Master Thesis

The Effects of Anodal tDCS of the vMPFC on Physiological Responses During a Fear Extinction Paradigm

Marius Husmann

S2120844

Faculty of Behavioural, Management and Social Science

University of Twente

Prof. Dr. Ing. Willem Verwey

Dr. Rob van der Lubbe

Dr. Harleen Chhabra

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Abstract

This study aimed to explore the effects of anodal transcranial direct current stimulation (tDCS) applied to the ventromedial prefrontal cortex (vmPFC) on fear extinction learning, with conditioned physiological responses as key outcome measures. Participants underwent fear acquisition, extinction, and extinction recall phases, separated by 24-hour intervals, with tDCS applied for the first ten minutes of the extinction phase. Physiological responses were recorded using skin conductance (SCR), respiration, pupillometry, and electrocardiography (ECG).

Results showed that SCRs during fear acquisition indicated significant stimulus differentiation, with stronger responses to the conditioned stimulus that was paired with the US (CS+) than the conditioned stimulus that was never coupled with the US (CS-), demonstrating successful fear learning. An interesting conditioned response emerged in the respiratory rate SD, with participants showing more stable breathing patterns during CS+ trials. In the extinction phase, SCRs for both CS+ and CS- decreased significantly across trials, suggesting extinction learning. Notably, participants who received tDCS showed significantly reduced SCRs to both CS+ and CS-, indicating enhanced extinction learning, though CS differentiation persisted. During recall, SCR differentiation had dissipated in both groups, while respiratory rate SD remained stable in the tDCS group, unlike the control group, which showed increased variability.

This study's findings align partially with previous research, suggesting that tDCS may enhance extinction learning through reduced fear responses. The results highlight tDCS's potential as a tool to improve extinction-based therapies, though future research with larger samples and controlled environmental conditions is warranted to clarify its mechanisms and effects.

Introduction

Anxiety disorders are the most prevalent mental health conditions globally, affecting a significant proportion of the population. According to the World Health Organization (WHO) in 2023, an estimated 301 million individuals, or approximately 4% of the world's population, suffer from anxiety disorders. This figure represents a significant increase from the 264 million reported just two years ago (WHO, 2017).

The ramifications of anxiety disorders extend far beyond individual suffering, encompassing significant societal and economic burdens. Those affected may experience reduced productivity, unemployment (Kessler et al., 2009; Simpson et al., 2010; Remes et al., 2016; Martino et al., 2019), and a high risk of mortality (Van Hout et al., 2004). Despite advancements in therapeutic interventions such as cognitive-behavioral therapy (CBT) and selective serotonin reuptake inhibitors (SRI), complete recovery remains uncommon, with a substantial portion of patients classified as non-responders to treatment (Taylor et al., 2012). To address the increasing prevalence and impact of anxiety disorders, novel therapeutic approaches must be investigated.

Cognitive model of fear conditioning

Classical conditioning is a common model to describe the cognitive processes involved in the development of anxiety disorders (Dittert et al., 2018). Fear conditioning, specifically, is an associative learning process. A neutral stimulus that would not elicit a fear response gets coupled with an aversive stimulus. The former is usually known as an unconditioned stimulus (US), as it produces fear responses without prior conditioning. Through repeated coupling, the neutral stimulus gets associated with the US. As a result, the recipient becomes conditioned to expect the US whenever the neutral stimulus is shown. Therefore, the stimulus is referred to as a conditioned stimulus (CS). If conditioning was successful, the CS will now trigger the fear response due to association with the US. Anxiety towards the CS is usually preserved through avoidance behavior. In operant conditioning, this behavior can be described as negative reinforcement. Due to the avoidance of the CS, no new associations can be formed (Mowrer, 1956). To overcome the fear of the CS it needs to be reassociated with e.g. a neutral experience. This process is known as extinction learning (Myers, 2007). The extinction learning model builds the foundation for exposure therapy (McNally, 2007). In exposure therapy, instead of avoiding the CS, it is actively pursued in a safe environment, without the concurrence of the US. If successful, through repeated exposure, a new neutral association of the CS will be formed.

To gain a better understanding of the mechanisms of fear extinction learning, researchers can conduct a fear extinction paradigm. This paradigm usually consists of a fear acquisition phase, a fear extinction phase, and one or more recall sessions. During the acquisition phase, a neutral stimulus (CS, e.g. a specific geometrical figure) is repeatedly paired with an aversive/unpleasant stimulus (e.g. an electric shock). Participants are often presented with an additional unpaired stimulus for effect comparison. During the extinction phase, participants are again presented with the CS, this time without the US, and the fear response is expected to decline until it is extinct gradually. Lastly, in the recall phase, the participants are again presented with the unpaired CS to assess extinction memory retention

(Milad et al., 2014). Optimally, all of these phases are conducted on separate days to ensure more ecological validity and a more realistic neurobiological model (Abend and Van ’t Wout, 2018).

Neurobiological basis of fear learning and fear extinction

Thanks to technological advancements and an increasing understanding of brain functions, researchers have a good understanding of the underlying circuits involved in fear conditioning. Gonzalez and Fanselow (2020) reviewed the literature and described how sensory information from the sensory cortex converges on neurons in the basolateral amygdala (BLA) during CS and US presentations. The BLA, as part of the amygdala, triggers a fear response through its projections to other regions within the amygdala and then to further brain areas that create the full fear response. This model is sufficient for simple Pavlovian fear conditioning tasks.

Partial CS+ reinforcement, in which the CS+ is only intermittently paired with the US, increases task complexity by introducing uncertainty about the association between the CS+ and the US. This added complexity requires the activation of additional brain areas to modulate the subcortical regions involved in fear processing (Gonzales and Fanselow, 2020). Current models suggest that the ventromedial prefrontal cortex (vmPFC) is activated to navigate the competing CS+ associations, as it receives signals from both the BLA and the hippocampus and projects back to the BLA (Gonzales and Fanselow, 2020). This allows the vmPFC to modulate amygdala activity based on the valence of the conditioned association and the context in which the stimulus is presented. In brain imaging research, the vmPFC has shown increased activation during extinction learning (Phelps et al., 2004; Milad et al., 2007). Furthermore, Phelps et al. (2004) found a decrease in vmPFC depression during an extinction learning paradigm while at the same time showing that there is a correlation between extinction retention and vmPFC activation. Accordingly, successful extinction learning requires coordinated processing across the hippocampus, the amygdala, and the vmPFC, with vmPFC modulating the subcortical processes of the amygdala (Gonzales and Fanselow, 2020). From this, increased activity of the vmPFC during extinction learning can help to improve the effectiveness and efficiency of learning.

tDCS in extinction learning

Enhancing cortical activation with non-invasive brain stimulation techniques represents a promising avenue in neuropsychological research. Currently, two primary modalities are used for targeted brain stimulation: magnetic and electrical. Transcranial direct current stimulation (tDCS) is a popular method for electric stimulation. During tDCS, a low-intensity direct current (typically 1~2 mA) is delivered from a cathode to an anode, traversing the cerebral cortex (Nitsche and Paulus, 2000). Electrodes are strategically placed on the recipient's scalp, to hyperpolarize or excite areas adjacent to the anode, while areas neighboring the cathode experience depolarization (Markovic et al., 2021).

Previous investigations have explored the application of anodal tDCS on the ventromedial prefrontal cortex (vmPFC) among healthy human participants (van't Wout et al., 2016; Dittert et al., 2018; Vicario et al., 2020b). Notably, van't Wout et al. (2016) and Vicario et al. (2020) administered stimulation at an intensity of 2 mA, whereas Dittert et al.

(2018) used 1.5 mA. These studies reported enhanced fear extinction and recall abilities. Conversely, Abend et al. (2016) reported different findings, suggesting that anodal stimulation over the medial prefrontal cortex (mPFC) may lead to an overgeneralization of fear responses.

Discrepancies in findings could be attributed to differences in electrode placement. Vicario et al. (2020b) used computational modeling to show that their AF3 electrode montage yielded stronger electrical fields in the vmPFC compared to the Fpz montage used by Abend et al. (2016). Consequently, van't Wout et al. (2016) also used the AF3 electrode montage in their study. Duration of stimulation could also significantly affect extinction learning. Stimulation can be administered either online during extinction or offline, during resting states before or after extinction. Remarkably, all three studies demonstrating enhanced extinction learning through tDCS on the ventromedial prefrontal cortex (vmPFC) employed online stimulation (van't Wout et al., 2016; Dittert et al., 2018; Vicario et al., 2020b). Among these, van't Wout et al. (2016) and Vicario et al. (2020) administered 2 mA stimulation for 10 minutes, whereas Dittert et al. (2018) administered 1.5 mA for 20 minutes. The critical importance of timing is supported by the findings of Ney et al. (2021), who observed that administering tDCS 10 minutes post-extinction task during consolidation processes impaired extinction retention. Thus, the current evidence suggests that extinction learning could be enhanced by administering tDCS during the extinction learning phase itself.

Assessing successful extinction learning

Extinction learning can be assessed through three primary approaches: self-assessment by participants using questionnaires or rating scales, analysis of brain activity using techniques such as fMRI or EEG, and evaluation of psychophysiological responses for example electrodermal activity (EDA) and heart rate variability (HRV) (Vuk et al., 2021). While self-assessment enables gathering insights directly from the participants, it is susceptible to biases and variations in semantic interpretation among participants (Wetzel et al., 2016).

On the other hand, analyzing brain activity offers objective assessments of fear extinction paradigms and insights into the functional aspects of fear learning phases. However, there are challenges associated with this method. In the case of MRI, access to the required technology is limited (Jalloul et al., 2023), and participants must minimize movement during data acquisition to avoid artifacts that could influence results (Savalia et al., 2017). Moreover, the immobility constraints of certain brain activity measurement devices, such as fMRI, further complicate the experimental setup, as participants need to remain still with their head immobilized (Savalia et al., 2017). In contrast, physiological measurements offer a balance between objectivity and practicality. They provide unbiased assessments while imposing fewer constraints on researchers in terms of resources and experimental setup. Four primary physiological markers, namely electrodermal activity, ECG, respiration, and pupil responses, are commonly used to infer participants' mental states in fear conditioning studies.

Electrodermal Activity in Fear Conditioning and Extinction Studies

Electrodermal activity (EDA) is a well-researched outcome variable in the context of fear conditioning, describing the continuous variation in the skin's electrical phenomena. Wallin (1981) has suggested EDA as an indicator of neurophysiologic arousal as well as an estimate of sympathetic nervous system activity. Skin Conductance Responses (SCR) are produced when a sympathetic nervous burst occurs, due to the temporal and spatial summation of spikes triggered by the sudomotor nerve (Lidberg and Wallin, 1981). SCR is primarily influenced by moisture levels, which are regulated by sweat production and blood flow, both controlled by the sympathetic nervous system. In fear conditioning paradigms, it is expected that fear responses produce higher levels of skin conductance (SC). This trend has been consistently observed across various manipulations of the paradigm.

In fear extinction research, SCR is often measured as baseline-corrected peak SCR during the presentation of conditioned stimuli (CS+ and CS-). Baseline correction involves subtracting the pre-stimulus skin conductance level from the peak SCR following stimulus onset, which ensures that changes in arousal are accurately attributed to the stimuli rather than to general fluctuations in skin conductance. A stronger peak SCR in response to the CS+ is typically interpreted as a stronger conditioned fear response. During extinction, repeated presentations of the CS+ without the US usually lead to a reduction in peak SCR, indicating that the fear response is weakening as participants learn that the CS+ no longer predicts the aversive outcome. By contrast, the CS- serves as a safety cue and typically elicits a weaker SCR or no response at all. In extinction learning, researchers expect to see a decline in peak SCR responses to the CS+ over the course of the extinction phase. A faster or more complete reduction in SCR suggests more effective extinction learning, as participants are adapting to the absence of threat.

