Cover Page

Title:

HIRREM for Mitigation of Symptoms of Military-Related Traumatic Stress

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Comments:

This is the IRB-approved study protocol that was approved 10/06/2017.

There have been no other substantive changes. Since this protocol approval, there have to date been nine continuing reviews by the IRB, with the most recent continuing approval being granted on 10/27/2022.

Title:

HIRREM for Mitigation of Symptoms of Military-Related Traumatic Stress

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Abstract:

Background: Post-traumatic stress disorder (PTSD), and traumatic brain injury (TBI) are the signature injuries of recent military conflicts (OIF/OEF/OND). Physical and emotional trauma due to blast injury exposures, 24/7 duty, sleep deprivation, and multiple deployments, are taking a toll on the functional brain capability of many soldiers, leaving lingering symptoms preventing return to duty or re-integration into civilian life. Effective noninvasive interventions for PTSD are lacking. High-resolution, relational, resonance-based, electroencephalic mirroring (HIRREM®) is a noninvasive, closed-loop neurotechnology to facilitate recipient-unique relaxation and auto-calibration of cortical neural oscillations by reflecting auditory tones in near real time. We have previously reported that HIRREM was associated with reduced symptoms of insomnia in a randomized clinical trial, and with reduced PTSD symptom scores in a feasibility trial, with no serious adverse events identified.

Objectives: The primary objective of this pilot clinical research study is to evaluate the effectiveness of HIRREM in combination with usual care for mitigating self-reported symptoms of military-related traumatic stress in military personnel and veterans. The primary outcome will be change in the PTSD Checklist-Military (PCL-M) symptom inventory, secondary outcomes including other self-reported symptom inventories, as well as physiological and functional measures.

Methods: This will be an open label, single-site, clinical research study. Up to 40 active duty military personnel, or recent Veterans, age 18 or older, who have been diagnosed with PTSD, have been treated for, are referred by military medical personnel for, or have active symptoms of military-related traumatic stress, with or without mild TBI, will be recruited to receive up to 24 HIRREM sessions over 2 weeks. The primary outcome will be differential change in the PCL-M from baseline to completion of HIRREM sessions. Secondary measures include differential change in the Insomnia Severity Index (ISI), the Center for Epidemiological Studies Depression Scale (CES-D), an anxiety measure (GAD-7), a quality of life measure (EQ-5D), an autonomic symptom measure (Compass 31), and a daily sleep diary, along with physiological measures including heart rate (HR), and blood pressure (BP), with calculation of heart rate variability measures (HRV), baroreflex sensitivity (BRS). Functional measures will include reaction time (drop-stick task), and grip strength (hydraulic dynamometer). If there is a history of TBI, a Rivermead Post-Concussion Symptoms Questionnaire (RPQ) will be added. There will be pre- and post-intervention data collection for all measures (baseline, V1, and at completion of HIRREM sessions, V2). Self-report measures will be repeated by phone at 1, 3, and 6 months after completion of sessions (V3, V4, and V5 respectively). The sleep diary will be maintained from V1 until V3. A brainwave assessment will be obtained at V1.

Sample Size and Statistical Analysis: The primary statistical intent of this pilot clinical research study is to generate estimates of effect size in order to plan subsequent randomized studies. Given that outcomes will be collected at multiple follow-up time points (V2, V3, V4, and V5), effect estimates will be based on generalized linear models (GLM), which can accommodate within-subject correlations due to repeated assessment over time. While no directly relevant, controlled study data is currently available, a recent, national study of prolonged exposure therapy in veterans with PTSD (N=1,888) indicated a mean decrease on the PCL-M of 15.2 points (standard deviation = 20.0). Assuming a similar level of variability

in our population and a sample of 10 subjects, we expect to generate a 95% confidence interval for the mean change on the PCL-M with a half-width (1.96 x standard error) of ± 12.4 points.

Importance: This pilot study addresses an urgent health need for military personnel and veterans. It extends the results of prior work, but in a more homogeneous, focused sample of active duty military personnel and Veterans, and also extends the period of follow up. Positive results would suggest a potential role for HIRREM as a noninvasive, non-drug alternative for symptom reduction with military-related traumatic stress, and justify larger studies, or controlled trials in this population.

Background:

Post-traumatic stress disorder (PTSD) may occur in response to a traumatic event with symptomatology related to re-experiencing of phenomena, avoidance/numbing, and increased states of arousal[1]. PTSD has a lifetime prevalence of 6.8% in the USA[2] and often co-exists with physiological and psychological comorbidities such as traumatic brain injury (TBI)[3] and depression[4]. Additionally, insomnia and frequent sleep disturbances are also considered core components of PTSD[5].

PTSD and TBI are the signature injuries of recent military conflicts (OIF/OEF/OND). Physical and emotional trauma due to blast injury exposures, 24/7 duty, sleep deprivation, and multiple deployments, are taking a toll on the functional brain capability of many soldiers, leaving lingering symptoms preventing return to duty or re-integration into civilian life. PTSD has been diagnosed in more than 150,000 military service members since the year 2000[6], is often associated with traumatic brain injury, and frequently includes symptoms of insomnia and depression.

Medications are often used to help control symptoms of PTSD, however pharmacologic treatments may involve side effects and intolerability in a portion of individuals. Cognitive-behavioral approaches are effective for many patients, yet these therapies may not generalize to every patient as the focus is limited to only a portion of the complex presentation of PTSD. These therapies do not directly address the autonomic and physiological dysfunction exhibited by PTSD patients. Additional interventions, especially noninvasive, non-drug approaches, addressing these autonomic and physiological pathways, particularly upstream sources, may provide important alternative therapeutic options for patients with PTSD.

