Cranial Electrotherapy Stimulation on Acute Stress

Study Protocol

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PROTOCOL TITLE:

Cranial Electrotherapy Stimulation (CES) Influences on Acute Stress Responses

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1.0 Purpose of the study:

The purpose of this study is to quantify the effects of 20 sessions of (Active vs Sham) Cranial Electrotherapy Stimulation (CES) on multiple measures of acute stress responses: biochemical (salivary alpha amylase and cortisol), physiological (e.g., heart rate, heart rate variability, respiration rate), emotional (state anxiety), and behavioral (i.e., cognitive task performance).

2.0 Background / Literature Review / Rationale for the study:

Acute Stress
Soldiers are continually exposed to acute stressors during training and operations, activating a cascade of biochemical changes in the body, including an initial sympathetic-adrenal medulla (SAM) catecholamine-related response, and a slower-moving hypothalamic-pituitary-adrenal (HPA) glucocorticoid-related response (Angelova et al., 2021; Axelrod & Reisine, 1984; Buchheim et al., 2019; Charmandari et al., 2005; Gagnon & Wagner, 2016; McEwen, 2007; Padgett & Glaser, 2003). The diverse effects of acute stress on neurotransmitters and the central nervous system are associated with similarly diverse perceptual and cognitive effects, generally related to the intensity of experienced stress and the processes demanded by specific mental tasks. For example, mild-to-moderate acute stress can improve performance on relatively basic (or well-learned and rehearsed) perceptual and cognitive tasks, such as simple reaction time and verbal memory retrieval, perhaps owing to activation of the ascending reticular activating system (ARAS) (Arnsten, 2009; Broadbent, 1971; Brunyé et al., 2021a, 2021b; De Cicco et al., 2018; Hupbach & Fieman, 2012; Shields et al., 2019). Moderate-to-high acute stress, in contrast, can negatively influence performance on tasks more demanding of executive processes and spatial memory retrieval, perhaps owing to HPA-related activation and brain regions (e.g., prefrontal cortex, hippocampus) with high glucocorticoid receptor densities (Arnsten, 1998, 2009; Brunyé et al., 2016, 2019; Cerqueira et al., 2007; Luine et al., 1994; Olver et al., 2015; Richardson & VanderKaay Tomasulo, 2011; Shields et al., 2015, 2016).

Individuals engaged in high-stakes occupations, such as military personnel, first responders, and emergency medicine physicians, are continually exposed to moderate-to-high stress, which can negatively influence their ability to sustain performance on job-related duties (Meredith et al., 2011; Orasanu & Backer, 1996; Reynolds & Wagner, 2007; Sood et al., 2011). As a result, scientists and practitioners alike seek methods to reduce the stress response and increase resilience to its short- and long-term effects. This includes efforts to train emotion regulation strategies, resilience, mindfulness, and biofeedback (Berking et al., 2010; Jha et al., 2015; Kennedy & Parker, 2019; Reivich et al., 2011). Methods also include non-invasive brain stimulation, including transcranial direct current stimulation (tDCS), transcranial alternating current stimulation (tACS), transcranial magnetic stimulation (TMS), and cranial electrotherapy stimulation (CES). Each of these techniques has received attention for its potential utility in altering biochemical and physiological manifestations of acute stress responses and thus altering behavior.

Cranial Electrotherapy Stimulation
Cranial electrotherapy stimulation (CES) differs from transcranial electrical stimulation (tES) in several ways. First, CES electrodes include one anode and cathode but are

typically attached to the temples, mastoids, or earlobes (rather than the scalp). Second, CES is typically administered using alternating rather than direct current, at relatively low intensities ranging from 50-500 μA and frequencies ranging from 0.5 to 100 Hz. Third, rather than targeting specific brain regions, CES is thought to exert nonspecific effects on both the central and peripheral nervous systems. Many mechanistic explanations regarding CES effects on the central and peripheral nervous systems, neurotransmitters and hormones, and emotion and behavior have been proposed (see Brunyé et al., 2021a, 2021b). Regarding central and peripheral nervous system effects, various publications have proposed that CES influences sympathetic versus parasympathetic drive, alters oscillatory brain activity, increases cerebral blood flow in subcortical brain regions, stimulates afferent projections of the vagus and/or trigeminal nerve, and modulates functional brain connectivity and plasticity. Regarding neurotransmitter and hormonal effects, studies have variably proposed that CES alters SAM and HPA axis and associated neurotransmitters such as cortisol, serotonin, norepinephrine, dopamine, acetylcholine, and Y-aminobutyric acid (GABA) (Barclay & Barclay, 2014; Datta et al., 2013; Ferdjallah et al., 1996; Kavirajan et al., 2014; Lee et al., 2019; McClure et al., 2015; Roh & So, 2017; Schroeder & Barr, 2001; Wagenseil et al., 2018; Winick, 1999; Yennurajalingam et al., 2018). Unfortunately, no comprehensive model of CES provides mechanistic linkages between these putative effects of CES or provides compelling associations to behavioral outcomes.

According to computational models of CES current propagation, CES has the strongest neuromodulatory effects on diverse cortical and subcortical brain regions including the temporal lobes, medulla oblongata, midbrain, thalamus, hypothalamus, pons, and insula (Datta et al., 2013; Ferdjallah et al., 1996). In research examining brain activity with electroencephalography (EEG) during and after CES administration, some studies find evidence for alpha and beta band power modulation, suggesting that CES may be expected to influence arousal states and attention (Lee et al., 2019; Schroeder & Barr, 2001; Wagenseil et al., 2018). Finally, two studies have examined brain hemodynamics during and after CES administration, using both functional magnetic resonance imaging (fMRI) and xenon-enhanced computed tomography (XeCT). In the fMRI study, the authors found that CES induced broad regional brain deactivation and altered default mode network activity and proposed that CES may induce transient downregulation of rumination or worry (Hamilton et al., 2011). In the XeCT study, the authors found that CES altered cerebral blood flow (CBF) specifically in the brainstem and thalamus, suggesting that CES plays a role in modulating anxiety and pain perception (Gense de Beaufort et al., 2012).

In addition to its effects on functional brain activity, CES has also been found to modulate salivary, urinary, blood, or cerebrospinal fluid (CSF) levels of stress hormones, inflammation and immune markers, and neurotrophic factors. These studies find highly mixed results. For example, while most studies suggest that CES does not influence serotonin, dopamine, β-endorphins, alpha amylase, cortisol, adrenocorticotrophic hormone (ACTH), C-reactive protein, interleukin-1, or interleukin-6 (Cho et al., 2016; Krupitsky et al., 1991; Roh & So, 2017; Yennurajalingam et al., 2018), an earlier study suggested that CES administration decreases cortisol and increases ACTH, serotonin, and β-endorphins (Liss & Liss, 1996). Note that the latter study shows a high risk of bias, with experimenters testing themselves as participants, financial conflicts of interest, and a lack of random assignment or blinding to CES conditions (Brunyé et al., 2021a, 2021b; Kavirajan et al., 2014).

