Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

Methods and Materials:

Participants: Participants STAI-T scores ranged from 45 to 63 (mean [M] = 53, standard deviation [SD] = 5). Written informed consent was obtained from all participants. Individuals with current depressive episode, current or past neurological disease, or family history of bipolar disorder were excluded, as were individuals on medication for anxiety or depression, or with any contraindications to MRI or tDCS. Participants who successfully met full screening requirements were invited to take part in two tDCS/MRI scanning sessions at the John Radcliffe Hospital, Oxford. Participants were compensated for their time at a rate of £10 per hour. For the present study we initially recruited sixteen participants, Data for two participants were partially lost due to server problems. These two participants were replaced to bring the total N recruited to eighteen and the dataset analysed to sixteen (Table S1).

Table S1: Baseline characteristics of participants recruited into the study

	All	**Sham-Active**	**Active-Sham**
Mean age (min-max)	23.3 (20-42)	24.4 (21-41)	23.4(19-27)
Sex	Female: 18	Female: 9	Female: 9
Mean trait anxiety (STAI-T)	53.3 (45-63)	52.8 (45-63)	53.6 (48-61)

Formal sample size calculation was precluded, because no prior study had determined the effect of tDCS on brain activity in a high anxious sample. Hence, we estimated the likely effect size of tDCS, and the likely minimum sample size, informed by two prior related studies. Our previous work[1] showed that prefrontal tDCS reduced behavioral threat vigilance in healthy volunteers, with an effect size of Cohen *d* = 0.8737. Another previous work, using fMRI and the identical task paradigm to that used here, reported higher amygdala and lower prefrontal activation in a high versus low anxious sample[2], with an effect size of Cohen *d* = 0.99. To detect effects of these magnitudes in the current repeated measures design[3], a priori sample size calculation yielded N = 8 as the minimum sample size required to detect a reduction in amygdala fMRI signal (difference between two dependent means: matched pairs, one tailed, alpha =.05, dz = 0.99, power = .8), and N = 10 to detect a reduction in attention to threat behavior (difference between two dependent means: matched pairs, one tailed, alpha = .05, dz = 0.87, power = .8).

Design: A randomisation list was prepared by a colleague (Department of Psychiatry, University of Oxford) separate from the study and kept in a locked cabinet. Based on this randomisation list the experimenter was given a code to enter into the tDCS device which determined whether real or sham stimulation was delivered. Thus, the experimenter was blind to the stimulation order. On the day of the study participants first filled out mood questionnaires before being introduced to the scanner environment and undergoing a structural scan, during which they practiced a training version of the attentional control task. Then participants vacated the scanner and received tDCS in a separate room while they sat at rest. This allowed the participants to practice the task and become comfortable in the scanner environment to minimise time spent entering the scanner after tDCS.

Attentional load paradigm: Visual stimuli were back-projected onto a translucent screen behind the bore of the magnet, visible via an angled mirror placed above the participant’s head. In the present study the face stimuli comprised four different individuals with fearful and neutral expressions taken from the

Pictures of Facial Affect[4] and cropped to remove extraneous background information. The neutral faces were morphed using computer graphics to have a neutral: happy expression mix of 30:70%, because wholly neutral faces have previously been found to be aversive[5]. The experiment was performed using Presentation® software (Version 14.0, Neurobehavioral Systems, Inc., Berkeley, CA).

On each trial, a string of 6 letters was superimposed on a task-irrelevant face distractor presented in the centre of the screen. Participants had to indicate whether the letter string contained an “X” or an “N”. A target was present on every trial.

The task was to decide whether the letter string contained an “X” or an “N”. In half the blocks – the *“high attentional load”* condition – the string comprised a single target letter (N or X) and 5 non-target letters (H, K, M, W, Z) arranged in random order. In the other half of blocks—the ‘‘*low attentional load*" condition—the letter string comprised 6 Xs or 6 Ns, reducing attentional search requirements. This manipulation of attentional load is identical to the one used in Bishop and others[2], Jenkins and others[6] and conforms to Lavie’s[7] description of heightening cognitive effort by: 1) increasing the number of different identity items that need to be perceived, or 2) making perceptual identification more demanding on attention. The rationale for these load conditions is that when the task is undemanding, greater distractibility puts higher demands on attentional control.

A mixed block/event-related design was used — the level of attentional load (high or low) was varied across blocks, while the expression of the task-irrelevant face distractors (fearful or neutral) was varied across trials. These 2 factors (attentional load × distractor emotion) resulted in 4 conditions: high load/fearful distractors; high load/neutral distractors; low load/fearful distractors; low load/neutral distractors. The key hypothesis-driven condition of interest was: low load/fearful distractors. Previous work has shown that amygdala response to threat is observed only in the low load condition in this task[2]. Therefore, by examining the effect of tDCS on brain regions selectively activated by this key hypothesis-driven contrast (fearful versus neutral face distractors under conditions of low load) it was possible to test the hypothesis that tDCS reduces vigilance to threat in trait anxiety by altering fronto-limbic activity; specifically, by reducing amygdala response to fearful distractors.