The sensitivity of SCR to experimental variations has been demonstrated in numerous studies (Waters et al., 2023; Marinos et al., 2022; Zhao et al., 2022; Mertens et al., 2021; Modecki et al., 2020; O`Malley et al., 2018; Merz et al., 2016; Milad et al., 2005; Vervliet et al., 2004). For instance, Vervliet et al. (2004) showed that presenting a generalization stimulus (GS) instead of the CS+ during extinction resulted in stronger SCR responses during recall, suggesting incomplete extinction learning. Similarly, Merz et al. (2016) found that delaying extinction by 24 hours (as opposed to immediate extinction) led to lower SCRs during recall, indicating more effective retention of extinction learning in the delayed group. Additionally, manipulations of attention during extinction, as shown in the work of O'Malley et al. (2018), affect SCR patterns. Participants instructed to avoid attending to the CS+ exhibited larger SCRs during extinction, and their fear responses declined less over time. This demonstrates that attention-related cognitive processes can influence the physiological markers of fear and safety learning.

These findings consistently show that SCR is not only sensitive to changes in conditioned fear responses but also to subtle cognitive and contextual factors that influence extinction learning. This makes it a valuable tool for assessing the effects of interventions such as online anodal tDCS to the vmPFC, which is hypothesized to enhance the neural circuits involved in fear extinction.

Respiration in Fear Conditioning and Extinction Studies

Respiration plays a role in fear conditioning studies, with changes in respiratory amplitude, rate, and cycle symmetry providing valuable insights into emotional arousal. Respiratory Sinus Arrhythmia (RSA), the natural fluctuation in heart rate with breathing, highlights the interdependence of respiration and heart rate variability (Yasuma & Hayano, 2004). While respiration is partly under conscious control, research suggests a clear link between the respiratory network and the sympathetic nervous system (SNS), making respiration a useful measure of fear responses (Molkov et al., 2014).

In fear conditioning studies, researchers typically measure respiratory responses during CS presentation, focusing on parameters like amplitude and cycle time. However, interpreting respiratory signals is complex due to the relatively slow frequency of respiration, which can pose challenges in short stimulus presentation windows (Schaffer and Ginsberg, 2017). Decreased respiratory amplitude has been observed in response to the CS+ during early phases of fear learning, but this pattern can reverse later in the trials (Castegnetti et al., 2016). Van Diest et al. (2009) found increased respiratory rate (shorter cycle time) and higher inspiratory duty time in response to CS+, reflecting heightened arousal. Wehrli et al. (2022) reported a significant difference in respiratory amplitude during fear recall, with smaller amplitudes for CS+ compared to CS-, suggesting that respiratory responses can capture subtle changes in fear expression over time.

These studies indicate that respiration is sensitive to fear learning but may vary depending on timing, trial length, and individual differences. Respiration's low frequency introduces challenges when analyzing short time windows, as each CS may coincide with different phases of the breathing cycle, complicating interpretation. Additionally, respiratory responses may take longer to stabilize after each trial, potentially affecting subsequent data. However, despite these limitations, there is potential for respiratory measures to serve as indicators of fear responses during extinction learning (Wehrli et al., 2022). For example, smaller respiratory amplitudes during CS+ trials may indicate greater emotional regulation, suggesting a promising direction for extinction research.

Given that respiratory patterns reflect both SNS activity and emotional arousal, tDCS could modulate these responses during extinction. Measuring changes in respiratory amplitude and cycle timings over the course of extinction could provide physiological evidence for tDCS's role in facilitating extinction learning.

ECG in Fear Conditioning and Extinction Studies

Studies in the medical domain, especially on hypertension, have shown that the cardiovascular system is regulated by sympathetic nervous system activity (Sinski et al., 2006). Additionally, heart rate variability (HRV) has been used as an indicator of sympathetic nervous system activity for several decades (Kiyono et al., 2016). Consequently, in several fear conditioning and fear extinction studies, participants' electrocardiograms (ECG) serve as outcome variables. Typically, the conditioned stimulus (CS) presentation interval is subdivided into 500ms time bins. Mean heart rates during these bins are calculated and adjusted against a baseline, commonly derived from the mean heart rate 1 to 2 seconds

preceding each trial. Multiple studies have consistently reported a distinct heart rate pattern associated with fear conditioning.

Generally, immediately following CS onset, there is a deceleration in heart rate lasting approximately two seconds, followed by a mid-interval acceleration. Subsequently, there's another deceleration, often leading to bradycardia, in anticipation of the unconditioned stimulus (US) onset, indicative of successful conditioning (Hugdahl et al., 1983; Diest et al., 2009; Castegnetti et al., 2016; Exner et al., 2021; Battaglia et al., 2022). This pattern has been reliably reproduced across various studies. Interestingly, for CS+, the second deceleration appears to be more pronounced, suggesting a stronger deceleration effect (Hugdahl et al., 1979; Diest et al., 2009; Castegnetti et al., 2016; Exner et al., 2021; Battaglia et al., 2022).

These findings show that ECG components such as heart rate and heart rate variability can provide evidence for extinction learning and extinction retention. As for respiration, tDCS might modulate these responses by enhancing extinction learning and/or extinction retention.

Pupillometry in Fear Conditioning and Extinction Studies

Pupillometry, the analysis of pupil diameter, emerges as a promising tool for assessing fear learning. Unlike respiration and heart rate, pupil dilation lacks a frequency component, allowing researchers to focus solely on diameter and timing. While relatively recent in its application, pupillometry has been successfully used as an outcome variable in fear conditioning and fear extinction paradigms (Greenberg et al., 2013; Jentsch et al., 2019; Leuchs et al., 2017; Visser et al., 2013; Visser et al., 2015). Furthermore, increases in the activity of the sympathetic nervous system (SNS) are directly linked to pupillary dilation in awake individuals (Packiasabapathy, Rangasamy, Sadhasivam, 2021).

In fear conditioning, the pupil dilates more in response to CS+, reflecting heightened arousal. This dilation is most pronounced in the seconds before the unconditioned stimulus (US) onset, where participants anticipate the aversive event (Jentsch et al., 2019; Leuchs et al., 2017). During extinction, the difference in pupil dilation between CS+ and CS- diminishes, indicating a reduction in fear as the association between the CS+ and the US weakens (Visser et al., 2015). In studies using pupillometry, pupil dilation is typically analyzed by subtracting the baseline pupil diameter, measured just before each trial, from either the peak or average pupil diameter during CS presentation. This baseline correction ensures that changes in pupil size reflect arousal triggered by the CS (Greenberg et al., 2013; Jentsch et al., 2019). Larger baseline-corrected pupil dilation in response to the CS+ compared to the CS- indicates successful fear learning. The strongest effects are seen just before the anticipated US (Greenberg et al., 2013). As extinction progresses, pupil dilation to the CS+ decreases, reflecting reduced arousal and successful extinction learning (Leuchs et al., 2017; Visser et al., 2015). This reduction in CS+ dilation over time is crucial, as it serves as a physiological marker of extinction.

Given the sensitivity of pupillometry to SNS-driven arousal, it could effectively capture tDCS-induced enhancements in extinction. By comparing conditioned pupil dilation between tDCS and control groups, we can quantify how quickly participants learn to dissociate the

CS+ from the anticipated threat, thereby providing robust physiological evidence for tDCS's role in promoting extinction.

Rationale of the current study

This study aims to investigate the effect of tDCS on fear learning and extinction in a blinded sham-controlled design. By simultaneously recording various physiological responses, the study offers a unique opportunity to explore the interrelationships among these variables. This approach not only facilitates the assessment of intervention effectiveness in fear extinction using different outcome measures but also sheds light on how tDCS influences physiological responses. Such insights will inform the methodology of future research in this domain.

The application of anodal tDCS on the ventromedial prefrontal cortex (vmPFC) during the extinction phase may enhance extinction learning by modulating neural circuits associated with fear regulation. The optimal timing for administering tDCS is hypothesized to be during the beginning of the extinction tasks, as evidence suggests that stimulation after the extinction phase impairs retention (Ney et al., 2021) and early stimulation can enhance later extinction trials (Van`t Wout et al., 2016). Furthermore, tDCS-induced effects on fear extinction might be measurable through physiological markers like skin conductance response (SCR), pupil dilation, ECG, and respiratory patterns, which are sensitive to changes in fear responses and emotional regulation. We hypothesize that participants receiving tDCS will exhibit more rapid and pronounced reductions in conditioned physiological responses during extinction, reflecting enhanced extinction learning. Additionally, we hypothesize that tDCS will improve extinction retention, as evidenced by reduced conditioned physiological responses during recall.

Methods

Participants

A total of 43 healthy participants (19 females and 24 males) aged between 20 and 31 years (mean age = 24.4 years) took part in the study. All participants were right-handed and non-smokers. Participants were healthy and did not receive hormonal or neurological medication, neither did they receive therapy for anxiety disorders. They were recruited through various channels, including the IfADo website, social media advertisements, the Sona credit system, and flyers posted on university blackboards. Participants were randomly assigned to either the real tDCS group (8 females and 15 males) or the sham tDCS group (11 females and 9 males). Prior to participating, written informed consent was obtained from all participants. The study protocol was approved by the IfADo Ethics Committee. Participants were compensated either monetarily or with Sona credits.

Two participants were excluded from the final analysis due to missing skin conductance data. Thus, the final analysis included 21 participants in the real tDCS group (14 males and 7 females; mean age = 24.76 years) and 20 participants in the sham tDCS group (11 females and 9 males; mean age = 24 years). A one-way ANOVA showed no

significant group differences regarding sex ($F(1, 41) = 1.148$, $p = 0.291$) or age ($F(1, 41) = 0.595$, $p = 0.445$).

Task

Participants underwent a fear extinction paradigm with partial reinforcement. Pictures of a blue light (CS+) and a yellow light (CS-) were used as CS (see Figure 1). An electric shock served as the US. The intensity of the US was individually set for each participant, targeting a rating of 8 on a scale of 1-9, where 1 is not painful at all and 9 is unbearably painful, so it should be very uncomfortable but not really painful. The shock was delivered to index and middle fingers of the right hand using a constant voltage stimulator - unipolar pulse system (Data Acquisition, Loggers, Amplifiers, Transducers, Electrodes | BIOPAC)

The first day was the acquisition phase. Participants observed 16 CS+ and 16 CS- trials, presented in a randomized order. The reinforcement rate was 62.5%. Each trial consisted of 1.2 seconds of a context cue (lamp without light) followed by 12 seconds of either CS+ or CS- presentation. For CS+ trials followed by the US, the US was administered 500 milliseconds before the end of the CS+ and co-terminated with it. The inter-trial interval jittered between 19 to 25 seconds.

Day 2 was the extinction phase, during which participants observed 8 CS+ and 8 CS- trials, with no CS+ trials reinforced by the US. The real tDCS group received online stimulation with a current amplitude of 2mA for a duration of 10 minutes.

Day 3 was the recall phase. Participants observed 8 unreinforced CS+ and 8 CS- trials, similar to Day 2, but no tDCS was administered.