Normally, oscillatory frequency amplitudes exhibit a roughly even distribution throughout the cortex ("balanced") and among component frequency bands in any given cortical region (in "proportion"). Though some research has failed to find any differences in EEG asymmetry between patients with PTSD and controls[7], specific spatial imbalances and suboptimal proportions of frequencies have been shown. Anxious-arousal, a symptom type within PTSD, has been associated with right hemisphere frontal and posterior asymmetry[8] [9]. Quantitative EEG (QEEG) analysis has shown increased theta and beta activity for patients with PTSD)[10]. Low-resolution electrical tomographic analysis (LORETA) of PTSD patients has exhibited significantly decreased 4-5 Hertz (Hz) theta band activity over the right temporal lobe as well as decreased 6-7 Hz theta band activity over the frontal lobes, bilaterally[11]. Similarly, imbalances have been reported in TBI[12], insomnia[13], and depressive disorders[14].

High-resolution, relational, resonance-based electroencephalic mirroring (HIRREM®, Brain State Technologies, LLC, Scottsdale, AZ) is a noninvasive, closed-loop feedback neurotechnology that monitors brain electrical activity at high spectral resolutions, using 2-channel recordings, and provides acoustic stimulation (auditory tones of variable pitch and timing) that is derived from real time changes in dominant frequencies. The intention of HIRREM is to facilitate relaxation and auto-calibration of neural oscillatory dynamics. Although the exact mechanism remains to be clearly defined, HIRREM allows the brain to move on its own towards a more balanced, relaxed state[15].

The HIRREM system uses unique sensors which are placed on the scalp and held in place using standard EEG conductive paste. The sensors precisely measure and map the surface EEG frequencies and amplitudes of the brain throughout its major lobes. The sensors utilize embedded computer chips that improve filtering of electromagnetic interference/artifact, allow collection of more precise frequency data, and thus demonstrate functional aspects of the brain in greater detail.

The choice of the specific auditory tone to be reflected back to the user is made through a mathematical algorithm which identifies the dominant frequency of the individual's EEG spectrum in a floating middle range, in a given instant of time. The dominant EEG frequency is translated to an auditory tone, specific to that brain EEG frequency, and is played back to the individual through ear buds, with a delay of as little as 8 milliseconds. Since the brain is a dynamic organ, with constantly changing frequencies, the subject hears a series of auditory tones. It appears that a phenomenon of resonance occurs between the tones being fed back, and the oscillating neural circuits in the recipient's brain. The operational theory is that resonance between the tones, provided as acoustic stimulation, creates an opportunity for the brain to either dissipate or accrete neural energy in an extremely subtle, noninvasive way. Neural-musical resonance may be a mechanism for auto-calibration of neural networks.

Like any polished mirror, HIRREM is extremely precise, and also "non-judgmental." There is no operant conditioning, no "learner-in-the-loop, and no imparting of normative information by the HIRREM provider, that would aim to explicitly reward, inhibit, entrain, instruct, re-program, or in any other way to over-write the brain's existing pattern of activity. Thus, HIRREM is fundamentally different from other available technologies such as binaural beats, auditory or photic stimulation, "synchronization" and other "brain-enhancement" methodologies.

Relevant Pilot Data:

Since 2011, the Department of Neurology has carried out a series of research studies to evaluate the effects, and potential benefits, of HIRREM for a variety of conditions. As of this submission, a total of 275 participants have been enrolled in one of four clinical research studies, and have received an estimated total of 3,400 HIRREM sessions. Some key preliminary results are summarized below, with relevance for symptoms expected in this military cohort with PTSD.

PTSD in the Developmental Study:

Participants with symptoms from a wide variety of conditions have been enrolled in an ongoing, IRB-approved, open label, Developmental (DE) Study, designed to evaluate the feasibility of HIRREM, to

provide pilot data that can be used as a foundation to both transition to controlled trials, as well as to seek outside funding for the same. To date, 158 participants have enrolled in the DE Study.

Fifteen participants (8 females, mean age 45.8) who were enrolled in the DE Study, identified PTSD symptomatology as a primary reason/clinical motivation for enrollment and met a diagnostic cut-off score of 44 on the PTSD Checklist - Civilian Version score (PCL-C). The table below demonstrates the improvements seen in the PCL-C, ISI, and CES-D, from before to after HIRREM intervention for these participants. HIRREM was a feasible intervention for those with PTSD, demonstrating significant, clinically-relevant improvements in symptom inventory scores for PTSD, as well as co-morbid depressive mood, and insomnia. (Presented in part as a poster at the annual meeting of the International Society for Traumatic Stress Studies, Los Angeles, CA, November 2, 2012).

Measure	n	Baseline Score Median	Post-HIRREM Score Median	Median Change	p value
PCL-C	15	57 (41 to 81)	33 (17 to 76)	-24 (-42 to 0)	$p < .0001$
ISI	14	19.5 (13 to 28)	11.5 (0 to 22)	-11 (-15 to 4)	$p < .0001$
CES-D	13	35 (16 to 49)	10 (0 to 36)	-18 (-40 to -1)	$p < .0001$

Insomnia Pilot Trial:

Twenty subjects, with an Insomnia Severity Index (ISI) score of >15 (14 women, mean age 45.4, mean ISI 18.6), were enrolled in a randomized, un-blinded, wait-list control, crossover, superiority study. Subjects were randomized to receive 8-12 HIRREM sessions over 3 weeks, plus usual care (H+UC), or usual care alone (UC). Pre- and post-HIRREM data collection included ISI (primary outcome), and many secondary, exploratory measures. ISI was also repeated 4-6 weeks post-HIRREM. All subjects completed the primary intervention period. Analysis for differential change of ISI in the initial intervention period for H+UC versus UC showed a drop of -10.3 points (95% CI: -13.7 to -6.9, $p < 0.0001$, standardized effect size of 2.68)[16].

