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Effects of Multisession Transcranial Direct Current Stimulation on Stress Regulation and Emotional Working Memory: A Randomized Controlled Trial in Healthy Military Personnel

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ABSTRACT

Objectives: Top-down stress regulation, important for military operational performance and mental health, involves emotional working memory and the dorsolateral prefrontal cortex (DLPFC). Multisession transcranial direct current stimulation (tDCS) applied over the DLPFC during working memory training has been shown to improve working memory performance. This study tested the hypothesis that combined tDCS with working memory training also improves top-down stress regulation. However, tDCS response differs between individuals. Resting-state electrophysiological brain activity was post hoc explored as a possible predictor of tDCS response. The predictive value of the ratio between slow-wave theta oscillations and fast-wave beta oscillations (theta/beta ratio) was examined, together with the previously identified tDCS response predictors age, education, and baseline working memory performance.

Materials and Methods: Healthy military service members ($n = 79$) underwent three sessions of real or sham tDCS over the right DLPFC (anode: F4, cathode: behind C2) at 2 mA for 20 minutes during emotional working memory training (*N*-back task). At baseline and within a week after the tDCS training sessions, stress regulation was assessed by fear-potentiated startle responses and subjective fear in a threat-of-shock paradigm with instructed emotional downregulation. Results were analyzed in generalized linear mixed-effects models.

Results: Threat-of-shock responses and emotional working memory performance showed no significant group-level effects of the real vs sham tDCS training intervention ($p > 0.07$). In contrast, when considering baseline theta/beta ratios or the other tDCS response predictors, exploratory results showed a trait-dependent beneficial effect of tDCS on emotional working memory training performance during the first session ($p < 0.01$).

Conclusions: No evidence was found for effectivity of the tDCS training intervention to improve stress regulation in healthy military personnel. The emotional working memory training results emphasize the importance of studying the effects of tDCS in relation to individual differences.

Clinical Trial Registration: This study was preregistered on September 16, 2019, at the Netherlands Trial Register (www.trialregister.nl) with ID: NL8028.

Keywords: Dorsolateral prefrontal cortex (DLPFC), emotional working memory, military, stress regulation, transcranial direct current stimulation (tDCS)

Conflict of Interest: The authors reported no conflict of interest.

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INTRODUCTION

Military personnel risk exposure to a variety of stressors, including physical danger and witnessing severe human suffering.[1,2] Prolonged and high levels of stress can impair operational performance[3] and contribute to the development of mental health problems such as anxiety and posttraumatic stress disorder (PTSD).[4,5] Adequate top-down regulation of stress-related reactions and emotions contributes to psychological resilience against these adverse effects of stress.[6–10] Cognitive strategies for top-down stress regulation involve, for instance, reevaluating the value or meaning of a stressful situation to reduce its emotional impact.[11]

However, the effectiveness of top-down stress regulation may be compromised when stress levels are too high.[12] Both acute and chronic stress levels interfere with the functioning of the prefrontal cortex (PFC), especially in the dorsolateral parts (DLPFC) that play a substantial role in stress regulation[13,14] and cognitive functions such as working memory.[15] Instead, better stress regulation has been associated with better working memory performance, specifically in the emotional domain.[16–19] Emotional working memory plays a role by actively keeping threat-related information available and allowing for the selection and updating of this information to deploy effective stress regulation strategies.[20] Accordingly, the right DLPFC has been identified as a target region for noninvasive brain stimulation to improve symptoms of stress-related disorders, including PTSD.[21] In addition, in healthy volunteers, several studies demonstrated that stress regulation improved after the application of transcranial direct current stimulation (tDCS) over the right DLPFC or the neighboring ventrolateral PFC.[22–27]

Transcranial direct current stimulation modulates subthreshold cortical excitability and plasticity by polarizing nerve tissue using low-intensity electrical currents (1–2.5 mA) administered over the scalp. Cortical excitability is generally assumed to increase by anodal tDCS and decrease by cathodal tDCS, although the exact mechanisms underlying tDCS effects are still unclear (further reading[28,29]). Single-session tDCS, however, yields transient neurophysiological effects that typically fade out within a few hours,[30] and single-session tDCS over the DLPFC does not always effectively modulate stress regulation.[31–33]

More consistent and sustained effects on higher-order cognitive functions and on symptoms of depression and PTSD are suggested to follow from multiple sessions of tDCS over the DLPFC or the ventrolateral PFC, particularly when applied during a neurocognitive training or therapy that activates the tDCS-targeted brain region.[34–41] One way to activate the right DLPFC is by a working memory task that is shown to activate several PFC regions involved in stress regulation.[18,42] The idea that working memory performance can be improved by anodal tDCS over the DLPFC is supported by converging evidence from a recent meta-analysis.[43] In addition, several studies suggest that multisession tDCS during working memory training may lead to long-lasting performance gains in working memory and other cognitive functions depending on working memory,[44–47] indicating a potential benefit for stress regulation capacity.

However, not all studies of combined tDCS with working memory training find these beneficial effects.[48,49] These negative findings could be related to the considerable variability between individuals in tDCS effects on cognitive functions such as working memory.[37,50] This variability has been associated with factors such as age,[51–53] baseline cognitive performance,[54–58] and education.[59]

Variability in tDCS response may be even better explained by neural processes that interact with the effects of tDCS. Such processes are, for instance, indicated by markers from electroencephalography (EEG).[60–62] One EEG marker that could be related to tDCS effects on emotional working memory is the power ratio between slow-wave theta band activity (4–7 Hz) and fast-wave beta band activity (13–30 Hz) in resting-state EEG, that is, the theta/beta ratio.[63–65] The theta/beta ratio is thought to reflect the balance between subcortical-based emotional and motivational drives and cortical-based cognitive control, based on associations between the theta/beta ratio and cognitive control over emotional input,[66–68] reward-motivated learning on cognitive tasks,[69–71] and working memory training gains.[72] In addition, the theta/beta ratio has been associated with effects on cognitive performance of a tDCS-related technique, transcranial random noise stimulation.[73,74]

Following these lines of evidence, the primary objective of this study was to test whether multisession anodal tDCS of the right DLPFC during emotional working memory (WM) training could improve top-down stress regulation in healthy military personnel. The secondary objective was to explore the predictive value of the theta/beta ratio on interindividual variability in tDCS effects, in addition to the previously identified tDCS response predictors age, baseline performance, and education.