In clinical contexts, CES devices are classified as class II (special controls) medical devices for the treatment of anxiety or insomnia and as class III medical devices for the treatment of depression (Docket No. FDA-2014-N-1209). In one of the largest placebo-controlled CES studies to date, active (versus Sham) CES (100 μA, 0.5 Hz, 35 days of treatment) was administered to 115 participants with an anxiety disorder; results showed an approximately 32% reduction in anxiety measured using the Hamilton Rating Scale for Anxiety (HAM-A-17) (Barclay & Barclay, 2014). However, results are generally mixed about CES effectiveness in treating clinical insomnia (Feighner et al., 1973; Lande & Gragnani, 2013; Wagenseil et al., 2018; Weiss, 1973) and depression (Kavirajan et al., 2014; McClure et al., 2015; Mischoulon et al., 2015).

In our own recent study (Brunye et al., 2022), we used a double-blind, placebo-controlled, crossover design to examine the effects of CES on emotional, physiological, biochemical, and behavioral responses to acute stress. Healthy male participants visited the laboratory for two sessions, one involving active CES administration (100 μA, 0.5 Hz, 20 min administration) and one involving Sham, inactive CES. During each session, participants were placed under stress (threat of torso shock) while performing challenging cognitive tests, and we measured emotional, biochemical (alpha amylase, cortisol), physiological (heart rate, respiration rate, heart rate variability, pupil diameter), and cognitive behavioral (memory, decision-making, spatial orienting) responses. Outcome metrics were compared using repeated measures analysis of variance (ANOVA) and planned comparisons. The stress induction reliably modulated measures of sympathetic adrenal medullary (SAM) activity but not hypothalamic–pituitary–adrenal (HPA) axis activity. Active versus placebo CES did not significantly influence any emotional, biochemical, or physiological outcome measure; there was, however a consistent numerical pattern wherein active CES did appear to reduce some of the physiological responses to stress relative to Sham CES. Furthermore, active CES did selectively increase performance on a recognition memory test and degrade performance on a perceptual decision-making test; however, overall performance on our cognitive tasks was very low, suggesting a potential floor effect that could be masking any additional effects of CES. Overall, our study found very limited evidence that CES reliably modulates the acute stress response.

The Present Study

The present study is designed to extend our recent study in three primary ways.

First, rather than a fixed intensity of CES (100μA), we will use a standardized procedure to identify each participant's sensory threshold (Bystritsky et al., 2008; Gong et al., 2016; Schroeder & Barr, 2001). Because 100μA was consistently sub-threshold (i.e., imperceptible) in our prior participants, it is likely this procedure will result in higher (e.g., 250-500μA) CES intensities administered. There are three primary reasons why we have chosen to use higher intensity CES, and why we believe it will make it more likely to find CES effects on physiology, biochemistry, and behavior:

1. Two extant research studies suggest that 300μA (Bystritsky et al., 2008; Overcash et al., 1989) and up to 1500μA (Smith et al., 1994) are effective at lowering anxiety, in line with the conclusions of a review examining CES effects on anxiety and stress (De Felice, 1997).
2. Most of the data from our prior research with the device shows the predicted pattern of results, for example lower heart rate variability and respiration rate with CES (vs sham); however, the pattern is numerical only and does not reach traditional significance levels. We believe that higher intensity CES may

amplify these previously noted patterns; of course, this is an empirical question and cannot be answered without additional data collection.

3. This procedure of individualized thresholding is congruent with manufacturer's recommendations, and they report seeing the most reliable anxiolytic-like effects with this procedure. Also, the procedure of individualized thresholding and minimum intensity (≥250μA) is congruent with the Special Operations Command's (SOCOM) current use of the device during military training and operations, where they report anecdotal success for stress mitigation.

Second, rather than a single 20-minute session of CES administration, we will use daily administration (20-minutes/day) over the course of 4-6 weeks.

These first two modifications are being made to better conform with the device manufacturer's (Electromedical Products International, Inc., Mineral Wells, TX) recommendations for CES administration.

Finally, to elicit a more robust stress response that activates both SAM and HPA activity, we will increase the intensity (to level 3 of 5) of our torso shock administration. With these changes, we make four primary hypotheses:

H1: Biochemistry: We hypothesize that Active vs Sham CES will reduce biochemical responses to stress, including salivary alpha amylase as an indicator of SAM activity and salivary cortisol as an indicator of HPA activity.

H2: Physiology: We hypothesize that Active vs Sham CES will reduce physiological responses to stress, including heart rate, heart rate variability, respiration rate, and pupil diameter.

H3: Emotion: We hypothesize that Active vs Sham CES will reduce subjective experiences of stress, including responses on the STAI.

H4: Behavior: We hypothesize that Active vs Sham CES will lead to increased performance on tasks typically degraded by stress exposure, including a recognition memory test and a spatial memory test.

3.0 Participant Population:

We expect to recruit up to 500 individuals, including Soldiers and civilians, to achieve a final sample size of 40 complete data sets. Our high recruitment number allows for Soldier group briefings (active-duty units at their home station and HRV cohorts at DEVCOM SC) which are routinely 30+ and can be upwards of 100 Soldiers.

We anticipate an approximately 40-50% attrition rate given our requirement to meet a minimum CES threshold of 300μA (i.e., a 250μA minimum CES intensity) and the prolonged study duration. To accommodate the potentially high attrition rate, we expect to recruit and consent up to 500 Soldiers and/or civilians, and schedule and enroll up to 100 Soldiers and/or civilians.

We are recruiting civilians in addition to military personnel because we have limited access to military personnel to conduct this research, and we have no a priori reason to believe that civilians will show markedly different stress responses, task performance, or CES responses relative to military personnel. In fact, most extant research examining the utility of CES for stress reduction is conducted with civilians. Because one of our tasks (marksmanship) could be considered geared towards military personnel, see

Section 15 for a description of possibly increased risk of psychological harm to civilians versus military personnel.

We will recruit participants 18-40 years of age, or 17 years if emancipated minor. This age range was chosen to represent the majority of Active Duty enlisted military personnel, which is under 41 years of age *(Defense, 2014)*. There is no gender-based enrollment restriction.

Inclusion Criteria: Eligible individuals include those who:

1. Can sit and stand freely.

2. Have not used or experienced CES administration in the past.

3. Agree to have their data stored in a repository (database) for future use. Peer-reviewed scientific journal may require data to be stored in a repository as a condition for publication. Such repositories are online databases, in which coded data is linked (no link to participants personally) to the journal article citation.

