitability with polarity dependent effects; anodal stimulation exerts an excitatory effect through neuronal depolarization whereas cathodal tDCS exerts an inhibitory effect through hyperpolarization. We performed two studies with reversed polarity of tDCS electrodes targeting reconsolidation of fear memory in the prefrontal cortex. In a third study, we investigated the effects of excitatory tDCS targeting the prefrontal cortex on cognitive reappraisal.

Results:
While tDCS (right prefrontal: anodal, left supraorbital: cathodal) enhanced fear memories, tDCS (right prefrontal: cathodal, left supraorbital: anodal) did not have any effect on fear memories. Anodal tDCS of the prefrontal cortex facilitated cognitive reappraisal during emotional upregulation as well as downregulation by either increasing or decreasing emotional responsiveness.

Conclusions:
Our results indicate that anodal tDCS of the prefrontal cortex is effective in modifying emotional memory and modulating cognitive reappraisal. However, cathodal tDCS of the prefrontal cortex did not affect emotional memory. More studies with additional control groups are needed to develop tDCS as an effective neuromodulatory tool to consistently influence emotional memory.

b) ABSTRACT (GERMAN)

Einführung:
Bisherige Studien haben verschiedene pharmakologische und nicht pharmakologische Interventionen untersucht, um das emotionale Gedächtnis zu beeinflussen. Die Modifizierung des emotionalen Gedächtnisses könnte wichtige therapeutische Konsequenzen haben, vor allem in Hinsicht auf die Behandlung psychiatrischer Erkrankungen. Viele Interventionen, die in Tierstudien gute Ergebnisse gezeigt haben, können aufgrund ihrer Invasivität in humanen Studien nicht durchgeführt werden. Deshalb ist es wichtig, nicht-invasive Verfahren, die nur geringe Nebenwirkungen haben und das emotionale Gedächtnis beeinflussen könnten, zu untersuchen.

Methodik:
Unser Ziel war es, mittels nicht-invasiver Hirnstimulation beziehungsweise Gleichstromstimulation (tDCS), diverse Gedächtnisprozesse in gesunden Probanden zu beeinflussen. TDCS beeinflusst kortikale Exzitabilität mit polaritäts-abhängigen Effekten: anodale Stimulation wirkt exzitatorisch durch neuronale Depolarisation; cathodale Stimulation wirkt inhibitorisch durch neuronale Hyperpolarisation. Wir haben zwei Studien mit reversierter Polarität durchgeführt, um die Rekonsolidierung des Angstgedächtnisses im präfrontalen Kortex zu untersuchen. In einer dritten Studie haben wir den Effekt auf anodal tDCS auf Neubewertung während der Emotionsregulation untersucht.

Ergebnisse:
Wir konnten zeigen, dass tDCS (rechts präfrontal: anodal, links supraorbital: cathodal) das Angstgedächtnis verstärkt, aber tDCS (rechts präfrontal: cathodal, links supraorbital: anodal) keinen Effekt auf das Angstgedächtnis hat. Die anodale Stimulation auf den präfrontalen Kortex führt zu einer Verstärkung der Neubewertung während die Emotionen hoch- und herrunterreguliert werden.

Schlussfolgerung:
Unsere Ergebnisse zeigen, dass anodale Stimulation auf den präfrontalen Kortex emotionales Gedächtnis und Neubewertung während Emotionsregulation beeinflusst. Allerdings hat cathodale Stimulation auf den präfrontalen Kortex keinen Effekt auf das emotionale Gedächtnis. Mehr Studien mit zusätzlichen Kontrollgruppen werden benötigt, um tDCS als effektiver Neuromodulator für das emotionale Gedächtnis zu entwickeln.

c) INTRODUCTION

Various interventions targeting different memory processes have been studied as a potential tool to disrupt negative emotional memory, which in turn could have therapeutic implications. Emotion regulation is one such technique, which can modify maladaptive emotional reactions. Previous studies have investigated cognitive reappraisal strategies to up- or downregulate negative emotions elicited for example, by pictures or videos (Eippert et al., 2007; Kanske, Heissler, Schönfelder, Bongers, & Wessa, 2011). Recently, a number of studies have investigated reconsolidation, which offers a unique window where the memory trace is thought to be labile after reactivation, and thus more susceptible to manipulation (Dudai, 2006). In animals, protein synthesis inhibitors directly injected into the basal and lateral nucleus of the amygdala have been successfully used to modify fear memories during the reconsolidation window (Duvarci & Nader, 2004; Nader, Schafe, & Le Doux, 2000). Such invasive interventions have an obvious limitation; they cannot be used in humans.

In humans, propranolol (Kindt, Soeter, & Vervliet, 2009; Wood et al., 2015) and cortisol (Meir Drexler et al., 2015; Meir Drexler, Merz, Hamacher-Dang, & Wolf, 2016) have been investigated but the effects are inconsistent. Schiller et al. (2010) reported that it is possible to non-invasively rewrite negative emotional memory in humans through behavioural safety learning during the reconsolidation window. Though this approach was promising, subsequent studies also failed to replicate these effects consistently (Golkar, Bellander, Olsson, & Ohman, 2012; Kindt & Soeter, 2013). Therefore, there is an urgent need to develop alternative interventions that are effective in modifying emotional memory safely in humans.

Transcranial direct current stimulation (tDCS), a form of non-invasive brain stimulation, is a promising tool, which could potentially modify emotional memory. However there are only a few published studies exploring the effects of tDCS on emotional memory till date. TDCS is known to modify cortical excitability with polarity dependent effects. Anodal tDCS results in depolarization of neurons, leading to an excitatory effect, whereas cathodal tDCS results in hyperpolarization, and thus inhibition of cortical neurons (Nitsche & Paulus, 2000). Based on these antagonistic effects on cortical excitability, effects have been shown on various forms of memory (Been, Ngo, Miller, & Fitzgerald, 2007). Our objective was to modify processes like reconsolidation and cognitive reappraisal through tDCS of the prefrontal cortex.

d) METHODS

We designed three experiments to modify emotional memory through tDCS in healthy human subjects. In the first study, we performed tDCS (right prefrontal: anodal, left supraorbital: cathodal) during the reconsolidation window (Mungee et al., 2013). In the second study, we reversed the electrode polarity and performed tDCS (right prefrontal: cathodal, left supraorbital: anodal) during the reconsolidation window (Mungee, Burger, & Bajbouj, 2016). We hypothesized that reversing the electrode polarity should achieve the opposite effect in line with the physiologically antagonistic effets of tDCS. In our third study, we stimulated the right dlPFC with anodal tDCS to investigate the effects of increased dlPFC excitability on cognitive reappraisal (Feeser, Prehn, Kazzer, Mungee, & Bajbouj, 2014). Table 1 summarizes the three experiments.

For all three studies, healthy individuals were recruited for participation. Individuals with contraindications to tDCS or a history of psychiatric or neurological disease were excluded from the study. The study was approved by the local ethics committee of Charité Universitätsmedizin, Campus Benjamin Franklin, Berlin. All participants were provided a complete oral and written description of the study and informed consent was obtained from each participant before participation.

