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Simultaneous Application of Transcranial Direct Current Stimulation during Virtual Reality Exposure

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Abstract

Transcranial direct current stimulation (tDCS) is a form of non-invasive brain stimulation that changes the likelihood of neuronal firing through modulation of neural resting membranes. Compared to other techniques, tDCS is relatively safe, cost-effective, and can be administered while individuals are engaged in controlled, specific cognitive processes. This latter point is important as tDCS may predominantly affect intrinsically active neural regions. In an effort to test tDCS as a potential treatment for psychiatric illness, the protocol described here outlines a novel procedure that allows the simultaneous application of tDCS during exposure to trauma-related cues using virtual reality (tDCS+VR) for veterans with posttraumatic stress disorder (NCT03372460). In this double-blind protocol, participants are assigned to either receive 2 mA tDCS, or sham stimulation, for 25 minutes while passively watching three 8-minute standardized virtual reality drives through Iraq or Afghanistan, with virtual reality events increasing in intensity during each drive. Participants undergo six sessions of tDCS+VR over the course of 2-3 weeks, and psychophysiology (skin conductance reactivity) is measured throughout each session. This allows testing for within and between session changes in hyperarousal to virtual reality events and adjunctive effects of tDCS. Stimulation is delivered through a built-in rechargeable battery-driven tDCS device using a 1 (anode) x 1 (cathode) unilateral electrode set-up. Each electrode is placed in a 3 x 3 cm (current density 2.22 A/m^2) reusable sponge pocket saturated with 0.9% normal saline. Sponges with electrodes are attached to the participant's skull using a rubber headband with the electrodes placed such that they target regions within the ventromedial prefrontal cortex. The virtual reality headset is placed over the tDCS montage in such a way as to avoid electrode interference.

Introduction

Posttraumatic stress disorder (PTSD) is a chronic and disabling condition that is especially prevalent among veterans. Despite its prevalence and devastating impact, many who receive evidence-based psychotherapy for PTSD have significant residual symptoms[1]. The synergistic application of non-invasive brain stimulation together with PTSD-focused principles of psychotherapy presents an opportunity to improve therapeutic gains and lower PTSD-related burdens.

A core component of PTSD is the inability to inhibit a maladaptive fear response[2,3]. Pathologically elevated activity in the amygdala and dorsal anterior cingulate cortex, regions that facilitate the fear response, has been consistently reported in PTSD. This is alongside reduced activity in the ventromedial prefrontal cortex (VMPFC), a region thought to down-regulate the fear response[3,4,5,6,7]. Accordingly, increasing endogenous VMPFC activity during the processing of fear-inducing stimuli may be a promising method to improve inhibition of fear and the effectiveness of exposure-based treatments.

Exposure-based psychotherapies, a first-line treatment for PTSD, aim to facilitate corrective learning by teaching patients that the hazardous experience (i.e., the cause of their PTSD) is no longer present or threatening in their current environment[8,9]. Emotional engagement in PTSD therapy is a crucial component of success[10] but is hampered by patients wanting to avoid experiencing distressing emotions and the presence of comorbid psychiatric disorders. One appealing approach to maximize and track emotional engagement over sessions is using immersive and contextually relevant virtual reality (VR) environments[11,12]. VR implementation is supported by prior data indicating that VR could generate efficacy rates comparable to those observed with standard cognitive-behavioral interventions[11,13,14]. VR has the additional benefit of providing a standardized environment for treatment development for specific hypothesis testing.

The VR environment furthermore allows for the integration of adjunctive non-invasive brain stimulation methods, such as transcranial direct current stimulation (tDCS). tDCS alters cortical excitability via subthreshold modulation of neuronal resting membrane potentials using a weak (typically 1 – 2 mA) constant electrical current[15]. Stimulation is typically provided over a 20 – 30-minute period. Effects of tDCS are dependent upon the current polarity. Although an oversimplification, in theory, positive current flow (i.e., anodal stimulation) increases the likelihood of neuronal depolarization, whereas negative current flow (i.e. cathodal stimulation) decreases the likelihood of neuronal action potentials[16,17]. As such, tDCS readies the brain for subsequent responses to external stimuli to facilitate learning and memory[18].

tDCS has a favorable safety profile as a low risk technique that is well tolerated and associated with minimal side effects[19,20]. tDCS is also inexpensive; tDCS devices cost around $9,000 compared to >$70K for clinically available non-invasive brain stimulation methods, such as transcranial magnetic stimulation. tDCS devices are also portable, as they are battery powered, as opposed to needing a dedicated electrical circuit. This portability allows use in any office location or room, including at home. These factors enable tDCS to be used in combination with therapeutic interventions including VR and existing models of PTSD treatment. Flexible use may be particularly important in the new landscape delivering psychiatric care and non-invasive brain stimulation in the post-COVID19 world.