Exclusion Criteria: The following exclusion criteria will be evaluated by self-report (on the Informed Consent Form) prior to beginning the study.

1. Use of prescription medications, other than oral contraceptives, as they may alter stress hormones (Granger, Hibel, Fortunato, & Kapelewski, 2009).
2. Women only:
    a. Pregnant or plan to become pregnant during the study, as the shock belt may be unsafe for pregnant women and their children.
    b. Nursing, as it may alter stress hormones (Ahn & Corwin, 2015).
3. History of:
    a. A neurological or psychological disorder (such as depression, anxiety disorders, migraines, cluster headaches, seizures, Post-traumatic stress disorder (PTSD) or panic attacks). Individuals with neurological and psychological disorders may exhibit altered stress responses, not representative of the population (Spijker & van Rossum, 2012).
    b. Cardiac disease (including arrhythmia or fast or skipped heart beats). The shock belt may be unsafe for individuals with cardiac disease (Steptoe & Kivimäki, 2012)
    c. Implanted medical devices, such as pacemakers. The Shock belt may be unsafe for individuals with an implanted medical device.
    d. Hypertension. The Shock belt may be unsafe for individuals with diagnosed hypertension (Spruill, 2010). This risk is low, and the prevalence of undiagnosed hypertension in our targeted cohort (ages 18-40) is also very low; for these reasons, we believe self-report of diagnosed hypertension is sufficient.
    e. Insomnia, as insomnia is associated with chronic physiological arousal (Bonnet & Arand, 2010)
    f. Head injury (including neurosurgery, concussion, TBI, skull fracture, hematoma)
    g. Illness that caused brain injury
    h. Any other brain-related condition (such as traumatic brain injury)

i. Metal in the head (outside of mouth, such as shrapnel, surgical clips, or fragments from welding or metalwork)
j. Implanted medical device (e.g., pacemaker, insulin pump)

Study Withdrawal Criteria:

We have five study withdrawal criteria, related to minimum CES intensities, failing to meet minimum performance during the criterial learning tasks, intolerance for torso shock, self-reported symptoms on the Daily Participant Questionnaire (Appendix A), and intolerance for CES.

First, during the individualized baselining phase (part of the Thresholding and Baselining session), if a participant does not reach our minimum CES intensity of 250μA (i.e., a 300μA minimum threshold), they will be dismissed from the study.

Second, during the baseline or follow-up session, if a participant cannot reach the accuracy criterion during the criterial learning tasks within 5 study-test cycles, they will be dismissed from the study.

Third, during the baseline or follow-up session, if a participant cannot tolerate the torso shock (psychologically or physically), we will reschedule their session for a different day. If during two consecutive sessions they cannot tolerate the level 3 intensity, they will be dismissed from the study.

Fourth, at the beginning of any CES session, if the participant answers a 3 or higher (on the scale from 1-4) to any of the Daily Participant Questionnaire items, their session will be rescheduled for a different day. If they report 3 or higher for any of these questions for two consecutive sessions, they will be dismissed from the study.

Fifth, during any CES session, if a participant cannot tolerate the CES administration at their individualized intensity (i.e., answering ≥ 3 to a single item on the “presence and intensity” *and* “relation to stimulation” sections), they will receive secondary CES stimulation as described in the Procedures section (6.0). If their reported symptoms do not successfully subside (i.e., falling below a 3 rating) after the secondary stimulation, and this occurs for two consecutive sessions, they will be dismissed from the study.

Each of these study dismissal criteria are described in more detail in the Procedures section (6.0), and detailed in the Appendix E flowchart.

4.0 Special Populations:

- ☐ Children
- ☐ Fetuses/Neonates
- ☐ Prisoners
- ■ Members of the military
- ☐ Non-English speakers
- ☐ Those unable to read (illiterate)
- ☐ Employees of the researcher
- ☐ Students of the researcher

- ☐ Adults lacking capacity to consent and/or adults with diminished capacity to consent, including, but not limited to, those with acute medical conditions, psychiatric disorders, neurologic disorders, developmental disorders, and behavioral disorders
- ☐ Disadvantaged in the distribution of social goods and services such as income, housing, or healthcare
- ☐ Fear of negative consequences for not participating in the research (e.g. institutionalization, deportation, disclosure of stigmatizing behavior)
- ☐ Approached for participation in research during a stressful situation such as emergency room setting, childbirth (labor), etc.

5.0 Research Locations and Sample Size:

5.1 Research Locations

Research will be conducted at two indoor, climate-controlled locations:

1. Center for Applied Brain and Cognitive Sciences (CABCS), Tufts University, 177 College Ave, Medford, MA.

   AND

2. U.S. Army DEVCOM Soldier Center, 10 General Greene Ave., Natick, MA 01760

5.2 Sample Size

Sample size estimation was based on effect sizes from Barclay & Barclay (2014) who found that CES reduced subjective anxiety with an effect size of f = 0.47. Using GPower, the total sample size was estimated to be 38 to achieve power of 0.80 with an alpha criterion of 0.05. Our goal is therefore 40 complete data sets, with 20 participants in each of the two groups.

6.0 Procedures Involved:

We will use a double-blind, placebo-controlled design with random assignment of participants to one of two experimental groups: Active versus Sham CES. Two versions of the criterial learning and virtual reality tasks have been developed (Brunyé & Giles, 2023; used in DEVCOM Armament Center IRB protocol 18-007, Tufts University protocol 1808016), and will be used herein, in balanced order across participants.

Military participants will be transported to and from the CABCS research performance site in Medford, MA, for participating in the baseline and follow-up session. If overnight accommodations are required for Soldiers, those will be provided along with other travel related items (e.g., per diem). Travel will be planned by the research team and coordinated with unit leadership (if troop request) or HRVPO (if HRVs). Civilian participants will not be provided with transportation, accommodations, or any other travel-related compensation.

All non-disposable devices used in the protocol will be disinfected in accordance with Occupational Health and Safety Administration and Centers for Disease Control

guidance, and product label instructions, paying attention to the required personal protective equipment and the contact time needed to achieve disinfection. In addition, all frequently touched surfaces in the research area, such as workstations, countertops, and doorknobs will be cleaned between participants.

Upon scheduling, participants will be randomly assigned to the Active or Sham CES condition, using the [=rand()] function in Microsoft Excel. All participants will complete a Thresholding & Baseline session, the CES administration sessions, and then a Follow-up session.

Testing Day 1: Thresholding & Baselining

The baseline session is designed to establish each participant's CES individualized threshold and their baseline stress responsiveness and performance in our virtual reality scenario. This session will always take place at a consistent time of day for individual participants (e.g., morning or afternoon), and always at the CABCS in Medford, MA, using the Soldier and Small Unit Ambulatory Virtual Environment (SUAVE) facility.

