Official Title: EEG Markers of Training-Induced Improvements in Cognitive Functioning

Brief Title: Brain Markers of Improvements in Cognitive Functioning

NCT: 03490110

Document Date: February 29, 2016

2a. Research Plan

Background and Significance

Traumatic brain injuries (TBIs) inflict an enormous toll on military personnel (Warden, 2006). TBI related impairments in goal-directed behavior can have a significant impact on social and occupational functioning. Common complaints include difficulties with holding information in mind, distractability, performing multiple tasks simultaneously, and feeling easily overwhelmed (Kennedy et al., 2008; Church, 2009; Chen & Loya, 2014). These cognitive difficulties are often exacerbated by symptoms of post-traumatic stress (PTS; Vasterling et al., 2009; Polak et al., 2012; Scott et al., 2015). An underlying source of these complaints relates to brain functions important for the goal-directed regulation of attention, learning, memory and organization (Larson et al., 2006; Chen et al., 2011, 2012). Veterans hampered by deficits in these areas may not learn as quickly or perform as effectively as they did prior to their injuries. There remains a major need for interventions specifically designed to improve goal-directed functioning.

The path towards developing improved interventions for these cognitive challenges may be aided by a rehabilitation neuroscience approach. This approach elucidates neural-behavioral mechanisms of improvements in cognition, and leverages that knowledge to improve existing interventions or develop novel ones. As an intermediate step in this developmental process, appropriate neurophysiological measurements should be included to probe mechanisms of training-induced plasticity associated with existing interventions targeting improved functioning. Ideally, interventions and measurements should draw from a unified neuroscience framework to facilitate empirical tests of theory.

To promote improvements in goal-directed functioning, we have developed a series of interventions (Chen et al., 2011; Novakovic-Agopian et al., 2011; Loya et al., 2014; Rodriguez et al., 2014). Training emphasizes learning the goal-directed application of self-regulation skills across multiple challenge contexts. Participants are trained in applying a Stop-Relax-Refocus (S-R-R) strategy to stop activity when distracted or overwhelmed, relax, and redeploy attentional resources for the task at hand. To facilitate the transfer of these skills to daily life, we developed scenarios that allow for greater intensity of practice across a wide range of challenging contexts. These scenarios were implemented in digital game format to increase access to well-designed and calibrated learning opportunities. Trainers provide intensive, individualized coaching that maximizes each patient's understanding of trained skills, strengthens skill use, and develops the ability to strategically apply these skills in situations outside the training environment. In sum, challenging experiences are integrated with coaching to provide intensive guided experiential learning. Thus, the intervention utilized in the proposed study will intensively train self-regulation skills via extensive and supported practice with their application across a continuum of simple to complex contexts and goals.

There is significant variability in response to any given rehabilitation intervention, and the neural bases of this variability are not well understood. Electroencephalography (EEG) is a research tool with an established history of clinical application following TBI (Rapp et al., 2015; Schmitt & Dichter, 2015). Previous reports of mild to moderate TBI have suggested alterations in the theta (4-8 Hz) and alpha (8-13 Hz) ranges (Thornton & Carmody, 2009). Given its availability in clinical settings, and utility in measuring the effects of attention and working memory (Luck et al., 2000), we propose to examine the neurophysiologic underpinnings of behavioral responses to training using well-validated event-related EEG markers, and novel application of EEG network connectivity measures to resting state data. In a related example, EEG markers for Attention-Deficit Hyperactivity Disorder (ADHD) suggested that processing deficits in the encoding and maintenance stages of working memory (WM) are differentially explained by variance in theta and alpha (Lenartowicz et al., 2014). Application of these methods to understand and even predict treatment outcomes could help direct interventions, save time, and improve patient qualify of life. Better understanding the neural bases of functional improvements may help guide intervention development by providing new markers for feedback or targets for electrical modulation.