In the first study dealing with fear memory reconsolidation, 74 subjects were included and randomly assigned to the tDCS or sham group. The study was designed as a within-subjects trial conducted over three consecutive days. On Day 1, all participants underwent fear conditioning with partial reinforcement. The conditioned stimuli (CS) were blue and yellow squares and the unconditioned stimulus (US) was a low-intensity electric shock applied to the right wrist. One stimulus was paired with the US in 38% of the trials (CS+) and the other was never paired with a shock (CS–). A Grass Medical Instruments stimulator (Grass Medical Instruments, Quincy, Massachusetts, USA) was used to deliver 50 pulses/s for 200 ms. The intensity of the electric shock was individually determined, so that the electric shock was uncomfortable but not painful. The starting stimulus was 10 V and we increased it depending on the individual threshold to a maximum intensity of 60 V. 10 presentations of each CS + and CS – were presented in a randomized order to the participants; six additional CS+ presentations were associated with a shock (US). Skin conductance responses (SCR) were recorded by the Schuhfried Biofeedback X-pert 2000 device (Schuhfried, Moedling, Austria). On the second day, all participants were

shown a CS + reminder using a single presentation of the coloured square paired with the shock on day 1 (CS +). Immediately after this, the participants in the tDCS group were stimulated for a total duration of 20 min by two saline- soaked surface sponge electrodes (15 cm^2) during the reconsolidation window. The participants in the sham group received only a brief current for the first 30 s to mimic the itching associated with real stimulation. The anodal electrode was placed over the right dlPFC (F4 location of the international 10 : 20 EEG system (Jasper, 1958) and the cathode over the contralateral supraorbital area close to the ventromedial prefrontal cortex (vmPFC) (Figure 1). We used a constant current battery-driven tDCS stimulator (CX6650; Rolf Schneider Electronics, Gleichen, Germany). On Day 3, fear responses in both the groups were assessed by measuring their SCR. We presented the participants with 10 CS + and 11 CS – presentations in a randomized order. An extra presentation of CS – was shown to maintain the total number of trials on all 3 days equally.

In our second study with fear memory reconsolidation, we included 25 subjects and followed the same protocol as in the first study with one important change: we reversed the polarity of the tDCS electrodes so that the cathodal electrode was placed over the right dlPFC with electrodes placed at the right frontolateral location (F4 of the international 10 : 20 EEG system (Jasper, 1958) and the anode over the contralateral supraorbital area close to the vmPFC.

In the third experiment involving cognitive reappraisal, 48 participants were included. The study was designed as a double blind, between-subjects, sham-controlled trial conducted on two separate days. On the first day, the subjects received extensive training to familiarise them with the use of cognitive reappraisal strategies. The participants were randomly assigned to receive either tDCS (n = 23, 1.5 mA anodal tDCS for 20 min over the right dlPFC) or sham stimulation (n = 25). Participants were shown negative and neutral pictures from the International Affective Picture System while they were instructed to either downregulate, upregulate or maintain their emotions. After every presentation, participants rated the intensity of their emotional arousal. TDCS was applied through a pair of saline-soaked surface sponge electrodes (anodal electrode surface area = 35 cm^2, cathodal electrode surface area = 100 cm^2) connected to a battery-driven constant current tDCS stimulator (NeuroConn GmbH, Ilmenau, Germany). For stimulating the right dlPFC, the anode was placed over F4 according to the 10 - 20 international EEG system (Jasper, 1958). The cathode was placed above the left supraorbital region. Skin conductance was recorded continuously during the emotion regulation task using the Schuhfried Biofeedback X-pert 2000 device (Schuhfried, Moedling, Austria).

Table 1 – Overview and timeline of the experiments

Experiment 1 (n = 74)

Day 1	Day 2	Day 3
Fear acquisition	Group 1 → tDCS (anodal) [F4] Group 2 → tDCS (sham) [F4]	Fear response assessment

Experiment 2 (n= 25)

Day 1	Day 2	Day 3
Fear acquisition	Group 1 → tDCS (cathodal) [F4] Group 2 → tDCS (sham) [F4]	Fear response assessment

Experiment 3 (n= 48)

Day 1	Day 3-4
Cognitive Reappraisal Training	Group 1: Task + tDCS (anodal) [F4] Group 2: Task + tDCS (sham) [F4]