MATERIALS AND METHODS

Participants

Active-duty military personnel (aged 18–60 years, uncorrected normal hearing) were recruited between January 2020 and April 2021. This study was part of a double-blind randomized controlled trial that was preregistered at The Netherlands Trial Register (www.trialregister.nl) with ID: NL8028. The a priori–computed required sample size to detect tDCS effects on stress regulation was 62. Supplementary Data section 1.1. describes how the required sample size was computed. Exclusion criteria were alcohol or drug dependence, psychoactive medication or drug use within the past two weeks, (history of) a psychiatric or neurologic disorder (except for attention-deficit/hyperactivity disorder) or serious head trauma, large or ferromagnetic metal parts in the head, implanted cardiac pacemaker or neurostimulator, pregnancy, neurostimulation in the past month, or skin damage or diseases at intended electrode sites. All participants provided written informed consent and received €65 for participation. The authors assert that all procedures contributing to this work complied with the ethical standards of the relevant national and institutional committees on human experimentation and with the Declaration of Helsinki of 1975, as revised in 2008. The medical ethical committee of the University Medical Center Utrecht approved the study. Table 1 shows demographics and baseline psychological characteristics for each tDCS group.

Noninvasive Brain Stimulation

Transcranial direct current stimulation was administered at an intensity of 2.0 mA (impedance < 10 kΩ) for a duration of 20 minutes with a DC-stimulator Plus (NeuroConn GmbH, Ilmenau, Germany). Anodal tDCS was concentrated on an area in the right DLPFC that has been shown to be activated by both WM performance and top-down emotion regulation.[13,18,75] The electrode montage to target this area was selected based on simulations of the electric field distribution in SimNIBS 3.2.3[76] (Figure 1b). A 3×3 cm saline-soaked sponge-covered anode was placed over EEG

Table 1. Participant Characteristics (Frequency or Mean ± SD).

Variable name	Real tDCS	Sham tDCS
Sex		
Male	35	33
Female	2	2
Age (years)	34.0 ± 10.7	36.1 ± 11.1
Educational level*		
High school Diploma	2	3
Vocational degree	20	21
Associate's degree	2	0
Bachelor's degree	6	4
Master's degree	7	7
Number of deployments		
Never deployed	16	11
1 deployment	6	8
2–3 deployments	8	9
≥4 deployments	6	7
Rank		
Officer	8	6
Student-officer	3	3
Senior NCO	17	20
Junior NCO	9	6
Handedness†		
Right-handed	33	30
Left-handed	4	4
No preference	0	1
ASI-3 (rating 0–4)		
Physical	0.3 ± 0.4	0.3 ± 0.4
Cognitive	0.2 ± 0.3	0.2 ± 0.3
Social	0.8 ± 0.5	0.9 ± 0.7
ACS (rating 1–4)		
Focusing	2.7 ± 0.4	2.6 ± 0.5
Shifting	2.8 ± 0.3	2.8 ± 0.4
ERQ (rating 1–7)		
Reappraisal	4.7 ± 0.9	4.8 ± 0.8
Suppression	3.6 ± 1.0	3.7 ± 1.0
PANAS (rating 1–5)		
PA	3.7 ± 0.5	3.7 ± 0.4
NA	1.5 ± 0.4	1.5 ± 0.5
Shock intensity		
Current (mA)	8.2 ± 3.5, range: 3–30	8.3 ± 4.6, range: 2–39
Duration (ms)	754 ± 452, range: 200–2000	644 ± 424, range: 200–2000
Fear of shock (rating 0–10)	2.7 ± 1.6	3.2 ± 1.8
Pain of shock (rating 0–10)	1.7 ± 0.9	2.8 ± 1.4
Start performance *N*-back task (block 1, session 1)		
d' ($z_{hits} - z_{false\ alarms}$)	1.35 ± 1.0	1.29 ± 1.0

ACS, attentional control scale; ASI-3, 18-item Anxiety Sensitivity Inventory; ERQ, emotion regulation questionnaire; NA, negative affect; NCO, noncommissioned officer; PA, positive affect; PANAS, Positive and Negative Affect Schedule.

*Educational levels were assessed based on the Dutch educational system and for international interpretability converted to the best corresponding American degree.

†Participants were asked to identify themselves as left-handed, right-handed, or no preference.

position F4, and a 5 × 7 cm cathode was placed dorsal of C2 (Fig. 1b). Sham tDCS involved a 16-second fade-in fade-out stimulation at the start and end of the stimulation period, interleaved by 15-millisecond pulses of 0.11 mA. Changes in emotional state (STAI-6[79]) and possible tDCS adverse effects[80] (scored from 1, "absent," to 4, "severe") were assessed in each session.

Emotional Working Memory Training

During tDCS, participants performed an emotional WM task based on the visuospatial/auditory *N*-back task from Schweizer et al.[18] In each trial, participants indicated whether the location of an angry face in a 4 × 4 grid on a computer screen or a one-syllable negative word (eg, "death," "fear," "hate") matched with *N* trials before (Fig. 1b). Based on response accuracy, *N* increased or decreased by one in the consecutive block. The task contained ten blocks of 20 + *N* trials per block, with six target trials. To further increase emotional arousal during the task, unpredictable aversive screams (~80 dB, ~1 second) and negative fictitious performance feedback were presented during six of the blocks,[81] as illustrated in Figure 1b and further described in Supplementary Data section 1.2.

At postassessment, *N*-back task performance was tested on four prespecified WM load levels (*N* = 1–4).