The UC group later crossed over to receive HIRREM in addition to usual care (H+UC-2). Key secondary outcomes included statistically identical differential change for the first HIRREM group (H+UC-1) and H+UC-2, and persistence of the effect on the ISI up to >4 weeks post-HIRREM. Differential change in the H+UC group was also statistically significant for Center for Epidemiologic Studies Depression Scale (CES-D, -8.8, 95% CI: -17.5 to -0.1, $p = 0.047$), but other exploratory outcomes were not statistically significant. For all receiving HIRREM (n = 19), decreased high frequency total power was seen in the bilateral temporal lobes. No adverse events were seen. This pilot clinical trial, the first using HIRREM as an intervention, suggested that HIRREM is feasible and effective for individuals having moderate-to-severe insomnia, with clinically relevant, statistically significant benefits based on differential change of the ISI. Effects persisted for 4 weeks following completion of HIRREM.

Relevant TBI in the Developmental Study:

Twenty-one individuals (mean age 34.7, range 15-64, 9 women) who reported a history of relevant TBI (6 related to sports, 5 related to military service), were drawn from an IRB-approved open label feasibility study of the role of HIRREM for diverse clinical conditions. Participants had a baseline assessment followed by an average of 17.4 (range 10 to 36) HIRREM sessions (90 minutes each) over a median of 13 days (range 9 to 93). Temporal high frequency electroencephalic asymmetry (TFHA) scores (percentage basis) were calculated at baseline and over the course of serial HIRREM sessions by measuring one minute epochs of high frequency (23-36 Hertz) amplitudes (microvolts) at bilateral temporal lobes (T3/T4), subtracting the value at T3 from the value at T4, and dividing by the lesser of the two. Blood pressure and heart rate was monitored at baseline and after HIRREM, to get HRV.

On average, subjects reported reduced symptoms of insomnia (pre to post-HIRREM change in the Insomnia Severity Index 13.7 to 7.1, $p < 0.0001$), depression (change in CES-D 24.3 to 12.7, $p < 0.0001$), and PTS symptoms (change in PCL-C 44.7 to 32.7, $p = 0.0001$). There was increased (improved) HRV after HIRREM (SDNN increased from 51.8 to 65.2 ms, $p = 0.009$). In subjects ($n = 8$) who were initially right-side (T4) dominant (amplitudes ≥10% rightward), TFHA changed from a median of 49.7% to -2.5% ($p = 0.06$). In those who were initially left-side (T3) dominant ($n = 9$), THFA scores changed from a median of -30.4% to 18.9% ($p = 0.0004$). For those who were initially <10% asymmetrical in either direction ($n = 4$), TFHA scores changed from a median of -4.6% to 10.1% ($p = 0.75$). Thus, in this case series, the use of HIRREM by individuals with prior relevant TBI was associated with statistically significant reductions in clinical symptoms of insomnia, depression, and PTSD, and increased HRV. Trends were found for reduced THFA among those who were ≥10% asymmetrical at baseline[17]. These results were presented as an oral platform presentation at the 10th World Congress on Brain Injury, San Francisco, CA, March 21, 2014.

Correlations of Brain Pattern and Autonomic Function, and Effect of HIRREM:

73 participants (49 women, median age 47, range 13-83) were enrolled in an ongoing, open label, single site, IRB-approved, developmental study of HIRREM for individuals with diverse psychophysiological conditions. They received a median of 14 HIRREM sessions (range 7-36, 90 minute duration) over 10.5 days (4-93). One minute epochs of temporal (T3/T4, eyes closed, EC) high frequency (23-36 Hz) electroencephalic amplitudes (microvolts, μv) were analyzed from baseline, the first four, and last four HIRREM sessions. Serial values for the sum of T3 and T4 amplitudes and temporal asymmetry scores were calculated. Mean arterial blood pressure and heart rate were recorded continuously for 10 minutes and analyzed using spectral analysis software to calculate heart rate variability (HRV) measures such as the standard deviation of the normal R-R interval (SDNN).

A strong negative correlation (-0.823, $p < 0.0001$) existed between asymmetry at baseline and change from baseline based on the penultimate minute of the final HIRREM session, indicating a reduction in asymmetry over the course of sessions. The sums of T3 and T4 high frequency amplitudes decreased from baseline to the final session ($p < 0.0001$). SDNN increased significantly from baseline to the

ultimate minute (p = 0.0006). Changes in heart rate and other autonomic metrics were not statistically significant.

In this case series, HIRREM technology was applicable as a way to 1) assess autonomic balance, 2) facilitate reductions in hemispheric electroencephalic asymmetry, 3) decrease total amplitudes at T3/T4, and 4) facilitate improved HRV (SDNN)[18]. The strength of the observed correlations and effect sizes may have been attenuated by the heterogeneity of the population. To our knowledge, these data are the first showing that cerebral hemispheric lateralization in autonomic management can be easily leveraged for clinical assessment and intervention (presented as a poster at the annual meeting of the American Neurological Association, October, 2013, New Orleans, LA).

Study Objectives:

Primary Objective:

The primary objective of this clinical research study is to estimate the effectiveness of HIRREM plus usual care in reducing symptoms of military-related traumatic stress as measured by the PCL-M, among military personnel and veterans who have been diagnosed with PTSD, have been treated for, are referred by military medical personnel for, or have active symptoms of military-related traumatic stress, with or without TBI.