The protocol detailed below is designed to integrate tDCS during VR administration (tDCS+VR) in individuals with warzone-related PTSD in order to augment anxious habituation. The VR sessions allow for the exposure to trauma-related events to be standardized across participants to ensure a consistent content for this habituation. Participants undergo six sessions of tDCS+VR over the course of two to three weeks, with each session consisting of three identical VR drive-throughs. Six sessions were selected to approximate the duration of VR in Rothbaum et al.[14] and Difede & Hoffman[21]. This number of sessions showed efficacy in typical, non-VR treatment studies (*e.g.* Bryant et al.[22]) and was further informed by feasibility data from the prior pilot study[23]. Throughout each session, psychophysiology (i.e. skin conductance) is measured. This allows for testing of within and between session changes in hyperarousal to virtual reality events and adjunctive effects of tDCS. tDCS intensity is set at 2 mA and is delivered through a built-in rechargeable battery-driven stimulator that provides a constant, direct current using a 1 (anode) x 1 (cathode) unilateral electrode set-up. Each electrode is placed in a 3 x 3 cm (current density 2.22 A/m^2) reusable sponge pocket saturated with 0.9% normal saline. Sponges with electrodes are attached to the participant's skull using a rubber headband with the anode placed over Fp1 and AF3 regions and the cathode over PO8 of the 10 – 20 EEG electrode coordination system in order to target the ventromedial prefrontal cortex while preventing cathodal stimulation over the prefrontal cortex. Similar electrode montages, aimed to target the VMPFC, have been used to modulate the extinction of conditioned fear responses by our lab[24,25] as well as others[26]. The virtual reality headset is placed over the tDCS montage in such a way as to avoid interference with tDCS electrodes. tDCS should start during the initiation of VR[23] and continue throughout. Participants return for 1- and 3-month post-treatment assessment visits to assess longer-term effects of tDCS+VR on changes in symptoms of PTSD, depression, anxiety, and anger as well as improvements in sleep and quality of life. Hypotheses to be tested are 1A) the prediction that active tDCS+VR, compared to sham+VR, results in greater change on PTSD symptoms and quality of life/social function at end of treatment, and 1B) sustained change at 1- and 3- months post-treatment, and 2) that change in psychophysiological responses, reflective of habituation, relates to changes in PTSD symptoms and quality of life/functioning differently after active tDCS+VR versus sham+VR. This clinical trial is registered under ClinicalTrials.gov Identifier: NCT03372460.

Protocol

Eligible participants sign written, informed consent prior to the start of any research procedures. Research is performed in compliance with institutional, national and international human research guidelines. All methods described have been approved by the Institutional Review Board of the Providence VA Medical Center.

NOTE: The tDCS+VR protocol requires two dedicated research staff members. One staff member is the VR Controller, who operates the VR and administers the VR stimuli at the various time-points outlined below. The second study staff member operates the computer on which the psychophysiology is collected.

1. Screening, Diagnostic Interviews, and Magnetic Resonance Imaging

1. Recruit participants consisting of male and female veterans, with a specific focus on Operation Enduring Freedom (Afghanistan), Operation Iraqi Freedom, and

Operation New Dawn (Iraq) based on the following eligibility. Inclusion criteria: (1) diagnosis of chronic PTSD with trauma related to warzone experience, (2) age between 18-70 years, and (3) if in treatment, symptomatic despite ongoing stable treatment regimens for at least 6 weeks prior to study procedures. Ongoing medications and psychotherapy are allowed to continue unchanged during the study. Exclusion criteria are as follows: meet established safety criteria for magnetic resonance imaging (MRI), as MRI procedures are a component of this study, and include cardiac pacemaker, implanted device (deep brain stimulation) or metal in the brain, cervical spinal cord, or upper thoracic spinal cord, pregnancy or planning to become pregnant during the study. Additional tDCS-specific exclusions are skin lesions at the site of stimulation that may alter impedance (e.g., vascular moles or angiomas). Other exclusion criteria are lifetime history of moderate or severe traumatic brain injury (TBI); current unstable medical conditions; current (or past if appropriate) significant neurological disorder, or lifetime history of a) seizure disorder b) primary or secondary CNS tumors c) stroke or d) cerebral aneurysm, any primary psychotic disorder, bipolar I disorder, active moderate/severe substance use disorders (within the last month, excluding nicotine/ caffeine), active suicidal intent or plan to attempt suicide within 6 months as detected on screening instruments or in the investigative team's judgment.

NOTE: Participants for this study were recruited from the Providence VA.

2. Obtain written informed consent prior to the initiation of any study procedures.

3. Administer diagnostic interviews and questionnaires to verify diagnosis and assess severity of PTSD using the Structured Clinical Interview for DSM 5 (SCID-5)[27], the Clinician Administered PTSD Scale (CAPS-5)[28], and the PTSD Checklist for DSM5 (PCL-5)[29].

NOTE: Administration of the SCID-5 further allows the detection of any comorbid diagnoses that may preclude study exclusion criteria outlined above. Additional assessments, such as the Quick Inventory of Depressive Symptomatology Self-Report (QIDS-SR)[30], are up to the individual research teams depending on hypotheses.

4. Screen participants for safety to undergo tDCS and MRI based on the exclusion criteria listed above.

NOTE: Pre-screening MRI safety forms can be obtained from www.MRIsafety.com

5. Schedule participants to complete six VR sessions over the course of two to three weeks, such that participants complete a VR session approximately every other weekday.

2. Randomization

1. Prior to initial study implementation of tDCS+VR, retrieve active tDCS and sham codes from the tDCS device manual and input them into a randomization program to ensure blinding of tDCS+VR or sham+VR administration.

