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Emotion Regulation Training for Treating Warfighters with Combat-Related PTSD Using Real-Time fMRI and EEG-Assisted Neurofeedback.

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14. ABSTRACT

PTSD is a chronic and disabling condition characterized by dysregulated fear, anxiety, anger, and depression. Neurocircuitry-based models of PTSD emphasize dysregulation of the amygdala, which is involved in the regulation of PTSD-relevant emotions. We are utilizing real-time functional magnetic resonance imaging neurofeedback (rtfMRI-nf) training with concurrent electroencephalography (EEG) recordings to directly target and modulate the emotion regulation neurocircuit. By using the multimodal data, we can determine which EEG signals/leads or their combination specifically predicts or correlates with clinical improvement associated with the rtfMRI-nf training. During year 1 we have secured IRB approval and a Certificate of Confidentiality for the project and are actively enrolling veterans to complete rtfMRI-nf neurofeedback training with simultaneous EEG recordings, and a pre-, post-training clinical assessment battery to evaluate improvement on the psychological and behavioral domains affected in combat-related PTSD (Aim1). Our extensive recruitment campaign is currently yielding several potential participant contacts per week, resulting in ongoing Veteran enrollment in the project at a rate consistent with our goals during this phase of the project. We also have begun developing the software/hardware system for a stand-alone EEG training paradigm as proposed (Aim2).

15. SUBJECT TERMS

PTSD; amygdala; fMRI; EEG; real-time fMRI neurofeedback; simultaneous EEG-fMRI; emotion regulation

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Our *main objective* is to determine whether rtfMRI- and rtEEG-assisted neurofeedback emotion regulation training protocols can reduce the symptoms of combat-related post-traumatic stress disorder (PTSD), a chronic and disabling psychiatric condition. Individuals with PTSD suffer from the dysregulation of several types of emotion, including fear, anxiety, anger, and depression [1–4]. Neurocircuit models of PTSD emphasize the role of the amygdala and its reciprocal interactions with the ventromedial prefrontal cortex (vmPFC) [5–9]. To advance understanding of the treatment of combat-related PTSD, the current state-of-the-art research aims to test ways to modulate the functions of the emotion circuit implicated in PTSD. We utilize the recent advances in real-time functional magnetic resonance imaging neurofeedback (rtfMRI-nf) to directly target and modulate amygdala activity [10–11]. This technique measures neuronal activity with sufficiently high temporal resolution that information from the amygdala is immediately available to form a feedback loop. In parallel with rtfMRI-nf, we obtain simultaneous measurement of electroencephalography (EEG) signals, which directly reflect brain activity in the cerebral cortex [12]. By using the multimodal imaging data we can determine which EEG signals/leads or their combination specifically predict or correlate with clinical improvement that has been associated with the rtfMRI-nf training [11,13–16]. This knowledge will enable us to establish a translational path toward the development of stand-alone real-time EEG neurofeedback (rtEEG-nf) training for emotion regulation, which can facilitate the widespread implementation of the treatment approach due to the high portability and relatively low cost of EEG systems.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

   Combat-related PTSD, fMRI, EEG, emotions, amygdala, neurofeedback

3. **OVERALL PROJECT SUMMARY:** Summarize the progress during appropriate reporting period (single annual or comprehensive final). This section of the report shall be in direct alignment with respect to each task outlined in the approved SOW in a summary of Current Objectives, and a summary of Results, Progress and Accomplishments with Discussion. Key methodology used during the reporting period, including a description of any changes to originally proposed methods, shall be summarized. Data supporting research conclusions, in the form of figures and/or tables, shall be embedded in the text, appended, or referenced to appended manuscripts. Actual or anticipated problems or delays and actions or plans to resolve them shall be included. Additionally, any changes in approach and reasons for these changes shall be reported. **Any change that is substantially different from the original approved SOW (e.g., new or modified tasks, objectives, experiments, etc.) requires review by the Grants Officer's Representative and final approval by USAMRAA Grants Officer through an award modification prior to initiating any changes.**

During the second year of the project period we were facing recruitment challenges resulting in delays with rtfMRI-nf and EEG data collection. Despite substantial recruitments efforts, with a detailed description following the preliminary data analysis section, we remain in phase 1 of the project targeting Aim #1: *Establish rtfMRI-nf training feasibility with concurrent EEG recordings in a combat-related PTSD population (Phase 1, month 1–18)*. Importantly, slow recruitment resulted in a 12-month delay relative to the originally proposed scope of work. Activities are currently underway to also meet Project Milestone #2: fMRI/EEG data collection of 8 subjects per group (control: veterans with no PTSD; neurofeedback, sham: veterans with PTSD) as proposed. To diminish and avoid further delays with rtfMRI-nf and EEG data collections, as of month 24 of the project we are developing the rtfMRI-nf and rtEEG-nf software for the purpose of Aim #2: *Develop a stand-alone rtEEG neurofeedback training protocol for PTSD*. We have already developed EEG communication software to reach Milestone #3, and based on preliminary data analysis suggesting rtfMRI-nf amygdala training feasibility in PTSD, we have identified a target (frontal EEG asymmetry, see preliminary data analysis below) for development of the stand-alone rtEEG neurofeedback training paradigm and EEG-nf system (Milestone #4). We are anticipating that for the purpose of Phase 3 of the project and Aim #3: *EEG-only neurofeedback training feasibility in combat-related PTSD* (with human subjects research approval already obtained, Milestone #5 reached) recruitment will be easier and faster since there will be no MR-related exclusions (e.g., shrapnel in the body).
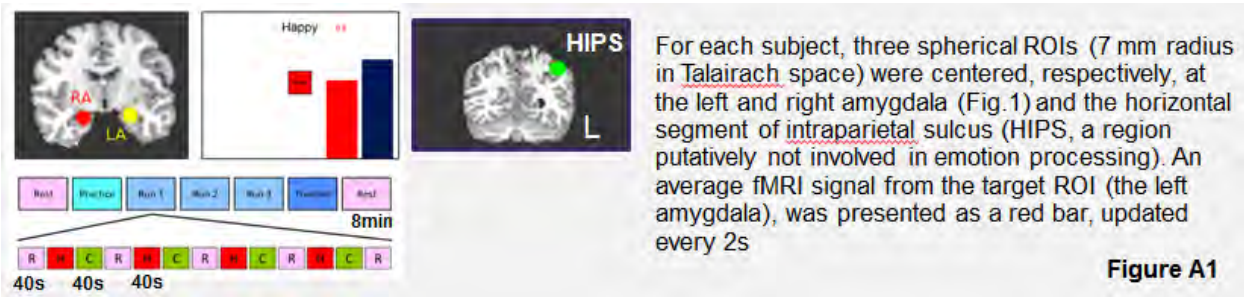
In order to monitor continuously recruitment progress, we have regular weekly meetings with recruitment staff and biweekly research meetings where current project needs, problems, method and software developments, and relevant activities are discussed with all investigators, including co-investigators Drs. Feldner (University of Arkansas) and Krueger (George Mason University), both joining via video or teleconference.