Secondary Objectives:

Secondary objectives include evaluation of the effect of HIRREM on:

- Autonomic nervous system functions, as manifested by blood pressure, heart rate, and HRV. We expect to see changes in autonomic activity and an improvement of sympatho-vagal balance, which would be reflected as changes in heart rate, and an increase of HRV parameters such as the standard deviation of normal R-R intervals (SDNN).
- Psychological symptoms such as depression, assessed by the CES-D symptom inventory. We expect to see improvement in this symptom score.
- Anxiety, as assessed by the GAD-7 symptom inventory. We expect to see reduced anxiety scores.
- Quality of life as evaluated using the EQ-5D measure. We hope to see improved overall quality of life scores.
- Autonomic symptoms will be evaluated with the Compass 31 inventory. We hope to see improved autonomic scores.
- The amount and quality of sleep as evaluated with a daily sleep diary, and with the ISI. The ISI allows pre- to post-HIRREM evaluation of insomnia, while the sleep diary allows evaluation of the timing and trajectory of any improvements in sleep. We expect to see improvement in the ISI, as well as parameters of the sleep diary.
- Reaction time, as evaluated by a drop-stick, clinical reaction time apparatus. We expect to see improved reaction time.
- Grip Strength, as evaluated by a hand dynamometer. We expect to see improved grip strength.
- Brain pattern as evaluated by analysis of electrical frequency and amplitude data, collected during the baseline HIRREM assessment, and during subsequent HIRREM sessions. For similar

locations, eye states, and frequencies, we expect to see improved balance and reduced hyperarousal.
- RPQ for those with TBI. We expect to see reduced scores.

Research Design and Method:

Overview:

This will be an open label, single site, pilot, clinical research study. Up to 40 active duty military personnel, or recent Veterans, age 18 or older, who have been diagnosed with PTSD, have received treatment for, are referred by military medical personnel for, or have active symptoms of military-related traumatic stress, with or without mild TBI, will be recruited to receive up to 24 HIRREM sessions over 2 weeks. For those who self-refer, and do not have a prior diagnosis or treatment for PTSD, active symptoms will be identified by a screening PCL-M score of 50 or greater. Recruitment of 40 participants will allow us to achieve the goal of 36 participants to complete the intervention, allowing for the possibility of dropouts. The primary outcome will be differential change in the PCL-M from baseline to completion of HIRREM sessions. Secondary measures include the Insomnia Severity Index (ISI), the Center for Epidemiological Studies Depression Scale (CES-D), an anxiety measure (GAD-7), a quality of life measure (EQ-5D), an autonomic symptom measure (Compass 31), and a daily sleep diary, as well as physiological measures including heart rate (HR), and blood pressure (BP), with calculation of heart rate variability measures (HRV), and baroreflex sensitivity (BRS). Functional measures will include reaction time (drop-stick paradigm), and grip strength (hydraulic dynamometer), and analysis of brain patterns. If there is a history of TBI, a Rivermead Post-Concussion Symptoms Questionnaire (RPQ) will be added. There will be pre- and post-intervention data collection for all measures (baseline, V1, and at completion of HIRREM sessions, V2). Self-report measures will also be repeated by phone at 1, 3, and 6 months after completion of sessions (V3, V4, and V5 respectively). The online sleep diary will be maintained from V1 until V3. A brainwave assessment will be obtained at V1.

Participants/Subjects

Men and women who are active duty military personnel or recent Veterans (OEF, OIF, OND) over the age of 18 who have been diagnosed with PTSD, received treated for symptoms of the same, or who have active symptoms of military-related traumatic stress, and who meet the following inclusion criteria and are interested to participate in the study will be offered participation. Subjects will be identified and recruited by the Care Coalition, collaborating with the US Special Operations Command, by physician referral from contacts at other military facilities, and from the community by physician referral, or self-referral. Each subject must be able to provide an informed consent.

Interested subjects will be informed with a more detailed description of the study and the extent of their commitment, through screening visits or phone interviews. If potential participants express continued interest in the project and have no major exclusions, they will be scheduled for an enrollment visit (V1) at which time an informed consent will be completed, brief medical history information

obtained, and baseline study measures obtained, prior to the start of the intervention. Those scheduled for an enrollment visit will also be provided a copy of Handout to Study Participants (Appendix).

Inclusion Criteria:

Active duty military personnel, or recent veterans (OEF, OIF, or OND), men and women, who have a diagnosis of PTSD, have received treatment for, are referred by military medical personnel for, or have active symptoms of military-related traumatic stress, with or without mild TBI, will be considered eligible to participate in the study. For those who self-refer, and do not have a prior diagnosis or treatment for PTSD, active symptoms will be identified by a screening PCL-M score of 50 or greater:

Exclusion Criteria:

- Unable, unwilling, or incompetent to provide informed consent
- Physically unable to come to the study visits, or to sit in a chair for several hours
- Known seizure disorder
- Severe hearing impairment (because the subject will be using ear buds during HIRREM)
- Ongoing need for treatment with opiate, benzodiazepine, or anti-psychotic medications, anti-depressant medications (SSRI, or SNRI's), sleep medications such as zolpidem or eszopiclone, stimulants such as Adderall, Provigil, or Ritalin, or thyroid hormone
- Anticipated and ongoing use of recreational drugs, alcohol, or energy drinks
- Lack of internet or smart phone access (will maintain remote access daily sleep diary through 1 month post-HIRREM visit)

Participants are encouraged to discuss their participation with their health care provider following completion of the study because HIRREM may alleviate some of the need for medications they were on previously. Participants are requested to abstain from using any alcohol or recreational drugs during the intervention, and until the one month follow-up phone data collection since use of these substances may cause reversal or cessation of the benefits of HIRREM. In addition, the participants are also advised to suspend chiropractic, cranial-sacral therapy, and bio-energy work during the intervention, until the one month follow-up phone data collection. Other instructions and requests may be given to the patient and will be contingent upon the condition(s) being treated and will be at the discretion of the investigator.