2. Using the randomization program, create randomization urns by assigning participants to receive either active tDCS or sham during virtual reality based upon sex (male; female) and PCL-5 symptom severity (low; high).

NOTE: The randomization program should generate a tDCS device code that can subsequently be entered into the tDCS device to ensure the tDCS administrator remains blinded to whether active or sham stimulation is applied. As such, this is a double-blind protocol where

both participants as well as tDCS administrators are blinded to stimulation status.

3. tDCS Device Set-up

1. Program the tDCS device with the following parameters and settings, listed under 3.1.1 and 3.1.2, by pressing both keys on the left side of the tDCS device to save each setting.
    1. Setting A: 30 s ramp up to 1 mA intensity, 1 mA stimulation for 30 s, and ramp down to off over 30 s.
    2. Setting B: 30 s ramp up to 2 mA intensity, 2 mA stimulation for a duration of 25 min, and a 30 s ramp down to off.
2. Set the tDCS device to **study mode**, or other double-blinding feature, following tDCS device instructions.
   NOTE: Setting A is used to obtain information regarding impedance prior to stimulation and assessment of tDCS tolerability prior to starting VR. Additionally, the application of a brief electrical current has previously been used to provide some degree of somatic sensation to assist in study blinding[24,25,31]. Setting B allows entering in the specific study code for randomization (active or sham) for each participant. Settings C and D are not used in this protocol.

4. Psychophysiology Set-up

1. Use hard- and software capable of recording and analyzing electrodermal activity (EDA)/galvanic skin response (GSR) on a dedicated psychophysiological recording computer that is different from the computer that runs the VR software.
2. Create a data acquisition template according to software specific procedures with the following data collection settings: 5 µV; 10 HZ; DC. Heart rate: 1000 gain, Norm, DZ, 0.05 Hz.
   NOTE: Creating a data acquisition template ensures consistency of data acquisition settings across sessions and participants.

5. tDCS Study Visit: Set-up and Administration

NOTE: For the steps below the addition of TM1 and TM2 refers to research “team member 1” and “team member 2” so that the various steps can be completed simultaneously.

1. When the participant arrives, gently clean, without vigorous rubbing, the participant’s skin at the approximate areas where the sponges/electrodes will be placed with an alcohol swab and let dry.
2. Measure and record the circumference of the participant’s head. Calculate 5% and 10% of the circumference to be used later for electrode placement.
3. Put the head strap on the participant, covering areas where sponges and electrodes will be placed, in such a way that it is still possible to fit one finger under the head strap.
4. Ensure that the rubber band connector is on the side of the head so that it is out of the way of the electrodes and does not interfere with the VR head-mounted display.
5. Fill each electrode sponge with 4 mL of saline using a syringe. Insert the electrodes into sponge pockets.
6. While positioned behind the participant, establish the location for the cathodal electrode using the previously calculated 10% of head circumference and measure this distance out from the inion of the head to the right. Place the cathodal electrode and verify measurements so that the cathode is approximately behind the right ear on the mastoid process.

7. Next, reposition to face the participant and establish the location for the anodal electrode by measuring out the previously calculated 10% of head circumference from nasion up, and then measuring out the previously calculated 5% of head circumference to the right. Place the anodal electrode and verify measurements so that the anode is touching 10 – 20 EEG electrode locations AF3/Fp1.

8. Turn the tDCS device on and then plug in the electrodes.

9. To load Setting A, exit out of study mode by pressing the top right button, then enter the master code of the device by using the top and bottom left buttons. After entering the master code, click **OK** by using the bottom left button. Next, make sure the arrow is pointing at **trigger**. Use the top right button to move through the settings until it reads, **load… setting**. Scroll the arrow to the bottom of the screen using the left arrows, then use the top right arrow to move through all the settings and back to setting A. Finally, click the top left arrow to load setting A.

10. Check the impedance by simultaneously pressing the top right and bottom left button to confirm that there is adequate contact between the tDCS electrodes and participant's skull. Record the initial impedance.

    1. Always make sure that the electrodes are not plugged into the device before turning it on. Similarly, make sure to always unplug electrodes before turning off the device.

       NOTE II: The tDCS device will shut off automatically if the impedance is above 55Ω. As a guideline, do not start the tDCS device if the impendence is greater than 35Ω in order to limit the chance of an automatic shut-off. If the impedance is too high, add a little saline to the sponges, move the participant's hair out of the way, or tighten the rubber headband if it appears to be too loose. Avoid dripping saline onto the participant – if this occurs, the sponges are too saturated.

11. Start stimulation under Setting A. Record the impedance prior, during, and after stimulation under Setting A. After completion of stimulation under Setting A, remove the electrodes from the tDCS device and turn the device off.

12. TM1: Place two self-adhesive, disposable EDA electrode patches on the thenar part of the participant's non-dominant hand.

13. TM1: Open the EDA/GSR data acquisition software to allow new data capture. Open the previously generated data acquisition template and click **Create/Record** a new experiment. Calibrate EDA signal following specific software instructions by first attaching one electrode to one electrode patch, calibrate, and then connecting the second electrode to the second electrode patch.

14. TM1: To ensure adequate GSR signal ask the participant to take a deep breath in and hold it for 10 s before breathing out.