We will use a novel combination of neurocognitive training and two categories of EEG data for these investigations. (a) Event related changes in brainwaves can dissociate components of working memory into discrete processes that are targeted by training (attention, encoding, maintenance). We will examine EEG with WM load under distraction pre- and post-training, and test whether these markers help to explain neural bases of variability in behavioral changes with training. We predict that training-induced changes in

working memory span and attention/executive functioning will be related to changes in established EEG markers. (b) Network connectivity models of dynamic brain networks will be synthesized from brief assays of tonic self-regulation. Our previous work has shown that baseline network modularity during a brief period of self-regulation, as measured by functional MRI, can predict response to cognitive training (Arnemann et al., 2015). In the current project, we will evaluate the use of EEG network connectivity analysis to predict response to cognitive training. Measures of brain network properties do not rely on averaging individuals with different anatomy (Tymofiyeva et al., 2014), and therefore may be particularly useful for real-world applications.

Preliminary Studies

Our preliminary data support the component foundations for the project, including the feasibility of implementing the proposed intervention; a model for determining the extent of neural changes; and the plausibility of the hypothesized mechanisms, with data to ground power calculations for testing these mechanisms.

(1) Goal-oriented attentional self-regulation training (GOALS) for Veterans with chronic TBI: In an ongoing study, *32* Veterans with chronic TBI and functional complaints received GOALS or brain health education (BH) training, matched for time and intensity over 5-6 weeks. Pre-to-post-training changes on a composite measure of attention and executive functions ($p<0.01$), as well on a simulated, ecologically-valid 'real-world' task ($p<0.01$) were observed following GOALS and not BH. These data support the potential value of training Veterans with TBI how to better self-regulate within the context of their individual goals.

(2) Guided experiential learning of goal-oriented self-regulation (intensive skill training supported by digital game experiences): Extending this work, we developed methods to provide more intensive training in the strategic application of self-regulation skills. This system was designed to "bridge the gap" between initial skill learning and transfer of skills to personal life (Loya et al., 2014, submitted; Rodriguez et al., 2014). In ongoing studies, *19* participants with history of brain injury and chronic cognitive symptoms have completed 7 training sessions in person or by tele-video, and approximately 30 minutes of daily practice over 6-8 weeks using custom-developed digital game experiences ('From Startup-to-CEO'). Objective measures of transfer to performance on non-trained tasks have been obtained using a range of measures. In preliminary analyses, improvements have been documented on composites of complex attention and executive functions *($t(18) = 2.66, p = 0.016, d = 0.63$),* verbal learning and memory *($t(18) = 2.02, p = 0.06, d = 0.48$),* and processing speed *($t(18) = 2.99, p = 0.008, d = 0.71$*). These results provide a promising foundation for training Veterans with brain injury and will allow investigation of the neural bases of improvements in cognitive functioning.

(3) Functional brain network organization and learning in response to training: The ability to regulate 'baseline' brain state likely influences performance during goal-directed cognitive tasks (Otten et al., 2006; Guderian et al., 2009). Brain state can be described by network parameters such as the modularity of brain network organization. Modularity is theorized to support learning and plasticity by enhancing the adaptivity, robustness, and evolvability of network function (Stevens et al., 2012). Modularity is a summary of the extent to which a large-scale brain network can be characterized by sub-networks (i.e. modules) with high within-module connectivity, and low between-module connectivity. We posited that parameters of brain state such as modularity may 'tune' how the brain handles tasks, influencing the responsiveness, efficiency, and plasticity of brain networks in response to experiences. As an initial test, we examined the extent to which network modularity might explain inter-individual variability in response to cognitive training for TBI patients (n=11) who participated in GOALS training (Arnemann et al., 2015). We acquired fMRI from brief pre-training sessions, in which patients were instructed to try to achieve a relaxed but focused resting state (an assay of brain state regulation). We examined the predictive value of baseline modularity during this 'focused rest' assay for pre- to post-training changes in measures of attention and executive control. Pre-training brain modularity predicted the degree of improvement in attention/executive function following training (*Pearson correlation $r=0.61$m $p<.05$*), such that those individuals with higher baseline modularity exhibited greater treatment response. These results provide preliminary support for the hypothesis that the regulation of functional brain network organization may influence learning. In the present proposal, we will use EEG in a similar manner to characterize network modularity before and after training.