Participants will first complete the demographics form, daily participant questionnaire, and emotional state (STAI-S, STAI-T; see Appendix A) questionnaires.

Because our individualized thresholding procedure may result in immediate exclusion from the study, participants will do it prior to baselining.

CES Individualized Thresholding: Participants will be asked to sit and relax in a chair, and we will assist them in donning the CES electrodes on the Alpha-Stim AID device (see Appendix D for specifications). The device has two electrode clips that attach to the left and right earlobes; each clip has two disposable pads that will be soaked with saline (to promote conductivity between the electrodes and skin). A single CES device labeled THRESHOLDING will always be used for this phase; this device will always be *active*. The device will be powered on and set to 0.5Hz at the lowest possible intensity (50μA). Participants will be told that we will increase the intensity by a very small amount every 30 seconds and ask them to answer each of the questions on the CES side effect questionnaire. At each 30-second interval, the experimenter will increase the stimulation intensity by 50μA.

If a participant has at least one symptom at 3 or higher (i.e., ≥ 3 in the Presence and Intensity section), *and* they believe it is "possibly" or "definitely" related to the stimulation (i.e., ≥ 3 in the Relation to Stimulation section), we will immediately stop the thresholding procedure. The stimulation intensity achieved will then be recorded by the experimenter.

During all subsequent CES sessions, 50μA below a participant's stimulation threshold will be used as that participant's individualized CES intensity. For example, if the participant achieves 350μA, then all subsequent CES sessions will use a 300μA intensity. If the participant never reports any symptom and relation to CES as ≥ 3, the device will be used at the maximum intensity of 500μA. Because this study intends to increase stimulation intensity beyond that of our prior study, any participant who does not achieve an individualized stimulation intensity of at least 250μA (i.e., ≥ 300μA threshold) will be dismissed from the study.

Baselining. Participants who achieve the minimum 300μA threshold will then complete a series of criterial learning tasks, then a series of cognitive tests while under threat of torso shock. Prior to, during, and after these phases, participants will provide saliva samples and complete the STAI-S questionnaire. These phases are detailed below:

1. Criterial Learning: Participants will learn and be tested to criterion on a series of experimental stimuli. These include a map of a fictitious virtual city, a list of suspicious items from a *be-on-the-lookout (BOLO)* list, and camouflage patterns or objects (e.g., things that are carried in the hands, such as a rifle, broomstick, pipe, etc) representative of friendly versus enemy forces. Participants will be required to reach a minimum of 80% accuracy on these three criterial learning tasks, and will repeat the study-test sequence until they reach that criterion. We will probe for confidence (e.g., *how confident are you in your answer, on a scale from 1-7?*) and metacognitive ratings (e.g., *how likely are you to be able to point to the theater when standing at the restaurant, on a scale from 1-7?*) after each study trial. See Appendix C for details on these three criterial learning tasks. In our prior study, all participants reached the 80% criterion for all tasks within 3 study-test cycles; this is expected to take about 30-35 minutes. If a participant cannot reach criterion within 5 study-test cycles, they will be dismissed from the study.

2. Cognitive Testing Under Stress: Participants will be brought into the virtual reality system and will stand in front of a large array of visual displays with surround sound and a vibrating platform (for multi-sensory immersion). Study personnel will then help the participant don the shock belt, bioharness, and eye tracking glasses (see Appendix D for details on these devices). The CES device will not be donned. After a brief eye tracker and weapon aim calibration, a practice/familiarization phase, and confirming that all devices are successfully communicating data, three cognitive tests will be performed in an interleaved manner. First, a recognition memory test will involve recognizing previously learned suspicious targets in the virtual environment while ignoring visually similar distractor objects; participants respond with a simulation rifle (see Appendix D) and an attached game controller. Second, a spatial orienting task that involves pointing towards a series of distant (out of visible range) target landmarks (e.g., bank, theater) in the virtual environment and estimating distance to those landmarks; participants reorient themselves and select a direction by using the simulation rifle and attached game controller.

   Finally, a decision-making task that involves a simulated entry control point: participants will judge whether an approaching person is friendly, or enemy based upon their camouflage pattern and/or objects being carried, and use this information to either let them pass or fire upon them (using the rifle). During this task, there may be one (i.e., low workload) or many (i.e., high workload) potential targets simultaneously appearing in the scene. Participants will also provide confidence ratings for their decisions during these tasks. During task performance, we will use remote TRACKPACK/E infrared cameras (ART, GmbH, Germany) to continuously track and log weapon position, head and eye gaze position, and physiological measures of the bioharness. Depending upon which rifle is used for the study (based on availability and functionality), it may or may

not also collect inertial measurement unit (IMU) data with an integrated sensor (i.e., roll, pitch, yaw, *xyz* position).

During the cognitive testing, if an incorrect response is provided by participants (e.g., > 45˚ absolute angular error during the spatial orienting task, incorrectly firing upon a friendly or incorrectly letting an enemy pass during the decision-making test), the system will automatically trigger a torso shock. The shock belt will be set at level 3, which (in our previous studies) participants tend to find uncomfortable and stressful, but not painful. If participants cannot tolerate the shock and ask for it to be disabled, we will pause data collection and give them a 5-minute rest period and ask whether they would like to try it again. If they respond *yes*, we will resume data collection (and repeat this rest-retry procedure as necessary). If they respond *no*, we will ask whether they are willing to be rescheduled and try again on another day; if they respond *no*, we will not reschedule. If they respond *yes*, we will reschedule the participant for another session. If during two consecutive sessions they cannot tolerate the shock, they will be dismissed from the study.

Upon completion of testing, participants will return to the private seating area, where they will complete the additional saliva sampling and STAI-S.

3. Saliva Sampling: Participants will provide saliva samples using the Salivabio Oral Swab (SOS) method from Salimetrics. This sampling method involves participants placing a hygienic absorbent swab under their tongue for 2 minutes, and then placing the swab into a vial. The advantage of the SOS method is that participants can do it entirely independently (opening the swab package, placing the swab under the tongue, removing swab from mouth, placing swab in the vial, closing the vial) and without direct contact from the experimenter. Using this method, saliva samples will be taken immediately prior to Questionnaires, immediately prior to Cognitive Testing Under Stress, immediately following Cognitive Testing Under Stress (accompanied by emotional state questionnaires), and at 20-minutes, 40-minutes, and 60-minutes after. In this manner, we will collect a total of 5 saliva samples during this Baseline session.