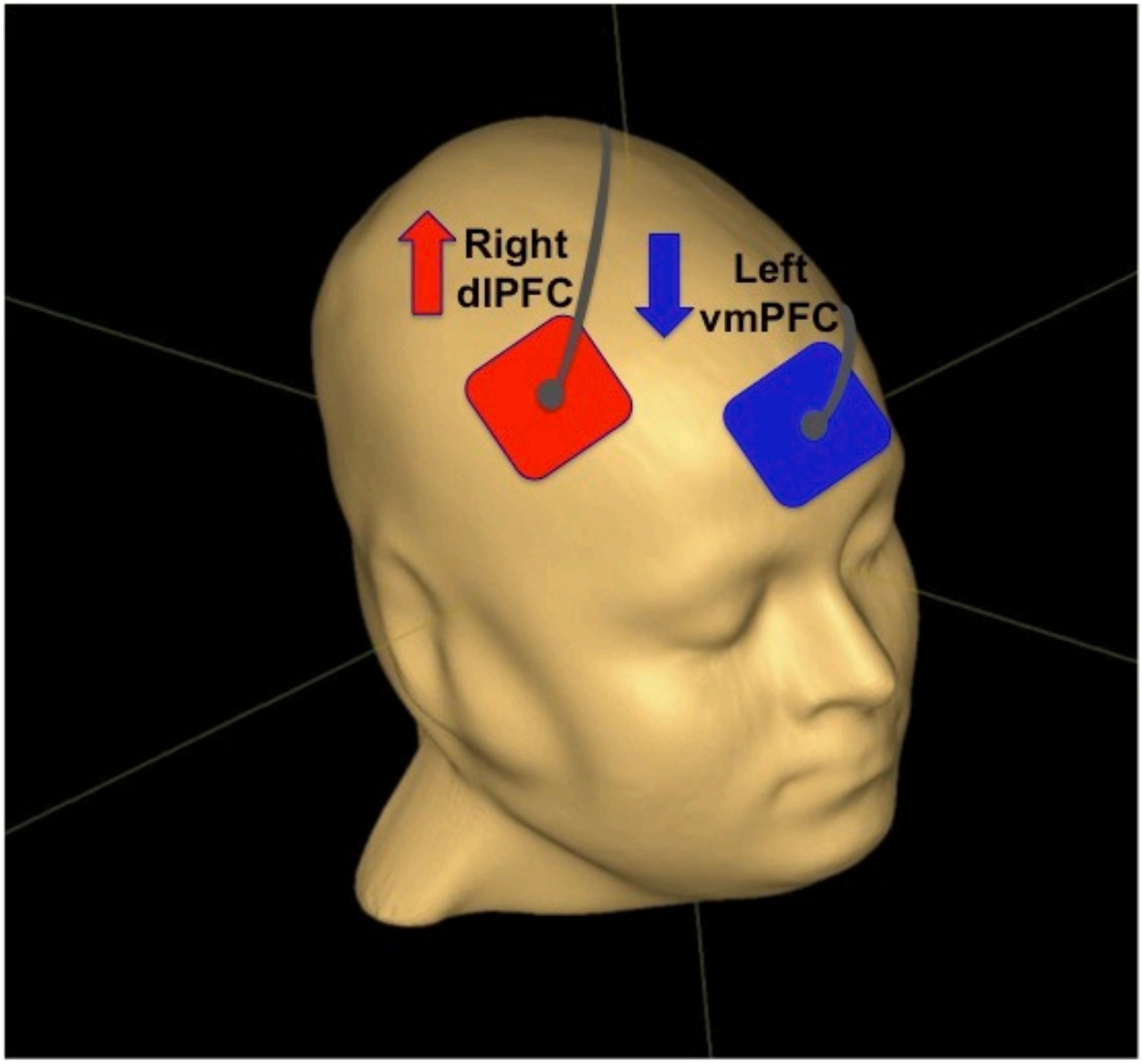

Figure 1: Electrode positions for transcranial direct current stimulation (right dorsolateral prefrontal: anodal, left supraorbital: cathodal), prepared using the navigated brain stimulation, NBS System (eXimia; Nexstim Ltd, Helsinki, Finland). dlPFC, dorsolateral prefrontal cortex; vmPFC, ventromedial prefrontal cortex (Mungee et al., 2013).

e) RESULTS

We included subjects that successfully acquired fear conditioning in the data analysis for the first and second experiments related to fear memory reconsolidation. For the third experiment investigating emotion regulation, we included all subjects that showed detectable SCR amplitudes in the analysis. SCR data were analysed using the MATLAB 7.11.1 (Mathworks Inc., Sherborn, MA) based software LedaLab (www.ledalab.de). SCR was decomposed by continuous decomposition analysis (CDA). This method extracts the phasic information underlying the skin conductance response, and aims at retrieving the signal characteristics of the underlying sudomotor nerve activity (Benedek & Kaernbach, 2010).

In the first study investigating fear memory reconsolidation, an independent-samples t-test was used to compare the mean differential SCR in the tDCS (right prefrontal: anodal, left supraorbital: cathodal) and sham group for the first three trials during fear memory assessment on day 3. We analysed the early phase because we expected the fear response to be at its peak here before habituation is expected to occur. The mean differential SCR was significantly higher for the tDCS group (mean = 0.06, SD = 0.31) compared to the sham group [mean = – 0.17, SD = 0.46; t (48) = 2.05, $p < 0.05$] with a moderate effect size (Cohen's d = 0.59). These findings indicate an enhancing effect of tDCS on fear memories.

Following a similar approach in the second study investigating fear memory reconsolidation with reversed polarity, we analysed the first three trials and found no significant effects for CS trial [F(1,15) = 2.05, $p > 0.05$, ηp2 = 0.12] or group (tDCS/sham) [F(1,15) = 3.38, $p > 0.05$, ηp2 = 0.18]; the interaction between CS trial and group was also not significant [F(1,15) = 0.55, $p > 0.05$, ηp2 = 0.04]. Next, we conducted a repeated measure ANOVA for the first two trials each of CS + and CS − on Day 3. Here, we found significant effects for CS trial [F(1,15) = 5.28, $p < 0.05$, ηp2 = 0.26], but no significant effects for group [F(1,15) = 3.60, $p > 0.05$, ηp2 = 0.19] or the interaction between CS trial and group [F(1,15) = 2.15, $p > 0.05$, ηp2 = 0.13]. These results indicate that the participants showed defensive responses up to the first two trials of CS + and CS –. The lack of defensive responses after three trials each of CS + and CS – is probably explained because of rapid habituation. To summarize, there was no significant effect of tDCS on fear responses on day 3.

In the third experiment investigating the effect of tDCS on emotion regulation, our results revealed lower arousal ratings in the downregulation condition for the tDCS group (M = 2.17, SD = 0.88) as compared to the sham group (M = 3.46, SD = 0.71; t = 5.22, $p < 0.001$). We also found significantly lower SCR in the downregulation condition for the tDCS group (M = 0.26, SD = 0.27) as compared to the sham group (M = 0.56, SD = 0.36; t = 3.05, $p = 0.004$). The opposite effect was observed for the upregulation condition in which higher arousal ratings in the upregulation condition were found for the tDCS group (M = 6.08, SD = 0.59) as compared to the sham group (M = 4.86, SD = 0.83; t = 5.50, $p < 0.001$) accompanied by marginally enhanced skin conductance responses (trend, but not statistically significant).

f) DISCUSSION

We observed that tDCS (right prefrontal: anodal, left supraorbital: cathodal) resulted in enhancement of fear memories. Two possible mechanisms involving the anode and cathode could explain this effect. Anodal tDCS of the right DLPFC could have resulted in cortical depolarization and thus led to a strengthening of the memory trace coding for conditioned fear memories. Secondly, the cathode positioned over the left orbit might have led to cortical hyperpolarisation in the left vmPFC, which in turn projects to the amygdala (Kim & Whalen, 2009). On a cellular level, it is probable that excitatory tDCS facilitated noradrenergic and glutamergic inputs to the amygdala, resulting in persistence of fear memory after reconsolidation (Otis, Werner, & Mueller, 2015).

While enhancing fear memories does not have direct therapeutic implications, a protocol with reversed polarity of electrodes might physiologically offer the opposite effect, i.e. inhibition of fear memories through cortical hyperpolarisation of the prefrontal cortex. Therefore, we designed our second experiment with reversed electrode polarity of tDCS (right prefrontal: cathodal, left supraorbital: anodal) during the reconsolidation window. Interestingly, this stimulation had no effect on fear memories, contrary to the expected physiological effect on cortical excitability. A potential reason for this could be the difficulty in achieving an inhibitory effect though cathodal stimulation when the right dlPFC is highly activated during the reconsolidation process (Jacobson, Koslowsky, & Lavidor, 2012). Another possibility is the complexity of the fear circuit, which could make it challenging for tDCS to consistently modify this circuit. A recent review by Ledoux (2016) proposed a two systems framework for the neural circuit underlying fear with the first circuit involving cortical areas which are responsible for generating feelings of fear and anxiety, and a second circuit involving subcortical areas like the amygdala which are responsible for physiological responses to fear. Moreover, it is suggested that the amygdala is not responsible for generating the experience of fear, but rather for detecting and physiologically responding to threats. Applying this model to our experiments, it is plausible that stimulating cortical regions like the dlPFC and the vmPFC with tDCS might rather influence the subjective feeling of fear than the physiological response to fear, since the second circuit is not directly influenced by tDCS. Future studies should address these limitations and use additional measures of fear to address both the physiological response and the subjective feeling of fear and anxiety.