    NOTE: An increase in GSR should be noticeable. If no change in GSR is detected, research staff can clap their hands without warning to elicit a GSR response. A baseline skin conductance level value lower than 2 µS might be problematic because it could indicate too low a skin conductance to measure GSR throughout the VR session.

15. TM2: Turn the virtual reality system on and open the Patient Application program. Check that the screen resolution is set to 1280 x 720 and click **play**. Then, open the Clinician Controller program and select the Iraq Rural driving or the Afghanistan Rural driving scenario based

on the scene that is most relevant to the participant's deployment(s). Under the patient avatar window, select the position of **Driver**. Set sound volume at 65% of maximum.

16. TM2: With assistance of the participant, place the head-mounted display on the participant's head, ensuring that the display does not dislocate the electrodes. Check for comfort. Then, place the headphones on the participant's head and check for comfort.

17. TM1: Start EDA data collection and record 2 min of baseline EDA by explaining to the participant that they'll need to sit quietly for 2 min. Press F1 on the keyboard to mark the beginning of baseline period and F3 to mark the end of baseline period.

    NOTE I: Using keys F1, F2, and F3 for markings are essential to allow for later data analyses. F5 can be used to mark participant generated interference throughout EDA data collection (*e.g.* coughing, movement, etc.).

    1. After completion of baseline EDA, do not stop EDA data collection but continue to run until all three drives have been completed.

18. Turn the tDCS device on and plug the electrodes back in. The device now reflects **study mode** and Setting B. Use the bottom right button to click **OK** to confirm that Setting B is programmed to apply a 2 mA intensity for a total of 25 min, with a 30 s ramp up and ramp down each.

    NOTE: During the VR session participants might express some discomfort from the headband or an itchy, prickly sensation. However, participants should be instructed to report any pain or an increasingly heating or burning sensation as this warrants the immediate shut-off of the tDCS device to avoid local skin burns.

19. Enter the participant specific randomization code retrieved from the randomization software and click **OK**, then start the stimulation by pressing the top left button to click **Y**.

    NOTE: Participants should be informed that some people experience cyber sickness from VR and that this feeling is similar to car sickness. If cyber sickness occurs, it should recede quickly. Before the participant leaves, inquire if they are able to operate a vehicle. If not, supportive care can be provided, and usually additional waiting time is sufficient.

20. To start the drive, click the **Off** button under driver control.

    NOTE: Each participant will do three drive-throughs per session, each lasting about 8 min in duration, amounting to 24 min total. The 25 min of active or sham stimulation programmed in the tDCS device allows for an additional minute to be used to check in with the participant in between drive throughs.

21. For the first session (VR1, day 1) the VR Controller must guide the participant through the occurrence of VR events using a verbal prompt during the first drive-through as follows: "Up ahead there will be a road ambush. In 3…2…1… go" (VR Controller selects the 'road ambush' in the VR menu).

    NOTE: This will only be done for the first VR drive-through on the first session. For all other VR drive-throughs or sessions, the participant must go through the drive without verbal prompting. However, the VR Controller can remind participants that they will see the same scenes as the previous drive-through, but no verbal warning of upcoming VR events will be provided.

22. VR Controller: Ensure that each drive-through starts with at least 30s of driving only in the VR environment. Then,

administer each VR event (with a minimum of 10s of driving between each event) by clicking the event as labelled in the clinician controller software environment. VR events will occur in the following order: gunshots, Blackhawk helicopter flying overhead, insurgent ambush and another insurgent ambush, followed by IEDs, a bridge ambush and an explosion of the vehicle in front of the participant's vehicle. See Appendix 1 for timing of various VR events in both the Afghanistan and Iraq driving scenarios.

NOTE: This sequence of VR events is repeated in the same order and VR events are repeated at the same time during each of the three VR drive-throughs during each VR session.

23. While the VR Controller administers the VR events, have the staff member monitoring the skin conductance data acquisition press F2 on the keyboard every time a VR event is administered.

24. When the car returns to the beginning of the drive, stop the car from driving by clicking the **Throttle** button under driver control.

25. After each drive-through, the VR Controller must check in with the participant to ensure the safety and comfort of the participant before continuing with the next drive-through. If the participant mentions potentially more serious tDCS side-effects, such as a burning or increasing heating sensation, please follow tDCS device manual guidelines for stopping tDCS.

26. Complete drives 2 and 3 using the same order of VR events as during drive 1.

27. After completion of all three VR drive-throughs for one session, check and record tDCS impedance by going out of **study mode** by first pressing the top right button and entering the master code of the device by using the top and bottom left buttons.

28. Unplug the electrodes from the tDCS device and turn off the device.

29. Query the participant for any potential side effects by administering a tDCS side-effects questionnaire[32].

30. Finally, clean the VR headset, headphones and rubber headband after use with alcohol swabs and disinfectant wipes. Take a screenshot of the fully collected EDA trace over time for quality control processing.

NOTE: Implementation of additional cleaning and preventative measures might be necessary as precautions to reduce the spread of COVID-19. For example, participants might need to wear surgical facemasks. The wearing of facemasks increases the likelihood of fogging of the VR lenses. Surgical tape can be used to tape the masks over the participant's nose to reduce fogging. Similarly, the availability of multiple headbands - for both tDCS and the VR headset - and headphones will ensure spaced out usage between participants for cleaning and disinfection.