Primary Outcome Measure: Threat-of-Shock Paradigm

Stress-related responses were assessed by a threat-of-shock test with three conditions: "no-shock" (N), "predicable-shock" (P) and "unpredictable-shock" (U), abbreviated as the NPU-threat test.[82] The test contained two seven-minute sequences. Per sequence, three no-shock blocks were alternated by two predicable- and two unpredictable-shock blocks. In a workup procedure, electrical shocks were tuned to an intensity rated by the participant as 4 on a 5-point Likert scale (1: "I feel no shock," 5: "the shock feels very uncomfortable but not painful"). During each 60-second block, three cues were presented (4 seconds), interleaved by variable intertrial-intervals (ITI) (3–30 seconds). One or two shocks were delivered per threat block at a computer-randomized moment during the last second of cue presentation (predictable shock) or during the ITI (unpredictable shock). In total, six shocks were delivered per threat condition. Supplementary Data section 1.3.1. describes further test details.

Physiological threat responses were assessed by the eyeblink fear-potentiated startle reflex.[83] Eyeblink startles were recorded by electromyography (EMG) of two active 4-mm flat surface Ag/AgCl electrodes (BioSemi BV, Amsterdam, The Netherlands) filled with conductive gel, placed ~1 cm apart on the left orbicularis oculi muscle.[84] A startle probe of 50 milliseconds of white noise at ~100 dB was delivered through 3A insert earphones (E-A-RTONE™, 3M™) at a computer-randomized moment during the first three seconds of each cue, and during each ITI. EMG data were preprocessed (Supplementary Data section 1.3.3.), and startle amplitudes were quantified as the maximum amplitude between 20 and 120 milliseconds after probe onset (baseline-corrected and within-subject standardized).[84] Subjective fear for each condition and context (cue, ITI) was self-reported after every sequence on a visual analogue scale from 0: "I did not feel nervous or anxious at all" to 100: "I felt very nervous or anxious."

In line with previous research of top-down stress regulation,[8,12] psychoeducation was provided before the NPU-threat test. Stress regulation strategies were explained by instructing participants to view a situation with a "detached, objective, impartial and scientific mindset" or think of more positive aspects of the situation,[11] as described in more detail in Supplementary Data section 1.3.2. Participants were instructed to use these strategies to downregulate threat-related emotional responses during the NPU-threat test.

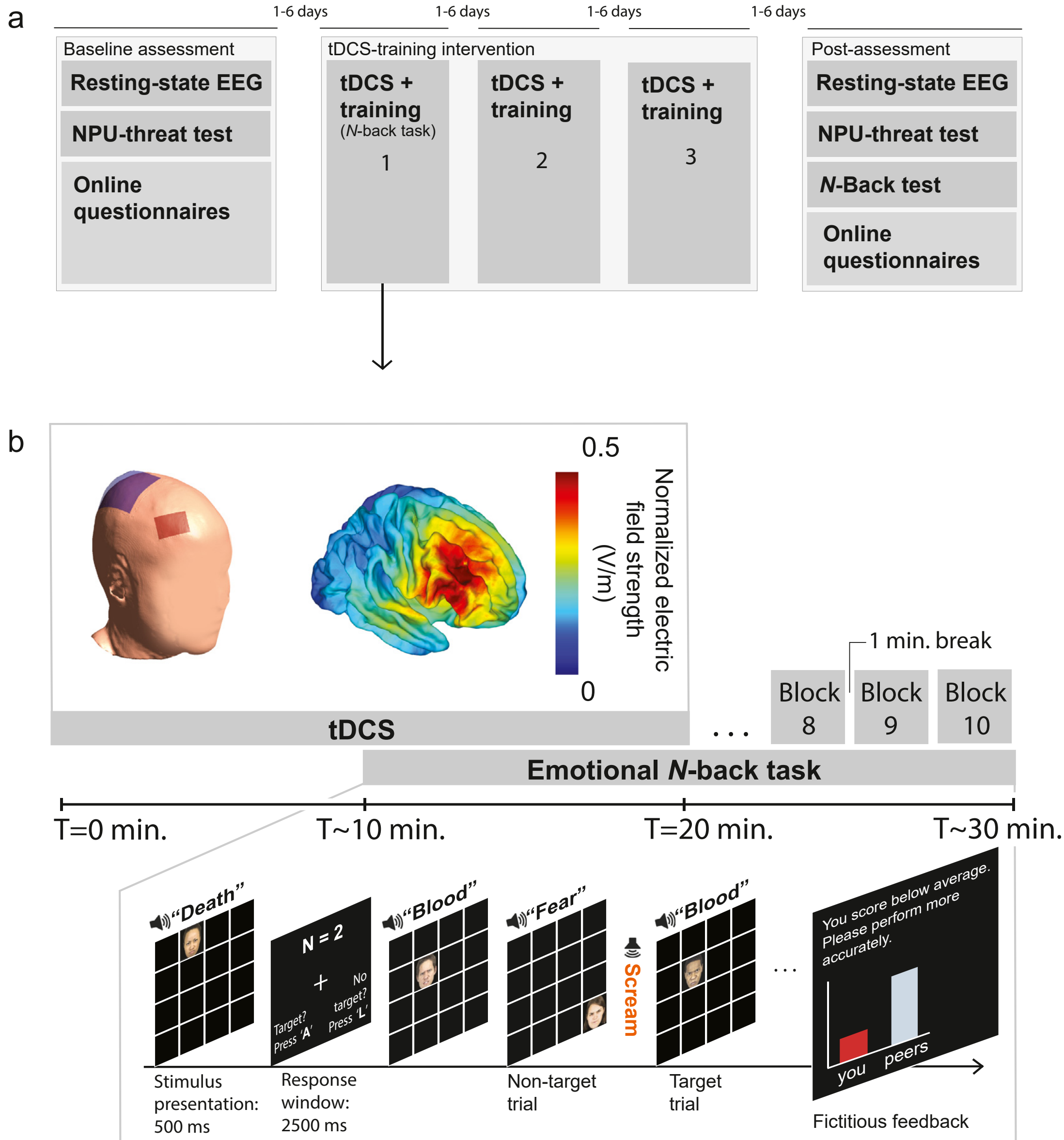

Figure 1. a. Overview of the study design. b. Overview of a tDCS training session. The tDCS montage and example of electrical field distribution are simulated in SimNIBS 3.2.3[76] with twenty brains obtained from a publicly available magnetic resonance imaging data set of neurologically healthy individuals.[77] The figure displays the average electrical field distribution across these 20 simulations. The example of an *N*-back trial sequence represents a WM load of *N* = 2. Angry face stimuli are derived from the Chicago face data base.[78] [Color figure can be viewed at www.neuromodulationjournal.org]