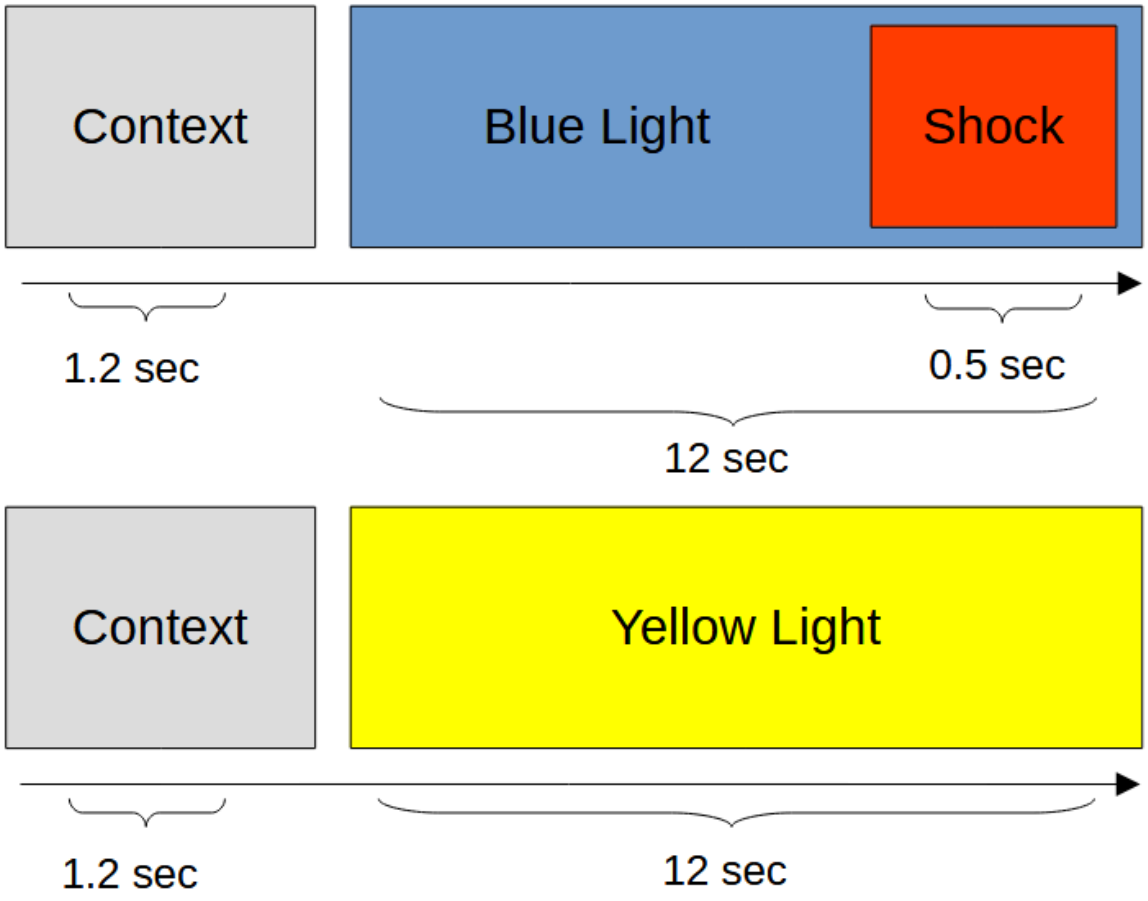

Figure 1: The top timeline shows a CS+ trial. The US was applied in 62.5% of trials during acquisition. The second timeline shows the CS- trials

Measurements and Stimulation

SCR

Participants' SCR during the experiment was measured using a Brian Vision Brain Amp ExG and the Brain Vision Recorder (Brain Vision - Solutions for neurophysiological research). Electrodes are placed on the palm on the middle phalanx of the index and middle finger of the participants' left hand (see Figure 2). EDALYT gel was applied to the electrodes. The signal was recorded at a sample frequency of 1000 Hz, a range of 16.4 mV, a high cutoff filter of 250 Hz, and a resolution of 0.5 µV.

SCR analysis. Brain Vision analyzer was used to downsample the data from 1000Hz to 100Hz and export the data in a .mat file. In-house matlab scripts were used to analyze the data (https://gitlab.ruhr-uni-bochum.de/ikn/eda-analysis/-/tree/master). For the analysis, onsets for each trial were defined and the SCR peaks were detected from the start of context (CS+/CS-) to 500msec after the stimulus end. The maximum peak value within this window for each trial was used as the SCR response. Four trials each for CS+ and CS- were averaged to obtain block-averaged values for all three days of the experiment. For acquisition there were 4 blocks of CS+ and CS- (16 trials each of CS+ and CS-) and for

extinction and recall there were 2 blocks of CS+ and CS- (8 trials reach of CS+ and CS-). Data was excluded if: i) participants were non-responders (no response to CS+), ii) CS+ response smaller than CS- response, or iii) CS+ with US response < 0.01ms.

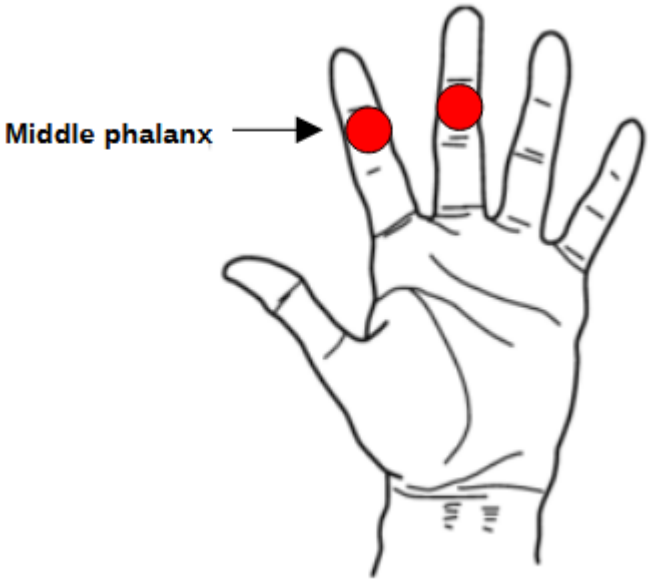

Figure 2: Left hand of the participant. The red dots indicate the position of the EDA electrodes on the middle phalanx of the index and middle finger

ECG

The cardiac activity of the participants is collected using an MRI ready ECG/Respiratory unit (https://www.siemens-healthineers.com/). The Unit was connected to 4 pre-gelled electrodes, three of which were placed on the participants' left lower chest and one reference electrode placed on the right upper chest (see Figure 3).

ECG analysis. Dicom data was first converted to readable ECG and log text files using the CMRR script (https://github.com/CMRR-C2P/MB) and was time logged with fMRI recording using the TAPAS PhysIO toolbox (https://www.tnu.ethz.ch/en/software/tapas/documentations/physio-toolbox). The obtained ECG time series was then used for data analysis. The heart rate variability analysis script used is a script of the HRVTool v.107 for Matlab https://de.mathworks.com/matlabcentral/fileexchange/52787-marcusvollmer-hrv). All the time and frequency parameters for HRV were calculated for each trial and the same time window, as defined for EDA analysis was used. The data was similarly block-averaged for all three days.

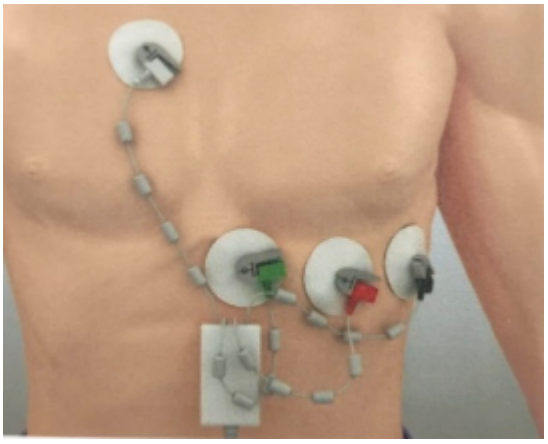

Figure 3: Position of the ECG electrodes on the participants chest.

Respiration

The respiratory activity of the participant was collected using a chest belt connected to the ECG/Respiratory unit (Siemens Healthineers | Corporate Home(siemens-healthineers.com)). The belt was placed according to where the participant's chest or belly moved most during breathing while lying down. The signal was collected at a frequency of 400 Hz. As for ECG recordings, Dicom data was first converted to readable ECG and log text files using the CMRR script (https://github.com/CMRR-C2P/MB) and was time logged with fMRI recording using the TAPAS PhysIO toolbox (https://www.tnu.ethz.ch/en/software/tapas/documentations/physio-toolbox).

Respiration analysis. After extracting the raw data, it was cleaned and prepared for statistical analysis using an in-house script utilizing the Python package neurokit2 (Makowski et al., 2021) (Appendix 1). For data cleaning, a 0.05-3 Hz bandpass Butterworth filter was applied (Khodad et al., 2018). RVT, which is basically a product of respiratory rate and volume, was calculated through the methodology suggested in Harrison et al. (2021). For each trial, the first 11.5 seconds starting from CS onset were analyzed. The baseline-corrected maximum and minimum rate during this time window were extracted. Additionally, the maximum and minimum respiratory signal amplitude, as well as respiratory cycle variables (for a comprehensive overview of all variables, see table…) were calculated and extracted. Further, we extracted the timepoints of the analysis window where each of the rate and volume related events occurred (e.g. the time of the maximal respiratory amplitude of the trial.

Pupillometry

The pupil response was recorded using the Eyelink 1000Plus Eye Tracker (SR Research Ltd. - Eye-Tracking Company (sr-research.com)) at 500 Hz or the Arrington Eye Tracker (http://www.arringtonresearch.com/) at 1000 Hz. For data collected using Arrington, eye-tracking goggles were attached to the MRI head coil, and data was collected from both eyes. For Eyelink, a tracking mirror was attached to the head coil, and the infrared camera was placed behind the MRI bore and positioned between the participant and the display monitor. The data was acquired only from the right eye. The eye tracker was calibrated just before the start of the task using the standard Eyelink 9-point calibration procedure.

Pupillometry analysis. For preprocessing the eyelink data an in-house script utilizing the pypillometry package for Python (https://ihrke.github.io/pypillometry) was used (Appendix 2). Blinks were detected as consecutive 0s that followed stark velocity changes in pupil diameter development. Detected blinks were linearly interpolated from 150 ms before the blink to 150 ms after the blink. Rapid blinks were merged together. Following the interpolation, the data was bandpass filtered between 0.02-4 Hz by application of a third-order Butterworth filter. Lastly, the signal was downsampled to 50 Hz. Participants with either more than 40% missing data throughout the whole recording or more than 16% missing data during the trials were excluded from analysis.

Preprocessing of the Arrington data happened automatically according to the standard Arrington preprocessing pipeline. Blinks were detected and missing data points were interpolated.

The variable of interest, baseline corrected max pupil dilation, was extracted in the same way for both Arrington and Eyelink data. For each trial, the baseline of the pupil diameter was defined by calculating the mean pupil dilation of the 500 ms interval prior to CS presentation. The baseline-corrected peak pupil dilation of the time interval 9.5-11.4 seconds after context onset was extracted for later statistical analysis for each trial.

tDCS

Brain stimulation was administered using a Starstim Neuroelectric tDCS device (ref). Anodal tDCS at vmPFC was administered by placing the anode on the Nasion. Return electrodes were placed on positions Ex19, Ex20, F7, and F8 using the 10-20 EEG system. Ten20 conductive paste and Emla crème were applied to the tDCS electrodes (see Figure 4). The electrodes were connected to the tDCS device inside the MRI scanner. Impedance was controlled to not be higher than 10 kΩ. The stimulation was applied during the 10 minutes of extinction presentation at 2 mA with a ramp-up and -down of 30 seconds each. The participants of the control/sham group only received brief stimulation for the first 30 seconds (with a ramp-up and -down of 30 seconds each) of the experiment. This was done to account for a possible placebo effect that might be induced by the itching sensation associated with the stimulation. tDCS-related side effects and perceived tDCS stimulation status (real/sham) were recorded after all three days of recording.