In our third experiment investigating the effect of anodal tDCS on cognitive reappraisal, we observed that stimulating the right dlPFC with anodal tDCS during downregulation resulted in lower arousal ratings and decreased skin conductance responses. For the upregulation condition, anodal tDCS resulted in higher arousal ratings accompanied by marginally enhanced skin conductance responses. Depending on the reappraisal condition (downregulation or upregulation) emotional arousal was either elevated or reduced. Hence, differential responses to tDCS seem to depend on the particular reappraisal process the participants are performing. Our results support current models of the neural circuits underlying cognitive reappraisal (Kevin N. Ochsner & Gross, 2005). Our findings also support existing literature showing a correlation in the stremgth of the prefrontal cortex-amygdala coupling and the attenuation of negative affect after reappraisal (Banks, Eddy, Angstadt, Nathan, & Phan, 2007). A potential explanation for our findings is that anodal tDCS of the right dlPFC resulted in an excitatory effect through cortical depolarization. A second explanation could be that modulating subcortical structures like the amygdala through prefrontal cortex-amygdala connections impacted autonomic responses. Our findings support existing literature and confirm that the dlPFC plays an important role in the neural circuit underlying emotion regulation. Our experiment is limited by the absence of imaging data to exactly pinpoint the neural circuit that was influenced by tDCS. Future studies need to replicate these effects with additional control groups to investigate alternative stimulation sites and additional control tasks.

In these three experiments, we modulated the neural circuit underlying the processes of memory reconsolidation and emotion regulation (Figure 2). While these neural pathways share some overlap, there are also important differences to consider. A recent meta-anaylsis reported that cognitive control regions like the dorsomedial, dorsolateral, and ventrolateral prefrontal cortex (dmPFC, dlPFC, vlPFC), which are previously known to regulate non-emotional memory, are also activated during cognitive reappraisal (Buhle et al., 2014). The authors also found bilateral activation of the amygdala during reappraisal, which supports the existing literature proposing the role of the amygdala in aversive stimuli; these are often used in the form of pictures and videos during cognitive reappraisal. However, the authors report that the vmPFC was not consistently activated during reappraisal. In contrast, the vmPFC is thought to play a critical regulatory role in fear extinction by projecting inhibitory connections to the amygdala (Milad & Quirk, 2002). Delgado, Nearing, Ledoux, & Phelps (2008) proposed that this regulatory role of the vmPFC extends across multiple emotional memory processes. The authors postulated that inhibitory connections from the vmPFC to the amygdala could be shared by the fear extinction

pathway and emotion regulation strategies. Further, in case of emotion regulation, the DLPFC might exert an indirect influence on the amygdala through its projections to the vmPFC. However, the neural circuit for extinction during the reconsolidation window appears to differ slightly from standard extinction. Schiller, Kanen, LeDoux, Monfils, & Phelps (2013) reported that the amygdala showed similar responses for both processes; however the vmPFC-amygdala connections showed enhanced connectivity during standard extinction as compared to extinction during reconsolidation. The authors go on to conclude that fear extinction during reconsolidation might bypass the prefrontal cortex.

Taking our findings from the first two experiments together with the existing literature, it appears plausible that in a mechanism similar to behavioral interference, the prefrontal cortex-amygdala connections might not be sufficiently activated before performing tDCS during fear memory reconsolidation. An activation of this circuit was probably triggered by excitatory tDCS, leading to enhancement of fear memories in our first experiment. However, the inability of inhibitory tDCS to disrupt the functionally weak prefrontal cortex-amygdala circuit might have contributed to the null effects in our second experiment. Future studies targeting reconsolidation to inhibit fear should focus on interventions that can directly modulate the amygdala, rather than prefrontal cortex-amygdala connections, which are not consistently active during reconsolidation and thus especially difficult to inhibit through cathodal tDCS, compared to activation through anodal tDCS. Finally, our results from the third experiment are in line with existing literature suggesting a key role for the prefrontal cortex in emotion regulation and a successful pathway to modulating this circuit through excitatory tDCS. Neuroanatomically and –functionally, these findings indicate that it might be easier to modulate processes like cognitive reappraisal, where the DLPFC plays a critical role and is technically easier to target using tDCS than reconsolidation, where indirect effects on the amygdala through tDCS might not be strong enough to achieve fear elimination.

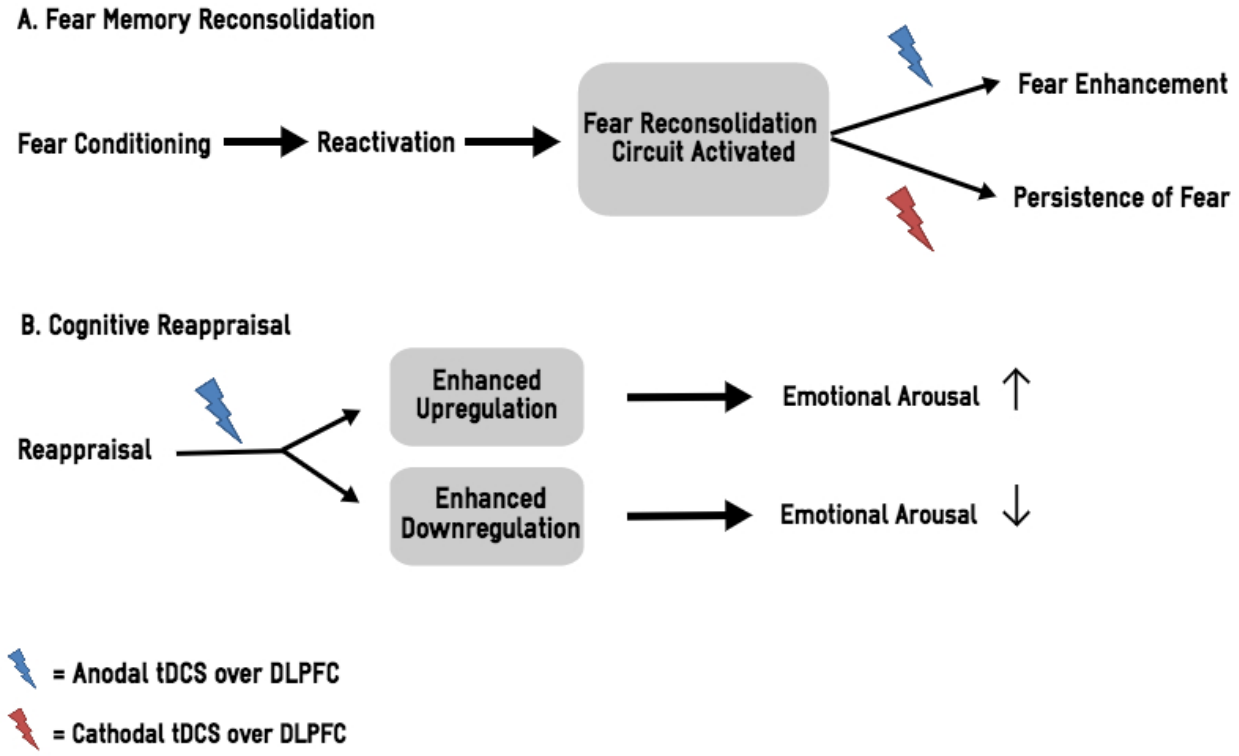

Figure 2: Summary of the effects of tDCS on emotional memory processes

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2. AFFIDAVIT

I, Aditya Mungee certify under penalty of perjury by my own signature that I have submitted the thesis on the topic „*Modifying Emotional Memory in Healthy Human Subjects Through Transcranial Direct Current Stimulation of the Prefrontal Cortex*". I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The sections on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) correspond to the URM and are answered by me. My contributions in the selected publications for this dissertation correspond to those that are specified in the following joint declaration with the responsible person and supervisor. All publications resulting from this thesis and which I am author of correspond to the URM (see above) and I am solely responsible.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