Additionally multimodal EEG and fMRI brain imaging capacity have expanded substantially. In particular, a second MRI 750 3 Tesla MRI scanner was successfully delivered and installed and is being equipped at this time with a custom real-time fMRI system allowing neurofeedback experiments with simultaneous EEG recording, state-of-the-art RF brain array coils, a 128-channel MR-compatible EEG system, and fMRI-related auxiliary equipment. The expansion of major research hardware and multimodal EEG and fMRI brain imaging capabilities will further enhance our capacity to move subjects through the experimental protocol during regular LIBR MRI weekday scanning operations (Mon–Fri 8 am–8 pm) as well as on Saturdays (8 am–8 pm).

Preliminary data analysis

We continuously increased the numbers of veterans enrolling in, undergoing, and finishing Phase 1 of the study. Hence, we continue to advance efficient data processing pipelines and conduct preliminary data analysis for Aim #1, which includes the following: (i) to validate whether veterans with PTSD are able to use rtfMRI-nf training to enhance their control of the hemodynamic response of the amygdala; (ii) to further assess specificity of neuromodulatory effects induced by the LA neurofeedback; (iii) to evaluate possible sustained neuroplastic changes induced by the procedure; and (iv) to conduct preliminary analyses identifying a single EEG feature that is optimally suitable for developing the stand-alone rtEEG-nf training protocol (Phase 2) and experimental evaluation of the protocol (Phase 3).

A. rtfMRI-nf amygdala training. Preliminary data analyses were conducted on the 8 veterans in the amygdala feedback group and six in the control feedback (HIPS) group (Fig. A1). These analyses include results from multiple rtfMRI-nf visits. A general linear model analysis of the fMRI data from the neurofeedback visits was conducted using AFNI. Statistical analysis for behavioral data was performed in SYSTAT.

For each subject, three spherical ROIs (7 mm radius in Talairach space) were centered, respectively, at the left and right amygdala (Fig.1) and the horizontal segment of intraparietal sulcus (HIPS, a region putatively not involved in emotion processing). An average fMRI signal from the target ROI (the left amygdala), was presented as a red bar, updated every 2s
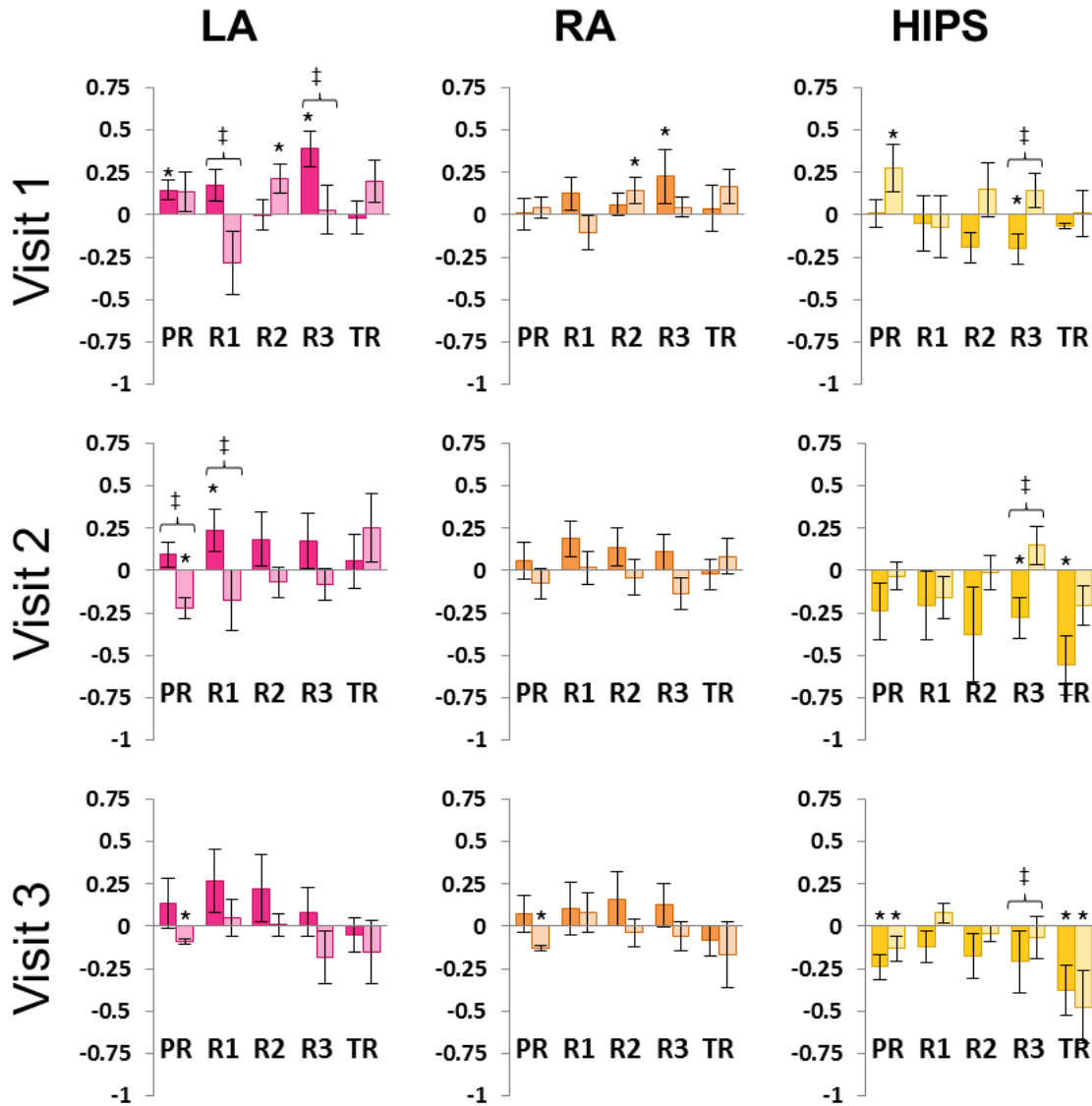
Figure A1

All subjects were male, age 18–55, right-handed U.S. military combat veterans. No significant baseline differences were observed in age, PTSD symptom severity, or depression symptom severity between the two groups (as described in Table A1).

	Experimental (LA)	Control (HIPS)
n	8	6
Age (std dev)	31 (8)	36 (10)
Initial CAPS (std dev)	52.0 (10.1)	56.8 (24.4)
Initial HDRS (std dev)	13.5 (5.4)	14.3 (8.1)

Table A1. Demographic information for experimental and control groups. CAPS = Clinician Administered PTSD Scale. HRSD = 21-Item Hamilton Rating Scale for Depression.

ROI analysis results.