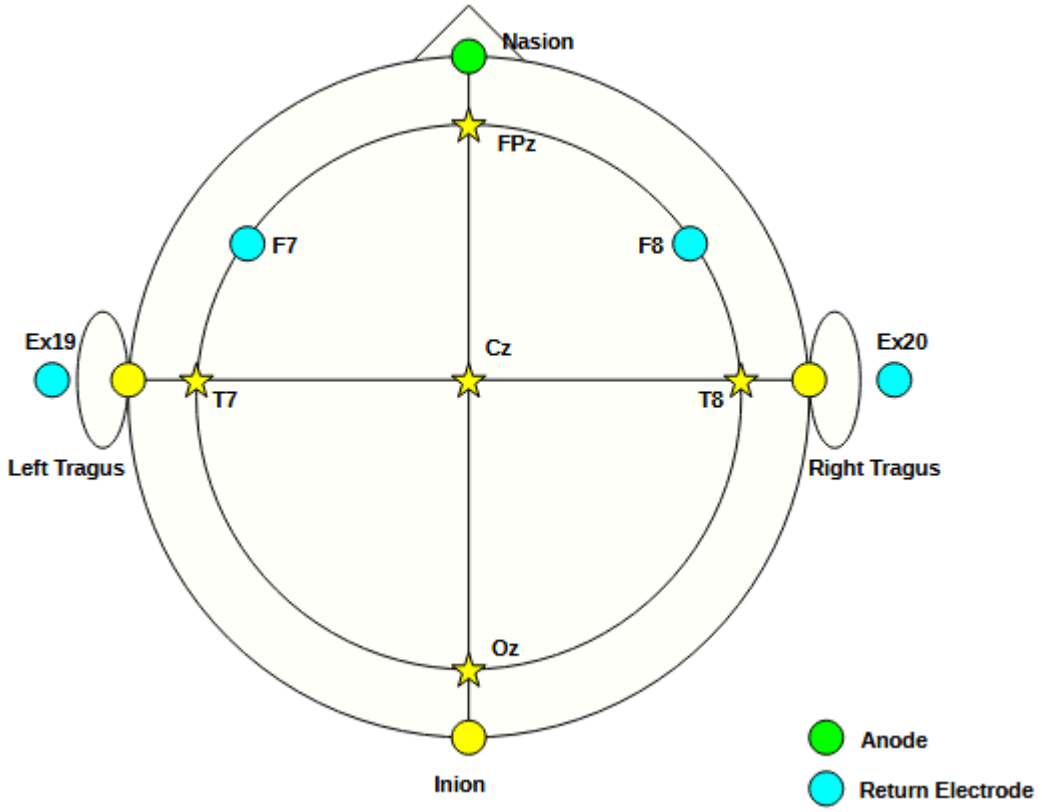

Figure 4: Position of the tDCS electrodes on the participants head. The 10-20 EEG system is used for mapping. The anode is placed on the nasion. The return electrodes are placed on Ex19, Ex20, F7, F8.

Procedure

The experiment took place over 1+3 days. The first recording (Baseline) took place within a week before Days 1-3, which were all separated by 24 hours.

Day 0

Day0 was not part of the main experiment. Here, medical checks were conducted to see if the participants were eligible for stimulation and fMRI recordings. The MRI recordings were conducted in a SIEMENS Magnetrodrom 3 Tesla MRI, using a 64-channel head coil. Additionally, participants had to give written consent and fill out different forms to assess handedness (Appendix 3) and check for exclusion criteria regarding brain stimulation and/or MRI recordings (Appendix 4). After filling out the forms, participants underwent a 30-minute MRI session. Different resting-state measures as well as structural scans were conducted. MRI was not part of this thesis work and therefore the details are beyond the scope of this study. In addition to the MRI scans, ECG and respiration were recorded. This baseline also served the purpose of getting the participants used to lying down in the MRI for a prolonged period of time.

Days 1-3

The experimental setup and procedure were consistent for Days 1-3, with the exception of the main task and the setup of the shock intensity for the US. Upon arrival, participants were led to the preparation room. They first completed a PANAS questionnaire (Appendix 5), followed by receiving the experiment instructions for the day. This was done in order to account for systematic errors and the effect of the participants' mental state going into the experiment. Once the instructions were clear, participants were equipped with the tDCS electrodes. In the MRI facility, they completed the standard MRI form, and the ECG, EDA, and stimulation electrodes were applied. Participants were also provided with earplugs. Inside the scanner, the tDCS, EDA, and stimulation electrodes were connected, the respiration belt was applied, the head coil was attached, and the participant was given the MRI-button box (see Figure 5 for the complete setup). Before entering the scanner, participants received final verbal instructions, and the impedance of the tDCS and EDA electrodes was checked.

Each day's scan began with approximately 15 minutes of resting, during which participants were asked to keep their eyes closed and relax. Following this, the eye tracker was calibrated, and the task specific to each day was conducted. After the task, participants answered a questionnaire using the button box, and questions about their feelings regarding the different CS were asked to assess subjective fear ratings. This was followed by another resting phase scan. All presentations, including the eye-tracker calibration, the main task, and the questionnaire, were displayed on a screen placed at the back of the MRI scanner, visible through a mirror on the 64-channel head coil. After the task and scan, all electrodes were detached. Participants then completed one last questionnaire regarding the tDCS stimulation to control for the effectiveness of the placebo stimulation and check for systematic effects on the final data.

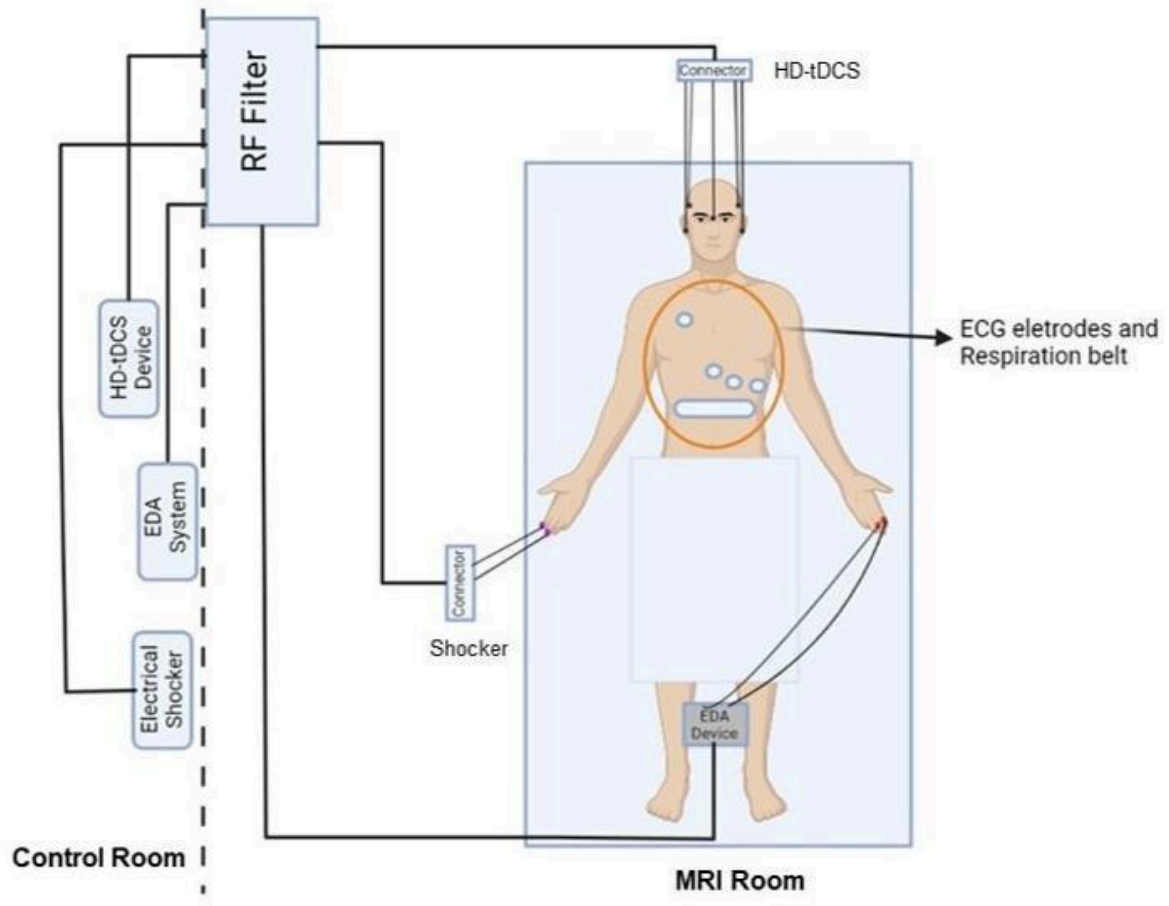

Figure 5: Final laboratory setup during recording. Participants' eyes were tracked via a camera standing behind the MRI-scanner through a mirror installed on top of the head-coil.

Statistical analysis

For analysis, trials were combined into blocks of 4 by taking the mean response for each of the preprocessed physiological response measures over four trials. For example, the first 4 CS+ trials were combined into one block. This resulted in 4 CS+ and 4 CS- blocks for the acquisition day and 2 CS+ and CS- blocks each on the extinction and recall days. These block averages served as the dependent variables.

Acquisition

Repeated measures analysis of variance (RM-ANOVA) was conducted for every physiological response measure of interest. CS-type (CS+ and CS-) as well as block (block 1, block 2, block 3, and block 4) were used as within-subject factors. Additionally, the experimental condition (real and sham tDCS) was used as a between-subject factor to determine if there were significant differences between the groups during conditioning for any of the physiological responses. Successful fear acquisition was indicated by statistically significant differences between CS+ and CS- trials for at least the later blocks of acquisition.

Extinction

To assess whether extinction learning was successful, RM-ANOVA was performed again for all physiological responses that showed fear acquisition. CS-type (CS+ and CS-) and block (block 1 and block 2) were used as within-subject factors, and experimental condition (real and sham tDCS) served as the between-subject factor. Successful extinction learning was indicated by no significant difference between CS+ and CS-. This can happen during early extinction (block 1) or late extinction (block 2).

Recall

To assess extinction retention, RM-ANOVA was conducted for all variables that showed successful fear acquisition. The analysis was like that of the extinction phase. Successful retention was indicated by no significant differences between the CS-types. A significant effect of tDCS was hypothesized.

Results

Sample characteristics

A Chi-Square Test of Independence was conducted to examine the association between gender and experimental group. The results showed no significant association between gender and group membership, $\chi^2(1, N=41)=1.172$, $p=0.28$. This indicates that gender was evenly distributed across the two groups. Further, an independent sample t-test showed no significant difference in age between the two experimental groups, $t(1,39)=3.796$ $p=0.06$. A further Chi-Square Test of Independence showed that the placebo application worked as there was no significant association between tDCS application and tDCS appreciation $\chi^2(1, N=41)=0.266$, $p=0.61$ during the extinction session.

SCR

Acquisition: SC peak response differences between CS+ and CS- during the CS presentation time interval excluding US presentation indicated successful fear learning with CS+ trials having significantly stronger SC responses, $F(1,20)=42.390$ $p<0.001$ $\eta^2=0.68$. Additionally there was a significant effect of Block on SC peak responses, $F(3,60)=10.581$ $p<0.001$ $\eta^2=0.37$. The interaction of CS and Block was not significant, $F(1,20)=2.424$ $p=0.075$ $\eta^2=0.11$. There was no significant interaction effect of CS*tDCS, $F(1,20)=3.427$ $p=0.079$ $\eta^2=0.15$ as well as for the interaction of Block*tDCS, $F(3,20)=0.897$ $p=0.448$ $\eta^2=0.04$.

Post hoc analysis revealed that the skin conductance peak response to CS+ was significantly stronger than to CS- with a mean difference of 0.382, 95% CI [0.260, 0.505], $p < .001$. Post hoc analysis of the effect of Block on the SC peak response revealed significant mean differences between Block 1 and Block 2 of 0.200, 95% CI[0.092, 0.307], $p<0.001$, Block 1 and Block three of 0.252 95% CI [0.072, 0.432] $p=0.003$, Block 1 and Block 4 of 0.304 95% CI [0.116, 0. 0492] $p<0.001$. No significant differences were revealed for the remaining Block combinations.