6. Analyses

1. GSR preprocessing

    1. Using GSR processing software, open the participant's stored GSR file and save a new copy of the file for preprocessing so that the original, raw data file remains conserved.

    2. Visually inspect the data for artifacts and general drift, then remove or correct them. Follow previously published guidelines on artifact removal and corrections for general drift which can be found at

https://www.birmingham.ac.uk/Documents/college-les/psych/saal/guide-electrodermal-activity.pdf

2. Skin conductance level baseline

    1. Record the average, minimum, and maximum values (in µS) across the 2 min baseline period by selecting the 2 min baseline period with the cursor. This information provides some index of the tonic skin conductance level and the level of EDA responsiveness.

        NOTE: Although a 2 min baseline period is used here, a longer time period of up to four or 5 min can be used.

3. Event-related skin conductance response (SCR) to VR stimuli

    1. Determine and create epochs related to VR events using the stimulus type event markings in the data by selecting the one second before each VR event and up to ten seconds following each VR event. The epoch width is the amount of time included to capture the SCR. Each psychophysiology equipment set will have its own set of instructions for creating epochs. Refer to the manual of your psychophysiology-collecting device for this information.

        NOTE I: Although SCRs typically have an onset, or latency, of 1-3 s after event presentation, VR events are not always presented immediately when initiated. For example, while an IED explosion and distant gun fire will occur immediately when initiated, the onset of gun fire as part of an insurgent ambush or the flyover of a Blackhawk is delayed by several seconds. As such, the 10 s window for SCR analyses should be liberal enough to capture SCRs in response to all VR events.

        NOTE II: Verify that events, not fixed time intervals, are selected for analysis. Here the events are user defined **type 2- event specific VR start** as entered by a research team member.

    2. Follow data processing procedures as outlined in the psychophysiology software used in order to mark the start and the end of each epoch of interest and extract event-related SCR data. See Appendix 2 for an example using a **Find Cycle** approach. Export preprocessed GSR data for further analyses.

4. Further analyses

    NOTE: Given the relatively large epochs related to VR events, namely from 1 s prior to 10 s following VR events, the preprocessed output file will contain both event-related SCRs and non-event related, or non-specific SCRs. To determine the event-related SCR, use the first positive deviation that surpasses a 0.02 µS threshold occurring after at least two seconds. A window of two seconds is chosen as the epoch contains the 1 s prior to VR event presentation, and event-related SCRs do not typically have a latency of less than 1 s.

    1. Using statistical analysis software, determine whether distribution of SCR data is normal. If not, apply a square-root or Log transformation to correct for skew/kurtosis following steps appropriate for the statistical analysis package used.

5. Use linear mixed models to test for the effect of active tDCS or sham on SCRs during VR, where group (active tDCS or sham) is a between-subjects variable, statistically controlling for baseline skin conductance level (SCL) and other demographic or clinical factors

(*e.g.* PTSD severity). In order to test the effect of tDCS on between-session habituation, use VR session (1 – 6) as a within-subjects variable. To assess the effect of tDCS on within-session habituation, use individual drive-throughs (1 – 3) within each VR session as a within-subject variable.

Representative Results

Representative results presented here reflect individual psychophysiological data tracings from four participants who completed the above outlined protocol. Enrolled participants are veterans with a diagnosis of PTSD and – in line with trial inclusion criteria – are between the ages of 18 and 70 years old. Given that this a currently ongoing double-blind, randomized sham-controlled trial (NCT03372460), it is not possible to present data pertaining to effectiveness of active tDCS versus sham. Therefore, individual raw, non-processed, skin conductance data tracings collected as part of this ongoing clinical trial are presented. This will provide preliminary insight into what could be expected, including obstacles when collecting psychophysiological data and skin conductance recordings in particular. Data on twelve veterans with warzone-related PTSD using the above protocol as part of a separate pilot study have previously been published[23].

Based on visual inspection of the skin conductance traces, participant A (**Figure 1**) appears to show signs of between-session habituation from the first VR session to midpoint of protocol, during the third VR session, to the last, sixth VR session.

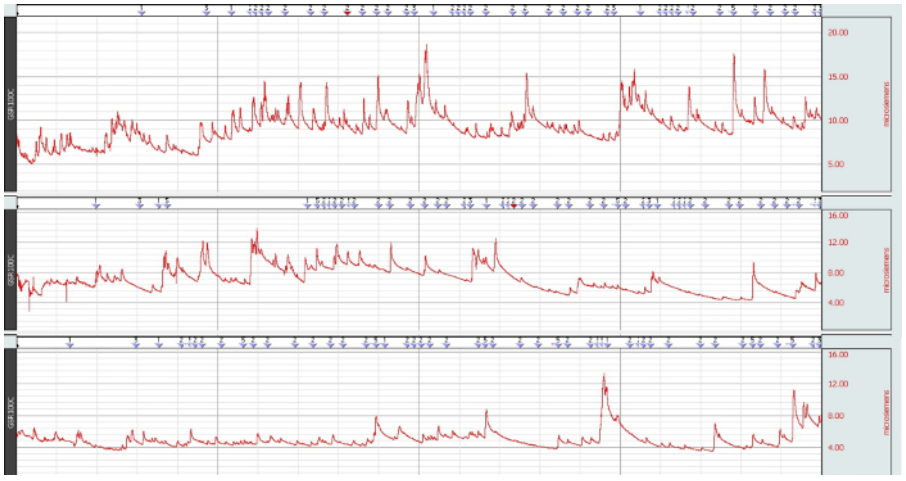

Figure 1: Example of raw skin conductance data tracing from participant A. Figure 1 shows screenshots of raw skin conductance data obtained during VR session 1 (top), VR session 3 (middle), and VR session 6 (bottom). Reductions in skin conductance reactivity indicate between-session habituation. VR sessions 2, 4, and 5 are not pictured to allow for better visual comparison of skin conductance tracings. Please click here to view a larger version of this figure.