(4) ERP measures of working memory and multitasking: Key elements of the training program aim to improve attention regulation as a gateway for working memory and the ability to multitask. Laboratory

measures of these cognitive functions have been studied using EEG recording (Gazzaley, 2011; Pratt et al., 2011). Our recent work demonstrated that Veterans with mild TBI and PTS showed a working memory deficit when cognitive control functions were more heavily taxed (Honzel et al., 2014). In that study, we examined working memory for letters maintained across a delay that was either unfilled, or filled with a challenging distractor task. The patients were slightly less accurate than controls on the unfilled (single task) version, but impaired to a disproportionate extent on the dual task version. Furthermore, a neural index of memory retrieval processes was unaltered in the patients for the single task version but decreased for the dual task *(difference wave at Cz, dual task: t(30)=2.704, p=.011, Cohen's d=.956).* This event-related potential (ERP) "old/new" effect differentiates correctly remembered old items from correctly rejected new items, beginning at 300 ms post-stimulus and continuing for several hundred ms (Rugg & Curran, 2007). This task will allow us to assess brain activity and task performance before and after training, to test the prediction of an increase in the ERP old/new effect after training.

Research Design and Methods

Participants: The participants will be Veterans with history of TBI: *n* = 20 will undergo the training intervention, and *n* = 20 will participate in repeated testing without intervention. Inclusion criteria: Veterans, ages 21 to 55, with a history of TBI (as defined by the American Congress of Rehabilitation Medicine and VA, with reported plausible mechanism of head injury, loss of consciousness with some period of post-traumatic alteration in cognition), in the chronic, stable phase of recovery (>6 months from injury), with residual cognitive complaints (Neurobehavioral Symptom Inventory, cognitive subscale >9). Participants will be on stable psychoactive medications (> 30 days); able and willing to participate in EEG, training and, assessments. *Common mental health comorbidities present amongst OEF/OIF/OND Veterans, such as depression and PTS (Carlson et al., 2011; Carter, 2014), will be permitted in order to address realistic aspects of recruitment (i.e. it would be unrealistic to expect a sample of 'pure TBI' amongst current generation combat Veterans) as well as a practical goal of intervention work in addressing needs of Veterans. However, participation will be limited to Veterans reporting PTS and depression symptoms below clinical thresholds (see self-report questionnaires section for more details: PCL-5 scores < 38, Weathers et al., 2013; BDI-II scores < 19; Homaifar et al., 2009).* Exclusion criteria: Severely apathetic/abulic, aphasic, or other reasons for being unable or unwilling to participate with the training tasks; severe cognitive dysfunction; history of neurodevelopmental abnormalities; ongoing illicit drug or alcohol abuse (AUDIT>8); schizophrenia; bipolar disorder; history of other neurological disorders; current medical illnesses that may alter mental status or disrupt participation in the study; active psychotropic medication changes. There will be no restriction in regard to gender, race and socioeconomic status.

Study design and participant flow*. Participation in the proposed study will span approximately 10-12 weeks. Participants will be recruited via IRB-approved methods, and interested candidates will undergo preliminary screening (~ 1 hr phone interview + VA records review). Eligible participants will be invited to enroll and provide informed consent approved by the IRB of VA Northern California.*

Baseline assessments of cognitive status and functioning, as well as EEG, will be conducted in weeks 1-2. Time commitment for baseline assessments is approximately 4-5 hours. Participants will then be assigned to (a) active training or (b) no training conditions in a pseudo-random order, matched across key demographic variables. The no training condition will serve to assess changes to EEG and other measurements with repeated testing in the absence of active training. This will lay a foundation for future studies that will incorporate an active comparison intervention.

Participants in active training will complete seven training sessions in weeks 3-10. Training sessions last 2 hours, and participants are requested to complete approximately 2.5 hours of additional skill practice over the course of each week outside of session (total ~4.5 hours per week). The same overall training time will be targeted (~32 hours) for all individuals, although the training schedule will accommodate variations in individuals' schedules and availability over the course of ~8-10 weeks to maximize engagement. In the 2 weeks after training concludes, participants will complete a second round of assessments (~ 3-4 hrs). Participants will be compensated $20/hr for their time during the assessment portions of the study as well as for their travel expenses at $0.55/mile for travel greater than 20 miles to and from the study site.

Enrollment will begin within 3 months of the initiation of the grant period, and continue through month 21. Data will be checked and pre-processed on a rolling basis and final analyses and reports will be completed in months 22-24.