Berlin, 12th December 2016

Aditya Mungee
Doctoral Candidate

3. DECLARATION OF CONTRIBUTION

Aditya Mungee made the following contribution to the selected publications:

Publication 1:
Mungee, A., Kazzer, P., Feeser, M., Nitsche, M. a, Schiller, D., & Bajbouj, M. Transcranial direct current stimulation of the prefrontal cortex: a means to modulate fear memories. *Neuroreport*, 1–5. 2013. http://doi.org/10.1097/WNR.0000000000000119

Contribution in detail:
As first author, I carried out this research work in the course of a three-year project funded by DAAD (Deutscher Akademischer Austauschdienst) in the working group headed by Prof. Malek Bajbouj. My preparation for this project included detailed literature review on brain stimulation techniques and fear memory processes to make myself familiar with the current state of research. After extensive discussions with Prof. Bajbouj and our collaborators in USA (Prof. Daniela Schiller) and Göttingen (Prof. Michael Nitsche), I conceptualized a study design to modify reconsolidation of fear memories. Other memory processes that were discussed included consolidation, cognitive reappraisal and sleep dependent memory processes. Alternative brain stimulation techniques that were discussed included transcranial magnetic stimulation (TMS). Since direct current stimulation (tDCS) offers a deeper and less focal stimulation, this was our method of choice to influence the fear memory circuit.

To get hands-on training with tDCS, I attended a workshop with our collaborator Prof. Michael Nitsche in Göttingen. I also got an opportunity to get hands-on training with TMS in collaboration with the Dahlem Institute of Neuroimaging as part of the excellence initiative "Languages of Emotion". Simultaneously, I wrote and presented our research proposal to DAAD in New Delhi, the Charité Medical Neuroscience Program Committee and the Charité Ethics Committee in Berlin. Weekly lab meetings in the working group headed by Prof. Bajbouj gave me a chance to get feedback from my colleagues on how to improve the study design. After finalizing the study design and obtaining ethical approval, I started conducting the experiments. I was responsible for obtaining informed consent and checking for inclusion and exclusion criteria for the study. Seventy-four participants were recruited for the study through poster advertisements in Berlin and altogether 222 experimental sessions (3 days per subject) were

conducted. After the experimental part of the project was completed, analysis and statistical tests were carried out with the help of software like SPSS and LedaLab. Preliminary data was presented in our weekly lab meetings and further statistical approaches were discussed within the working group.

During the course of the study, I had the opportunity for extensive supervision through meetings with Prof. Bajbouj and Skype meetings with Prof. Nitsche and Prof. Schiller. After the data analysis was completed, I wrote and submitted our manuscript including figures and tables to the journal *Neuroreport*. As corresponding author, I was responsible for addressing the concerns of the peer reviewers and revising the manuscript in its final form for publication.

Publication 2:

Feeser, M., Prehn, K., Kazzer, P., Mungee, A., & Bajbouj, M. Transcranial Direct Current Stimulation Enhances Cognitive Control During Emotion Regulation. *Brain Stimulation*. 2013. http://doi.org/10.1016/j.brs.2013.08.006

Contribution in detail:

Together with the first author Ms. Feeser, I conceptualized the study and conducted pilot experiments to train subjects in emotion regulation. Additionally, I assisted her in screening subjects based on inclusion and exclusion criteria; especially in excluding subjects that had medical contraindications to tDCS. I also assisted Ms. Feeser in performing tDCS during the experiments as well as writing the manuscript and revising the paper after peer review in the journal *Brain Stimulation*.

Publication 3:

Mungee, A., Burger M., Bajbouj M. No Effect of Cathodal Transcranial Direct Current Stimulation on Fear Memory in Healthy Human Subjects. *Brain Sci. 6*, 55. 2016. doi:10.3390/brainsci6040055

Contribution in detail:

As first author, I conceptualized this follow up project after our first study with memory reconsolidation resulted in facilitation of fear memories. After extensive literature research including two new studies that had also reported that tDCS can modify fear memory, I presented a study design with reversed polarity of tDCS electrodes with the aim of achieving the opposite

effect, i.e. inhibition of fear memories. I underwent further training in non-invasive brain stimulation at a summer school in Oxford and presented this study design in our weekly lab meeting.

After incorporating suggestions from Prof. Bajbouj and other members of our working group, I started conducting the experiments. I was responsible for obtaining informed consent and checking for inclusion and exclusion criteria for the study. Twenty-Five participants were recruited for the study through poster advertisements in Berlin and altogether 75 experimental sessions (3 days per subject) were conducted. After the experimental part of the project was completed, analysis and statistical tests were carried out with the help of software like SPSS and LedaLab. Preliminary data was presented in our weekly lab meetings and further statistical approaches were discussed within the working group.

After the data analysis was completed, I wrote and submitted our manuscript including figures and tables to the journal *Brain Sciences*. As corresponding author, I was responsible for addressing the concerns of the peer reviewers and revising the manuscript in its final form for publication.

Berlin, 12th December 2016

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Aditya Mungee

Doctoral Candidate

4. PRINTED COPIES OF SELECTED PUBLICATIONS

Mungee, A., Kazzer, P., Feeser, M., Nitsche, M. A., Schiller, D., and Bajbouj, M. (2014). Transcranial direct current stimulation of the prefrontal cortex: a means to modulate fear memories. Neuroreport 25, 480–484. doi: 10.1097/WNR.0000000000000119

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Feeser, M., Prehn, K., Kazzer, P., Mungee, A. & Bajbouj, M. Transcranial direct current stimulation enhances cognitive control during emotion regulation. Brain Stimul. 7, 105–112 (2014). https://doi.org/10.1016/j.brs.2013.08.006

brain sciences

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Article

No Effect of Cathodal Transcranial Direct Current Stimulation on Fear Memory in Healthy Human Subjects

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Abstract: Background: Studies have demonstrated that fear memories can be modified using non-invasive methods. Recently, we demonstrated that anodal transcranial direct current stimulation (tDCS) of the right dorsolateral prefrontal cortex is capable of enhancing fear memories. Here, we examined the effects of cathodal tDCS of the right dorsolateral prefrontal cortex during fear reconsolidation in humans. Methods: Seventeen young, healthy subjects were randomly assigned to two groups, which underwent fear conditioning with mild electric stimuli paired with a visual stimulus. Twenty-four hours later, both groups were shown a reminder of the conditioned fearful stimulus. Shortly thereafter, they received either tDCS (right prefrontal—cathodal, left supraorbital—anodal) for 20 min at 1 mA, or sham stimulation. A day later, fear responses of both groups were compared. Results: On Day 3, during fear response assessment, there were no significant differences between the tDCS and sham group ($p > 0.05$). Conclusion: We conclude that cathodal tDCS of the right dorsolateral prefrontal cortex (right prefrontal—cathodal, left supraorbital—anodal) did not influence fear memories.