Visual inspection of participant B raw skin conductance tracing (**Figure 2**) appears to indicate within-session habituation when comparing the first drive-through (red square) to the third drive-through (green square). Prior studies suggest that although within-session habituation is important, between-session habituation may be a better

predictor of prolonged exposure-based treatment success for PTSD[33,34].

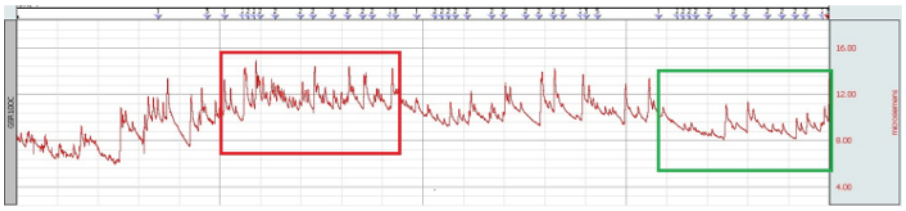

Figure 2: Example of raw skin conductance data tracing from participant B. Figure 2 shows screenshots of raw skin conductance data obtained during the first drive (red square) and third drive (green square) of one VR session. Data represented in this figure may indicate within-session habituation from the first drive-through to the third drive-through. Please click here to view a larger version of this figure.

Visual inspection of participant C raw skin conductance data (**Figure 3**) appears to show a less stark habituation profile compared to participant A (**Figure 1**), this participant nonetheless demonstrates both between- and within-session habituation. Furthermore, and similar to participant A, the skin conductance level is numerically higher during the first VR session as compared to the remaining five sessions.