There were 3 imaging acquisition runs, each comprising 12 blocks of 4 trials. There was a 2 s interval between blocks. Within blocks, the inter-stimulus interval was randomly jittered using an exponential function with a mean of 4.5 s and a minimum of 3 s.

Transcranial Direct Current Stimulation (tDCS): Stimulation was delivered using a battery powered device (DC Stimulator Plus, Neuroconn, Germany[8]). The rubber electrodes (5cm x 5cm) were placed in saline soaked sponges and affixed to the scalp with a rubber band. We used a bipolar-balanced electrode montage which positioned the anode (positive) electrode on the left dorsolateral prefrontal cortex and the cathode (negative) electrode on the right dorsolateral prefrontal cortex (F3 and F4 respectively, 10/20 system of electrode placement). In the real/active tDCS condition, stimulation (20 minutes at 2mA) was applied while the participant sat at rest. In the sham condition participants received 30 s of direct current, followed by impedance control with a small current pulse every 550 ms (110 µA over 15 ms) instead of the stimulation current, resulting in an instantaneous current of not more than 2 µA or 40 sec of active stimulation. This method of sham stimulation produced the physical sensations typical of real tDCS and displayed realistic impedance values on the device display. The experimenter was thus blind to the stimulation condition, facilitated by a ‘study’ mode for blinding on the device. Modelling of the electric field evoked by tDCS was carried out using SimNIBS software[9] and a standard anatomical brain model. As previously reported[10], the modelling suggests that that the strongest field is evoked in the medial prefrontal cortex (see Fig. S1).

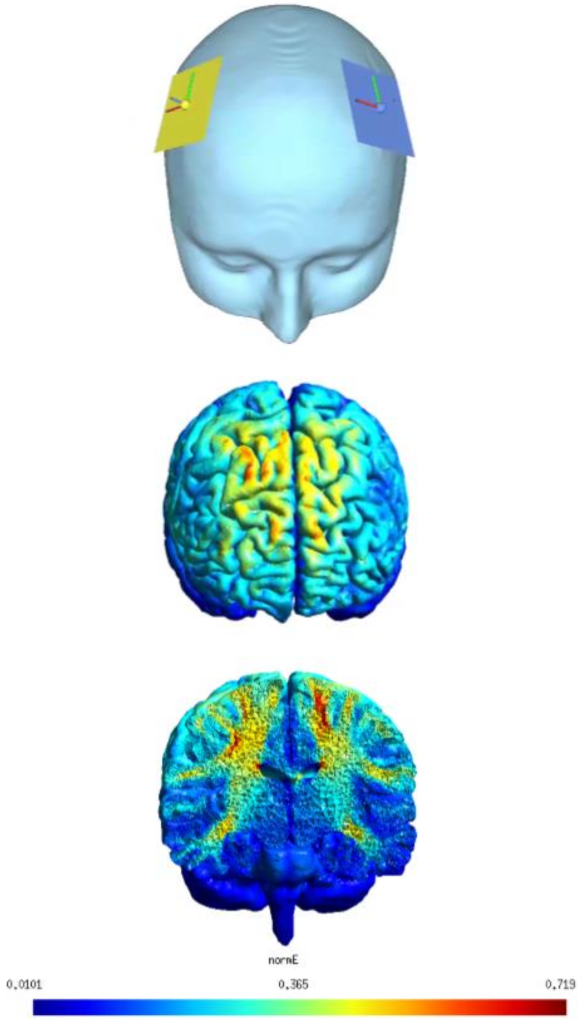

Figure S1. TDCS montage and corresponding simulated electric field distribution. Bipolar balanced dorsolateral prefrontal cortex configuration: Sponge electrodes over the F3 (anode, blue sponge) and F4 (cathode, yellow sponge) EEG sites. The electric field was simulated with a current amplitude of 2mA. Electric field simulation was performed using SimNIBS 2.0.1[9]

Image Acquisition: Blood oxygenation level dependent (BOLD) contrast functional images were acquired with echo-planar T2*-weighted (EPI) imaging using a Siemens 3T Magnetom TrioTim syngo MRB17 with a head coil gradient set. Each image was made up of 45 interleaved 3mm thick slices, inter-slice gap, 1mm, field of view 25x25cm; matrix size, 64 x 64; flip angle 87° echo time (TE), 30ms; voxel bandwidth, 2368 Hz/Px; acquisition time (TA), 2.3 s; repetition time (TR), 2710ms. Slice acquisition was interleaved and covered the whole brain with an additional z shim to reduce distortion in the orbitofrontal cortex. Data were acquired in 3 scanning runs of ~5 min each. The first 5 volumes of each run were discarded to allow for T1 equilibration effects.

Results:

Behavioral results: Accuracy and reaction time data were analyzed using repeated measures ANOVA, with within-subjects factors of load (low, high), emotion (fear, neutral) and tDCS (real, sham). As expected, participants were faster to identify the target letter and made fewer errors in low perceptual load condition (M = 755 ms, SD = 160 ms) compared to the high perceptual load condition (M = 1064 ms, SD = 240 ms) $F(1, 15) = 77.060$, $p < .001$; $F(1, 15) = 58.885$, $p < .001$ respectively. There were no other significant main effects or interactions with tDCS and emotion (all $p > .05$).

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