Questionnaires

Top-down stress regulation tendencies, anxiety sensitivity, emotional symptoms, and cognitive control of attention were assessed by self-report on the Dutch translation of the Emotion Regulation Questionnaire,[85,86] the 18-item Anxiety Sensitivity Inventory (ASI-3),[87,88] the Positive and Negative Affect Schedule,[89] and the Attentional Control Scale (item 12 about attention during "lectures" was adapted to "lessons").[90]

Exploratory Measures

Educational level and age were recorded during the baseline visit. Baseline WM performance was defined as a Start Performance score based on the sensitivity ($d' = z_{hits} - z_{false\ alarms}$) at the start of the training (block 1, session 1, where all participants performed a 1-back task). Start performance scores did not significantly differ between groups (mean ± SD: real tDCS 1.35 ± 1.0, sham tDCS 1.29 ± 1.0, $t_{70} = 0.26$, $p = 0.799$).

The theta/beta ratio was extracted from 4-minute resting-state EEG (alternating 1-minute eyes open, 1-minute eyes closed), recorded at the start of the baseline and postassessment visits (experimenters left the room). EEG data were recorded and amplified with a BioSemi ActiveTwo system (BioSemi BV, Amsterdam, The Netherlands) at 2048 Hz, relative to a Common Mode Sense active electrode in combination with a Driven Right Leg passive electrode, from channels Fp1, Fp2, AF3, AF4, F7, F8, F3, F4, Fz, Cz, FC1, FC2, FC5, FC6, C3, C4, CP1, CP2, CP5, CP6, P7, P8, P3, P4, Pz, PO3, PO4, O1, O2, and Oz. Offline preprocessing was done with custom MATLAB scripts, EEGLAB v2021.0[91] and ERPLAB v8.10.[92] Continuous data were segmented in 1-second epochs. Eye blinks were identified and removed based on the EEGLAB ICA function. Epochs containing artifacts caused by movement or facial muscle contractions were automatically marked (> 30 mV difference between adjacent samples, > 100-mV difference per 200-millisecond signal, or absolute amplitude larger than ± 75 mV) and deleted after visual inspection. A fast Fourier transform was applied per epoch using Welch's method (Hanning taper, 50% overlap, 0.25-Hz resolution). The power spectral density (mV^2/Hz) was averaged over epochs and log-transformed. Following previous research, the frontocentral theta/beta ratio was calculated as the average theta power (4–7 Hz) divided by the average beta power (13–30 Hz) from channels Fz and Cz (data collapsed across eyes-open and eyes-closed conditions).[66,69] Theta/beta ratios tended to be higher in the real tDCS group than in the sham tDCS group (mean ± SD: real tDCS 7.7 ± 3.1, sham tDCS 6.5 ± 2.4, $t_{70} = 1.82$, $p = 0.074$).

Procedure

Participants were recruited, and study visits were carried out at several military bases in The Netherlands. After provision of study information, screening, and obtaining of informed consent, eligible individuals were randomly allocated to real or sham tDCS (1:1) by selecting the next-available stimulator-activating code from a list. This list contained 20 codes for real tDCS and 20 codes for sham tDCS, which were randomized with the MATLAB function "randsample." Experimenters and participants were blind for code-to-condition correspondence. Baseline and postassessment visits took place one to six days before and after the tDCS training intervention and included a resting-state EEG recording and the NPU-threat test. Self-report questionnaires were completed online. Three tDCS training sessions took place one to six days apart during working hours (between 6 AM and 9 PM, depending on working shift). Because it is not yet clear whether online tDCS or offline tDCS is most effective to modulate cognitive performance,[93–95] tDCS (20 minutes) was turned on approximately 10 minutes before the emotional *N*-back training was started such that tDCS continued during the first half of *N*-back task performance and was turned off during the second half of the task, shown in Figure 1b. The emotional *N*-back test version was carried out during the postassessment visit. Participants were debriefed about their tDCS condition (real or sham) and the fictitious nature of the *N*-back performance feedback after data collection was completed.

Data Reduction and Statistical Analysis

Data were analyzed in generalized linear mixed-effects models (GLMMs) based on a gamma distribution in R[96] using the "lme4" package.[97] Within-subject outliers in dependent variables (> 3 standard deviations from the mean) were excluded. Effects are reported as significant when $p < 0.05$ (two-tailed). Significant interaction effects were followed up by post hoc comparisons of the estimated marginal means using the "emmeans" package[98] and were reported with Cohen's *d* effect sizes

Emotional Working Memory

The effects of tDCS were examined for both *N*-back training performance and *N*-back postassessment performance. *N*-back training performance was operationalized as the achieved WM load level (*N*) per block and analyzed by fixed effects for Group (real vs sham tDCS), Session (sessions 1, 2, and 3), Block (1–10), and the quadratic term $Block^2$ to model the typical nonlinear learning curve during training sessions. Interindividual variability in performance levels and in learning rates was modeled by a random intercept for Participant and a random slope for Block. *N*-back postassessment performance was operationalized as the correct-response median reaction time (RT) and the sensitivity ($d' = z_{hits} - z_{false\ alarms}$) reflecting the ability to distinguish target trials from nontarget trials,[99] analyzed by fixed effects for Group, WM load ($N = 1$–4), and the maximum WM load during training (Train Max), together with a random intercept for Participant.