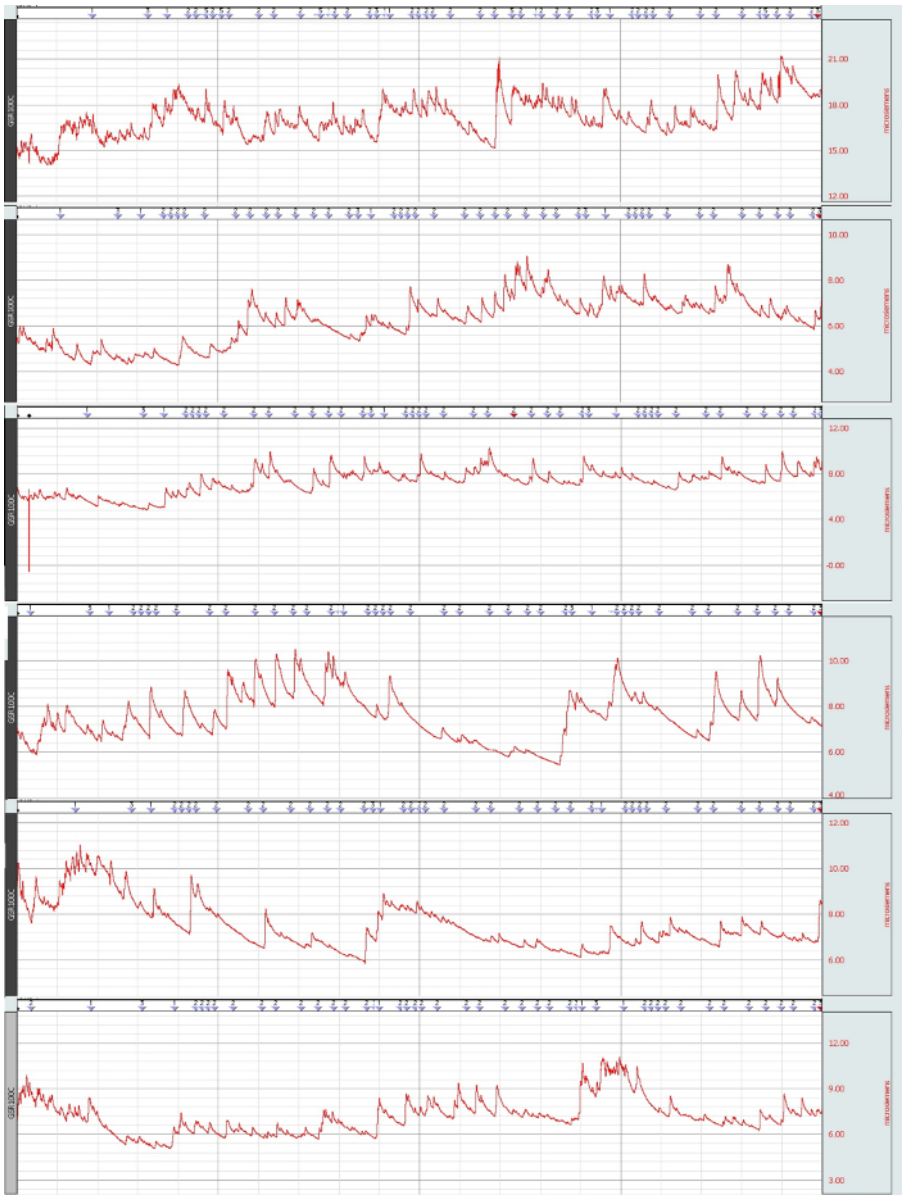

Figure 3: Example of raw skin conductance data tracing from participant C. Figure 3 shows raw skin conductance data screenshots from participant C for VR sessions 1 through 6 ordered from top to bottom. Participant C appears to demonstrate both between- and within-session habituation. Please click here to view a larger version of this figure.

Raw skin conductance data from participant D (**Figure 4**) demonstrate a skin conductance level that can be considered too low for proper analyses with an absence of visually detectable skin conductance responses. As such, these data represent data collection failure. Although the raw data also reveal the presence of artifacts and electrode signal loss, the persistently low skin conductance levels and absence of visually detectable skin conductance responses across all six VR sessions is apparent for this individual.

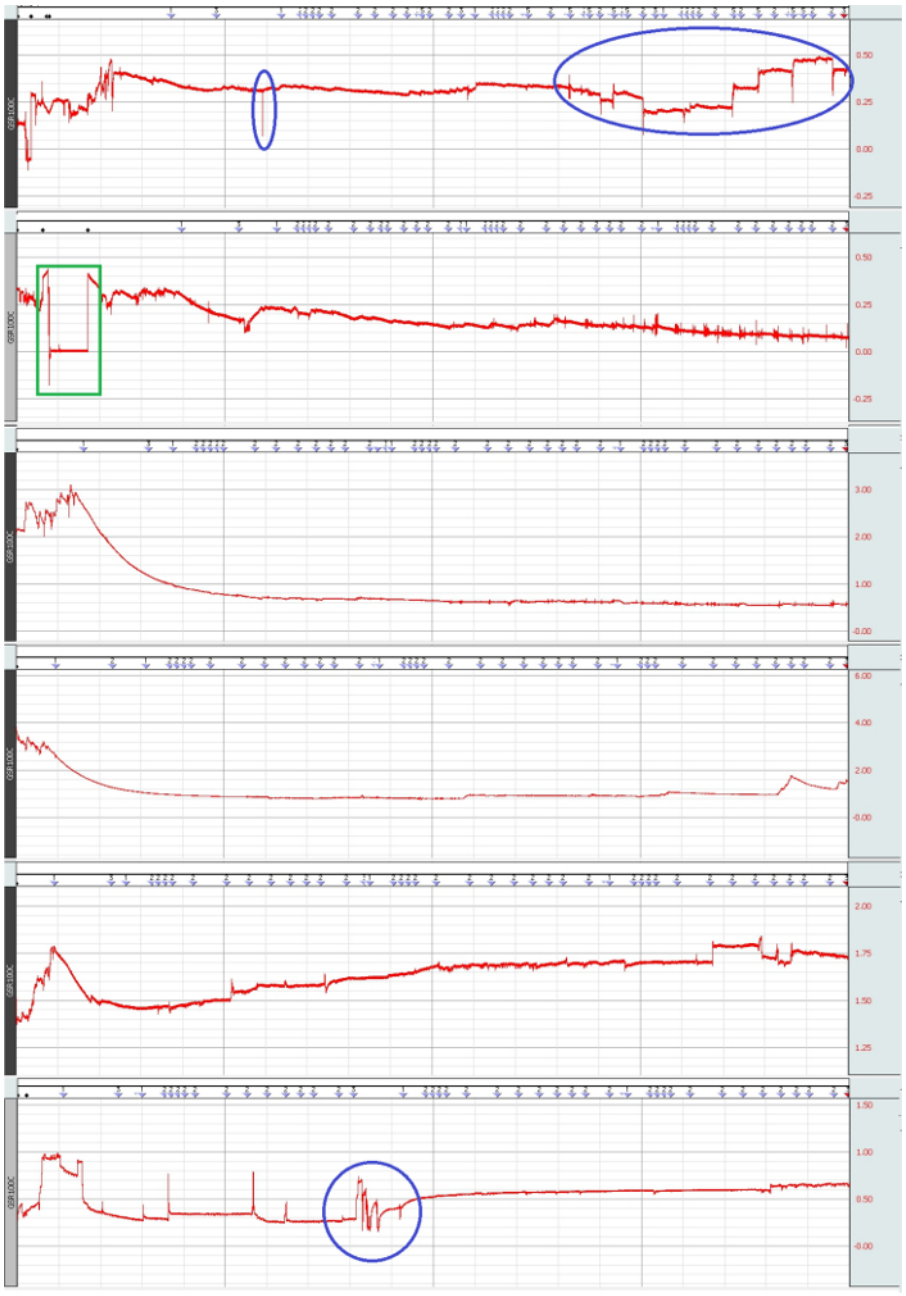

Figure 4: Example of raw skin conductance data tracing from participant D. Figure 4 shows raw skin conductance data screenshots from participant D during VR sessions 1 through 6, ordered from top to bottom, demonstrating unmeasurable skin conductance levels and responses, as well as artifacts (blue ovals) and EDA electrode signal loss (green square).