Source	Type III Sum of Squares	df	F	Sig.	Partial Eta Squared
CS	6.383	1	42.390	**<.001**	.679
CS * tDCS	.516	1	3.427	.079	.146
Error(CS)	3.012	20			
Block	2.315	3	11.801	**<.001**	.371
Block * tDCS	.176	3	.897	.448	.043
Error(Block)	3.923	60			
CS * Block	.340	3	2.424	.075	.108
CS * Block * tDCS	.267	3	1.899	.139	.087
Error(CS*Block)	2.808	60			

Table 1: RM-ANOVA output table of within subject effects on SCRs at alpha=0.05 during acquisition

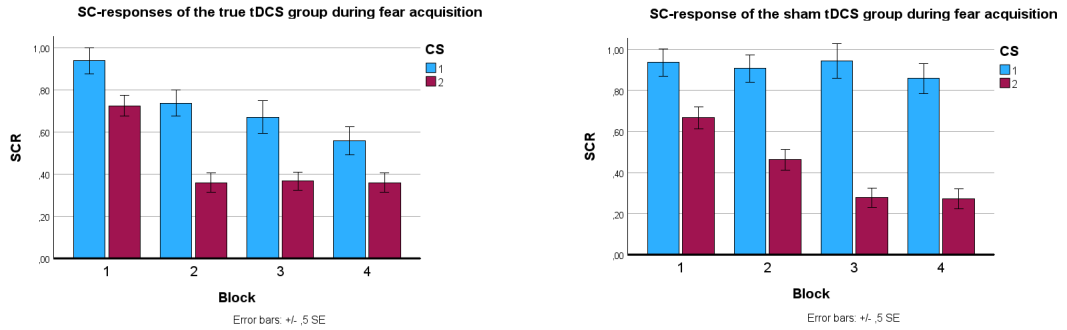

Figure 6: SCRs to the CS+(blue) and CS-(red) during the acquisition session. The left figure shows the results of the true tDCS group. The right figure shows the results of the sham-tDCS control group. The error bars indicate 0.5 SD. CS 1 (blue) represents cs+ trials, CS 2 (red) indicates CS- trials

Extinction: The response difference between CS+ and CS- persisted throughout extinction, $F(1,20)=14.184$ $p=0.001$ $\eta^2=0.42$. Additionally, analysis of variance revealed a significant main effect of Block, $F(1,20)=20.825$ $p<0.001$ $\eta^2=0.51$ but no significant effect of the CS*Block interaction, $F(1,20)=3.998$ $p=0.059$ $\eta^2=0.18$. Further, tDCS had a significant effect on overall SC peak response, $F(1,20)=7.708$ $p=0.012$ $\eta^2=0.28$ throughout the extinction session.

Post hoc analysis revealed a significant mean difference between CS+ and CS- for both Block 1 of 0.255 95% CI [0.090, 0.419] $p=0.004$ and Block 2 of 0.117 95% CI [0.050, 0.184] $p=0.002$. Additionally between Block 1 and Block 2 there is a significant mean difference in SC peak of 0.263 95% CI [0.143, 0.383] $p<0.001$. The significant effect of tDCS lead to a mean difference of -0.360 95% CI [-0.630, -0.089] $p=0.012$ in SCR peak compared to sham tDCS.

Source	Type III Sum of Squares	df	F	Sig.	Partial Eta Squared
CS	.753	1	14.184	**.001**	.415
CS * tDCS	.029	1	.554	.465	.027
Error(CS)	1.061	20			
Block	1.508	1	20.825	**<.001**	.510
Block * tDCS	.056	1	.778	.388	.037
Error(Block)	1.448	20			
CS * Block	.103	1	3.998	.059	.167
CS * Block * tDCS	.014	1	.537	.472	.026
Error(CS*Block)	.517	20			

Table 2: RM-ANOVA output table of within subject effects on SCRs at alpha=0.05 during extinction

Source	Type III Sum of Squares	df	F	Sig.	Partial Eta Squared
Intercept	11.586	1	31.606	<.001	.612
tDCS	2.826	1	7.708	**.012**	.278
Error	7.332	20			

Table 3: RM-ANOVA output table of between subject effects on SCRs at alpha=0.05 during extinction

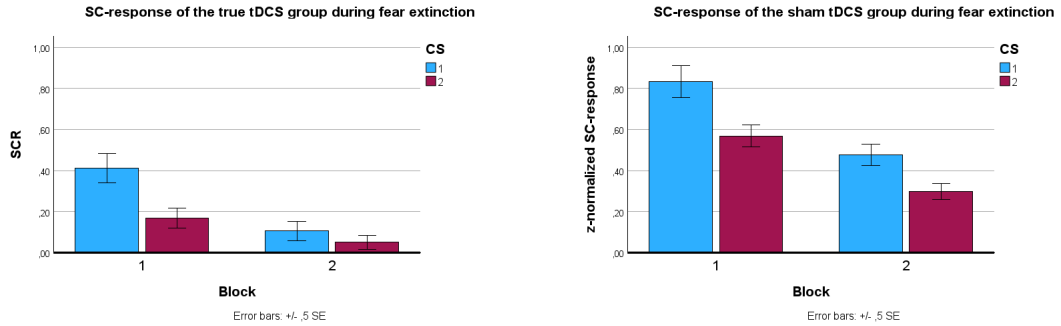

Figure 7: SCRs to the CS+(blue) and CS-(red) during the extinction session. The left figure shows the results of the true tDCS group. The right figure shows the results of the sham-tDCS control group. The error bars indicate 0.5 SD. CS 1 (blue) represents CS+ trials, CS 2 (red) indicates CS- trials

Recall: Analysis of variance showed no main effect of CS type, $F(1,20)=3.281$ $p=0.085$ $\eta^2=0.14$. Throughout the session the overall SC response decreased, indicated by a significant effect of Block on overall performance, $(F1,20)=23.929$ $p<0.001$ $\eta^2=0.55$. The effect of tDCS on SC peak was insignificant during recall, $F(1,20)=0.483$ $p=0.495$ $\eta^2=0.02$. The interaction of CS*Block was insignificant, $F(1,20)=1.191$ $p=0.288$ $\eta^2=0.06$, as well as the CS*tDCS interaction, $F(1,21)=0.805$ $p=0.380$ $\eta^2=0.04$ and the Block*tDCS interaction, $F(1,20)=1.024$ $p=0.324$ $\eta^2=0.05$.

Post hoc analysis revealed a mean difference of 0.217 95%CI [0.124, 0.309] $p<0.001$ between Block 1 and Block 2

Source	Type III Sum of Squares	df	F	Sig.	Partial Eta Squared
CS	.100	1	3.281	.085	.141
CS * tDCS	.024	1	.805	.380	.039
Error(CS)	.608	20			
Block	1.024	1	23.929	**<.001**	.545
Block * tDCS	.044	1	1.024	.324	.049
Error(Block)	.856	20			
CS * Block	.022	1	1.191	.288	.056
CS * Block * tDCS	.005	1	.275	.606	.014
Error(CS*Block)	.366	20			

Table 4: RM-ANOVA output table of within subject effects on SCRs at alpha=0.05 during recall

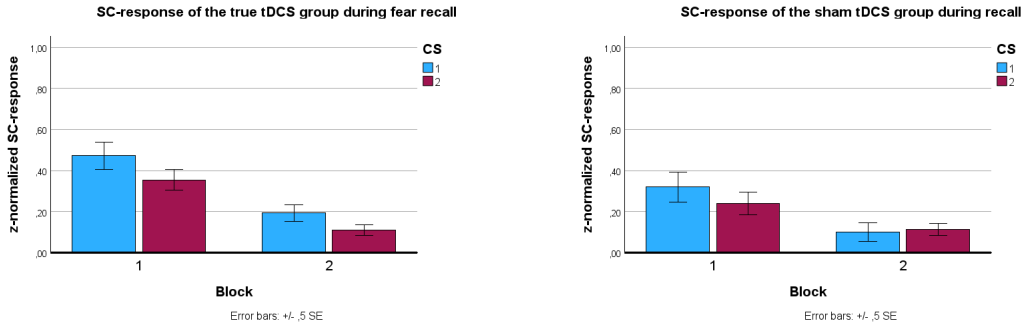

Figure 8: SCRs to the CS+(blue) and CS-(red) during the recall session. The left figure shows the results of the true tDCS group. The right figure shows the results of the sham-tDCS control group. The error bars indicate 0.5 SD. CS 1 (blue) represents CS+ trials, CS 2 (red) indicates CS- trials

Pupillometry

There was no significant difference in Pupil diameter response between CS+ and CS- trials during acquisition, indicating no conditioned fear response, $F(1,25)=2.350$ $p=0.138$. This persisted throughout extinction, $F(1,25)=0.731$ $p=0.401$ $\eta^2=0.03$ and recall, $F(1,25)=0.000$ $p=1.000$ $\eta^2=0.000$.

Respiration

Acquisition: There was no conditioned response found for maximal respiratory amplitude, $F(1,33)=1.489$ $p=0.231$ $\eta^2=0.04$, minimal respiratory amplitude, $F(1,33)=0.770$ $p=0.387$ $\eta^2=0.02$, maximal respiratory rate, $F(1,33)=2.941$ $p=0.096$ $\eta^2=0.08$, minimal respiratory rate, $F(1,33)=0.352$ $p=0.557$ $\eta^2=0.01$, mean respiratory rate, $F(1,33)=0.001$ $p=0.973$ $\eta^2=0.000$ and consequently RVT, $F(1,33)=0.022$ $p=0.883$ $\eta^2=0.00$ during the CS presentation interval. Interestingly, respiratory rate standard deviation significantly differed between different CS types, $F(1,33)=4.658$ $P=0.038$ $\eta^2=0.12$ with stronger deviation for CS- trials compared to CS+ trials.

Post hoc analysis revealed a significant mean difference in respiratory rate SD between CS+ and CS- of -0.143 95% CI [-0.279, -0.008] p=0.038.

Source	Type III Sum of Squares	df	F	Sig.	Partial Eta Squared
CS	1.439	1	4.658	**.038**	.124
CS * tDCS	4.771E-5	1	.000	.990	.000
Error(CS)	10.196	33			
Block	2.281	3	1.456	.231	.042
Block * tDCS	1.489	3	.951	.419	.028
Error(Block)	51.686	99			
CS * Block	.679	3	.627	.599	.019
CS * Block * tDCS	2.138	3	1.974	.123	.056
Error(CS*Block)	35.735	99			

Table 5: RM-ANOVA output table of within subject effects on respiratory rate SD at alpha=0.05 during acquisition

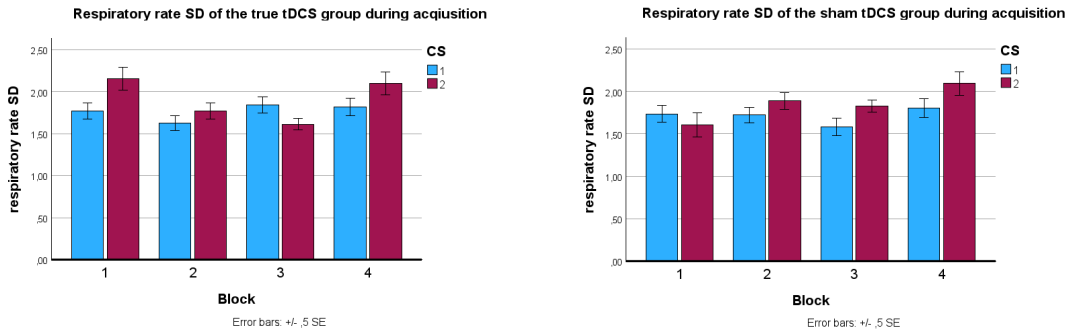

Figure 9: Respiratory rate SD response to the CS+(blue) and CS-(red) during the acquisition session. The left figure shows the results of the true tDCS group. The right figure shows the results of the sham-tDCS control group. The error bars indicate 0.5 SD. CS 1 (blue) represents CS+ trials, CS 2 (red) indicates CS- trials

Extinction: maximal amplitude, F(1,33)=0.689 p=0.413 η^2=0.02. Minimal amplitude, F(1,33)=0.027 p=0.871 η^2=0.00. Rate max, F(1,33)=0.182 p=0.673 η^2=0.01. Rate min, F(1,33)=0.296 p=0.590 η^2=0.01. Rate mean, F(1,33)=0.284 p=0.598 η^2=0.01. RVT, F(1,33)=0.556 p=0.462 η^2=0.02.