After completing this series of saliva samples, questionnaires, and tasks, participants will be briefed on the CES session procedures. Participants will then be released for the day. Overall, we expect the Baseline session to last approximately 3 hours, as detailed in the below example schedule:

0800-0815	Questionnaires (daily checklist, STAI-S/T, demographics)
0815-0820	CES Thresholding
0820-0825	Saliva Sampling
0825-0900	Criterial Learning
0900-0920	Don Bioharness & Shock Belt; Don & Calibrate Eye Tracking Glasses & Rifle
0920-0945	Cognitive Testing Under Stress
0945-0950	Saliva Sampling & STAI-S
1005-1010	Saliva Sampling & STAI-S
1025-1030	Saliva Sampling & STAI-S

1045-1050	Saliva Sampling & STAI-S
1050-1100	CES Session Familiarization
1100	Dismissal

The CES Sessions

Participants will then return to the laboratory at CABCS or visit the laboratory at the DEVCOM Soldier Center on a daily basis (Monday through Friday) for up to six consecutive weeks, or until they achieve a total of 20 sessions (whichever occurs first). They will be scheduled at a semi-consistent time every day (i.e., in morning or afternoon), and complete a session expected to last about 30-60 minutes. During each session, participants will complete the daily participant questionnaire, the STAI-S, a CES stimulation period involving Active or Sham stimulation, a CES secondary administration (as needed), and the STAI-S.

If a participant answers 3 or higher on any question on the daily participant questionnaire, they will be rescheduled for a different day. If they report 3 or higher on any of the questions for two consecutive sessions, they will be withdrawn from the study.

1. Active or Sham CES Administration: Participants will be asked to continue sitting and relaxing in the chair and the electrode cable (still attached to their earlobes) will be connected to a different CES device. Two types of CES devices will be used: an Active device or a Sham device. The Sham devices are manufactured by EPII and look and behave exactly like the Active devices, however they do not actually send any electrical current to the electrodes. The two versions of the devices will be coded as devices A and B, and neither the experimenter nor participant will know which is Active and which is Sham; only the Principal Investigator will keep the key to decode whether the device is Active or Sham, to be revealed only during data analysis (or to a medical professional if there is an adverse event). The experimenters and participants will only know which coded device (device A or B) to use during CES sessions. In both conditions, the device will be powered on and set to the intensity identified during the CES Individualized Thresholding phase, and a countdown timer (displayed on the device screen) will begin.

   After the 20 minutes of stimulation have elapsed, the device will automatically turn off. Immediately after the 20-minute CES administration, participants will complete the STAI-S questionnaire a second time, and the CES side effects questionnaire.

2. CES Secondary Administration: If a participant answer is ≥3 to one or more questions on the CES side effects questionnaire (in *both* the “presence and intensity” and the “relation to stimulation” sections), we will follow the manufacturer’s guidelines to administer additional CES until symptoms subside. According to the manufacturer’s internal research (derived from an analysis of over 10,000 CES sessions), brief secondary CES administrations can help

alleviate most side effects in most circumstances. Specifically, we will provide successive 5-minute secondary CES administrations at the individualized intensity. After each secondary CES administration, there will be a 1-minute break when the participant will again complete the CES side effects questionnaire. The successive 5-minute CES administrations with interleaved CES side effects questionnaire will be repeated up to twice, or until the negative sensations subside (i.e., all ratings ≤ 3), whichever occurs first. The number of secondary CES administrations necessary to resolve vestibular sensations or anxiety (i.e., 1 or 2) will be recorded by the experimenter. If the participant's reported symptoms do not subside with secondary CES administration, and this occurs for two consecutive CES sessions, they will be withdrawn from the study. Participants will then be dismissed until their next session.

Below is an example schedule for a CES session:

0800-0810	Questionnaires (daily checklist, STAI-S)
0810-0830	CES Administration
0830-0835	Side Effects Questionnaire
0835-0850	Secondary CES Administration (as necessary)
0850-0900	STAI-S Questionnaire
0900	Dismissal

Our goal is to have each participant complete a total of 20 CES sessions, though we do expect some attrition (see Section 3.0). We will prioritize the number of sessions (i.e., reaching 20) over the duration of the study; for example, some participants may complete the 20 sessions study in a 5-week period (rather than 4-week period) due to scheduling issues. We will allow up to 6 weeks to complete the study; if the 20 sessions are not completed in 6 weeks, the participant will be removed from the study. Variability in completion time (i.e., inter-session lag) will be recorded and used as a covariate in statistical analyses.

At the end of the final CES session (session 20), participants will complete the Final CES Session Questionnaire (Appendix A) to assess whether our blinding was successful, and their opinions about CES.

Final Testing Day: Follow-up

The final testing day will serve as the follow-up session to assess whether 20 sessions of Active (versus Sham) CES will impart any changes to the biochemical, physiological, affective, or behavioral responses to stress induction. This session will be held at approximately the same time of day as the thresholding and baselining session, within 5 days of the last daily CES session. The procedures for this session will precisely match the thresholding and baselining session but without the thresholding phase. After completing the final testing day, participants will have completed the study.

Data Analysis

We will analyze data from our biochemical, physiological, affective, and behavioral measures using Generalized Estimating Equations (GEEs) and/or Analyses of Variance (ANOVAs) to examine changes over the 20 CES sessions, changes from baseline to follow-up, and changes due to Active versus Sham CES. An effect will be deemed statistically significant if the likelihood of its occurrence by chance is $p < 0.05$; to reduce the likelihood of a Type I error, any follow-up comparisons (e.g., t-tests) will use a Bonferroni-corrected alpha criterion.

6.1 Additional Safeguards for Special Populations:

This research is funded by the Department of Defense, and the funding agency is interested in how military personnel respond to CES administration; for that reason, we will prioritize the recruitment of military personnel, and secondarily recruit from civilian populations as required to fulfill our sample size goal.

When recruiting military personnel to participate in this research, we will conform to additional safeguards for the protection of human subjects outlined in DoD Instruction (DoDI) 3216.02. These include additional safeguards to protect participants who may be more vulnerable to coercion or undue influence.

7.0 Investigational Medical Devices:

For the CES sessions, we will be using the Alpha-Stim AID cranial electrotherapy stimulator (CES) medical device, which is an FDA cleared, handheld medical device used for the treatment of anxiety, insomnia, and depression (i.e., AID; see Appendix D).

Because our study is using the device with healthy participants, the device is being used off-label in a manner that is for *investigational use only*. As detailed in the Informed Consent Form, this means that the device is being used in a study examining its safety or effectiveness, and not for the treatment, diagnosis, or prevention of any disorder.

Our intent is to test the effectiveness of the device; its safety has been documented in over 100 independent clinical research studies.

8.0 Incomplete Disclosure or Deception:

We will not use incomplete disclosure or deception. Participants will be informed that they will be randomly assigned to a Sham or Active condition.