Training Intervention: Training follows a manualized protocol, with seven in-person two-hour sessions (Loya et al., 2014; Rodriguez et al., 2014) and instructions to practice skill use while playing the Startup-to-CEO game for 30 minutes/day, five times per week, and to practice skill use within the context of their daily life. Game scenarios are utilized to highlight contexts where goal-directed functioning is susceptible to disruption and where skill use might be helpful. They further help facilitate discussions about skill application in personal life. In addition, twice weekly semi-structured phone check-ins are conducted to provide motivational support and reinforce skill use.

Pre- and Post-training Assessments of Neuropsychological Performance: Standardized tests will be administered before and after training (Novakovic-Agopian et al., 2011). The Assessor will be blind to treatment status. All instruments have well-established psychometric properties, and many are included in NINDS Common Data Elements for TBI (indicated with '*'). Attention and executive function will be assessed using Letter Number Sequencing* (WAIS-IV); Auditory Consonant Trigrams; Digit Vigilance Test; Design and Verbal Fluency Switching; Trails B*; DKEFS* Color-Word Interference: Inhibition and Inhibition/Switching subtests (Delis & Kaplan, 2001). To account for practice effects, alternative test forms will be used when possible, and/or norms for repeated testing will be used. The major outcome measures will be a composite score (averaged *z* scores) of the attention/executive function domain (Arnemann et al. 2015) and the Automated Operation Span Task, which assesses WM in the presence of distractions (Unsworth et al., 2005). Training effects on primary outcomes will be assessed by computing pre-post change scores. Participants will complete questionnaires of self-perceived changes in cognitive functioning.

Self-Report Questionnaires: Each participant will be given two additional questionnaires to assess psychological symptoms as potential covariates. The PCL-5 is a 20-item self-report measure of DSM-5 PTSD symptoms used for screening or monitoring symptoms of PTSD (Weathers, et al., 2013). *A cut-off score of 38 has been recommended as a threshold for diagnosis of PTSD (Weathers et al., 2013).* The Beck Depression Inventory (BDI) is one of the most commonly used self-reported screens for major depressive disorder. *A cut-off score of 19 has been used as a threshold for major depressive disorder for Veterans with mild TBI history (Homaifar et al., 2009).* Participant replies will be monitored for suicidal ideation or intent. If there is any indication that a participant is a danger to self or others, a protocol has been established to ensure that he or she will be further evaluated by Dr. Loya or Dr. Chen to determine what level of intervention is necessary. VANCHCS Mental Health Service provides emergency walk-in evaluations, if it is believed that immediate or a greater level of intervention is necessary. In addition, all participants are provided information about the Suicide Hotline number for Veterans (1-800-273-8255).

Pre- and Post-Training EEG: Participants will attend testing sessions pre- and post-training. The first phase of each session measures several minutes of resting state brainwaves for use in connectivity modeling (Langer et al., 2013). Participants will complete a brief assay of self-regulation, with instructions to relax and focus on the cycle of breathing, re-directing attention if the mind wanders, as in our prior fMRI study (Arnemann et al., 2015). In the second phase, participants will perform our well-validated working memory dual task while EEG is recorded and averaged using standard methods. EEG time-frequency analyses will target the theta and alpha bands based on prior studies (Thornton & Carmody, 2009). This experiment is ideal for use in evaluating potential neural mechanisms of our targeted training, because of its sensitivity to working memory load and distractions. Data will be collected by staff in Dr. Swick's lab, and EEG analysis carried out by Dr. Schalles, who has experience describing EEG markers and evaluating novel training approaches in clinical populations (Pineda et al., 2014).

ERP Recording: Continuous EEG will be recorded from 64 scalp electrodes and two electrodes placed on the left and right mastoids using the ActiveTwo Biosemi electrode system. Four electrodes placed laterally and below both eyes will record blinks and eye movements. The EEG will be sampled at 512 Hz. BioSemi uses a "common mode sense" active electrode and "driven right leg" passive electrode to form a feedback loop that serves as the "ground" electrode. All signals will be recorded using ActiView software.