The effect of Rate SD got extinguished, F(1,33)=0.015 p=0.903 η^2=0.00, but neither block F(1,33)=0.32 p=0.859 η^2=0.00, tDCS, F(1,33)=0.306 p=0.584 η^2=0.01 nor the CS*Block interaction, F(1,33)=2.262 p=0.132 η^2=0.07 had a significant effect on the outcome.

Recall: Amplitude Max, F(1,32)=0.156 p=0.696 η^2=0.01. Amplitude Min, F(1,32)=2.343 p=0.136 η^2=0.07. Maximum rate, F(1,32)=0.495 p=0.487 η^2=0.02. Minimum rate, F(1,32)=3.179 p=0.085 η^2=0.10. Mean rate, F(1,32)=0.583 p=0.451 η^2=0.02. RVT, F(1,32)=0.399 p=0.533 η^2=0.01.

Rate standard deviation again was not affected by CS type, F(1,30)=0.647 p=0.427 η^2=0.02. But an effect of the Block*tDCS interaction was evident, F(1,30)=9.484 p=0.004 η^2=0.02. Post hoc analysis revealed a significant mean difference from real to sham tDCS for block 2 of -0.829 95% CI [-1.612, -0.046] p=0.039. This was driven by a significant increase in respiratory rate SD by the sham tDCS group from Block 1 to Block 2 of 0.658 95% CI [-1.081, -0.234] p=0.003.]

Source	Type III Sum of Squares	df	F	Sig.	Partial Eta Squared
CS	.628	1	.647	.427	.021
CS * tDCS	.710	1	.732	.399	.024
Error(CS)	29.122	30			
Block	1.538	1	2.386	.133	.074
Block * tDCS	6.113	1	9.484	**.004**	.240
Error(Block)	19.335	30			
CS * Block	.000	1	.001	.974	.000
CS * Block * tDCS	.131	1	.306	.584	.010
Error(CS*Block)	12.805	30			

Table 6: RM-ANOVA output table of within subject effects on respiratory rate SD at alpha=0.05 during recall

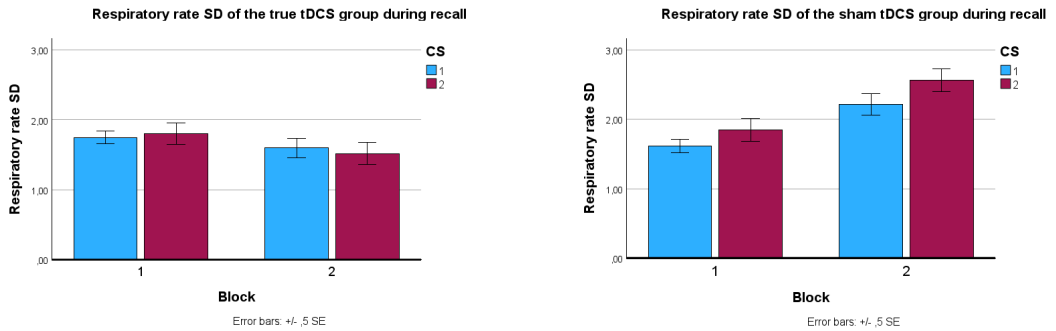

Figure 10: Respiratory rate SD response to the CS+(blue) and CS-(red) during the recall session. The left figure shows the results of the true tDCS group. The right figure shows the results of the sham-tDCS control group. The error bars indicate 0.5 SD. CS 1 (blue) represents CS+ trials, CS 2 (red) indicates CS- trials

Cardiac activity

Acquisition: Neither of the recorded variables exhibit successful conditioning during acquisition. Inter beat intervall, F(1,32)=0.001 p=0.980 η^2=0.00. BPM, F(1,32)=0.002 p=0.969 η^2=0.00. Area under the curve, F(1,32)=1.262 p=0.269 η^2=0.04. SDSD, F(1,32)=1.867 p=0.181 η^2=0.05. RMSSD, F(1,32)=1.769 p=0.193 η^2=0.05. HPV, F(1,32)=1.208 p=0.280 η^2=0.04. SD_ratio, F(1,32)=0.011 p=0.919 η^2=0.01.

Extinction: Inter beat intervall, F(1,32)=2.294 p= 0.141 η^2=0.07. BPM, F(1,32)=0.459 p=0.504 η^2=0.02. Area under the curve, F(1,32)=1.772 p=0.193 η^2=0.05. SDSD,

F(1,32)=1.756 p=0195 η^2=0.06. RMSSD, F(1,32)=1.718 p=0.200 η^2=0.06. HPV, F(1,32)=1.770 p=0.194 η^2=0.06. SD_ratio, F(1,32)=0.002 p=0.966 η^2=0.00.

Recall: Interestingly for the recall session there was a significant effect of CS type on IBI, F(1,28)=4.294 p=0.048 η^2=0.13 with CS+ showing marginally longer IBI compared to CS-. This effect was consistent throughout the whole session and between the two groups, indicated by no significant effect of Block, F(1,28)=0.024 p=0,878 η^2=0.001 nor tDCS, F(1,28)=0.543 p=0.467 η^2=0.02. Expectedly the same held true for Heart Rate, F(1,28)=4.355 p=0.046 η^2=0.14 and BPM, F(1,29)=6.584 p=0.016 η^2=0.19.
AUC, F(1,28)=2.868 p=0.101 η^2=0.09. SDSD, F(1,28)=0.134 p=0.717 η^2=0.01. RMSSD, F(1,28)=0.193 p=0.664 η^2=0.01. HPV, F(1,28)=1.056 p=0.313 η^2=0.04. SD_ratio, F(1,28)=0.114 p=0.738 η^2=0.00.

Post hoc analysis of the difference between CS+ and CS- for IBI revealed a mean difference of 3.182 95% CI [0.036, 6.327] p=0.048. For BPM the mean difference was -0.848 95% CI [-1.524, -0.171] p=0.016. Lastly there was a significant mean difference of heart rate of -0.002 95% CI [-0.003, -0,00002954484173] p=0.046.

Source	Type III Sum of Squares	df	F	Sig.	Partial Eta Squared
CS	303.695	1	4.294	**.048**	.133
CS * tDCS	8.369	1	.118	.733	.004
Error(CS)	1980.532	28			
Block	2.842	1	.024	.878	.001
Block * tDCS	136.766	1	1.149	.293	.039
Error(Block)	3331.811	28			
CS * Block	81.043	1	1.134	.296	.039
CS * Block * tDCS	34.596	1	.484	.492	.017
Error(CS*Block)	2001.232	28			

Table 7: RM-ANOVA output table of within subject effects on IBI at alpha=0.05 during recall

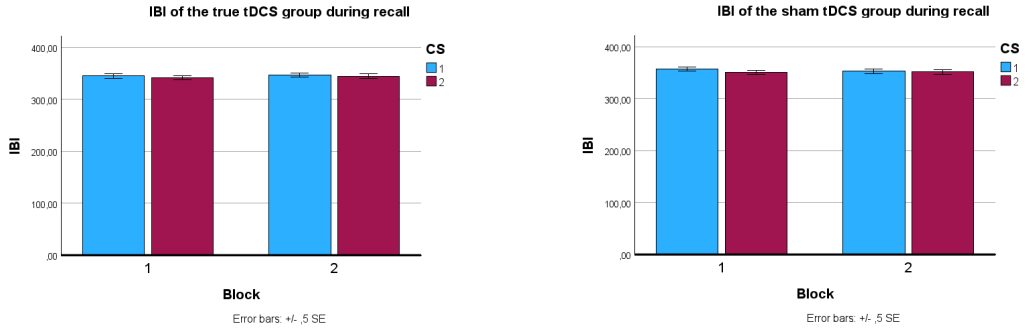

Figure 11: IBI response to the CS+(blue) and CS-(red) during the recall session. The left figure shows the results of the true tDCS group. The right figure shows the results of the sham-tDCS control group. The error bars indicate 0.5 SD. CS 1 (blue) represents CS+ trials, CS 2 (red) indicates CS- trials

Source	Type III Sum of Squares	df	F	Sig.	Partial Eta Squared
CS	21.555	1	6.584	**.016**	.190
CS * tDCS	1.148	1	.351	.558	.012
Error(CS)	91.661	28			
Block	.014	1	.004	.951	.000
Block * tDCS	2.395	1	.659	.424	.023
Error(Block)	101.752	28			
CS * Block	3.189	1	.657	.425	.023
CS * Block * tDCS	2.395	1	.493	.488	.017
Error(CS*Block)	135.990	28			

Table 8: RM-ANOVA output table of within subject effects on BPM at alpha=0.05 during recall

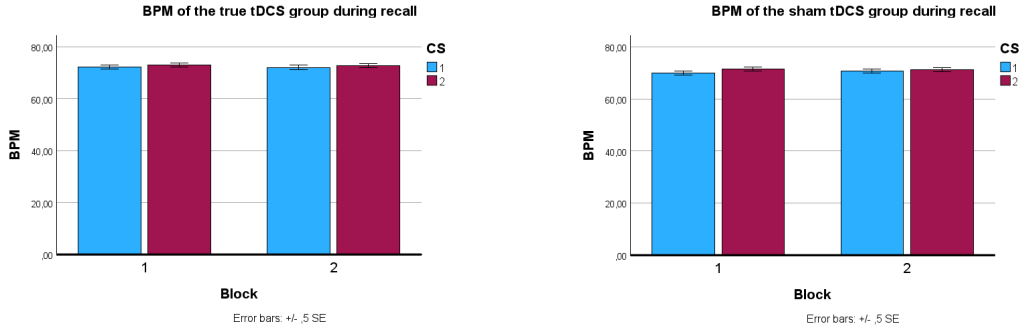

Figure 12: BPM response to the CS+(blue) and CS-(red) during the recall session. The left figure shows the results of the true tDCS group. The right figure shows the results of the sham-tDCS control group. The error bars indicate 0.5 SD. CS 1 (blue) represents CS+ trials, CS 2 (red) indicates CS- trials

Source	Type III Sum of Squares	df	F	Sig.	Partial Eta Squared
CS	7.703E-5	1	4.355	**.046**	.135
CS * tDCS	1.405E-7	1	.008	.930	.000
Error(CS)	.000	28			
Block	1.928E-7	1	.007	.933	.000
Block * tDCS	2.156E-5	1	.811	.375	.028
Error(Block)	.001	28			
CS * Block	1.383E-5	1	.695	.411	.024
CS * Block * tDCS	1.179E-5	1	.593	.448	.021
Error(CS*Block)	.001	28			

Table 9: RM-ANOVA output table of within subject effects on HR at alpha=0.05 during recall

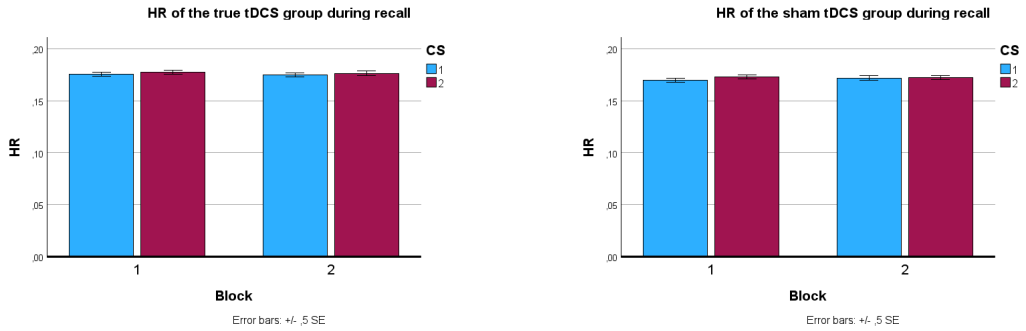

Figure 13: HR response to the CS+(blue) and CS-(red) during the recall session. The left figure shows the results of the true tDCS group. The right figure shows the results of the sham-tDCS control group. The error bars indicate 0.5 SD. CS 1 (blue) represents CS+ trials, CS 2 (red) indicates CS- trials

Discussion

The goal of the study at hand was to investigate the effects of anodal tDCS of the vmPFC during fear extinction by investigating the conditioned responses of different physiological systems. The experiment consisted of three phases, fear acquisition, fear extinction, and extinction recall. Each phase was conducted with a 24 hour break in between. Stimulation was applied during the first 10 minutes of the fear extinction phase. The physiological signals collected include EDA, pupillometry, respiration, and ECG. Results were compared to the results of a control group that only received placebo stimulation. Notably, the experiment was conducted inside an MRI scanner. The imaging data was not of interest to the current study and was therefore not analyzed. We hypothesized that participants who received stimulation would exhibit enhanced extinction learning, which should lead to a difference in conditioned physiological responses between the experimental and control group during the extinction and recall session.