1) Number of Subjects

   The primary statistical intent of this pilot clinical research study is to generate estimates of effect size in order to plan subsequent randomized studies. While no directly relevant, controlled study data is currently available, a recent, national study of prolonged exposure therapy in veterans with PTSD (N = 1,888) indicated a mean decrease on the PCL-M of 15.2 points (standard deviation=20.0)[33]. Assuming a similar level of variability in our population, we expect to generate a 95% confidence interval for the mean change on the PCL-M with a half-width (1.96 x standard error) of ± 12.4 points.

2) Number of HIRREM Sessions and Length of Study

All baseline measures, along with a brainwave assessment, will be obtained during a Monday morning enrollment visit (V1), and a daily sleep diary will be started. All V1 measures will require 3-4 hours to complete. The online sleep diary will be maintained until the one month post-HIRREM follow-up phone call. The HIRREM sessions will begin in the afternoon following the V1 data collection, and can be done with 2 sessions during a half day period. The sessions will typically be about 1.5-2 hours in length. Each session will typically include between 4 to 10 protocols, lasting from 6 to 40 minutes each. Some protocols will be administered while the participant is sitting upright, with eyes open, while others will be with eyes closed, in a reclining position. Participants will receive up to 24 HIRREM sessions over a two week period, although most will receive 20, during 10 half days, over a two week period.

A post-HIRREM data collection (V2) will occur after the final HIRREM sessions, typically on Friday afternoon, of the second week. All measures obtained at the V1 data collection visit, except for the brainwave assessment, will be repeated. The V2 data collection will take about 2.5 to 3.5 hours to complete. Additional follow-up data collection, for self-reported symptom inventories, will be done by telephone at 1 month (V3), 3 months (V4), and 6 months (V5) following completion of HIRREM sessions. Thus, all participants will have 5 data collections (V1-5), and will receive HIRREM intervention during a two week period between V1 and V2. Total time of study participation will thus be about 9.5 months.

Summary of Projected Typical Study Flow:

- Sunday: Arrival in Winston-Salem
- Monday (week 1): 0800 Enrollment/baseline data collection (V1), followed by HIRREM sessions (1-2)
- Tuesday-Friday (week 1): Two HIRREM sessions per day (sessions 3-10)
- Sat-Sun: R & R (potential for up to 4 additional sessions if needed)
- Monday-Thursday (week 2): Two HIRREM sessions per day (sessions 11-18)
- Friday (week 2): Last 2 HIRREM sessions (sessions 19-20), and post-HIRREM data collection (V2)
- Departure: Late Friday afternoon, or Saturday
- Post-HIRREM telephone data collections at one month (V3), three months (V4), and six months (V5) after completion of HIRREM sessions

A) Enrollment Visit

Informed consent obtained, a brief medical history obtained (Appendix), and collection of baseline measures, to include a HIRREM assessment, evaluating brain frequencies and amplitudes, as well as an MRI scan, and blood/saliva samples, are obtained at the enrollment visit (V1). The Clinical Research Unit (CRU) will assist with the blood collection for this study. This will occur prior to the start of HIRREM sessions, and will require about a total of 3-4 hours. When possible the completed medical history form may be requested and received prior to the enrollment visit. Instructions will be provided regarding completion of a daily sleep diary. This will be collected using an

online survey (REDCap), and will be continued through the V3 follow-up phone call, one month after completion of the intervention.

B) HIRREM Sessions

Participants may receive two HIRREM sessions in a half day period for an anticipated total of 20 HIRREM sessions per participant, with 10 half days spent during the two weeks of HIRREM. Some participants may receive up to 24 HIRREM sessions, over the course of 12 half days, if sessions are recommended to be done over the intervening Saturday and Sunday. Sessions last about 1.5-2 hours, and two can be done in a half day, with a short break (20-60 minutes) between sessions.

C) Post-Intervention Data Collection Visit

On the afternoon following the final HIRREM session, a post-HIRREM data collection visit will occur (V2). This will typically be on Friday afternoon of the second week of participation. All measures will be repeated, but no brainwave assessment will be done. This visit will take 2.5-3.5 hours.

D) Late Follow-Up Data Collection

One month after completion of V2, participants will be contacted by telephone to collect repeated symptom inventories (V3). The daily sleep diary will be discontinued after this call. This call should take about 30 minutes.

Three months after completion of V2, participants will be contacted on the phone to collect repeated symptom inventories (V4). This call should take about 30 minutes.

Six months after completion of V2, participants will be contacted on the phone to collect final repeated symptom inventories (V5). This call should take about 30 minutes, and will complete participation in the study.

3) High-resolution, relational, resonance-based, electroencephalic mirroring (HIRREM)

High-resolution, relational, resonance-based, electroencephalic mirroring (HIRREM®) is a computer-based technology created by Brain State Technologies, LLC, Scottsdale, AZ, designed to facilitate relaxation and auto-calibration of neural oscillations through reflecting back musical tones in near real time.