NPU-Threat Test

Startle amplitudes and fear ratings were significantly higher in the threat than in the safe conditions, as shown in Figure 2a and Supplementary Data section 2.4.1. To test the effects of tDCS, threat cue responses were analyzed by fixed effects for Group, Threat condition (predictable or unpredictable shock), Time (baseline, postassessment), Sequence Number (1 or 2), and Probe Number (1–6). Variability in threat responsivity and startle habituation was modeled by a random intercept for Participant (fear ratings) and a random slope for Probe Number (startle amplitudes).

Exploratory Analyses

To explore the predictive value of the theta/beta ratio on tDCS effects during the tDCS training sessions, the theta/beta ratio was entered to the GLMM analyzing *N*-back training performance as described above. Interactions between the theta/beta ratio and effects of tDCS group over time (over blocks or sessions) would indicate a predictive value of the theta/beta ratio. In addition, the predictive values of age, education, and start performance were evaluated following the same procedure.

RESULTS

Participants tolerated tDCS well. We noticed a small skin lesion on the anode location in one participant, likely resulting from an insufficiently soaked sponge pad during administration of tDCS in session 3. The lesion healed within six days. Some participants reported mild burning, itching, and tingling sensations, rated on average between 1 ("absent") and 2 ("mild"). No significant group differences appeared in these or other tDCS adverse effects or emotional state fluctuations during tDCS sessions, as shown in Supplementary Data section 2.1.

Three of the 79 included volunteers failed to comply with the *N*-back task instructions, and four dropped out prematurely because of restrictions related to coronavirus disease-2019 ($n = 2$), lack of time ($n = 1$), or no reason provided ($n = 1$). Ten participants showed insufficient (< 30%) valid startle responses ($n = 2$) or encountered technical issues during NPU-threat test recordings ($n = 8$), resulting in a sample of $n = 62$ for analysis of the primary outcome measure. For the other outcome measures, data from 72 participants were available for statistical analyses (real tDCS: $n = 37$, sham tDCS: $n = 35$). Supplementary Data Figure S1 shows the full CONSORT (Consolidated Standards of Reporting Trials) flow diagram.

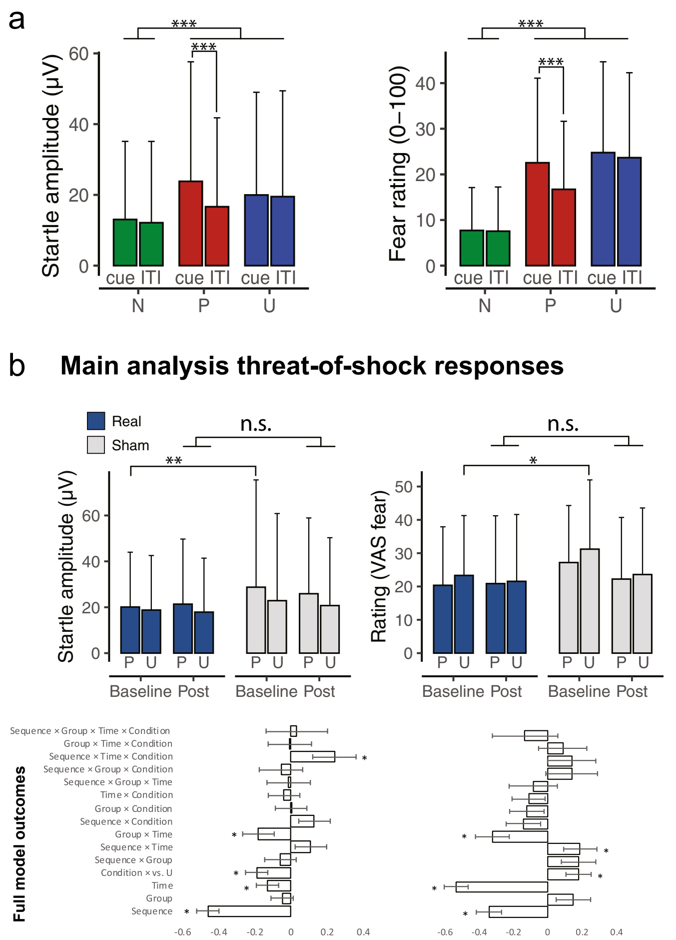

Figure 2. Startle response amplitudes and fear ratings per condition of the NPU-threat test (a) and per tDCS group (b). Error bars represent the standard deviation. ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. Full model outcomes represent the estimated effects (*b*) with associated standard errors. Bars with a star (*) represent statistically significant effects. n.s., not significant. [Color figure can be viewed at www.neuromodulationjournal.org]

Emotional Working Memory Performance

TDCS Training Sessions

Results showed significant interaction effects of Group × Session and Group × Block^2 (p's < 0.016, Supplementary Data Table S2.2.1 and Fig. 3a). In separate GLMMs per session, the Group × Block^2 interaction showed a trend-like effect in tDCS training sessions 1 and 3 (p's < 0.059, Supplementary Data Table S2.2.2). Figure 3a shows that *N*-back training performance tended to be higher in the real tDCS group than in the sham tDCS group in the first tDCS training session ($p = 0.081$). This trend was not observed in the subsequent sessions (p's > 0.40, Supplementary Data Table S2.2.3). Results did not significantly differ when data were analyzed separately for performance during online tDCS (blocks 1–5) or offline tDCS (blocks 6–10), as shown in Supplementary Data section 2.2.1. Together, these results show no significant group difference but suggest that the real tDCS group tended to improve *N*-back performance faster compared with the sham tDCS group during the first tDCS session.