Please click here to view a larger version of this figure.

Discussion

The protocol detailed above describes the concurrent application of tDCS and VR, as opposed to the serial application of either technique. With respect to existing methods, the simultaneous application of tDCS with VR is important. While the VR provides a contextually rich and immersive environment for fear-related processing, the subthreshold stimulation provided by tDCS allows for the modulates of intrinsic neural activation associated with this fear-related processing. There are multiple critical steps in this protocol that can be divided into those that relate to tDCS+VR implementation and those related to psychophysiological data capture for analyses. With respect to tDCS+VR, it is of critical importance to ensure correct randomization and simultaneous application of tDCS throughout the entire VR session. Another blinded staff member can perform further confirmation of randomization.

As for ensuring simultaneous tDCS+VR two aspects are important; 1) the impedance achieved during tDCS set-up and 2) starting the tDCS device in close proximity to starting VR. The latter issue is relatively straightforward and should ensure that tDCS is continuously applied throughout the VR presentation while remaining well within the safety limits of tDCS when a 2 mA intensity is applied over a 25-minute duration[20]. With respect to impedance, low impedance is desirable. Knowing whether adequate impedance, or contact quality, is achieved depends on the tDCS device that is used. Some devices will display impedance in Ohms, where lower is better, whereas other devices use a 10- or 20-point display scale representing contact quality, where higher is better. Regardless of the specific device, the use of normal saline, 0.9% NaCl solution, as opposed to regular tap water to moisten the electrode sponges improves impedance[35]. The use of regular tap water should further be avoided because it associated with the occurrence of small skin lesions[35,36], one of the more serious possible side-effects of tDCS. Skin lesions can also occur if the skin under the electrodes is vigorously abraded prior to tDCS[37] or if a conductive gel is used, which can dry out[35,38], and should therefore also be avoided. Finally, a high impedance prior to starting tDCS can result in reaching or surpassing the prescribed safety parameters of the device, which will trigger the device to shut down mid VR administration. Although it is important to sufficiently moisten the electrodes sponges to ensure adequate impedance, this should be balanced by not excessively soaking the electrodes, as this may result in leaking, or dripping, of saline when the VR headset is placed. Leaking of saline may allow the electrical current to 'spread' over a larger area resulting in a lower, but unknown current density[39], which depends on tDCS intensity (in mA) and size of electrodes (in cm^2). Likewise, it is important that the VR head mounted display does not physically touch the sponges/ electrodes in order to avoid disruption of current flow and shifting of electrodes as participants move their head.

In this protocol, skin conductance is considered a primary outcome measure. Skin conductance is a psychophysiological measure of sympathetic nervous system activity[40]. Typical factors associated with skin conductance acquisition, such as effects of environmental temperature and humidity, aging, smoking status, caffeine use, and use of medications with anticholinergic effects[41], will need to be considered, but cannot always be eliminated. For example, it is possible to ask participants to abstain from using caffeine-containing products prior to VR sessions, but it is not ethical to ask them to discontinue antidepressant medications. Moreover, for reasons that are not always clear, a portion of individuals demonstrate very low or unmeasurable skin conductance levels and/or skin conductance responses,

which is highlighted in **Figure 4**. It is therefore important to enroll a sufficient sample size to tolerate the loss, or absence, of data. Specific to the implementation of this protocol, it should also be mentioned that event markers are currently entered manually during the psychophysiological data capture. Although this is a limitation, it is not uncommon in hospital systems that a non-hospital managed computer, in this case the computer that runs the VR environment, cannot be connected to the encrypted hospital information technology network. This means that it is not possible to have the computer that runs the VR environment send signals (*e.g.* through a TTL pulse) to the psychophysiological data capture computer that is on the hospital network. Although less elegant, one solution is to have two research team members be present during each VR session; one that controls the VR administration and one that manually enters event markers to the psychophysiological tracing, as can be seen at the top of each figure (see **Figure 1**, **Figure 2**, **Figure 3** and **Figure 4**). However, this does not address the presence of a slight time difference, less than half a second, from when VR events are initiated by the VR controller and entering the event marker by the second person. Future studies might want to mitigate this so that event markers can automatically be registered. Yet, the presence of a second research team member – different from the person who operates the VR environment – who can observe the participant throughout sessions is highly recommended. It should be expected that some participants might have strong emotional reactions during the study or experience cyber sickness-related side effects. The ability of the research team to quickly respond to these situations ensures the best possible care.

In summary, this protocol uses simultaneous tDCS during VR to augment habitation to trauma-related scenarios. The principal advantage of this approach is the use of an immersive trauma-related context and the application of a non-invasive brain stimulation technique during a clinically relevant cognitive process, as opposed to doing either consecutively. While the protocol described here uses in-office application in a veteran sample with PTSD, this approach of simultaneous non-invasive brain stimulation and virtual reality can translate to other fear-based and anxiety disorders as well as at-home applications of exposure-based approaches.

Disclosures

The authors have nothing to disclose.

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