A GLM analysis for each of the three visits was performed to determine the training effect of the neurofeedback procedure. The analyses were implemented in AFNI and SYSTAT. Pre-processing included cardiac and respiratory waveform correction, volume registration, and slice timing correction. Standard GLM analysis was then applied separately for each of the five neurofeedback runs on each of the three visits including the following regressors: two block stimulus conditions (Happy Memories, Count), six motion parameters, and five polynomial terms. After deconvolution, percent signal change for Happy vs. Rest conditions were calculated. In preparation for the whole-brain statistical group analysis, the spatially-normalized fMRI percent signal change maps were spatially smoothed using a Gaussian kernel with full width at half maximum (FWHM) of 5 mm. One-sample t-tests were performed separately for each run and for each group to determine whether activation was significant. Difference between groups was determined using a 3 (visit: 1, 2, 3) × 3 (ROI: LA, RA, HIPS) × 5 (run: PR, R1, R2, R3, TR) × 2 (group: EG, CG) analysis of variance (ANOVA).

Figure A2. Mean percent signal change between Happy and Rest conditions for each of the neurofeedback runs. PR = practice, R1–3 = training runs 1–3, TR = transfer. Left/dark bar = experimental group, right/light bars = control group. * = significant difference from 0, ‡ = significant difference between experimental and control. For run visit 3, experimental group n=7 due to scanner error.

ROI analysis results are shown below in Figure A2. Each row of graphs represents results from one of the three neurofeedback visits. Each column of graphs shows results for one of three ROIs: the left amygdala (target ROI for the experimental group), right amygdala (shown to explore the effects of laterality), and left HIPS (target ROI for the control group). In each graph, bar height represents mean percent signal change between Happy and Rest conditions for a

single run. The dark/left bars represent the experimental group while the light/right bars represent the control group. These results indicate that across each of the three visits subjects in the experimental group were able to better elevate activity in the left amygdala during neurofeedback scans, while subjects in the control group were unable to do so. A similar activation pattern occurred in the right amygdala, though the effect for the experimental group was not as strong as in the left. Neither group was able to up regulate activity in the left HIPS.

Clinical score change results

Clinical ratings were taken at the beginning of each visit and used to assess the effects of the neurofeedback training on PTSD and depression symptoms (Table A2). Subjects in the experimental group showed decreased PTSD and depression symptoms that were both statistically and clinically significant. Significant reduction in depression symptoms (according to the HRSD) were seen after only one neurofeedback visit and significant PTSD symptom reductions (according to the PCL-M) were observed after two neurofeedback visits. No significant changes in symptom measures were observed in the control group.

	Experimental (LA)			Control (HIPS)		
Scale	Initial score	Mean change	t	Initial score	Mean change	t
CAPS	47.7	*−21.5**	t(5) = −6.1	56.8	-	-
PCL-M	36.6	*−6.1**	t(7) = −3.1	46.2	−5.8	t(4) = −1.4
HRSD	14.6	*−2.4*	t(7) = −1.8	13.0	−6.6	t(4) = −2.1
MADRS	15.8	*−4.0*	t(7) = −3.7	15.3	−7.6	t(4) = −2.3

Table A2. Clinical score change results for both experimental and control groups. PCL-M = PTSD Checklist – Military Version (17–85). CAPS = Clinician Administered PTSD Scale (0–136). HRSD = 21-Item Hamilton Rating Scale for Depression (0–52). MADRS = Montgomery–Åsberg Depression Rating Scale (0–60). Initial ratings taken before first neurofeedback scan. Final ratings taken at final Stroop scan (after 3rd neurofeedback scan). * Indicates a significant change from pre- to post-scan ratings at $p < 0.05$. CAPS scores for the control group unavailable due to subject drop-out.

Functional connectivity results

A GLM-based functional connectivity analysis was applied using a seed ROI in the left amygdala region to determine functional connectivity of the amygdala network (Figure A2). The

seed ROI was defined as a sphere of 5 mm radius in the Talairach space with the same central point as the target ROI for the experimental group neurofeedback (21, 5, −16). The volume-registered and slice-timing-corrected single-subject fMRI data from the transfer run were high-pass filtered at 0.01 Hz and low-pass filtered at 0.08 Hz. The time course of the mean fMRI signal from the seed ROI only during the Happy condition was used as a stimulus regressor.
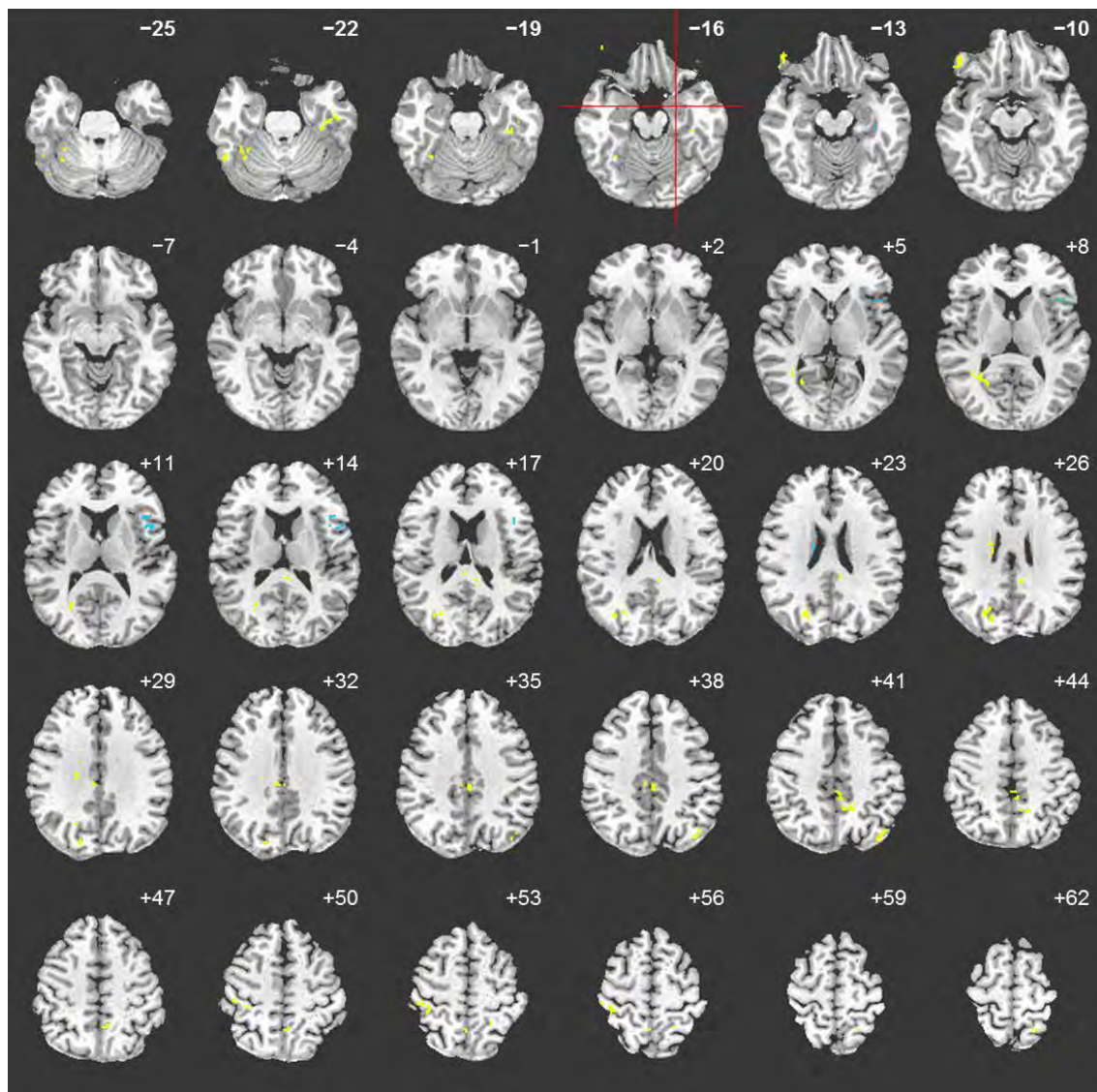
Figure A2. Functional connectivity analysis (visit 3 transfer run) using a seed ROI in the left amygdala (experimental vs. control). The connectivity maps are projected on a representative single-subject T1 template in the Talairach space. The red crosshairs mark the center of the seed ROI for the connectivity analysis (21, 5, −16). Warm colors indicate greater connectivity in the experimental group, cool colors indicate great connectivity in the control group. $p < 0.05$, cluster size > 30.