A) Brainwave Assessment

This is the first step in the HIRREM process. It occurs prior to the initial HIRREM session. The assessment creates a map of frequencies and amplitudes, and informs the choice of protocols for the initial HIRREM sessions. Our pilot data also suggest that this information is also useful for correlating with autonomic function (heart rate variability), and that changes can be observed in frequencies and amplitudes from pre- to post-

HIRREM. With the participant in a sitting position, sensors are sequentially placed over eight areas of the scalp to record one minute epochs of data while the brain is at rest, or on task, with eyes open and with eyes closed. For the assessment, measurements were taken at homologous regions of the bilateral hemispheres according to the 10-20 International System (Jasper HH, 1958) at F3/F4, C3/C4, P3/P4, T3/T4, FZ/OZ, and O1/O2 with both eyes closed (EC; one minute), eyes partially open (one minute), and eyes open (EO; one minute) conditions. For EO assessments, subjects are given standardized tasks involving numerical digit recall (F3/F4), reading silently (C3/C4), math calculations (P3/P4), listening comprehension (T3/T4), and to relax with eyes open (O1/O2). A midline measurement is taken at FZ/OZ, with an EO task to count number of appearances of a specific word as they read a standardized printed passage. Measurements will also be obtained at FP1/FP2, and CB1/CB2, with eyes open tasks being similar to F3/F4, and O1/O2, respectively. The reference sensors are connected at A1/A2 and linked for assessments. The data are processed to identify patterns and imbalances of frequencies and amplitudes, which are used to generate specific protocols for the initial HIRREM session. The assessment takes about 45-60 minutes to complete.

B) HIRREM Sessions

Each session requires about 1.5-2 hours, and will include between 4-10 individual protocols, working with different locations on the scalp. Each protocol will typically last from 6-40 minutes. For the sessions, with the subject comfortably at rest, sitting or reclining, the sensors are placed over the specific target areas on the scalp corresponding with brain regions/lobes to be observed. Frequencies and amplitudes function are monitored in real time, and the dominant frequency within a chosen target frequency band, e.g. delta (0.5-3 Hz) is identified. The dominant frequency is assigned an auditory tone which is played back to the subject via ear phones with as little as 8 milliseconds delay. Thus, the subject listens to the energetic "song" being played in the brain from moment to moment, providing the brain with a mirror of itself, and its frequencies and amplitudes.

Some sessions will occur with eyes closed, for which the subject will be instructed to relax, and recline as much as is comfortable. Some sessions will occur with eyes open, during which the subject can read, or do other activities such as a word search, or just relax.

Although similar to methods such as neurofeedback, HIRREM uses an algorithm-based observation for the brain to view itself, which provides an opportunity for participant-unique auto-calibration and movement towards a more balanced state. Compared to other methods, there is no operant conditioning, no learner-in-the-loop, and no attempt to try to force the brain toward a standardized, population-based normal, ideal pattern

of frequencies and amplitudes. No active, cognitive involvement by the participant is needed to accomplish this process.

C) Safety

Evidence to date indicates that this intervention, HIRREM, is potentially high benefit and low risk. Based on experience reported by Brain State Technologies, garnered from provision of case management support, feedback from their clients, and feedback from the HIRREM provider community, as well as the IRB-approved studies at WFSM, we are not aware of any serious adverse events resulting from HIRREM sessions. On an anecdotal basis, some individuals undergoing HIRREM have reported an apparent "release of emotions" or paradoxical effects especially during initial sessions, which can manifest as brief periods of increased awareness of emotional states, both positive and negative. For example, some participants have cried as they reported feelings of joy, or of sadness. These experiences are typically transient, i.e. lasting intermittently over the course of one to several days. In the course of provision of over 5,000 HIRREM sessions to 467 subjects enrolled in one of four IRB-approved studies at WFSM, sub-threshold changes in emotional symptomatology or other paradoxical effects have been estimated to occur in less than ten percent of subjects. These were relatively brief episodes not requiring additional clinical intervention or necessitating discontinuation of sessions. All HIRREM sessions are administered by Technologists who have been certified in the procedure, including guidelines for addressing emotional releases that may occur. In the event that emotional releases are prolonged or intense, individuals would be advised to see a mental health professional for additional evaluation or treatment. There have been no instances in the 467 subjects participating to-date of prolonged or intense changes in emotional state, or paradoxical effects.

A PCL-M will be administered as a screening tool, and outcome measure. A score of 50 or higher on the PCL-M correlates with an increased likelihood a person would be diagnosed with PTSD, but it does not establish the diagnosis. That requires evaluation by a physician or behavioral health professional, and typically, more extensive testing. The PCL-M is only being used here as a screening tool, and outcome measure, and we will not be diagnosing participants with PTSD. Some may have strong feelings about not wanting to be diagnosed as such. We expect that all participants will have a PCP, or that they will have seen a behavioral health provider prior to enrolling. By definition, and in order to enroll, all participants will either have a diagnosis of PTSD, have received treatment for, been referred by military medical personnel for, or have active military-related symptoms of traumatic stress. All will be encouraged to follow up with their physician or behavioral health provider upon returning home. If they do not have a PCP, or behavioral health provider, they will be encouraged to seek one.

4) Other Data Collection and Process

A series of measures will be collected at the enrollment visit, as well as at three post-intervention time points for all participants. In addition, all will maintain a daily sleep diary starting with the V1 visit, continuing through the V3 visit. Symptom inventories (Appendix) and the daily diary will be collected electronically, using REDCap.

Self-report symptom scales/symptom diaries:

PCL-M

The primary outcome measure will be change in the PCL-M, which measures the American Psychiatric Association's Diagnostic and statistical manual of mental disorders (DSM-IV) Criteria B, C, & D of PTSD symptoms based on traumatic life experience related to military service. Seventeen items are rated on a Likert scale with a composite score range of 17 to 85. A score of 50 or higher correlates with probability of military-related PTSD[34, 35].