Before discussing the results, it has to be stated that due to exclusion criteria and unclean data, the final sample size for SCR and pupillometry analysis was comparatively small. Analysis of these variables was hence underpowered and possible effects might have not emerged from statistical analysis because of this.

Fear Acquisition

Conditioned responses were evident from the SCR as well as the rate SD of the respiratory data. Both pupillometry and ECG did not show any conditioned responses during fear acquisition and will therefore not be discussed further.

SCR

During acquisition SCR for CS+ were significantly stronger than for CS-, which is in line with previous research and indicates successful fear learning and stimulus differentiation (Waters et al., 2023; Marinos et al., 2022; Zhao et al., 2022; Mertens et al., 2021; Modecki et al., 2020; O`Malley et al., 2018; Merz et al., 2016; Milad et al., 2005; Vervliet et al., 2004). The effect was present throughout all four trial blocks. Interestingly, the safety cue CS-

elicited an SCR as well, even though it was significantly weaker compared to CS+. While stronger for early trials, this response remained throughout the session.

Respiration

From previous research, we expected to see conditioned responses in respiratory amplitude and/or respiratory rate (Castegnetti et al., 2016; Wehrli et al., 2022; van Diest et al., 2009). Instead, the results of the experiment suggest a conditioned response of the respiratory rate SD. More specifically, participants' respiratory rate deviated less during CS+ trials compared to CS- trials indicating a more stable breathing pattern during CS+ trials. The difference between our findings and the findings presented in previous studies might be attributed to the body position of the participants, as they had to lie down inside the MRI scanner. Kyle Rehder (1998) has shown how patients' functional residual capacity changes in different ways based on body position. He further shows that the inspired gas is distributed in a different way throughout the lungs if patients are in the prone position compared to in other body positions. Additionally, different body positions result in different effects on pulmonary blood flow (K. Rehder, 1998). It has further been shown that body position can have a significant effect on how the respiratory and cardiac system react to stress, rest, and exercise (Perini and Veicsteinas, 2003; Anderson et al., 2015). Therefore body position might explain the difference of the conditioned responses found in our experiment compared to previous research.

Fear Extinction

The results of the conditioned physiological responses, SCR and respiratory rate SD, both indicate extinction learning. We further observed a significant effect of tDCS from the SCR data.

SCR

Previously, most researchers conducting fear extinction experiments reported a complete depletion of the CS differentiation visible from SCRs to indicate successful extinction learning (Waters et al., 2023; Modecki et al., 2020; O`Malley et al., 2018; Merz et al., 2016; Milad et al., 2005; Vervliet et al., 2004). While SCR still showed significant CS differentiation in our experiment, the overall responses to both CS+ and CS- decreased significantly throughout the session for both participant groups. For later trials, SCRs to CS+ were even lower than SCRs to the safety cue CS- during the earlier extinction trials. This suggests that participants learned to fear both CS+ and CS- significantly less throughout the extinction session. It also has to be noted that the results of the CS*Block interaction showed a trend ($p=0.059$ $\eta^2=0.167$). The trend was mostly driven by a decrease in SCRs to CS+.

As hypothesized, the extinction learning pattern was enhanced by the application of tDCS. Participants who received stimulation exhibited significantly weaker SCRs to both CS+ and CS- compared to the participants who only received placebo stimulation, indicating more effective extinction learning. CS differentiation was not affected by stimulation. These results are not in line with what was previously reported. All experiments utilizing online tDCS targeting the mPFC or vmPFC of healthy participants reported depletion of CS

differentiation during extinction (Abend et al., 2016; Van`t Wout et al., 2016; Dittert et al., 2018; Vicario et al., 2020).

Dittert et al. (2018) as well as Abend et al. (2016) attribute the differentiation depletion partially to fear generalization, indicated by an increase in SCR to CS- in their experiments. Notably, Dittert et al. (2018) only had a 10-minute break between acquisition and extinction and started stimulation during this short break. As McGough (2000) argues, this short time period between acquisition and extinction does not allow for fear memory consolidation and may therefore influence the results. A further difference between our study and the studies of Dittert et al. (2018) and Abend et al. (2016) is the electrode placement. Dittert et al. (2018) placed their electrodes on the positions F7 and F8, while Abend et al. (2016) placed their anode on FPz and the return electrode on Iz.

The effect that electrode placement can have becomes clear when looking at the results of Van`t Wout et al. (2016) and Vicario et al. (2020). In both studies, the anode was placed on AF3. In both experiments fear responses extinguished during late extinction with no fear generalization present in the results. Interestingly, they only report a significant effect of stimulation for late extinction, while in our experiment we observed a significant difference from the start.

The effect of tDCS during our extinction phase might be better explained by looking at the results presented by O´Malley et al. (2018), who compared the effects of different attention manipulations on the fear extinction process. They have shown that participants who were instructed not to pay close attention to the CS showed higher SCR for both CS+ and CS- during extinction in comparison to controls who did not receive any instructions (O'Malley et al. 2018). This pattern is inverse to the significant difference in SCR due to anodal tDCS of the vmPFC during extinction in the study at hand. Here participants who received stimulation exhibited significantly smaller SCRs to both CS+ and CS- in comparison to controls who did not receive any stimulation, indicating a modulation of attentional processes. Supporting this theory, Nejati et al. (2021) conclude in their review on the effects of tDCS on attentional bias that tDCS of the vmPFC enhances attention bias towards positive emotional material. They further argue that this is in line with evidence that suggests that the vmPFC downregulates emotionally negative responses of the amygdala (Nejati et al., 2021).

Respiration

The conditioned response of the respiratory rate SD got extinguished for both the stimulation and the placebo group. None of the independent variables, nor any of the interactions had a significant effect on respiratory rate SD during extinction. Going against our hypothesis, this also includes the application of tDCS, even though an effect was evident from the SCR results. The lack of an effect of tDCS might be explained by a return to natural breathing in response to both CSs by both experimental groups. Previously no one has conducted fear extinction paradigms utilizing respiratory responses, making it impossible to compare our results.

Recall

The recall phase demonstrated successful extinction retention for both physiological measures, with a significant effect of tDCS evident only in respiratory data.

SCR

During recall, SCR differentiation between CS+ and CS- dissipated for both groups, although a trend towards differentiation remained. Notably, while SCR responses decreased over time, no significant effects of tDCS were found during this phase, nor any relevant interactions. This result diverges from studies like Vicario et al. (2020), who reported CS differentiation only in their placebo group, but their effects were already present during extinction, making direct comparison difficult.

The absence of a tDCS effect during recall may suggest that the stimulation enhanced extinction learning during the session but did not significantly influence retention of that learning over time. This contrasts with findings by Abend et al. (2016), who observed no CS differentiation in the tDCS group during the first recall trial, which they attributed to increased SCR to CS-.

Respiration

Respiratory rate SD during recall indicated successful extinction retention, with no significant within-subject effects. However, a tDCS*Block interaction was observed, where the control group showed a significant increase in respiratory rate SD between early and late recall trials, while the stimulation group maintained stable patterns. This may reflect more consistent physiological regulation in the tDCS group, but without prior research on respiratory rate SD in this context, the implications remain uncertain.

Environmental Factors

Conducting the experiment inside an MRI scanner introduced unique challenges that may have influenced our results. The scanner's environment, including body position, space constraints, and loud noises, could induce stress (Eatough et al., 2009; Madl et al., 2022; Tessner et al., 2006; Leuken et al., 2012), potentially affecting physiological responses. Stress has been shown to impede fear extinction (Maren & Holmes, 2016), which may explain the persistence of CS differentiation in both the extinction and recall phases.

Moreover, the scanner's electromagnetic interference (EMI) introduced noise into the physiological data, leading to the exclusion of participants and potentially underpowered analyses. This is particularly relevant for SCR and pupillometry, which were the most affected by signal degradation, necessitating extensive preprocessing. As a result, smaller signal changes might have been lost, reducing the sensitivity of our analyses.

Conclusion

In conclusion, this study aimed to investigate the effects of anodal tDCS of the vmPFC during fear extinction, focusing on physiological responses across SCR, respiration, pupillometry, and ECG. It was hypothesized tDCS of the vmPFC during extinction learning enhances extinction learning and retention. The results support our hypothesis to some extent, indicating that tDCS enhances extinction learning as shown by significantly reduced SCRs in the stimulation group compared to the placebo group. Notably, while the SCR data revealed a clear stimulation effect, respiratory data did not show similar results, potentially due to the natural reversion of breathing patterns and the novel nature of this measurement in fear extinction paradigms.

Most notably, the effects present in our experiment diverge from previous research on the effects of tDCS in fear extinction learning. While CS differentiation persisted during extinction, SCRs to both CS+ and CS- significantly decreased as a result of stimulation, indicating enhanced learning of both, the new CS+ contingency and the safety cue (CS-). Also, there was no return to CS differentiation during the recall session. The findings suggest that tDCS might be a promising tool to improve exposure therapy by enhancing extinction learning.

Future Research

Overall, while the study provides evidence for the effectiveness of tDCS in modulating extinction learning, especially through SCR, further research with larger samples is needed. Future studies should also consider controlling for the environmental and physiological complexities introduced by the MRI setting to better understand the role of e.g. body position and other unique aspects of conducting experiments inside the scanner.