The GLM model for each run also included six motion parameters, five polynomial terms for modeling the baseline and time courses from two additional ROIs defined, respectively, within the deep white matter and the CSF of the lateral ventricles. The resulting maps were transformed into Talairach space and spatially smoothed at 5 mm FWHM. The GLM-based R-squared statistics were converted to correlation coefficient values r, which were subsequently converted to z scores using the Fisher r-to-z transformation. A paired samples t-test was then used to compare connectivity between the experimental and control groups.

	Region	Center	Cluster size
	R. cerebellum	−43,63,−26	69
	R. cuneus	17,−77,30	64
	L. precuneus	−15,−49,42	62
	L. cingulate gyrus (BA 31)	−1,−29,34	53
	L. BA 20	−45,−11,−20	52
EG>CG	L. BA 7	−19,−53,62	42
	R. BA 30	27,−53,8	41
	R. cingulate gyrus	17,−7,28	39
	R. postcentral gyrus	43,−29,50	36
	R. inferior frontal gyrus (BA 47)	45,33,−12	30
CG>EG	L. insula	−39,17,12	41

Table A3. Functional connectivity analysis of visit 3 transfer run using a seed ROI in the left amygdala as function of group (experimental [EG] vs. control [CG]).

Correction for multiple comparisons was based on FDR.Results of the functional connectivity analysis showed that activity in the left amygdala was significantly more correlated with activity in other brain regions during happy memory recall for the subjects in the experimental group receiving LA neurofeedback than it was for subjects in the control group receiving left HIPS neurofeedback. In fact, only one region was shown to be more connected with the left amygdala in the control group vs. the experimental group (Figure A2, Table A3).

B. Functional connectivity analysis pre- and post rtfMRI-nf amygdala procedure.

We have also conducted a functional connectivity analysis on resting state fMRI scans acquired before and after the three-session rtfMRI-nf procedure. We found in combat veterans with PTSD, rtfMRI-nf amygdala training during happy autobiographical memory recall induced changes in resting state DMN functional connectivity (Figure B1).

Subjects reported significantly decreased PTSD symptoms (PCL-M), anxiety and depression after rtfMRI-nf training (see Table B1). In the post-training resting state scan, functional connectivity was found to have decreased in the insula (BA13), pregenual anterior cingulate cortex (pgACC including parts of BA32 and BA10), cingulate gyrus (BA31), precuneus (BA7), and thalamus. Interestingly, previous studies reported that patients with PTSD showed increased connectivity in insula, and BA32 compared to health controls [19]. Also connectivity in BA10 has been found to positively correlate with PTSD symptoms.

After - Before

PTSD Experimental group (active rtfMRI-nf)

PTSD Control group (control rtfMRI-nf)

Healthy control group (active rtfMRI-nf)
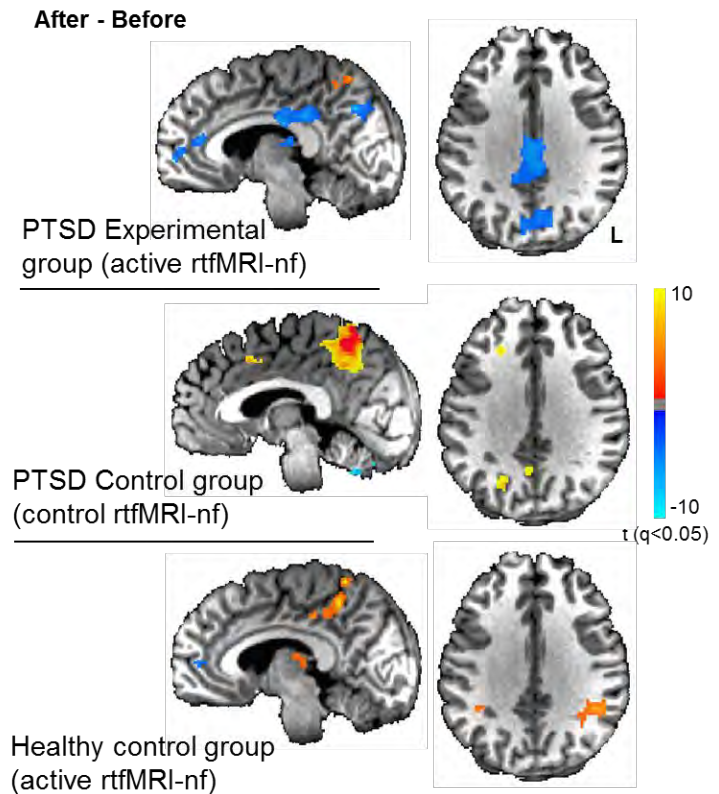
Figure B1. DMN functional connectivity difference between before and after rtfMRI-nf training

	PTSD Active (n=8)		PTSD Control (n=5)	
	Before	After	Before	After
PCL-M (Mean ± SD)	41.2 ± 11.5	31.5 ± 7.9 **	53.8 ± 16.6	44.6 ± 23.2
HARS (Mean ± SD)	16.0 ± 8.2	10.6 ± 5.7 *	21.2 ± 8.7	13.6 ± 5.3
HDRS (Mean ± SD)	13.5 ± 5.4	10.1 ± 4.2 *	16.4 ± 7.0	8.2 ± 3.6
MADRS (Mean ± SD)	17.5 ± 8.7	8.3 ± 4.5 *	18.8 ± 10.6	9.2 ± 5.4
SHAPS (Mean ± SD)	31.1 ± 1.4	28.5 ± 2.9 *	32.2 ± 3.2	25.3 ± 7.5