9.0 Recruitment Methods:

Participants will be recruited through three mechanisms: (1) Recruitment of Soldier Center Human Research Volunteers (HRVs), (2) Recruitment of Active Duty Units utilizing the United States Army Forces Command (FORSCOM) troop request process, and (3) Recruitment of Civilians. These are detailed below:

1. Recruitment of Soldier Center Human Research Volunteers (HRVs): Participants will be recruited from Soldiers in the Human Research Volunteer (HRV) program

assigned to Headquarters Research and Development Detachment (HRDD) at DEVCOM SC. Personnel from the Human Research Volunteer Program Office (HRVPO) will schedule a recruitment briefing about the study for potential HRV participants. Superiors of the potential HRV participants (e.g., unit officers, senior NCOs, and equivalent civilians) in the chain of command shall not be present at the recruitment briefing in which members of units under their command are afforded the opportunity to participate as human subjects. This briefing will be conducted in a group setting of potential HRV participants. The briefing will be completed either in-person or remotely (with the PI or AI briefing remotely or via a recorded slideshow, see PowerPoint notes for script).

A representative of the HRVPO may be present during this briefing. Potential participants will be asked to participate in the study after being informed of the purpose of the study, the nature of the test conditions, the risks associated with the study, all procedures affecting a participant's well-being, a participant's right to discontinue participation at any time without penalty, and that there is no penalty for deciding not to participate in the study.

Potential participants will have the opportunity to ask questions and will receive copies of the Informed Consent Form to review. After the briefing, potential participants will be given time to determine whether or not they wish to participate. Subsequent to the briefing, the HRVPO will coordinate with the Principal Investigator (PI) to obtain any additional information about the study requested by the potential participants. Those individuals interested in participating, will communicate this to the HRVPO so a consent meeting with the PI or appropriate research team member can be scheduled.

2. Recruitment of Active Duty Units: Participants will be recruited from active duty units utilizing the United States Army Forces Command troop request process or other appropriate request processes for troop support. Principal Investigators (or Associate Investigators) will provide information about the study to support these requests. The Unit Commander's support for Soldiers being recruited to voluntarily participate in research will be documented in a letter of support. The PI will obtain this letter prior to the study briefing, maintain it as part of study records, and send to the DEVCOM SC Human Research Protection Program Office.

   Participants from active duty units who agree to support the study will be briefed on the purpose of the study, the nature of the test conditions, the risks associated with the study, all procedures affecting a potential participant's well-being and fitness for duty, a potential participant's right to discontinue participation at any time without penalty, and that there is no penalty for deciding not to participate in the study. Superiors of the potential participants (e.g., unit officers, senior non-commissioned officers (NCOs), and equivalent civilians) in the chain of command shall not be present at the recruitment briefing in which members of units under their command are afforded the opportunity to consider participation as human subjects. The briefing will be completed either in-person (at Soldiers' home stations) or remotely (with the PI or AI briefing remotely or via a recorded slideshow, see PowerPoint notes for script).

   Potential participants will be asked to participate in the study after being informed

of the purpose of the study, the nature of the test conditions, the risks associated with the study, all procedures affecting a participant's well-being, a participant's right to discontinue participation at any time without penalty, and that there is no penalty for deciding not to participate in the study.

Potential participants will have the opportunity to ask questions and will receive copies of the Informed Consent Form to review. After the briefing, potential participants will be given time to determine whether they wish to participate.

3. Recruitment of Civilians: Civilians will be recruited from the community through websites advertising research [e.g., Tufts Paid SONA (i.e., not linked to course credit)], see Appendix B for recruitment language. This study has no ties to classroom grades and/or performance. Potential participants will be informed that there is no penalty for deciding not to participate. Interested participants will contact an associate investigator listed in the recruitment material to schedule a consent discussion.

   Participants who agree to learn more about the study will be informed about the purpose of the study, the nature of the test conditions, the risks associated with the study, all procedures affecting a participant's well-being, a participant's right to discontinue participation at any time without penalty, and that there is no penalty for deciding not to participate in the study.

   Potential participants will have the opportunity to ask questions and if they are interested in participating, they will sign the Informed Consent Form. Participants may directly contact the Principal Investigator or Associate Investigators for additional information at any time after the consent discussion. A signed copy of the Informed Consent Form will be provided to the participant following the consent discussion.

10.0 Consent Process:

Recruitment is detailed above in the Sample Recruitment section. Herein we will describe the next phase: consenting.

1. *Consent of Soldier Center Human Research Volunteers (HRVs):* For the HRV participants that expressed interest in participating in the study, a representative from the HRVPO will schedule an informed consent meeting between the potential HRV participants and the Principal Investigator or a designated research team member(s) responsible for consenting participants. The Principal Investigator or study team member consenting the HRV participants will meet with them to review the consent document and answer any questions the participant may have. The HRV participants will have an opportunity to meet individually with the study team member if they would like to discuss or ask questions about the study. Those individuals who agree to participate in the study will express their understanding by signing an Informed Consent Form. The study team member obtaining the consent will also sign the form. The study team member obtaining the consent will retain a signed Informed Consent Form and a signed copy of the Informed Consent Form will be provided to the participant. A representative from the HRVPO may also be present during consenting although

their presence is not required for consenting. Consenting will take place at DEVCOM SC; the consent form includes screening questions. Participant IDs are assigned upon beginning study participation and are never included in email communication. The PI and associate investigators communicating via email safeguard this information by storing all in a designated folder within their email inbox.

2. *Consent of (non-HRV) Active Duty Units:* For the active duty participants from FORSCOM, that expressed interest in participating in the study, an informed consent meeting will be scheduled between the potential active duty participants and the Principal Investigator or a designated research team member(s) responsible for consenting participants. The Principal Investigator or study team member consenting the participants will meet with them to review the Informed Consent Form and answer any questions the participant may have. The active duty participants will have an opportunity to meet individually with the study team member if they would like to discuss or ask questions about the study. Those individuals who agree to participate in the study will express their understanding by signing an Informed Consent Form. The study team member obtaining the consent will retain a signed Informed Consent Form and a signed copy of the Informed Consent Form will be provided to the participant. Consenting will take place at the participants' home unit. Participant IDs are assigned upon beginning study participation and are never included in email communication. The PI and associate investigators communicating via email safeguard this information by storing all in a designated folder within their email inbox. The consent form includes screening questions; consenting will take place either at Tufts University, the DEVCOM Soldier Center, or at the units' home station. Participants receive a study in-brief when they travel to participate, explaining the study once more.

3. *Consent of Civilians*. For civilians who respond to the research advertisements (in the form of website postings and emails), the Principal Investigator, associate investigators, or research assistants will schedule the informed consent interview on proposed research and be present at the interview. The PI will distribute the Informed Consent Form for the study so that the attendees have the form in hand at the time of the interview. At the interview, the PI will provide a briefing to the potential participants, using the approved briefing slides. After the briefing, potential participants will have the opportunity to ask questions. Those individuals who agree to participate in the study will express their understanding by signing an Informed Consent Form. The study team member obtaining the consent will retain the original completed Informed Consent Form, and a copy will be offered to the participant.