ERP Data Analysis: Off-line analysis will be completed using Brain Vision Analyzer software. Data will be re-referenced to the average of the mastoid electrodes and bandpass filtered from 0.1 to 30 Hz (48 dB/octave). The EEG will be segmented for each trial beginning 100 ms pre-stimulus and extending to 900 ms post-stimulus onset. EEG will be corrected for blinks using the Gratton procedure; artifacts exceeding 150 μV will be rejected. Segments will be baseline corrected and averaged with similar trials. ERPs will be

quantified by computing mean amplitudes in defined latency windows relative to a 100 ms pre-stimulus baseline. The primary measure will be the ERP "old/new effect" elicited during memory retrieval, time-locked to probe onset. This will be quantified by subtracting mean amplitude for Old – New probes (300-500 ms post-stimulus) at midline electrodes (Honzel et al., 2014).

EEG Time-Frequency and Network Analysis: Off-line analysis for resting state and dual task data will be performed in the EEGLAB Matlab toolbox (Delorme & Makeig, 2004). Data will be filtered with bandpass limits of 0.1 to 55 Hz using a standard FIR filter and re-referenced to the average of mastoid electrodes. Periodic artifacts, such as eyeblinks, will be removed using the Infomax Independent Component Analysis (ICA) algorithm. Remaining data will be further band filtered into four frequency bands (theta: 4-8 Hz, alpha: 8-13 Hz, beta: 15-30 Hz, & gamma: 30-55 Hz), and power envelope and phase angle will be extracted with a Hilbert transform.

Resting state connectivity will be calculated over two second epochs using a graph theoretical approach comparing each pair of electrodes in each frequency band, based on correlation of band power changes, and degree of phase coherence. *As we are working with resting state data, we have no a priori hypothesis about directionality of information flow. Therefore, correlations of these measures will suffice (Bastos & Schoffelen, 2016), thereby decreasing computation demands*. Spurious connectivity will be corrected by eliminating neighboring electrode connections and ignoring electrode pairs with 0 degrees of phase lag. Remaining electrode pairs will be adjusted to a common threshold to control number of network connections across patients. From these binarized connectivity matrices, modularity and characteristic path length (CPL) will be calculated using *the Matlab toolbox, EEGNET (Hassan, et al. 2015).* Time-frequency responses to dual task conditions will be calculated using Hilbert transformed data, epoched and averaged over the same time window and baseline as ERP analysis.

Sample Size Calculation: We powered the study based on prior group differences (mTBI/PTS vs. Controls). We used data from the Sternberg task (Honzel et al., 2014) to obtain an estimate of the standard deviation of the ERP old/new effect, and expected effect size based on a mixed effects ANOVA (subject as random effect) with group as the sole explanatory variable. To estimate sample size, we used the two sample *t* test as an approximation to the mixed effects ANOVA and used the method of variance inflation to account for the additional subject factors (Hsieh et al., 2003). We powered based on the primary experimental question at 80% and will control false discovery rate at 5% (Benjamini & Hochberg 1995) at the time of analysis for tests of other design and subject factors. The Sternberg data showed a mean difference between the patient and control groups of -1.097 with residual error of 0.956 from the mixed effects ANOVA. Using these values in a two-sample *t* test for mean difference, to obtain 80% power we need a sample size of 13 per group. *This pilot study will allow us to collect data to inform power calculations for future studies.'*

Experimental Design

EEG of Working Memory with and without a secondary attention task

Rationale: Previous studies in this population have shown a decline in WM performance that is exacerbated by increased task demands (Honzel et al., 2014). An established EEG marker of WM retrieval, the ERP old/new effect, indexed this impairment. Another EEG marker, frontal midline theta, is a sensitive correlate of working memory load (Gevins et al., 1997) that exhibits increases in power during cognitive demands for WM (Onton et al., 2005). This experiment will test the hypotheses that (a) patients will show changes in these markers with training; and (b) the EEG changes will explain variability in behavioral changes as measured by the operation span score and the attention/executive function composite score. We will examine EEG and behavioral performance on the Sternberg WM task alone and when the flanker interference task is performed during the maintenance period. Preliminary results from the training intervention suggest that improvements in working memory, attention, and executive functions can occur. Temporally sensitive EEG measures may elucidate the neural correlates of these changes, and help to explain individual differences in treatment response.