Postassessment

No significant main or interaction effects of Group were found for RT and d' (p's > 0.29), as shown in Figure 3b and Supplementary Data Table S2.3.1. Excluding data from participants who achieved a relatively low maximum WM load level (Train max < 4, real tDCS: $n = 9$, sham tDCS: $n = 3$) did not significantly change the results.

NPU-Threat Test

Significant Group × Time interactions were found for startle amplitudes and fear ratings (p's < 0.038, Fig. 2b and Supplementary Data Table S2.4.3). Follow-up comparisons revealed that the real tDCS group showed lower baseline startle amplitudes (p's < 0.035) and lower baseline fear ratings ($p = 0.050$) in response to the Predictable shock cues compared with the sham tDCS group, as shown in Figure 2b and Supplementary Data Table S2.4.4. Postassessment results showed no significant effects of real vs sham tDCS on startle amplitudes (p's > 0.141) or fear ratings (p's > 0.075).

Exploratory Analyses: Predictors of tDCS Effects

Exploratory results indicated that the baseline theta/beta ratio influenced the effect of real tDCS on *N*-back training performance. In the full model, significant three-way interactions were observed of Theta/beta ratio with Group and Session (Group × Session × Theta/beta ratio: $b(SE) = -0.11(0.04)$, $p = 0.013$) and with Group and Block (Group × Block^2 × Theta/beta ratio: $b(SE) = -0.11(0.04)$, $p = 0.013$, Supplementary Data Table S2.5.1). To interpret these interactions, *N*-back training performance was plotted per session, separately for median-split subsamples based on baseline theta/beta ratio (Figure 3c). Follow-up group comparisons indicated that the real tDCS group only showed significantly improved performance relative to the sham tDCS group during session 1 (not during later sessions, Supplementary Data Table S2.5.3) in participants with a higher baseline theta/beta ratio. Results showed no evidence for changes in theta/beta ratios from baseline to postassessment; no significant effect on theta/beta ratios was observed for Time, Group, or their interaction (Time: $b(SE) = 0.52(0.36)$, $p = 0.149$; Group: $b(SE) = 0.06(0.06)$, $p = 0.284$; Time × Group: $b(SE) = -0.13(0.08)$, $p = 0.111$).

In addition, the results of the second part of these exploratory analyses followed previous findings by showing an influence of age, education, and start performance on the effect of real tDCS on *N*-back training performance. For all three predictors, results showed significant four-way interactions with Group, Session, and Block (p's < 0.010, Supplementary Data Table S2.5.2). Visual inspection of *N*-back training performance per median-split subsamples and follow-up group comparisons indicated that improved performance in the real vs sham tDCS group during session 1 was shown by participants with lower educational level, younger age, and higher start performance (Supplementary Data Table S2.5.3). This predictor-dependent group difference was most pronounced for age (Supplementary Data Table S2.5.3), as illustrated in Figure 3c.

DISCUSSION

This study examined changes in top-down stress regulation in healthy military personnel after three sessions of anodal tDCS applied over the right DLPFC at 2 mA combined with emotional WM training. Contrary to our hypothesis, results indicated no significant effect of real vs sham tDCS combined with WM training on stress regulation; stress-related responses to a threat-of-shock paradigm with instructed emotional downregulation did not