Table B1 Clinical characteristics before and after rtfMRI-nf training.
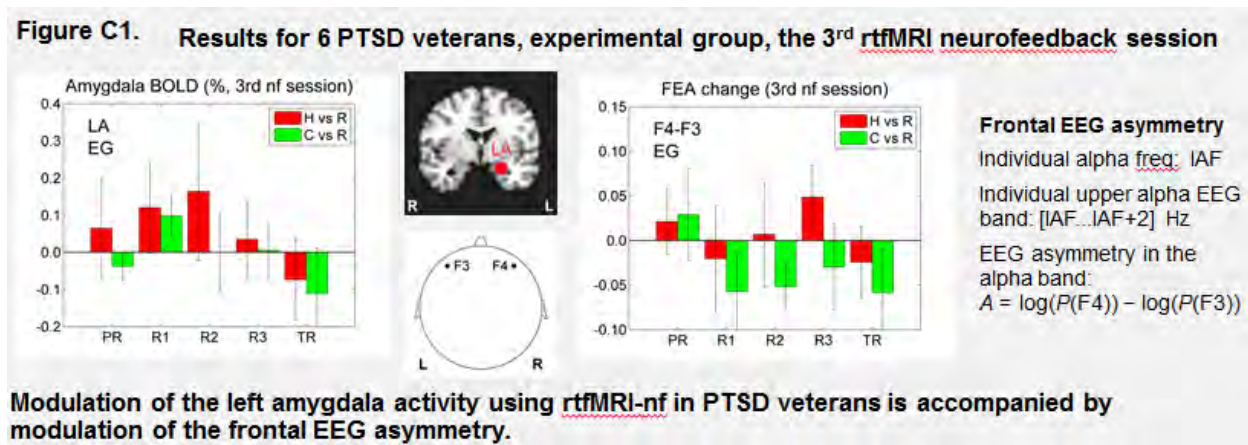
*or** indicate significant decrease after neurofeedback relative to before neurofeedback (*: $p<0.05$, **: $p<0.01$).

PCL-M: the PTSD Checklist, military version
HARS: the Hamilton Anxiety Rating Scale
HDRS: the Hamilton Depression Rating Scale
MADRS: the Montgomery–Åsberg Depression Rating Scale
SHAPS: the Snaith-Hamilton Pleasure Scale

In both the PTSD and health control groups the connectivity was decreased in the pgACC, which is implicated in recall of autobiographical memory. Decreased pgACC connectivity has also been observed in patients with major depression [20] and has been associated with the alleviation of depression severity [21]. However, the decrease in pgACC connectivity was not observed in the PTSD group who received control neurofeedback from the HIPS region. Thus, our results are consistent with previous reports, and also suggest that after rtfMRI-nf training the abnormal brain connectivity in PTSD is reversed. This suggests a sustainable plasticity effect due to the amygdala-targeted rtfMRI-nf training in veterans with PTSD.

C. rtfMRI-nf amygdala training effect on simultaneously collected EEG data.

At this time, we are developing data analysis methods for preliminary collection of simultaneously collected EEG data during rtfMRI-nf experiments alongside preliminary combined analysis of EEG and fMRI. To evaluate electrophysiological correlates of the rtfMRI-nf training targeting the amygdala, we examined average changes in frontal EEG asymmetry (FEA) in the upper-alpha EEG band for the Happy conditions with rtfMRI-nf relative to the Rest conditions (Figure C1).

Figure C1. **Results for 6 PTSD veterans, experimental group, the 3rd rtfMRI neurofeedback session**

Modulation of the left amygdala activity using rtfMRI-nf in PTSD veterans is accompanied by modulation of the frontal EEG asymmetry.

The analysis followed the procedure employed in our previous study on MDD patients (described in Appendix 1). The analysis was conducted among PTSD patients (*n=6*) who underwent the rtfMRI-nf training with the active nf condition (left amygdala target), and completed the study (including the final CAPS evaluation). The average FEA changes for EEG

channels F3 and F4 were determined for each run, and further averaged for the four rtfMRI-nf runs (Practice, Run 1, Run 2, Run 3) in a given training session.

Results of the FEA analysis are reported in Figure C2, where they are compared to the PTSD patients' CAPS ratings. Fig C2A shows correlation between the average individual FEA changes during the 1st rtfMRI-nf session and the initial CAPS scores. Fig. C2B displays correlation between the average FEA changes during the 3rd (last) rtfMRI-nf session and the patients' final CAPS scores. Figure C2C shows the correlation between the individual differences in the average FEA changes for the 3rd and the 1st rtfMRI-nf sessions and individual changes in the CAPS ratings (final vs initial).

The FEA changes in both Fig. C2A and Fig. C2B show positive correlations with the corresponding CAPS ratings that are approaching statistical significance. This result is consistent with the inverse correlation between the FEA values at rest and individual CAPS scores [18]. This effect and its tentative interpretation are similar to those for MDD patients in our previous study [Appendix 1]: more positive FEA *changes* during the rtfMRI-nf task (Happy) reflect more negative FEA *values* during the baseline condition (Rest). It should be noted, however, that diminished FEA levels may be associated with somewhat different neural mechanisms in MDD and PTSD. The results in Fig. C2C suggest that normalization in the PTSD patients' mental state (i.e. reduction in the CAPS ratings) is associated with reduction in the average FEA changes during the rtfMRI-nf task. Such behavior would be consistent with an increase in baseline FEA levels following the rtfMRI training course, but this would need to be verified separately.

Figure C2.

Based on our preliminary data analysis we have already identified frontal asymmetry in the alpha band (FA-alpha) as a promising target for rtEEG-nf experiments (Aim #3). In addition, our preliminary results suggest that variations in
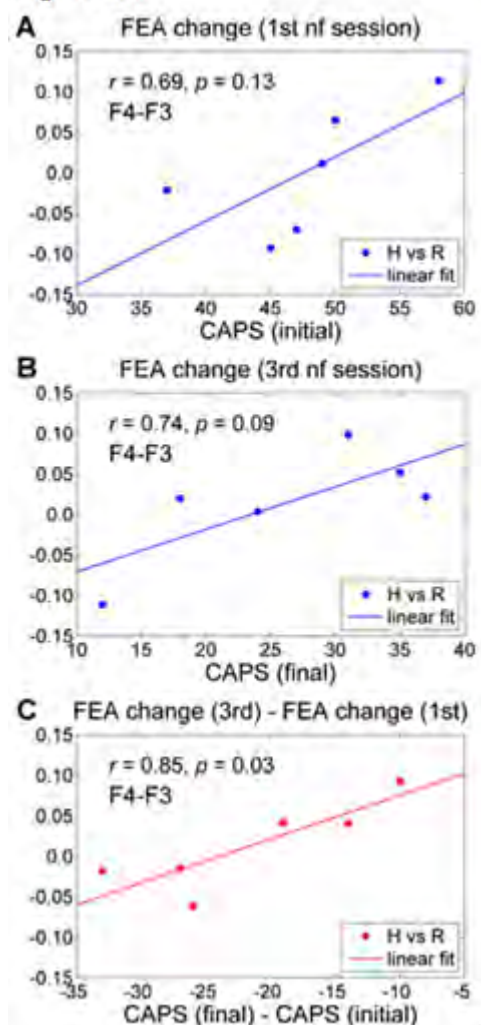
FEA during rtfMRI-nf training might independently provide valuable information about PTSD severity and treatment response.