11.0 Compensation:

Military personnel who choose to participate in this study will not be compensated. Civilians who choose to participate in this study will be compensated at a rate of $20/hour.

12.0 Economic Burden:

There is no economic burden imposed by this study.

13.0 Recording with Audio, Video, or Photographs

This study may optionally involve recording video and/or taking photographs of participants performing tasks. If participants agree (on the Informed Consent Form) to have these collected, digital videos and/or photographs would be used for educational, reporting, or illustration purposes and will be stored for at least 3 years after closure of the protocol. The Principal Investigator (PI) will store these digital media files on their password-protected computer, and no other study team members will have access.

14.0 Potential Benefits to Participants:

There are no direct benefits to the participants participating in this research. The results may help better inform future research on how CES may influence sympathetic nervous system responding under conditions of stress.

15.0 Risks to Participants:

We anticipate three primary risks:

1. Cutaneous Irritation due to StressX Belt: Mild electrical shock to the torso is commonly used as an effective, safe method of inducing acute stress in a laboratory context. The StressX Pro Belt meets strict safety regulation standards set forth by the International Electro Technical Commission (document IEC 479-2:1987). Specifically, an excess of 5000mJ of transient or capacitive discharge is required to produce direct serious health risk and the electrical stimulus system in the study (SETCAN StressX Pro Belt) has a maximum output of 92mJ (< 1mA over 150ms), more than 98% lower than the threshold to cause health risk (IEC 479-2: 1987). The shocks we deliver will be unpleasant but not painful, set to intensity level 3 (of 5) which delivers shock for only 60-80ms. In our recently completed study (DEVCOM AC IRB protocol 18-007), we completed over 100 sessions using this shock belt, with over 1,000 shocks delivered on (self-selected) intensity levels 1 through 3. With these settings, only one participant reported mild skin irritation, which the AC IRB determined did not qualify as an UPIRTSO. In the present study, we will use a fixed shock intensity of 3; while low probability (we estimate 1-2% incidence given our prior study), if at any time a participant can no longer tolerate the shock (either psychologically, or they experience anything more intense than mild skin irritation), we will reschedule their session. See Study Withdrawal Guidelines (Section 3.0) for details. Participants will be informed of shock belt-related risk, and that they can opt-out at any time, in the Informed Consent Form.

2. Side Effects due to CES Administration: Cranial electrotherapy stimulation (CES) has very low incidence of side effects; when they do occur, they tend to include transient dizziness, vertigo, headache, nausea, tinnitus, lightheadedness, or skin irritation under the electrodes. The manufacturer of the Alpha-Stim AID (EPII) suggests that side effects are seen in less than 1% of people using the device, and they are “all mild and self-limiting.” To gain a

more objective assessment, we reviewed some CES studies that were not associated with (i.e., funded by, conducted by) the manufacturer. In one of the largest CES studies to date, none of the 115 participants (N = 60 in Active CES group) reported any side effects with six weeks of daily (one hour per day; 0.5Hz at 100μA) CES administration (Barclay & Barclay, 2014). In another similarly designed study using CES at 0.5Hz and 300μA, 3 of the 12 participants dropped out of the study due to mild side effects (2 with dizziness, 1 with headache) (Bystritsky et al., 2008). In another study, participants receiving Active CES treatment for 2 weeks (20 minutes per day; N = 7; 2mA at mixed frequencies) showed similarly low rates of reported side effects as the Sham CES group (McClure et al., 2015). In our own recent study (0.5Hz, 100μA), reports of side effects were also no more frequent in the Active versus Sham CES group (Brunye et al., 2022). Given these results, we estimate that the rate of side effects with our proposed design will be very low (i.e., less than 25%), especially given our individualized daily thresholding procedure. See Study Withdrawal Guidelines (Section 3.0) for details. Participants will be informed of the risk of CES side effects, and that they can opt-out at any time due to discomfort with CES administration, in the Informed Consent Form.

3. Psychological distress due to rifle marksmanship: During the baseline and follow-up sessions, participants will use a simulated rifle to shoot targets in virtual reality. This is like a first-person shooter video game, which research shows can variably activate positive (i.e., reward; Koepp et al., 1998) or negative (i.e., sadness; Ravaja et al., 2008) emotions. There are vast inter-individual differences in the emotions experienced during or after video game play (Avila et al., 2008; Ravaja et al., 2008), making it difficult to predict which individuals will experience positively versus negatively valenced emotion. While we believe the risk of psychological distress due to this experience is low, it is possible that participants will experience distress during or after the rifle marksmanship task (such as sadness, remorse, fear, or anxiety). It is unknown whether the rate of distress will be higher or lower in civilians versus military personnel; it is also unknown whether the rate of distress will be higher or lower in participants with prior video game experience (i.e., evidence for desensitization in this manner is equivocal; Regenbogen et al., 2010). Most importantly, if any participants report negative emotion to the research team, we will refer Soldier participants to the Office of Medical Support and Oversight (OMSO) at the DEVCOM Soldier Center, refer Tufts University student participants to the Tufts University Heath Service, and recommend other participants (non-Soldier, non-Tufts affiliated) to speak with a qualified mental health practitioner.

We have no reason to believe that the acute stress response will differ between military personnel and civilian participants. In fact, research suggests that military personnel do not necessarily show higher levels of resilience to stress than civilians (Sohail & Ahmad, 2021). Furthermore, the primary population of military personnel recruited for this study (human research volunteers) has very limited military experience; furthermore, many will have a military occupational specialty (MOS) that is not infantry (e.g., engineer, aviation, transportation, maintenance), and most (if not all) will have never been deployed outside of the US or experienced combat. For these reasons, we do not have any compelling reason to believe they will have higher resilience to acute stress relative to civilians.

We do not anticipate any issues with virtual reality sickness/simulator sickness given that there is no virtual movement or optic flow through the virtual environment. Rather, participants are stationary in the environment; indeed, in our recently completed protocol, we experienced no reports (in over 200 sessions) of any typical symptoms of simulator sickness (e.g., nausea, headache).

If at any time, a **civilian** participant feels that they have been injured by the shock belt or CES and needs emergency care, Emergency Medical Care will be provided by calling 911, consistent with the Tufts University emergency response. It cannot be determined in advance which hospital or clinic will provide care. The participant and/or their insurance will be responsible for medical expenses.

If at any time, a **Soldier (i.e., DOD healthcare beneficiary)** participant feels that they have been injured by the shock belt or CES and needs emergency care, Emergency Medical Care will be provided by calling 911, consistent with the Tufts University and DEVCOM Soldier Center emergency response. This care includes but is not limited to free medical care at Army hospitals or clinics.