Insomnia Severity Measurements

The severity of insomnia symptoms is measured using the ISI with each data collection visit. The ISI is a 7 question measure, with responses from 0-4 for each question, yielding scores ranging from 0-28[36, 37].

Participants will also be asked maintain an online daily sleep diary from V1 through V3. This measure has ten questions to evaluate the quantity and quality of sleep. Data collection of the daily sleep diary will be accomplished via internet data entry using the REDdCap system.

CES-D

The CES-D is a 20-item survey assessing affective depressive symptomatology to screen for risk of depression[38]. Scores range from 0-60, with a score of 16 commonly used as a clinically relevant cut-off [39].

GAD-7

The GAD-7 is a seven item screening tool for anxiety that is widely used in primary care[40].

EQ-5D

Health-related quality of life is measured by the EQ-5D with each data collection visit[41]. The EQ-5D consists of 5 items assessing an individual's current health status (values from 0-2), yielding scores ranging from 0-10. Overall health status is also assessed with values from 0-100.

Compass 31

The Compass 31 is a 54 item, abbreviated quantitative measure of autonomic symptoms[42].

RPQ

The RPQ is a 16-item survey that assesses the severity of the most common post-concussion symptoms on a scale of 0 to 4. Items are compared to levels before the head injury and are reported as a 24 hour recall[43].

Physiological measures (HR/BP/HRV/BRS)

Blood Pressure (BP), Heart Rate (HR), Heart Rate Variability (HRV), and Baroreflex Sensitivity (BRS)

Continuous BP and HR are acquired from noninvasive finger arterial pressure measurements and ECG for a minimum of 10 minutes in subjects lying down quietly, supine. Systolic BP and beat to beat, RR, intervals (RRI) files generated via the data acquisition system (BIOPAC acquisition system and software, Santa Barbara, CA) at 1000 Hz are analyzed using Nevrokard BRS software (Nevrokard BRS, Medistar, Ljubljana, Slovenia) for measures of BRS, HRV and BPV as follows: Frequency Method. Power spectral densities of SBP and RRI oscillations are computed by 512 points Fast Fourier Transform (FFT) and integrated over specified frequency ranges (LF: 0.04-0.15 Hz; HF: 0.15-0.4 Hz). A Hanning window is applied and the squared-coherence modulus is computed if coherence is > 0.5 as reported. The square-root of the ratio of RRI's and SBP powers is computed to calculate LF, HF alpha indices, which reflect BRS. Power of RRI spectra in LF, HF range (LFRRI and HFRRI) are calculated in normalized units and the ratio of LFRRI/HFRRI is used as a measure of sympatho-vagal balance. Power of SBP spectra calculated as LFSAP is used as a measure of BPV. Sequence Method - BRS calculated by this method is based on quantification of sequences of at least three beats (n) in which SBP consecutively increases (UP sequence) or decreases (DOWN sequence), which are accompanied by changes in the same direction of the RRI of subsequent beats (n+1). The software scans the RRI and SBP records, identifies sequences, and calculates linear correlation between RRI and SBP for each sequence. If the correlation coefficient exceeds a pre-set critical value (0.85), the regression coefficient (slope) is calculated and accepted. The mean of all individual regression coefficients (slopes), a measure of sequence BRS, is then calculated for Sequence UP, DOWN and TOTAL. Time-Domain Analysis - Three time-domain parameters are used for hemodynamic variability. HRV is determined by computing the standard deviation of beat-to-beat interval (SDNN) and the root mean square of successive beat-to-beat differences in R-R interval duration (rMSSD).

a) HRV Data Processing and Interpretation

   Heart rate is measured as beat-to-beat intervals (RRI) recorded by pulse-wave recording, and will be analyzed using custom software developed by Biopac. Data can be loaded and viewed, and a subset of the data can be selected to avoid artifacts during device placement or removal. Outlier identification is performed by determining all inter-beat intervals which demonstrate a 30% difference from the mean of the previous four samples. Such outliers are removed from the data set. HRV statistics that are generated include mean, variance, SDNN, RMSSD, pNN50, VLF, LF, HF, TP, LF/HF, sample

asymmetry, sample entropy, and coherence. All of the algorithms for computation of these parameters are derived from information or source code from the Physionet archive. Data are saved to Excel spreadsheets for further statistical analysis by study team members.

b) Blood Pressure (BP)

Blood pressure (BP) measurements will be taken with a finger cuff on two fingers of the left hand while lying down on an examination table.

Functional Measures:

Reaction Time

Reaction time testing will be measured by a drop-stick, clinical reaction time apparatus. It is constructed from a meter stick covered in the friction tape with gradations. The modified meter stick is fixed to a weighted rubber cylinder. The apparatus is placed between the thumb and index finger of the subject and released at a random time during a countdown. The subject catches the apparatus and the distance fallen is converted to reaction. Following two practice trials, subjects perform eight trials, and a mean distance value is used for analysis. This simple clinical measure has been evaluated by Eckner et al, and demonstrated utility in testing comparable to computerized testing methods[28]. [44]

Grip Strength

Grip strength will be evaluated using a hydraulic hand dynamometer (Baseline Hydraulic Hand Dynamometer). The greatest force generated during three trials will be used for analysis[29].[45]

Brain Pattern Analysis

The proprietors of HIRREM have repeatedly observed that EEG asymmetries in the temporal lobes (specifically at T3 and T4 in the 10-20 International System for EEG correspond to autonomic dysfunction. When T4 signals are dominant to T3 signals a sympathetic state is favored which is manifested as symptoms of anxiety, cardiovascular over-drive, and hyperarousal. When T3 signals are excessively dominant to T4 signals a parasympathetic state is favored which is manifested as symptoms of emotional numbness, cardiovascular underactivity, GI dysfunction, and under-arousal. Lateralization of autonomic activity is not a novel finding, it is in accord with a number of previously published studies which support a hypothesis of autonomic lateralization[46].