Keywords: direct current stimulation; fear conditioning; memory; prefrontal cortex

1. Introduction

Different interventions have been studied as possible pathways to modify fear memories in animals as well as humans, since this could lead to new treatment methods for anxiety disorders and post-traumatic stress disorder (PTSD). The reconsolidation window has been a popular target for manipulating fear memories, since consolidated memories return to a labile state after reactivation, and reconsolidation requires de novo protein synthesis in the amygdala [1]. In animals, protein synthesis inhibitors like anisomycin have been used to inhibit fear memory reconsolidation [2]. Glucocorticoids have also been shown to impair fear memory reconsolidation in mice; this effect depended on the muscarinic cholinergic receptors, and was blocked by atropine [3]. A recent study showed that ketamine—a *N*-methyl-D-aspartate receptor antagonist—impaired reconsolidation of contextual fear memory in rats [4].

There have also been pharmacological studies to diminish fear in humans. Propranolol has been shown to interfere with fear and trauma memory reconsolidation [5]; however, these effects have not been consistently replicated [6]. Cortisol has been shown to influence fear reconsolidation in men, but this effect could not be replicated in women [7]. Aside from such pharmacological interventions, various non-invasive methods have also been studied to interfere with fear memories.

Schiller et al. used the reconsolidation window to behaviourally rewrite fear memories with non-fearful information [8]. Burger et al. showed that tVNS (transcutaneous vagus nerve stimulation) accelerates explicit fear extinction; however, it did not lead to better retention of extinction memory 24 h later [9]. A common problem with most of these techniques is the lack of replicability and consistent long term effects. Hence, it is important to systematically investigate different fear modulating interventions.

Non-invasive brain stimulation—more specifically, transcranial direct current stimulation (tDCS)—has also been shown to influence fear memories [10]. tDCS has been shown to alter cortical excitability; anodal tDCS results in neuronal depolarisation, leading to an excitatory effect, whereas cathodal tDCS results in hyperpolarisation, and thus has an inhibitory effect [11]. Based on these antagonistic effects, many studies have demonstrated that tDCS affects cognition, mood, and working memory [12].

To date, three studies targeting different processes have demonstrated that tDCS can affect fear memories in humans. Van't Wout et al. [13] stimulated the left dorsolateral prefrontal cortex (DLPFC) with anodal tDCS during extinction learning, and reported enhanced subsequent extinction of conditioned fear. Asthana et al. [14] reported that cathodal tDCS of the left DLPFC resulted in inhibition of fear memory consolidation, whereas anodal tDCS did not lead to the enhancement of fear memory. Previously, we have shown that tDCS (right prefrontal—anodal, left supraorbital—cathodal) during the reconsolidation window resulted in enhancement of fear memories [10]. The fear circuit underlying memory reconsolidation is unclear, and finding a window for tDCS to manipulate this circuit is challenging. It has been proposed that noradrenergic and glutamergic inputs to the basolateral nucleus of the amygdala (BLA) might restabilize glutamate-dependent plasticity during reconsolidation [15]. Furthermore, the authors propose that BLA output neurons to the central nucleus of the amygdala control aversive responses during emotional memory expression. tDCS can only induce changes in cortical excitability, and effects on deeper areas like the amygdala are indirect [16]. Hence, we chose to target the prefrontal cortex–amygdala circuit to disrupt fear memory reconsolidation. It is known that the DLPFC is involved in cognitive regulation of conditioned fear, and projects to the ventromedial prefrontal cortex (vmPFC), which in turn has inhibitory connections to the amygdala [17]. Since our previous study influencing the same neural circuit resulted in the enhancement of fear memories [10]—in line with the AeCi hypothesis (anodal: excitatory, cathodal: inhibitory)—we reversed this protocol and performed right prefrontal—cathodal, left supraorbital—anodal tDCS during the reconsolidation window. We chose the right dorsolateral prefrontal cortex as our target region for inhibitory cathodal stimulation because it is known that the right prefrontal cortex is activated during the processing of negative emotions [18]. The reference electrode for anodal stimulation was positioned on the left supraorbital area, close to the ventromedial prefrontal cortex, which possibly has an inverse relationship with the amygdala [19]. Enhancing the inhibitory connections from the vmPFC to the amygdala through anodal tDCS could inhibit fear memory. Hence, we propose that inhibitory stimulation of the DLPFC, together with excitatory stimulation of the vmPFC, would result in the inhibition of fear memories.

2. Methods and Materials

We followed an identical protocol as in our previous study [10], but for one important change; the electrode positions of the anode and cathode were reversed during tDCS stimulation in order to achieve the opposite effect.

2.1. Subjects

Twenty-five healthy subjects aged between 18 and 40 years were recruited by poster advertisements for the study. Subjects with metal implants inside the skull or eye, severe scalp skin lesions, cranial bone fractures, known history of epilepsy or previous seizures, pregnant or breast-feeding women, and subjects with a known psychiatric disorder or on CNS-acting medications were excluded from the study. The Charité institutional ethics committee approved the protocol, which

was conducted in accordance with the Declaration of Helsinki. All subjects were given a complete oral and written description of the study, and informed consent was obtained from each subject prior to participation. The subjects were compensated for their participation in the study.

2.2. Procedure

The experiment was conducted over 3 days, with a wash-out period of 24 h between the sessions. A quiet room was used to conduct the sessions in order to minimize the influence of external stimuli on skin conductance. The protocol for all three sessions is summarized in Table 1. The experiment was performed using Presentation® software (Version 7.0, [20]).

Table 1. Overview and timeline of the experiment. tDCS: transcranial direct current stimulation.

Day 1	Day 2	Day 3
Fear acquisition	Group 1 → tDCS (cathodal) [F4] Group 2 → tDCS (sham) [F4]	Fear response assessment

Two randomly assigned groups (tDCS and sham) underwent a Pavlovian fear conditioning paradigm with partial reinforcement. The conditioned stimuli (CS) were blue and yellow squares, and the unconditioned stimulus (US) was a low intensity electric shock to the wrist. One stimulus was paired with the US on 38% of the trials (CS+), and the other was never paired with a shock (CS−). We used a partial reinforcement schedule to avoid rapid extinction [7]. A Grass Medical Instruments stimulator was used to deliver 50 pulses/second for a duration of 200 ms. The intensity of the electric shock was adjusted to every individual subject, the threshold stimulus being uncomfortable but not painful. We used a starting stimulus of 10 V, and went up to a maximum intensity of 60 V. The participants were shown 10 randomized presentations of the CS+ and CS− each, and additionally 6 CS+ presentations, which were associated with a shock (US). The order of appearance of the colour paired with the shock was also randomized in order to avoid bias. We presented the stimuli for 4 s, with a 10–12 s gap between stimuli. The duration between stimuli was randomized in order to avoid false responses because of habituation. Skin conductance responses were measured using the Schuhfried Biofeedback X-pert 2000 device (Schuhfried, Moedling, Austria). The electrode was connected to the ring finger of the left hand.