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Appendix

Appendix 1: Respiratory data analysis tool

```
import os
import tkinter as tk
import scipy.io
import pandas as pd
import neurokit2 as nk



def run_analysis():
    intervallStart = entry1.get()
    intervallEnd = entry2.get()
    start = int(intervallStart)
    end = int(intervallEnd)
    root_dir = os.getcwd()
    print(root_dir)
    folder_name = "analysis"
    file_paths = create_new_folder(root_dir, folder_name)
    for file_path, folder_path in file_paths:
            pipeline(file_path, folder_path, start, end)  # Pass both file and folder paths to the
pipeline function

def create_new_folder(root_dir, folder_name):
    file_paths = []
    for subdir, dirs, files in os.walk(root_dir):
        if not dirs:
            for file in files:
                if file.endswith('.mat'):
                    # Construct the full path to the .mat file
                    file_path = os.path.join(subdir, file)
                    folder_path = os.path.join(subdir, folder_name)
                    os.makedirs(folder_path, exist_ok=True)
                    file_paths.append((file_path, folder_path))  # Store both file and folder paths
    return file_paths

def create_plots(rsp, target_directory):
    signals = pd.DataFrame({
        "RSP_Raw": rsp,
        "RSP_Khodad2018": nk.rsp_clean(rsp, sampling_rate=400)})
    fig = signals.plot().get_figure()
    fig.savefig(os.path.join(target_directory, 'clean_raw.png'))

def pipeline(dataset_directory, target_directory, start, end):
    print(dataset_directory)
    mat_data = scipy.io.loadmat(dataset_directory)
```

```
    print(".mat file successfully loaded")

    # Check if 'respiration_task' exists in the .mat file
    filename = os.path.basename(dataset_directory)
    print(filename)
        if not any(substring in filename for substring in ['Acq.mat', 'Ext.mat', 'Rec.mat', 'Rec2.mat']):
        print(f"Skipping '{dataset_directory}' - not respiration data.")
        return

    respiration_task = mat_data['data'].flatten()

    signal, info = nk.rsp_process(respiration_task, sampling_rate=400)

    # Determine the event markers based on the filename

    if "Acq" in filename:
        print("Acquisition")
        # Event markers for the "acquisition_respiration_physio.mat" type
          eventmarkers = [801, 4281, 8122, 11600, 15681, 19161, 23001, 26481, 30442, 34282, 37640, 41481, 45321, 48801, 52881, 56362, 59962, 63800, 67401, 71481, 75081, 78801, 82402, 86302, 89720, 93561, 97521, 101121, 104721, 108562, 112062, 116120]  # Replace with your event markers for this type
    elif "Ext" in filename:
        print("Extinction")
        # Event markers for the "acquisition_respiration_physio.mat" type
          eventmarkers = [801, 4281, 8122, 11600, 15681, 19161, 23001, 26481, 30442, 34282, 37640, 41481, 45321, 48801, 52881, 56362]  # Replace with your event markers for this type
    else:
        print("Recall")
        # Event markers for other types of files
          eventmarkers = [802, 4282, 8120, 11601, 15681, 19161, 23001, 26482, 30442, 34281, 37641, 41481, 45321, 48801, 52882, 56362]  # Replace with your event markers for other types

    num_rows = len(signal)
    markers = [0] * num_rows
    for index in eventmarkers:
        markers[index*4] = 5
    events = nk.events_find(markers)
        epochs = nk.epochs_create(signal, events, sampling_rate=400, epochs_start=start, epochs_end=end)
    processedDF = nk.rsp_eventrelated(epochs)



    rsp_cleaned = nk.rsp_clean(respiration_task, sampling_rate=400)
```

```
    rsp_rate_onsets = nk.rsp_rate(rsp_cleaned, sampling_rate=400, method="trough")
    print(rsp_rate_onsets)
    print(len(rsp_rate_onsets))
        signalepochs = nk.epochs_create(rsp_rate_onsets, events, sampling_rate = 400,
epochs_start=start, epochs_end=end)
    nk.epochs_plot(signalepochs)



    infodf = pd.DataFrame(info)
    infodf.to_csv(target_directory + '\\info.csv')

    processedDF.to_csv(os.path.join(target_directory, 'Trials.csv'))

    create_plots(respiration_task[:18000], target_directory)


starttime = float()
endtime = float()

greeting = tk.Label(
    text="Welcome to RDAT, your Respiration Data Analysis Tool",
    width=50,
    height=5)
greeting.pack()

entry1Label = tk.Label(
    text="Please enter the start of the interval (in seconds)",
    width=50,
    height=2)
entry1Label.pack()
entry1 = tk.Entry(width=50)
entry1.pack()

entry2Label = tk.Label(
    text="Please enter the end of the interval (in seconds)",
    width=50,
    height=2)
entry2Label.pack()
entry2 = tk.Entry(width=50)
entry2.pack()

button = tk.Button(
    text="start analysis",
    width=20,
    height=3,
    bg=("grey"),
    fg=("black"),
```

```
    command=run_analysis)
button.pack()

tk.mainloop()
```

Appendix 2: pupil data analysis tool

```
import pandas as pd
import numpy as np
import os
import pypillometry as pp
import pylab as plt

# Function to calculate z-values and append them to each participant's DataFrame
def calculate_and_append_z_values(df):
    # Extract pupil response column
    pupil_responses = df['response']

    # Calculate z-values
    mean_value = pupil_responses.mean()
    std_value = pupil_responses.std()
    z_values = (pupil_responses - mean_value) / std_value

    # Mark rows with z-values higher than 2 or lower than -2 as outliers
    #outliers = np.abs(z_values) >

    # Assign NaN to z-value column for rows corresponding to outliers
    #z_values[outliers] = np.nan

    # Append z-values as a new column
    df['z_values'] = z_values

    return df

# Function to process eye-tracking data for each session
def process_session(samples_file, events_file):
    # Read samples file
    df = pd.read_table(samples_file, index_col=False, names=["time", "ps", "x", "y"])

    # Replace dot with zero
    df['ps'] = df['ps'].replace('     -y', 0)

    # Read events file
    with open(events_file) as f:
        events = f.readlines()

    # Keep only lines starting with "MSG"
    events = [ev for ev in events if ev.startswith("MSG")]

    # Extract relevant events
    experiment_start_index = np.where(["!V CLEAR" in ev for ev in events])[0][0]
    events = events[experiment_start_index+1:]
    df_ev = pd.DataFrame([ev.split() for ev in events])
```

```
    df_ev = df_ev[[1, 5]]
    df_ev.columns = ["time", "event"]

    # Filter events containing "context"
    df_ev = df_ev[df_ev['event'].str.contains('context', case=False, na=False)]

    # Process pupil data using pypillometry
                d = pp.PupilData(df['ps'], time=df['time'], event_onsets=df_ev['time'],
event_labels=df_ev['event'], name="test")
    d = d.reset_time()
     d = d.blinks_detect(min_duration=5,blink_val=0,winsize=150,vel_onset=10, vel_offset=10,
min_onset_len=5, min_offset_len=5, units="ms")
    d = d.blinks_merge().blinks_interpolate().lowpass_filter(0.1).downsample(50)

    # Calculate baseline and response
    baseline = d.stat_per_event((-12500, -12000))
    response = d.stat_per_event((-2000, -500), statfct=max) - baseline     #, statfct=max

    # Create DataFrame with baseline and response values
    final_df = pd.DataFrame(list(zip(baseline, response)), columns=['baseline', 'response'])

    # Add trial number column
    final_df.insert(0, 'trial_number', range(1, len(final_df) + 1))
    # Calculate and append z-values
    final_df = calculate_and_append_z_values(final_df)

    return final_df

# Path to the directory containing subject data
eyeLink_dir = "C:/Users/mariu/OneDrive/Desktop/eyetracking/Eyetracking/EyeLink"

# Iterate through each subject directory
for subject_dir in os.listdir(eyeLink_dir):
    subject_path = os.path.join(eyeLink_dir, subject_dir)
    if os.path.isdir(subject_path):
        # Iterate through each session directory for the subject
        for session_dir in os.listdir(subject_path):
            session_path = os.path.join(subject_path, session_dir)
            if os.path.isdir(session_path):
                samples_file = os.path.join(session_path, "signal.asc")
                print(samples_file)
                events_file = os.path.join(session_path, "events.asc")
                session_df = process_session(samples_file, events_file)
                session_df.to_csv(os.path.join(session_path, "responses.csv"), index=False)
```

Appendix 3: Handedness Questionnaire

SFB 1280 Versuchspersonencode: _ _ _ _ _ _ _ _ _ _ _ _

Edinburgh Handedness Inventory

Bitte geben Sie für die folgenden Aktivitäten oder Objekte an, welche Hand Sie hierfür gebrauchen, indem Sie sich für "links+" oder "rechts+" entscheiden. Wenn Ihre Präferenz so stark ist, dass Sie niemals versucht haben, die andere Hand zu gebrauchen, dann wählen Sie "links++" oder "rechts++". Nutzen Sie die Kategorie "neutral" bitte nur, wenn Sie wirklich unentschlossen sind. Einige von den nachfolgenden Aktivitäten erfordern beide Hände. In diesem Fall steht der Teil der Aufgabe in Klammern, für den die Handpräferenz gesucht ist. Bitte versuchen Sie alle Punkte zu beantworten. Lassen Sie einen Punkt bitte nur dann unbeantwortet, wenn Sie überhaupt keine Erfahrung mit dem Objekt oder der Aufgabe haben.

	Links++	Links+	Neutral	Rechts+	Rechts++
Schreiben	☐	☐	☐	☐	☐
Zeichnen	☐	☐	☐	☐	☐
Werfen	☐	☐	☐	☐	☐
Schere	☐	☐	☐	☐	☐
Zahnbürste	☐	☐	☐	☐	☐
Messer (Ohne Gabel)	☐	☐	☐	☐	☐
Löffel	☐	☐	☐	☐	☐
Besen (obere Hand)	☐	☐	☐	☐	☐
Dose öffnen (Deckel)	☐	☐	☐	☐	☐
Streichholz anzünden	☐	☐	☐	☐	☐

Appendix 4: Stimulation/Imaging Criteria Questionnaire

SFB 1280 Versuchspersonencode: _ _ _ _ _ _ _ _ _ _ _ _

extinction learning | SFB 1280

Nehmen Sie regelmäßig Medikamente ein (im Zeitraum der letzten 6 Monate)?

☐ Ja ☐ Nein

Falls ja, welche?

Leiden Sie an einer psychischen Erkrankung (z.B. Angststörung, Depression, Schizophrenie, Abhängigkeit von Alkohol oder Drogen)?

☐ Ja ☐ Nein

Falls ja, welche?

Leiden Sie an einer neurologischen Erkrankung (z.B. Epilepsie, Migräne, Parkinson, Demenz, Multiple Sklerose)?

☐ Ja ☐ Nein

Falls ja, welche?

Wie viele Stunden pro Tag spielen Sie durchschnittlich Videospiele?

Auf welchem Niveau spielen sie Videospiele?

☐Zum Zeitvertreib
☐Als Hobby
☐Fortgeschritten
☐Wettbewerb

Bitte geben Sie Ihre Händigkeit an

☐Rechtshändig
☐Linkshändig
☐Beidhändig

SFB 1280 Versuchspersonencode: _ _ _ _ _ _ _ _ _ _ _ _

Der folgende Abschnitt richtet sich nur an Frauen

Hat Ihre Menopause bereits stattgefunden?

☐ Ja ☐ Nein

Falls ja, in welchem Alter hatten Sie ihre letzte Menstruationsblutung?

Verwenden Sie hormonelle Verhütungsmittel (z.B. Pille, Hormonspirale, Vaginalring, Depotspritze, Hormonimplantat)?

☐ Ja ☐ Nein

Falls ja, welche?

Wann haben Sie dieses hormonelle Verhütungsmittel das letzte Mal eingenommen, sich einsetzen oder geben lassen?

Datum (TT.MM.JJ)

Uhrzeit (HH:MM)

Wann werden Sie dieses hormonelle Verhütungsmittel das nächste Mal einnehmen, sich einsetzen oder geben lassen?

Datum (TT.MM.JJ)

Uhrzeit (HH:MM)

Wann war der erste Tag Ihrer letzten Menstruationsblutung?

Datum (TT.MM.JJ)

Wann wird der erste Tag Ihrer nächsten Menstruationsblutung sein?

Datum (TT.MM.JJ)

Appendix 5: PANAS questionnaire

Deutsche Version der Positive and Negative Affect Schedule: PANAS (GESIS Panel)

Name: **Date:**

Nun möchten wir gerne von Ihnen wissen, wie Sie sich fühlen. Die folgenden Wörter beschreiben unterschiedliche Gefühle und Empfindungen. Lesen Sie jedes Wort und tragen Sie dann in die Skala neben jedem Wort die Intensität ein. Sie haben die Möglichkeit, zwischen fünf Abstufungen zu wählen. Geben Sie bitte an, wie Sie sich **im Moment** fühlen.

	gar nicht	ein bisschen	einigermaßen	erheblich	äußerst
Aktiv	1	2	3	4	5
bekümmert	1	2	3	4	5
interessiert	1	2	3	4	5
freudig erregt	1	2	3	4	5
verärgert	1	2	3	4	5
stark	1	2	3	4	5
schuldig	1	2	3	4	5
erschrocken	1	2	3	4	5
feindselig	1	2	3	4	5
angeregt	1	2	3	4	5
stolz	1	2	3	4	5
gereizt	1	2	3	4	5
begeistert	1	2	3	4	5
beschämt	1	2	3	4	5
wach	1	2	3	4	5
nervös	1	2	3	4	5
entschlossen	1	2	3	4	5
aufmerksam	1	2	3	4	5
durcheinander	1	2	3	4	5
ängstlich	1	2	3	4	5