16.0 Withdrawal of Participants:

As in all human use protocols, participants will be fully briefed on the purpose, risks and procedures of the study before being asked to participate; they will also be informed of their right to withdraw from the study at any time without prejudice. During all sessions, participants will be allowed to stop participation at any time if they experience any side effects (or for any other reason).

As detailed in Section 3.0, we will also withdraw participants from the study based on several criteria (see Appendix E for flowchart). All data collected up to the point of withdrawal will be destroyed and not analyzed.

17.0 Data Management and Confidentiality:

All data and personal information obtained during this protocol will be considered privileged and held in confidence. Each participant will be assigned a unique subject ID number. This subject ID number will not contain any personal identifiers such as: name, social security number, address, date of birth, zip code, etc. This subject ID number will be the only identifier on all data collection instruments (i.e. questionnaires, collection forms, data files, and computer records), and used during data collection and analysis. Only the study investigators will have access to the Informed Consent Forms containing participant names and signatures. A master list linking the participants' personal identifiers with the study participant ID number (i.e., a link) will be kept as an electronic document on a password-protected government computer.

Only the PI will have access to the master file (link). Associate investigators and study staff will have access only to the coded data and not to personally identifiable information. The document that links participants' names to their ID number will be kept for three years after closure following verification and validation of the data, when it will be destroyed. The Informed Consent Forms will be destroyed three years after the study is closed. When the results of this protocol are published or disseminated in any manner,

no information will be included that could reveal identity. Complete confidentiality cannot be promised to military participants because information bearing on the military participants' health may be required to be reported to appropriate medical or command authorities.

Data will be backed-up after each session and inspected by a member of the research team. All data and personal information obtained during this protocol will be considered privileged and held in confidence. Each participant will be assigned a unique subject ID number. This subject ID number will not contain any personal identifiers and will be the only identifier on all data collection instruments (i.e. questionnaires, collection forms, and computer records). Furthermore, when the results of this protocol are published or disseminated in any manner, no information will be included that could reveal identity.

Participants will complete all questionnaires via Qualtrics, which is GDPR (General Data Protection Regulation) compliant, and/or tablet computers running a local software package (e.g., PsychoPy, SuperLab, or custom C# software). All data will be coded, with the only identifier being the participant's participant ID number assigned at the beginning of the study. Access to Qualtrics data is secure and individual permissions set prior to the study with access privileges. Once data have been processed, organized, and provided to the researchers from both Qualtrics and tablet interfaces, all electronic locally stored data will be deleted.

The Informed Consent Forms and any paper copies of the collected data will be stored in a locked file cabinet in the Principal Investigator's office (DEVCOM SC, Building 45, L002) for at least 3 years after closure of the protocol. Data will be transferred via DOD SAFE, OneDrive, and/or locally approved electronic data transfer sites or cloud applications to password-protected computers or held on secure, password-protected servers and hard drives for subsequent data analysis. Once the protocol is complete, data will be verified and archived to a storage medium (e.g. approved data repository or encrypted hard drive) or in the case of hard copies stored in locked file cabinets at the Soldier Center for at least 3 years after closure of the protocol. This data will not contain identifying information.

18.0 Provisions to Protect the Privacy and Confidentiality of Participants and the Research Data:

Participants will be informed of the possibility of future use for the data that is collected as part of the consenting process. Data files may also be shared with other government and non-government collaborators and contractors for future use.

The data collected in the current study will be used in future studies. There is a statement in the Informed Consent Form to obtain consent for future use of the data collected. Consent to future use of all data collected will be required for participation in the current study and is included under the inclusion criteria.

Participants in this study will consent to allowing their samples to be saved for future research that may or may not be directly related to the hypotheses set forth in this protocol. Therefore, once the protocol is closed, samples will be retained for future analyses that may or may not align with the aims set forth in the present protocol. In addition, the data may be used as inputs for developing models to assess measures of

performance, readiness, recovery, and/or lethality at the individual, small unit and platoon-level. DEVCOM Soldier Center or other approved entity may employ a machine learning framework within the database to identify groups of individuals who show an outcome of interest, and untargeted analysis will search for a set of dependent measures that are sensitive to conditional changes (i.e., with versus without an environmental stressor). In addition, machine learning algorithms may be used to down-select dependent measures that correspond to other classification standards.

In all cases, future use of data collected as part of this protocol will be restricted to deidentified data after the master linking document (linking participant identity to participant ID) has been destroyed.

19.0 Provisions to Monitor the Data to Ensure the Safety of Subjects:

The PI will periodically inspect the data, especially the CES side effect questionnaire responses, to ensure that research staff are following all mitigation procedures outlined in this protocol.

20.0 Compensation for Research-Related Injury:

There will be no financial compensation offered for research-related injury.

21.0 Data Sharing and Specimen Banking:

See Section 18.0.

22.0 International Research:

N/A

23.0 Multiple sites:

This study is a collaboration between the Center for Applied Brain and Cognitive Sciences (CABCS) at Tufts University (Medford, MA) and the DEVCOM Soldier Center (Natick, MA). Following initial and continuing Tufts University IRB approvals, the research team will electronically transmit all study protocol documents to the DEVCOM Soldier Center HRPP office and seek secondary Human Research Protections Office (HPRO) approvals prior to commencing participant recruitment or any other study procedures. Any modifications required by the Soldier Center HRPO to secure concurrence will be submitted in an amendment to the Tufts University IRB. The PI will be responsible for sharing (via email) the most recent protocol, approvals, and consent documentation. Any modifications will be done through the Tufts University IRB and be communicated to the Soldier Center HRPO, in accordance with institutional agreements. The PI will closely monitor research staff progress at both data collection sites.

24.0 Reliance Agreements/Single IRB:

The Tufts University IRB will serve as the IRB of record.

25.0 Qualifications to Conduct Research and Resources Available:

Because we recently completed data collection on a very similar study (DEVCOM Armament Center IRB #18-007), research assistants have been trained by project investigators on the execution of all tasks. Furthermore, investigators attended a virtual training seminar offered by the manufacturer of the Alpha-Stim AID device (EPII), which included practical and safety-related topics for the effective use of the device.

All study personnel have completed CITI training required for social and behavioral research. The PI, Dr. Okano is a Cognitive Neuroscientist and Principal Investigator at the Tufts Center for Applied Brain and Cognitive Sciences, and has over 10 years of research experience planning, conducting, and managing research in the cognitive sciences. She holds a PhD from Tufts University in Cognitive Neuroscience and completed post-doctoral training at Massachusetts Institute of Technology (MIT). At CABCS, she successfully manages the execution of large-scale research projects involving data collection from neurophysiological and physical sensors and has substantial experience applying advanced analytical approaches to multimodal data streams.