2.2.1. Day 2: tDCS

On the second day all subjects were shown a reminder using a single presentation of the coloured square (CS+) which was paired with a shock on Day 1. However, no shock was administered on Day 2. Immediately after this, the subjects in the tDCS group were stimulated with tDCS (1 mA) for a total duration of 20 min using two saline-soaked surface sponge electrodes (15 cm^2) with a current density of 0.67 A/m^2. The subjects in the sham group received only a brief current for the first 30 s in order to mimic the itching associated with real stimulation. The cathodal electrode was placed on the region of interest, the right DLPFC with electrodes (5 × 3 cm) placed at the right frontolateral location (F4 of the international 10:20 electroencephalogram system) [21], and the anode on the contralateral supraorbital area. We used a constant current battery driven stimulator (CX6650, Rolf Schneider Electronics, Gleichen, Germany). The current was ramped up to 1 mA over a period of 30 s to minimize side effects.

2.2.2. Day 3: Fear Response Assessment

To test the effect of tDCS, we presented both groups with the conditioned stimulus without the unconditioned stimulus to assess their fear responses on Day 3. We presented the subjects with 10 CS+ and 11 CS− presentations, and the order of appearance was randomized to prevent bias. Since the

subjects were shown a reminder of CS+ on the second day, we used one extra presentation of CS− on Day 3 in order to keep the total number of trials on all three days equal.

2.3. Data Analysis

The response to an average of all trials during fear acquisition on Day 1 (except the first trial) was used as a criterion to decide if the subjects had successfully acquired fear conditioning. It has been proposed that human participants habituate to the CS+ during conditioning, and that fear responses might be better observed in the earlier CS+ trials [22]. We first assessed fear conditioning in all subjects (n = 25) before discarding any data. Subjects (n = 8) where CS+ was equal or less than CS− for an average of all the trials during acquisition were excluded from the analysis. Hence, 17 subjects were included in the final sample [tDCS = 7 (m = 3, f = 4); sham = 10 (m = 2, f = 8)]. We used Ledalab, a MATLAB (Mathworks Inc., Sherborn, MA, USA) based software, more specifically the CDA (Continuous Decomposition Analysis) method to analyse the skin conductance data. This method extracts the phasic information underlying the skin conductance response, and aims at retrieving the signal characteristics of the underlying sudomotor nerve activity [23]. Since we expected the fear responses to be most pronounced in the early phase on Day 3, we restricted our analysis to the first three presentations of the CS+ and CS−. Since approximately one-third of the CS+ trials were paired with a shock (US) on the first day, we expected the conditioned subjects to show a fear response to at least the first three trials of CS+ on the third day. However, since no shocks are actually administered, a gradual learning effect and thus diminishing of the fear responses is expected after the early phase. We compared the mean differential SCR (skin conductance response) between the tDCS and the sham groups in the 0.5 to 4.5 s time window after stimulus onset (CS+ minus CS−). Square root transformation of the raw data was performed to normalize distributions. Each subject's normalized score was then divided by the mean square-root-transformed US response of that subject. Statistical analysis was performed using SPSS 20 (SPSS Inc., Chicago, IL, USA)

3. Results

All subjects tolerated the tDCS stimulation well, and no adverse effects were reported.

3.1. Day 1—Fear Acquisition

Fear responses were analysed for all subjects in the late phase on Day 1 (last three CS+ and last three CS− trials) using a repeated measures ANOVA with CS as the within-subjects factor and group (tDCS/sham) as the between-subjects factor. We found significant main effects of CS trial [$F(1,15) = 15.22$, $p = 0.001$, $\eta p^2 = 0.50$], but no significant effects for group [$F(1,15) = 1.09$, $p > 0.05$, $\eta p^2 = 0.07$], or the interaction between group and CS [$F(1,15) = 4.19$, $p > 0.05$, $\eta p^2 = 0.22$]. The significant difference between CS+ and CS− indicates that participants successfully acquired fear conditioning on Day 1, and there was no significant difference between the sham and the real group.

3.2. Day 3—Fear Memory Test

We first analysed the first three CS+ and the first three CS− trials on Day 3, since we expected gradual habituation after the initial phase. There were no significant effects for CS [$F(1,15) = 2.05$, $p > 0.05$, $\eta p^2 = 0.12$] or group (tDCS/sham) [$F(1,15) = 3.38$, $p > 0.05$, $\eta p^2 = 0.18$]; the interaction between CS and group was also not significant [$F(1,15) = 0.55$, $p > 0.05$, $\eta p^2 = 0.04$]. Next, we conducted a repeated measures ANOVA for only the first two trials of CS+ and CS− on Day 3. We found significant effects for CS [$F(1,15) = 5.28$, $p < 0.05$, $\eta p^2 = 0.26$], but no significant effects for group [$F(1,15) = 3.60$, $p > 0.05$, $\eta p^2 = 0.19$] or the interaction between CS and group [$F(1,15) = 2.15$, $p > 0.05$, $\eta p^2 = 0.13$]. These results indicate that the participants showed defensive responses up to the first two trials of CS+ and CS− each. Subsequently, rapid habituation took place so that there were no significant defensive responses after three trials each of CS+ and CS−. There was no significant effect of tDCS on

this habituation; both the tDCS and the sham groups showed habituation after 3 trials each of CS+ and CS−.

4. Discussion

In this study, we investigated the effects of tDCS (right prefrontal—cathodal, left supraorbital—anodal) on fear memories. Our results show no significant differences in the fear response between the tDCS and sham group. Recently, we reported that tDCS (right prefrontal—anodal, left supraorbital—cathodal) results in enhancement of fear memories [10]. According to the assumption that anodal tDCS enhances cortical excitability, while cathodal tDCS diminishes it [11], we hypothesized that reversing this stimulation protocol would result in an inhibition of fear memories. However, our results do not confirm this hypothesis.

There are two possible explanations why tDCS (right prefrontal—cathodal, left supraorbital—anodal) did not inhibit fear memories. Firstly, because the dual-polarity effect (more specifically, the cathodal inhibitory effect) is more difficult to replicate while investigating memory in comparison to motor cortex [24]. The hypothesis that anodal tDCS promotes cortical excitability while cathodal tDCS inhibits it has been established mainly through motor cortex studies [25]. It has been proposed that the lack of a cathodal inhibition effect in cognitive studies could be state-dependent due to the brain areas involved already being highly activated during a task [24]. This explanation is plausible in the case of our study; since the participants saw a reminder of the conditioned stimulus (connected to the electrical shock) just before tDCS, this probably resulted in the fear circuit being activated during the stimulation, hence making it difficult for the inhibitory cathodal stimulation to diminish these reactivated fear memories, as opposed to enhancement through anodal tDCS, where reactivation might play a facilitatory role [10]. Previous cognitive studies have also highlighted the difficulty in replicating polarity-dependent effects. Marshall et al. [26] reported that both cathodal and anodal tDCS intermittent stimulation of the lateral prefrontal cortex during a working memory task slowed reaction time. Kincses et al. [27] reported that anodal tDCS improved implicit learning; however, cathodal tDCS did not significantly impair implicit learning. Our results are in line with these findings, since cathodal tDCS of the prefrontal cortex had no effect on fear memories, while anodal tDCS has been shown to enhance fear [10].