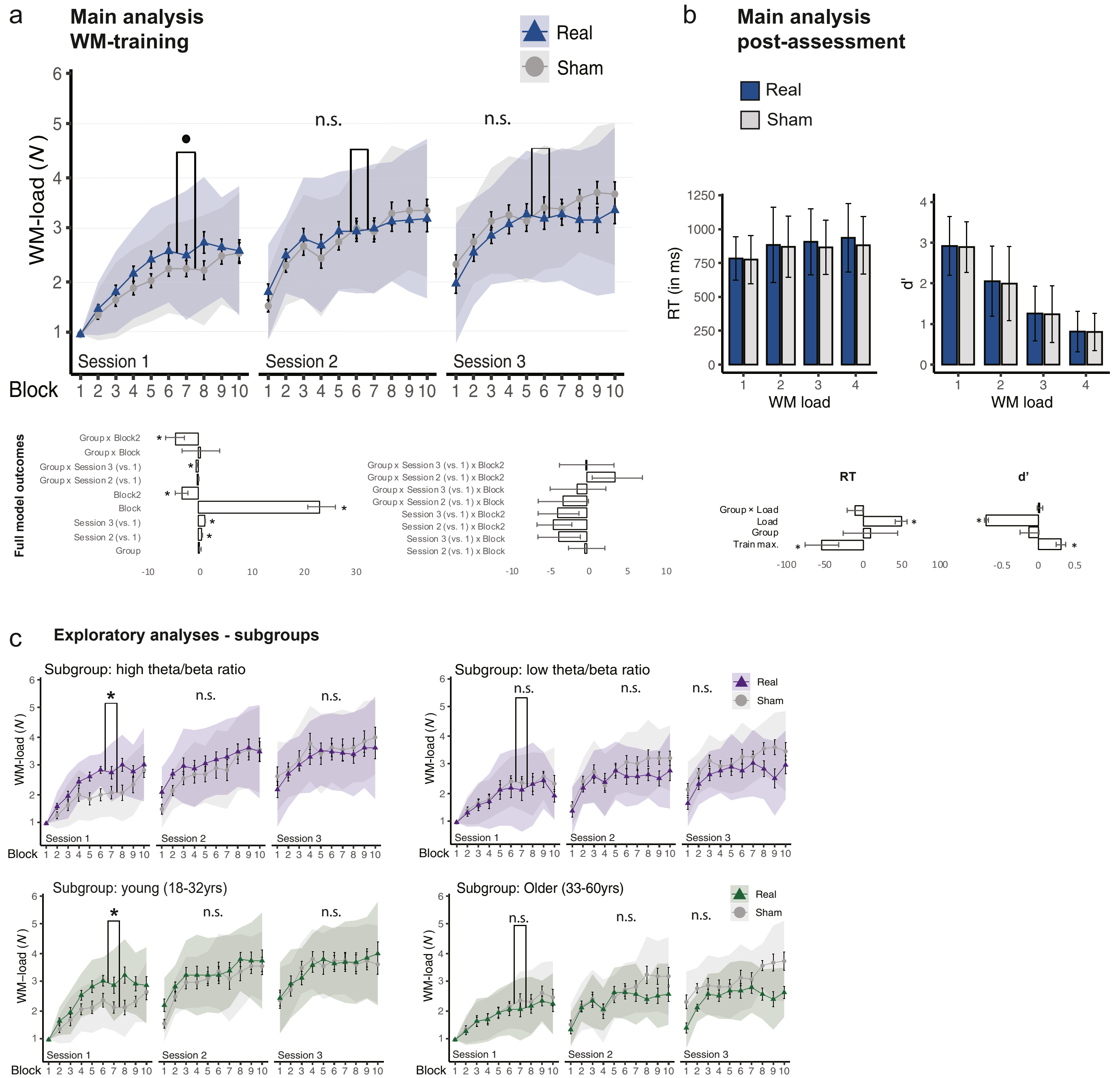

Figure 3. a. *N*-back performance level during training per session. Error bars represent the standard error around the mean. Ribbons represent the standard deviation. b. RT and d' per WM load in the postassessment *N*-back test. Error bars represent the standard deviation. c. WM load level during emotional *N*-back training per session, separated for median-split subsamples based on the theta/beta ratio (above) and age (below). $^{*}p < 0.005$; $\cdot p < 0.10$. Full model outcomes represent the estimated effects (*b*) with associated standard errors. Bars with a star (*) represent statistically significant effects. n.s., not significant. [Color figure can be viewed at www.neuromodulationjournal.org]

differ between groups. Moreover, at group level, results yielded no significant effect of real vs sham tDCS on emotional WM performance during the training or at postassessment. Interestingly, however, post hoc exploratory analyses of potential predictors of the tDCS response, including the theta/beta ratio, suggested a trait-dependent effect of tDCS on performance during the first tDCS WM training session. These findings suggest that tDCS as applied here may have only a short-lasting and trait-dependent effect during the early stages of the tDCS training intervention.

tDCS Effects on Stress Regulation

No significant real vs sham tDCS effects were observed at postassessment in the intensity or habituation of threat-related responses during the NPU-threat test. Although the NPU-threat manipulation was successful, our sample showed on average relatively low startle amplitudes (< 30 mV) and fear ratings (< 30 on a 0–100 scale) in response to the threat conditions, compared with other healthy participant samples (average startle amplitudes of almost 50 mV, fear ratings between 4 and 5 on a 0–10 scale).[100,101]

Although the stress regulation instructions may have lowered the intensity of threat responses in this study,[8,9] the low threat responses also could be a result of overall lower anxiety sensitivity in our sample (ASI-3 total scores mean ± SD: 8.1 ± 6.0) than in other nonmilitary Dutch healthy participant samples (mean ± SD: 10.7 ± 8.1).[88] Hence, our participants may have required little improvement in top-down stress regulation to attenuate the already low threat responses. Considering that stress resilience is part of military training and selection, threat manipulations that elicit stronger stress responses may be necessary for studies in healthy military populations. Moreover, it should be noted that our results may not generalize to individuals with higher threat sensitivity or stress regulation problems.

tDCS Effects on Emotional Working Memory Training

When considering predictive factors of tDCS response, tDCS showed beneficial effects on emotional WM performance, but this effect was limited to the first training session. This short-lived tDCS effect contrasts with results from four previous studies showing significant tDCS-induced performance gains that accumulated during WM training and that were sustained in the days or even months after the tDCS training intervention.[44–46,102] These studies administered anodal tDCS over the right[44,45,102] or left[44–46] DLPFC over multiple (four to seven) sessions in healthy volunteers, as in this study. Unlike this study, the effects of tDCS were tested in students, and different reference (cathode) locations were used (over the parietal cortex, contralateral to the anode, or surrounding the anode, ie, high-definition tDCS). However, because two of the same research groups did not replicate their results in later studies using similar samples and electrode montages,[48,49] these differences in study characteristics do not seem to explain the difference in study results.

The short-lasting tDCS effect in this study also could reflect that participants already reached ceiling performance during the first *N*-back training session. It has been shown that *N*-back ceiling performance can be achieved within 20 minutes of the task,[103] and we observed a significant flattening of performance improvement toward the end of the first session. In contrast, the emotional dual *N*-back task was based on a WM training study by Schweizer et al.[18] Maximum performance levels in that study (*N* between 4 and 5) were higher than the performance levels reached in our sample at the end of the first tDCS training session (*N* between 2 and 3, Fig. 2a). In addition, our participants further increased in *N*-back performance from the first to the second and third session (session 3: *N* between 3 and 4, Fig. 2a). These results suggest that participants did not reach ceiling performance during the first tDCS training session and therefore do not support the idea that ceiling performance explains the lack of tDCS effects on WM performance beyond the first tDCS training session.

Interestingly, our results concur with several previous studies showing similar short-lasting tDCS effects. For example, three studies in healthy students showed that multiple (two, three, or ten) sessions of anodal tDCS over the left DLPFC (1–2 mA) significantly enhanced WM performance during the first session, but not in subsequent sessions.[104–106] Another study showed that anodal tDCS over the left DLPFC (1.5 mA) only significantly enhanced cognitive task performance when real tDCS was applied in the first of two experimental sessions in a crossover design.[107] The authors of this study proposed that tDCS may primarily facilitate performance at the onset of learning new cognitive skills. In line with this idea, our results show that the early-stage tDCS effect was driven by a steeper learning curve, suggesting accelerated learning. Evidence from motor cortex stimulation research supports the idea that performing a learning task during tDCS mediates tDCS-related changes in task performance.[108,109] Moreover, effects of noninvasive brain stimulation, including tDCS effects on WM performance, have been shown to depend on arousal and stress levels.[110–113] In this study, central nervous system arousal could have been elevated in particular during the first session owing to the novelty of tDCS and the training task. Altogether, factors such as novel learning processes and arousal could have interacted with the neurophysiological effects of tDCS specifically during the early stages of the tDCS training intervention. More insights into these potential interactions may help to unravel how beneficial tDCS effects can be extended beyond the short-lasting performance enhancement observed here.