EEG balance at T3 and T4 suggests autonomic balance and benefit, and our preliminary results suggest that utilization of HIRREM at T3 and T4 has demonstrated success in ameliorating symptoms associated with autonomic disruption[31].[47] Preliminary data also demonstrate correlation between a T3/T4 temporal asymmetry score and objective physiological changes reflective of autonomic signaling to the heart at the baseline assessment (AAN T3/T4 2013

poster). Reduction in the sum of high frequency amplitudes at T3/T4 (reduced hyperarousal), has also been observed in subjects with hot flashes[48]. Comparison of such activity at T3 and T4 over time will provide objective physiological outcome data regarding the effect of HIRREM on autonomic balance and function.

5) Statistical Analysis

Data will be analyzed using the most recent versions of SAS (SAS Institute, Inc., Cary, NC) or the R Statistical Computing Environment. Histograms and descriptive statistics will be examined to evaluate the distribution of study outcomes. Because this proposal is designed to estimate effect sizes for future randomized trials, analyses will primarily consist of providing point estimates of effect size for each outcome along with corresponding 95% CIs. Given that outcomes will be collected at multiple follow-up time points (V2, V3, and V4), effect estimates will be based on generalized linear models, which can accommodate within-subject correlations due to repeated assessment over time. Critically, estimates produced from this pilot study will need to be used cautiously for planning future research, as it is well known that estimates from small sample sizes tend to be upwardly biased (overestimated effect sizes, underestimated variability[53].

6) Participant Compensation

Participants in this research project will not receive any monetary compensation.

Human Subjects Protection:

1) Consent and Assent

Written informed consent, or assent and parental permission will be obtained by the research staff from each competent subject.

2) Confidentiality and Privacy

Confidentiality will be protected by collecting only information needed to assess study outcomes, minimizing to the fullest extent possible the collection of any information that could directly identify subjects, and maintaining all study information in a secure manner. To help ensure subject privacy and confidentiality, only a unique study identifier will appear on the data collection form. Any collected patient identifying information corresponding to the unique study identifier will be maintained on a separate master log. The master log will be kept secure, with access limited to designated study personnel. Following data collection subject identifying information will be destroyed at the earliest opportunity, consistent with data validation and study design, producing an anonymous analytical data set. Data access will be limited to study staff. Data and records will be kept locked and secured, with any computer data password

protected. No reference to any individual participant will appear in reports, presentations, or publications that may arise from the study.

Brain State Technologies, LLC (BST) will assist with brain pattern analysis. To accomplish this, BST will be provided with the first 8 characters from the randomly generated, 36 character identifier that the HIRREM software generates for each participant's brain frequency and amplitude data, along with the participant's age and gender, which are believed important for understanding brain patterns. No other participant-specific information is provided.

3) Data and Safety Monitoring

The principal investigator will be responsible for the overall monitoring of the data and safety of study participants. The principal investigator will be assisted by other members of the study staff. Results will also be regularly reviewed with representatives of OSD, the funding agency.

Any collected patient identifying information corresponding to the unique study identifier will be maintained on a separate master log. The master log will be kept secure, with access limited to designated study personnel. Following data collection subject identifying information will be destroyed at the earliest opportunity, consistent with data validation and study design, producing an anonymous analytical data set. Data access will be limited to study staff. Data and records will be kept locked and secured, with any computer data password protected. No reference to any individual participant will appear in reports, presentations, or publications that may arise from the study.

A Medical Monitor, with knowledge of MRI, as well as HIRREM, will be identified. The Medical Monitor will review progress of the study, and any safety issues, with the PI on a monthly basis.

4) Reporting of Unanticipated Problems, Adverse Events or Deviations

Any unanticipated problems, serious and unexpected adverse events, deviations or protocol changes will be promptly reported by the principal investigator or designated member of the research team to the IRB and sponsor or appropriate government agency if appropriate.

Addendum (10.05.17):

This addendum briefly outlines the history, and the current status of this protocol and project to provide context for the revised protocol outlined above. Based on funding provided by a contract with US SOCOM, the initial 15 participants had all symptom, functional, physiological, imaging, biomarker outcomes. A subset also had evaluation of epigenetic markers. Following completion of the contracted work, the project was continued using other internal funding so that at total of 18 participants had all outcomes except for the biomarkers, and epigenetics. Thereafter, in order to expand the pilot data set, and to add participants from other branches of the military and Special Operations community, the study was continued with goal of enrolling up to 40 participants. However, due to lack of outside funding, all participants beyond number 18 did not receive MRI, biomarker, or epigenetic outcomes.

The protocol has now been modified to reflect the current study procedures and outcomes, removing mention of those prior outcome elements described above.

Appendix:

Data Collection Measures

- Post-Traumatic Stress Disorder Checklist-Military (PCL-M)
- Insomnia Severity Index (ISI)
- Center for Epidemiological Studies Depression Scale (CES-D)
- Generalized Anxiety Disorder 7-Item (GAD-7)
- EQ-5D
- Compass 31
- Daily Sleep Diary
- Rivermead Post-Concussion Symptoms Questionnaire (RPQ, for those with history of TBI)

Handout for Study Participants

Medical History Form

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