Experimental Design: The study will use the methods of Honzel et al. (2014), briefly described below.

Single-Task Condition (Sternberg Memory Task): Participants will be asked to remember either one or four consonants. After an 8500 ms delay, another consonant will be presented (probe). Participants respond with a button press to indicate whether the probe was part of the previous memory set (old) or whether the

probe was not part of the memory set (new).

Single-Task Condition (Arrow Flanker Task): Participants will be instructed to respond with a button press to indicate whether the central arrowhead points to the left or the right. Flanking arrows, positioned either above, below, or both above and below the central arrow, can point in either the same (congruent) direction (40% of trials) or different (incongruent) direction (60% of trials). Each flanker stimulus is presented for 200 ms, with the next trial beginning 300 to 500 ms after a response was made.

Dual-Task Condition (Sternberg Memory Task + Arrow Flanker): Participants will be required to perform the arrow flanker task during the delay interval of the Sternberg memory task. Nine flanker trials begin 300 to 500 ms following the presentation of each memory set. The stimulus parameters are the same as for the single-task flanker. The Sternberg probe is then presented 500 ms following the final flanker trial, and participants respond to indicate whether this item was in the previous memory set.

Statistical Analysis: The ERP old/new effect will be analyzed using a mixed effects ANOVA controlling for task (single, dual), set size (one, four), and time point (pre, post). The primary neuropsychological outcome measures will be included in the models as appropriate to assess changes from pre- to post-training. To examine the effects of repeated testing, differences in ERP mean amplitudes between patient groups (trained, untrained), task, and set size will be also be tested using a separate mixed effects ANOVA to control for random effects due to subject. Pre/post differences in Sternberg WM accuracy will be tested using a hierarchical generalized linear model. Time-frequency responses will be analyzed with the same mixed and linear models, with separate calculations for each frequency band, and a repeated measures model including all frequency bands.

Predicted Results: We predict that Veterans who show cognitive gains with training (based on neuropsychological measures) will show an increase in the ERP old/new effect in the dual task condition. Additionally, as prior work has shown that WM impairment in ADHD is characterized by decreased levels of alpha power during encoding, and compensatory increases in theta during maintenance (Lenartowicz, et al. 2014), we predict that cognitive gains will be reflected by changes in theta and alpha power over frontal scalp. Importantly, we can determine with precise temporal resolution whether the neural manifestations of training improvements occur during WM encoding, maintenance, and/or retrieval. Additional analyses will determine whether these EEG markers can predict which patients will improve with training.

Resting State EEG and treatment outcome prediction

Rationale: Our previous work showed that network modularity derived from a form of resting state fMRI was predictive of changes in cognitive functioning from training (Arnemann, 2015). Using a data driven network analysis approach, we propose to evaluate the potential use of EEG biomarkers for predicting the cognitive gains that individuals achieve through training. This will be the first study to apply the graph theoretic metric of network modularity using EEG recordings in participants with TBI.

Experimental EEG Design: Same task as described in Preliminary Study #3.

Statistical Analysis: The first stage will identify possible EEG biomarkers (frequency band, connectivity metric) using a Spearman Correlation with attention/executive function behavioral measures (baseline and change due to training). We will employ a stepwise regression to create a model from the potential biomarkers that maximizes the explanation of variance in behavioral measures.

Predicted Results: *Building on our past observations from MRI functional connectivity, we predict that patients with high modularity, and low characteristic path length, will exhibit the greatest positive behavioral changes with training, and these differences between subjects will be greatest in the theta and alpha bands. Patterns of connectivity in previously reported EEG measures suggest higher modularity scores in mTBI patients than controls (Cao & Slobounov, 2009)*, and that disruptions in long distance connectivity are primarily in the theta and alpha bands *(Kumar et al., 2009).* The study will allow a dual approach, combining data-driven analysis of resting state EEG data to identify potential predictive markers, and hypothesis-driven testing of cognitive performance to explain the relation of these biomarkers to the variability in response to training. This approach allows us to develop a model of brain connectivity on baseline resting state data, and test it on tasks that engage working memory and attention processes. *We further hypothesize that pre-training resting state modularity and CPL scores in mTBI patients will predict the degree to which theta and alpha power during working memory tasks normalize towards control responses*.