Summary of preliminary data analysis

Taken together this preliminary analyses of our rtfMRI-nf amygdala training results, demonstrates that veterans with PTSD can learn to self-regulate their amygdala BOLD responses during recall of positive autobiographical memories (i.e., confirmation of Ami #1). Notably, rtfMRI-nf training of the left amygdala resulted in improvements of PTSD and depression symptoms. Significant reduction in depression symptoms (according to the HRSD) were seen after just one neurofeedback visit and significant PTSD symptoms reductions (according to the PCL-M) were seen after just two neurofeedback visits in veterans with PTSD in the experimental group but not in control controls. Functional connectivity analysis of the amygdala during the neurofeedback procedures revealed substantial differences between the experimental (feedback from LA) and control groups (feedback from HIPS), proving additional evidence of a specific neuromodulatory effect induced by the LA neurofeedback procedure during positive memory recall. Brain regions co-activated with the LA feedback procedure (forming an amygdala-related network) were consistent with the broader literature regarding the amygdala-related neural network involved in emotion processing.

Evaluation of brain effects induced by the rtfMRI-nf procedure revealed changes in resting state DMN connectivity in veterans with PTSD. The LA rtfMRI-nf training procedure reversed abnormal connectivity observed in subjects with PTSD and as compared to healthy controls. Specifically in the post-training session, functional connectivity decreased in the insula, pregenual ACC, BA31, precuneus and thalamus. Veterans in the experimental group reported significantly reduced PTSD symptoms (PCL-M), anxiety and depression after rtfMRI-nf training. This analysis also suggests sustained neuroplastic changes induced by the rtfMRI-nf LA training and happy autobiographical memory recall.

Preliminary analysis of the concurrently acquired EEG data during rtfMRI-nf revealed that modulation of BOLD LA activity during the neurofeedback procedure was accompanied by changes in frontal EEG asymmetry (FEA) in the upper alpha band (power(F4)-power(F3) electrodes). The direction of changed in the FEA (e.g. more positive FEA) induced by the

rtfMRI-nf LA training was consistent with more approach-oriented responses and traits as well more positive emotions.

Indeed we observed that reduction in CAPS ratings was associated with reduction in the average FEA changes during the rtfMRI-nf task, indicating that variations in FEA during rtfMRI-nf training might independently provide valuable information about PTSD severity and treatment response. Those preliminary results identified the FEA as a promising target for EEG-only neurofeedback training among veterans with combat-related PTSD population (Aim #3).

Recruitment efforts description

For the second year (October 2013 through October 2014), LIBR recruitment staff developed methods of regular outreach in the community for the study. Recruitment efforts included attending events in the community such as mental health events and events that were targeted towards the study population. Recruitment staff also visited community organizations to attempt to build partnerships with our institution and to display recruitment supplies such as business cards and brochures. Staff continued to engage with community organizations in already established ongoing partnership with our institution. The recruitment supplies were replenished monthly or as needed. Since July 2014, the recruitment staff has also increased from one member to four teams of three. This change will allow for us to outreach a broader area in the community on a more consistent basis.

Recruitment efforts continued utilizing media outlets to introduce as well as provide information to the veteran population about research initiatives and needs. These media resources included: radio advertisements on at least a monthly basis, Facebook, and Craigslist. The latest Facebook ad ran in 50-mile radii of Tulsa and Oklahoma City and reached 120,000 males 18-55 year olds.

In July 2014, aiming to further improve our most effective recruitment tools, recruitment staff rewrote the radio advertisements and redesigned the brochures. The aim was to provide a more general advertisement. We hope that based on the new advertisements more veterans will call in to obtain study-specific information. We also made an effort to research which radio stations in the area were most popular and which time slots garnered the most listeners. We contacted the popular radio stations and have been in ongoing communication with them through September 2014. Results from each radio ad are being collected and compared. We are continually using the results to help make our recruitment efforts more efficient. Further efforts

were extended to continually researching new recruitment possibilities online or through other social avenues. For instance, recruitment investigated a gun show in the area and contacted their personnel to see if there was a possibility to set up a booth at their event.

In August 2014, in an effort to further validate local station genres, recruitment ran ads on airways that were reevaluated as promising. We began with a classic rock station, KJSR with Cox Media, from the 23rd to the 25th. We aired 15 total spots during weekday and weekend drive-times with a result of 6 newly established calls. Afterwards, we looked into a higher ranked rock station via the Arbitrends rankings, running 18 spots over three days with a return of 2 calls. In September 2014, in order to reach out to a broader population, recruitment staff contacted a Spanish radio station. Negotiations of price were made with KBEZ and Clear Channel radio for a PTSD ad running for 42 spots from the over six days. We established 4 new calls from this series. Recruitment staff most recently ran a radio ad with a classic country station, KXBL with the Journal Broadcast Group. We aired during 40 weekday-only, morning and evening drive-time spots. Beginning in October 2014, we have also begun airing radio ads on two radio stations in the Oklahoma City area.

Our recruitment efforts included an on-going monthly relationship with certain medical and veteran organizations such as the Veterans Initiative, Family and Children Services, Veteran's Advisory Council, and Laureate Psychiatric Clinic and Hospital. Each month, our recruitment-focused staff attended meetings and provided presentations along with providing over 2500 pieces of study-focused literature to various social workers, mental health counselors, psychologists, and physicians. These recruitment efforts included meetings and presentations with particular selected clinicians, which work directly with the target population. We also continued our efforts as we directly recruited patients/potential participants from Family and Children Services monthly and the Laureate Psychiatric Clinic and Hospital weekly. Starting in January, staff also began meeting with local physicians' offices, apartment complexes, motorcycle shops, and gun stores, in order to arrange for distribution of recruitment materials at these locations.