Influence of Individual Characteristics on tDCS Response

In addition to the confirmatory group-level analyses, post hoc analyses were performed to explore sources of individual differences in tDCS response. These exploratory analyses indicated that the early-stage tDCS effect on emotional WM performance was stronger in relation to higher theta/beta ratios. Higher theta/beta ratios have been repeatedly associated with higher reward motivation.[69,70,114] Earlier findings showed that reward motivation increases working memory performance and related PFC activity[115] and may promote tDCS effects on working memory,[116,117] which might explain the predictive value of the theta/beta ratio in this study. Moreover, higher theta/beta ratios have been associated with lower cognitive control, possibly indicating that individuals with dominant subcortical-based drives relative to cortical-based cognitive control benefited more from tDCS.[67,68] In addition, in line with previous findings, higher baseline WM performance,[54,118] younger age,[52,53] and lower educational level were associated with a stronger early-stage tDCS effect. Accordingly, several findings suggest that factors such as a shorter brain-to-skull distance[119] and higher levels of neural plasticity[120,121] may contribute to the higher effectivity of tDCS observed in the younger adults. Instead, results on the influence of education and baseline WM performance on tDCS response are mixed.[55,57,58] Our finding of larger tDCS response in individuals with higher baseline WM performance is in line with some previous studies,[54,59] and may indicate that higher recruitment of the targeted frontoparietal pathways facilitates tDCS effects.[54] However, such explanations remain largely speculative because this study does not identify the processes underlying the interaction between tDCS and these factors. Moreover, our results do not elucidate to what extent the four examined predictors reflect overlapping or distinct factors that influence tDCS response.

Clearly, replication of these exploratory results is required before firm conclusions can be drawn. Nevertheless, these findings highlight the importance of involving individual state- and trait-dependent factors in understanding the effects of tDCS. Interestingly, by replicating the association between the theta/beta ratio and effects of transcranial electric stimulation on cognitive performance,[73,74] our results motivate further research to establish whether this resting-state EEG readout is a useful predictor of tDCS response.

Limitations

This study is disadvantaged by some methodological drawbacks. First, ramp-up-ramp-down stimulation was applied in the sham tDCS condition. Although this sham method is commonly used,[122] results about blinding success have been mixed.[123–125] Blinding success was not formally assessed in this study, and a possible effect of unsuccessful blinding can therefore not be completely ruled out. Second, the effectivity of anodal tDCS applied over the DLPFC on working memory tasks performance depends on the electric field strength and consequent excitability changes in a relatively ventrally located area of the DLPFC.[38,43] Based on a priori electric field modeling estimations (Fig. 1b), the applied electrode montage should induce peak level electrical field strength in this region. Unfortunately, the actual electrical field strength in this region in individual participants in our study is not known and could not be estimated because of the lack of anatomical scans. Third, a tDCS training intervention with three sessions is comparable to previous tDCS intervention studies (two–ten sessions, as discussed above). However, not all studies show a significant effect of tDCS starting from the first session onward. For example, careful examination of findings in two previous studies shows that a clear effect of tDCS on WM performance started to manifest after three or more sessions.[44,46] Together with the potential ceiling performance issues on the NPU-threat test and *N*-back task, this suggests that future studies may benefit from applying more tDCS training sessions and using more sensitive outcomes measures (eg, more variety or cognitive challenge in the tasks) to get better insight into the effect of multisession tDCS on WM training. Finally, this study applied tDCS both online and offline, that is, tDCS administration only covered the first half of the *N*-back training in each session. Online and offline tDCS were combined because it is not yet clear which timing of tDCS has better effects on cognitive performance.[93–95] However, many previous tDCS WM training studies in healthy individuals applied tDCS online only[44–46,104,105] or offline only.[102] The generalizability of our results may therefore be limited to this specific online/offline tDCS application.

CONCLUSIONS

This study in healthy military personnel showed no evidence for the hypothesized beneficial effect on top-down stress regulation of multisession anodal tDCS over the right DLPFC during emotional WM training. Instead, the results suggest that tDCS had a short-term beneficial effect on emotional WM performance in the early stages of the training. This effect was moderated by the theta/beta ratio and other previously identified predictors of tDCS response, emphasizing the importance of state- and trait-dependent factors in effects of tDCS on cognitive performance.

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Authorship Statements

Fenne M. Smits, Elbert Geuze, Jack van Honk, and Dennis J.L.G. Schutter designed the study conceptually. Fenne M. Smits and Guido J. de Kort designed the practical aspects of the study. Fenne M. Smits, Karlijn Kouwer, and Lisa Geerlings conducted the study, including participant recruitment and data collection, with the supervision of Elbert Geuze and Dennis J.L.G. Schutter. Fenne M. Smits performed data analysis with the supervision of Elbert Geuze and Dennis J.L.G. Schutter. Fenne M. Smits drafted the manuscript with important intellectual input from Elbert Geuze, Jack van Honk, and Dennis J.L.G. Schutter. All authors reviewed the drafted manuscript. All authors approved the final manuscript.

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SUPPLEMENTARY DATA

To access the supplementary material accompanying this article, visit the online version of *Neuromodulation: Technology at the Neural Interface* at www.neuromodulationjournal.org and at https://doi.org/10.1016/j.neurom.2022.05.002.

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COMMENT

This negative study is an important addition to the literature, reminding the field of the importance to study the interaction between brain stimulation and various cognitive tasks. Although this intervention did not improve stress regulation, lessons learned from this work can help develop future intervention studies in patients.

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