Secondly, it is possible that the complex fear neural circuitry involved in reconsolidation is difficult to manipulate by stimulating superficial cortical areas like the DLPFC and vmPFC with a single session of tDCS, as opposed to deeper subcortical regions like the amygdala; however, these deep regions are technically challenging to stimulate using tDCS. At best, tDCS is capable of indirectly modifying cortico-subcortical connections [16]—a single session of tDCS might not be enough to manipulate physiological defensive responses reliably and consistently. To our knowledge, there are no published studies on the effect of repetitive sessions of tDCS on fear memory reconsolidation. Recently, LeDoux et al. [28] proposed a two systems framework with the first circuit involving cortical areas which are responsible for generating feelings of fear and anxiety, and a second circuit involving subcortical areas like the amygdala which are responsible for behavioural and physiological responses to fear. Further, it is suggested that the amygdala itself is not a fear center generating the experience of fear, but rather responsible for detecting and responding to threats. According to this model, stimulating cortical regions like the DLPFC and the vmPFC with tDCS might have more influence on the subjective feeling of fear rather than the physiological response to fear, since the second circuit is not directly influenced by tDCS. On similar lines, Schiller et al. [29] reported that while standard extinction involves areas of the prefrontal cortex like the vmPFC, extinction during the reconsolidation window appears to bypass the prefrontal cortex. Taking these findings together, we recommend that future studies with manipulations during the reconsolidation window should try to develop new techniques to target the amygdala, rather than the prefrontal cortex.

It is important to carefully consider the differences between our protocol and earlier studies which have manipulated fear using tDCS. Firstly, the studies differed on the target and reference electrode

positioning for tDCS. Asthana et al. [14] stimulated the F3 area according to the 10–20 EEG system corresponding to the left DLPFC with cathodal and anodal tDCS in two different groups; the reference electrode was located over the left mastoid. Van't Wout et al. [13] stimulated the AF3 region with anodal tDCS, and the reference electrode was on the contralateral mastoid. The authors argue that this protocol allows the best stimulation of the vmPFC. In our earlier study [10], we stimulated the F4 region corresponding to the right DLPFC with anodal tDCS and the left supraorbital area with cathodal tDCS. Secondly, let us consider differences in current density and the intensity and duration of tDCS. Van't Wout et al. stimulated with 2 mA, because the target and reference electrodes were situated further away from each other and the authors hoped to stimulate deeper areas with a higher intensity. Asthana et al. and Mungee et al. applied 1 mA intensity for the stimulation. While our duration of stimulation was 20 min, Asthana et al. applied tDCS for 12 min, and Van't Wout et al. for 10 min. Current density differed between the studies, with Van't Wout et al. reporting the highest value at 1.33 A/m^2, Mungee et al. at 0.67 A/m^2, and Asthana et al. at 0.29 A/m^2. Fourthly, the nature of the US also differed between the studies. While Asthana et al. used an auditory stimulus (screaming), Van't Wout et al. and our study used an electric shock to condition the participants. Finally, the studies targeted different pathways to influence fear. Both our current and previous study targeted reconsolidation 24 h after fear acquisition; hence, we performed tDCS during the reconsolidation window after reactivating the memory with a reminder. On the other hand, Asthana et al. performed tDCS during the consolidation phase a few minutes (10–20 min) after fear acquisition on the first day itself. Van't Wout et al. targeted extinction of conditioned fear, and hence performed tDCS during extinction learning.

These contrasting stimulation protocols highlight the challenges that we face while attempting to influence different emotional processes with a complex neural circuit through tDCS. Replication studies are needed for targeting each of these pathways to manipulate fear to check for consistent effects, before a conclusive statement can be made on a standard protocol that offers the best pathway to modify processes like fear extinction, consolidation, or reconsolidation.

Our study is limited by the small sample size (n = 17) and gender predominance towards females. This makes a direct comparison with our first study difficult, since we had a larger sample size (n = 50) and better gender balance [10]. Because of the relatively large electrode sizes used for tDCS, we cannot rule out the possibility of having stimulated other cortical areas involved in the neural circuit modulating fear. Since we used a bipolar stimulation design, we cannot discern between the effects of the left and right prefrontal electrodes. We used skin conductance response to measure fear, which is susceptible to noise due to spontaneous fluctuations in SCR that constitute within-subject variance [30]. We tried to minimize noise by using the CDA (Continuous Decomposition Analysis) method to analyse SCR; nevertheless, we cannot rule out the effect of residual noise. We did not measure additional physiological responses like heart rate or respiratory rate. On Day 2, we did not measure skin conductance after showing the reminder to measure fear response before tDCS.

5. Conclusions

In summary, we found no effect of tDCS (right prefrontal—cathodal, left supraorbital—anodal) on fear memories, in contrast to our earlier study [10] where we found that tDCS (right prefrontal—anodal, left supraorbital—cathodal) resulted in the enhancement of fear memories. Using alternative protocols targeting other pathways to manipulate fear, Asthana et al. [14] and van't Wout et al. [13] reported an inhibition of fear memories. To our knowledge, no negative studies with regard to tDCS and fear memories have been published yet. Our results emphasize the need to conduct more studies with diversity in target electrode positions, reference electrode positions, laterality, and intensity in order to identify appropriate stimulation protocols for influencing fear memories. The lack of such studies in healthy participants makes it difficult to develop standard stimulation protocols for patients with PTSD or anxiety disorders.

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Author Contributions: Aditya Mungee and Malek Bajbouj conceived and designed the experiments; Aditya Mungee performed the experiments; Aditya Mungee and Max Burger analyzed the data; Aditya Mungee, Malek Bajbouj and Max Burger wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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5. CURRICULUM VITAE

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

6. LIST OF PUBLICATIONS

1. Mungee, A., Zieger, A., Schomerus, G., Ta, T.M.T., Dettling, M., Angermeyer, M.C., Hahn, E., 2016. Attitude towards psychiatrists: A comparison between two metropolitan cities in India. *Asian J. Psychiatr*. 22, 140–4.
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4. Zieger A, Mungee A, Schomerus G, Ta TMT, Dettling M, Angermeyer M, Hahn E., 2016. Perceived stigma of mental illness: a comparison between two metropolitan cities in India. *Indian Journal of Psychiatry*, in press
5. Mungee, A., Kazzer, P., Feeser, M., Nitsche, M. a, Schiller, D., Bajbouj, M., 2013. Transcranial direct current stimulation of the prefrontal cortex: a means to modulate fear memories. *Neuroreport* 1–5
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8. Goraya Harmeen, Mungee Aditya, 2009. Ill Health as Factor of Poverty. *Australas. Med. J*. 1, 75–77

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