In August 2014, recruitment contacted and attended colleges in the local area to introduce our research and to discuss the possibility of displaying study brochures and other recruitment supplies on their campuses. We obtained permission and distributed recruitment materials on tables, cork boards, and information booths at the student unions on the Tulsa campuses of the

University of Oklahoma and Oklahoma State University. Permission was also obtained to distribute materials at the University of Tulsa student union, which is currently under construction. We are awaiting further approval to hand out recruitment materials on the campuses of Oral Roberts University, Tulsa Community College, and Tulsa Technology Center. We are also in the process of trying to contact a professor at University of Tulsa in hopes that by developing a partnership with her we may be able to meet local clinicians who work directly with veterans. We also hope to meet with clinicians who will be able to send us referrals for veterans who are receiving non-medicated treatments.

During the month of October 2014 alone LIBR has placed over 400 pamphlets, flyers, and business cards at local apartments, gyms, laundry facilities, liquor stores, colleges, and technology schools. Recruitment staff have reached out to a number of organizations in order to coordinate lectures, placement of recruitment materials, or participation in activities. These include the psychological health director at the 138th fighter wing in Tulsa, the psychological health director at the OKC National Guard, and the Rolling Thunder event coordinator. We have also looked into expanding our advertising options during events at the BOK Center in downtown Tulsa.

During the past year, recruitment staff members have attended a large number and variety of veteran-focused events in addition to the regular monthly meetings listed above:

11/05/13	Veterans Town Hall Meeting with Mayor Dewey Bartlett and Congressman Jim Bridenstine
01/25/14	National Guard 30 Day Yellow Ribbon Event
02/15/14	Oklahoma National Guard Join Force meeting
02/18/14	Veterans Treatment Court
02/22/14	National Guard 60 Day Yellow Ribbon Event
02/27/14	Tulsa Tech's Career Tech CT 4 Vets
03/08/14	Veterans Families United Foundation meeting
04/12/14	Jenks Southeast Families Run for Fun
04/21/14	LIBR Open House
05/02/14	National Guard 60 Day Yellow Ribbon Event
05/02/14	Memorial Veterans Arena dedication
05/29/14	Jack C. Montgomery VA "Moral Injury" workshop

06/14/14	Horses for Heroes "Operation Free Ride"
06/27/14	Jack C. Montgomery VA Oklahoma Communities for Veterans Resource Fair
8/29/14– 8/31/14	Patriots Day at Lafortune Park Golf Course and South Lake Golf Course
08/18/14	VA Behavioral Health Clinic visit
09/11/14	Coffe Bunker grand opening
9/18/14– 9/19/14	Zarrow Mental Health Symposium

In the upcoming months, recruitment will continue to research new ways to help recruit the target population. Current recruitment possibilities include recruiting in areas where there is a large veteran population. We were able to obtain a list of zip codes where many veterans reside. We will be attending community organizations in those areas to build partnerships that will help with recruitment. For example, new organizations we would like to connect with are post offices, tag agencies, unemployment agencies and laundry facilities in each of these areas.

We also are continuing to hand out surveys to the veterans that participate in the study. The surveys ask the veterans for recruitment suggestions. One recent idea we received from a survey was to attend drill weekend at the National Guard. A veteran suggested for us to teach a class about PTSD during drill weekend which would allow us to have direct contact with veterans and directly recruit from the base. We are currently trying to connect with bases in the area to see if this may be a possibility.

Enrollment information
Since the study began, LIBR has received 300 phone calls. We have completed 227 phone screens with veterans. Out of all the phone calls we received, only 36 so far have been unreachable after multiple contact attempts. 37 phone screens are still currently pending.

· 88 were excluded by phone screen

· 139 were eligible for the screening assessment at LIBR

There are about 30 pending assessments, and those subjects being contacted or are already scheduled for an assessment

· 85 were consented to the screening assessment as of 10/27/2014

· Out of the 85, 46 were eligible, 26 excluded, 7 didn't complete the assessment, 5 were medicated

· Out of the 46 that were eligible, 41 were consented to the study and 5 are in the process of being scheduled to be consented to the study

· 40 completed the initial CAPS

· Out of the 41 that were consented to the study, 8 were screen failures, 5 withdrew, and 1 was a lost to follow up

· 32 completed the 1st scan

· 29 completed the 2nd scan

· 27 completed the 3rd scan

· 25 completed the 4th scan

· 22 completed the final scan

· 19 completed the final CAPS

8 veterans are currently active in the study. Out of the 8 consented, 2 are in the process of scheduling their first scan.

4. **KEY RESEARCH ACCOMPLISHMENTS:** Bulleted list of key research accomplishments emanating from this research. Project milestones, such as simply completing proposed experiments, are not acceptable as key research accomplishments. Key research accomplishments are those that have contributed to the major goals and objectives and that have potential impact on the research field.

Due to recruitments delays, and continuing data collection phase there is nothing to report for the period covered by this report.

5. **CONCLUSION:** Summarize the importance and/or implications with respect to medical and /or military significance of the completed research including distinctive contributions, innovations, or changes in practice or behavior that has come about as a result of the project. A brief description of future plans to accomplish the goals and objectives shall also be included.

Our efforts during year 2 have resulted in substantial progress toward accomplishing our aims for this project. We have advanced necessary methodological aspects of the study, with a current primary focus on developing and implementing data analysis pipelines, and conducting data analysis on collected multimodal fMRI and EEG data. Multiple subjects have now successfully completed Phase 1, and preliminary data analysis further suggests its feasibility, and clinically relevance in reducing PTSD symptoms. For the purpose of accomplishing Aim #2, we are actively developing a software environment for real-time EEG neurofeedback (rtEEG-nf). Feasibility of rtfMRI-nf amygdala training (with simultaneous EEG recordings) in the combat-related PTSD population (Aim #1), allowed for identification of an EEG signal feature (frontal EEG asymmetry) for the purpose of establishing rtEEG neurofeedback, At this time, we are developing an EEG-nf training paradigm (Aim #3 and Phase 3), that involves novel algorithms for real-time artefacts correction in EEG signals for the purpose of improving EEG neurofeedback experiments and establishing its feasibility in veterans with PTSD. Besides those accomplishments; however recruitment of veterans continued to be challenging which has resulted in data collection delays. To resolve the recruitment challenges, we secured even more institutional support for our already very substantial recruitments efforts in order to further increase our recruitment campaign focused on the veteran's population in Oklahoma. Those substantial efforts (as described in this report) are now yielding an improved subject enrollment rate. We anticipate that those efforts will be sufficient and realistic to accomplish our pending aims of this project. Moreover, we anticipate that our accomplishments in Years 1 and 2 have situated our successfully collaborating team for further satisfactory progress throughout the remainder of the project period. Therefore, we remain well-positioned to develop and initially test a novel intervention that has the potential to advance both understanding of PTSD and our ability to successfully treat this chronic and costly condition.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

a. List all manuscripts submitted for publication during the period covered by this report resulting from this project. Include those in the categories of lay press, peer-reviewed scientific journals, invited articles, and abstracts. Each entry shall include the author(s), article title, journal name, book title, editors(s), publisher, volume number, page number(s), date, DOI, PMID, and/or ISBN.

    (1) Lay Press:
    (2) Peer-Reviewed Scientific Journals:
    (3) Invited Articles:
    (4) Abstracts:

b. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.

Conference presentation's abstracts:

1) Phillips, R., Zotev, V., Yuan, H., Young, K., Wong, C.K., Wurfel, B., Krueger, F., Feldner, M., Bodurka, J. (2014). Self-Regulation of Amygdala Activation with Real-Time fMRI Neurofeedback in Combat-Related PTSD. Oral presentation presented at the Joint Annual Meeting International Society of Magnetic Resonance in Medicine (ISMRM) and European Society of Magnetic Resonance in Medicine and Biology (ESMRMB) 2014, Milan, Italy.

2) Yuan, H., Wong, C.K., Phillips, R., Zotev, V., Misaki, M., Feldner, M., Bodurka, J. (2014). Default mode changes in veterans with PTSD after real-time fMRI neurofeedback training of amygdala. Poster presented at the 20th Annual Meeting of the Organization for Human Brain Mapping, Hamburg, Germany.

3) Wong, C.K., Zotev, V., Yuan, H., Bodurka, J. (2014). Cardiac cycle detection in EEG with residual MRI gradient artifact by multiple-scale peak detection. Poster presented at the 201th Annual Meeting of the Organization for Human Brain Mapping, Hamburg, Germany.

4) Bodurka, J. (2014). Emotional regulation training for treating warfighters with combat-related PTSD using real-time fMRI and EEG-assisted neurofeedback.
Progress report presented at the MOMRP PTSD Biomarkers IPR annual meeting, Ft. Detrick, MD.

7. **INVENTIONS, PATENTS AND LICENSES:** List all inventions made and patents and licenses applied for and/or issued. Each entry shall include the inventor(s), invention title, patent application number, filing date, patent number if issued, patent issued date, national, or international.

Nothing to report.

8. **REPORTABLE OUTCOMES:** Provide a list of reportable outcomes that have resulted from this research. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. This list may include development of prototypes, computer programs and/or software (such as databases and animal models, etc.) or similar products that may be commercialized.

1) Automation of motion correction using a method developed in house that utilizes the simultaneously acquired EEG data (E-REMCOR) [17]. E-REMCOR employs the EEG array as a sensitive motion detector and advances motion corrections in fMRI by allowing for corrections of even fast motions diffentially affecting individual slices of a collected brain volume. E-REMCOR utilizes high-temporal-resolution EEG data to correct for motion in fMRI images. It consists of independent component analysis on the preprocessed EEG data, identification of independent components (ICs) corresponding to the head motion, and utilization of EEG-based motion ICs as regressors to correct for head motion in the fMRI dataset. The essential part of E-REMCOR is to identify the ICs corresponding to the head motion, which involves analysis of their spectra, topographical maps, and contributions to the EEG signal. While E-REMCOR was shown to substantially remove head motions in fMRI dataset [17], its implementation requires experienced personnel for the identification of the motion ICs. To enhance the usability of E-REMCOR, Matlab code was developed to automatically preprocess the EEG data, calculate and analyze the properties of the ICs, and identify the motion ICs. This is accomplished by identifying and categorizing the special features of motion artifacts imposed on the spectra and the topographical maps of the ICs as well as the EEG signals. The automatic E-REMCOR procedure was applied to 305 fMRI scans. The automation is shown to be capable of substantially removing the head motion in

fMRI images. Depending on the subject's motion, the temporal signal-to-noise ratio (TSNR) in the corrected fMRI image with E-REMCOR application improves up to an average of 24% over the brain and a maximum of over 50% in certain brain region. In most cases when subjects have no significant motion, the average TSNR may increase slightly or decrease by less than 1%.The method can also reduce spurious correlations between the EEG and fMRI data caused by large and rapid head movements, greatly improve data quality, and reduce the number of subjects excluded from analysis due to motion artifacts. We applied the method on all currently acquired fMRI and EEG data from veterans with combat-related PTSD.

2) We are in the process of software development for the purpose of project Aim #2: development of stand-alone real-time EEG neurofeedback system. As part of this development a novel algorithm for better detection and correction of motion artefacts in real-time EEG data for the purpose of EEG-nf applications was implemented. We utilized moving window standard deviation and spline interpolation for better detection and correction of motion artefact in EEG data. This algorithim will be an important addition to the real-time EEG data correction pipeline for the purpose EEG neurofeedback experiments (Aim #3).

9. **OTHER ACHIEVEMENTS:** This list may include degrees obtained that are supported by this award, development of cell lines, tissue or serum repositories, funding applied for based on work supported by this award, and employment or research opportunities applied for and/or received based on experience/training supported by this award.

Nothing to report.

10. REFERENCES: List all references pertinent to the report using a standard journal format (i.e., format used in *Science*, *Military Medicine*, etc.).

1. Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, 2000).
2. Milliken, C. S., Auchterlonie, J. L. & Hoge, C. W. Longitudinal assessment of mental health problems among active and reserve component soldiers returning from the Iraq war. Jama 298, 2141-8, (2007).
3. Kessler, R. C. Posttraumatic stress disorder: the burden to the individual and to society. J Clin Psychiatry 61 Suppl 5, 4-12; discussion 13-4, (2000).
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16. Yuan, H., Zotev, V., Phillips, R., Drevets, W.C., Bodurka, J. Spatiotemporal dynamics of the brain at rest – exploring EEG microstates as electrophysiological signatures of BOLD resting state networks. Neuroimage 60(4), 2062-2072, (2012).
17. Zotev, V., Yuan, H., Phillips, R., Bodurka, J. EEG-assisted retrospective motion correction for fMRI: E-REMCOR. Neuroimage 63, 698-712, (2012).
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21. Yuan, H., Young, K.D., Phillips, R., Zotev, V., Misaki, M., Bodurka, J. Resting State Functional Connectivity Modulation and Sustained Changes after Real-Time fMRI Neurofeedback Training in Depression. Brain Connect. (2014, in press)

11. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

NOTE:

TRAINING OR FELLOWSHIP AWARDS: For training or fellowship awards, in addition to the elements outlined above, include a brief description of opportunities for training and professional development. Training activities may include, for example, courses or one-on-one work with a mentor. Professional development activities may include workshops, conferences, seminars, and study groups.

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on this eReceipt System https://cdmrp.org/Program_Announcements_and_Forms/ and under "Forms" on https://www.usamraa.army.mil) should be updated and submitted with attachments.

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1) Zotev, V., Yuan, H., Misaki, M., Phillips, R., Young, K.D., Feldner, M.T. Bodurka J.. Correlation between amygdala BOLD activity and frontal EEG asymmetry during real-time fMRI neurofeedback training in patients with depression. 2014 *NeuroImage* (under review),

Preprint available at: http://www.arxiv.org/abs/1409.2046