***PROTOCOL: Translational Research Center for Traumatic Brain Injury and Stress Disorders: Structural and Functional Neuroimaging Studies (Core B; Project 3).***

***IRB #2389***

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**1. BACKGROUND AND SIGNIFICANCE.**

Background:Prior work from this team and others have highlighted the prevalence of clinical diagnoses in Veterans of Iraq and Afghanistan, including mild Traumatic Brain Injury (mTBI), Post-Traumatic Stress Disorder (PTSD), Major Depressive Disorder (MDD), chronic pain, substance abuse, etc. Often, individuals suffer from multiple deployment-related conditions that seem to co-occur, as revealed by statistical modeling procedures (e.g., mTBI+PTSD+MDD) that have yet to be fully understood from both a psychological and biological point of view. In this project, we address the biological gap using highly advanced brain imaging techniques to understand and characterize how such clinical conditions can affect the brain. We will use multimodal neuroimaging procedures, as opposed to any single procedure, to provide the most informative data for differentiating the multifaceted symptoms of deployment trauma.

Significance: With advances in neuroimaging technologies, we are now able to image at high-resolution with varying contrasts using Magnetic Resonance Imaging (MRI). Multispectral procedures allow for morphometric, diffusion, and functional measures to be acquired within a single session. This approach provides a synergistic opportunity compared to any single advanced technologies in isolation to characterize degeneration of neural system in TBI across multiple levels of neural organization.

Relevance to Veterans Health: Many of the current diagnoses rely on subjective reports and are limited to binary categories. With advances in brain imaging, we are hoping to develop a better capacity for characterization and understanding of the burden of TBI and PTSD. This may lead to better diagnoses, therapeutic efforts, and treatment evaluation for Veterans.

**2.WORK PROPOSED NEUROIMAGING PROCEDURES.**

***Recruitment of subjects.*** Research personnel involved in Core B (IRB #2354) will perform recruitment of all TRACTS participants. We aim to enroll approximately 750 participants in total. Some participants may be asked to return for a follow-up visit. A first follow-up visit may take place approximately, but not limited to (depending on a participant’s availability) 12 months after the initial visit. A second follow-up visit may take place approximately, but not limited to (depending on a participant’s availability) 5 years after the initial visit. These follow-up visits will allow us to gather longitudinal data on the impact of TBI and PTSD on the brain.

Participants will not be included in the study if they match the following criteria:

- Metallic implants or foreign objects deemed unsafe by the MRI technologist (e.g., pace-maker, shrapnel, metallic screws, etc.)

- Surgery in the past 2 months except as approved by the MRI technologist (e.g., dental work, colonoscopy, etc.)

- Weight or size exceeding the capacity of the scanner table

Safety screening will be conducted using the “*Magnetic Resonance (MR) Procedure Screening Form for Patients”* provided by the department of Radiology of the VA Boston Healthcare Center. If there is a question about an individual’s eligibility for MRI scanning (i.e., if there is a question of metal anywhere in the body), then a clinical x-ray will be conducted in the Radiology department at the VA if the participant agrees. The x-ray will only be of the part of the participant’s body in which there is a question about metal. If there is more than one part of the participant’s body in which metal is questionable, the individual will not be able to have the x-ray, and he/she will not be able to have the MRI. If the clinical x-ray determines the presence of metal or other issues that deem ineligibility, the participant will not be included in the study. Or, if with or without an x-ray there are still further questions about whether there is metal present in their body, they will not be able to take part in the study to ensure their safety. A final decision regarding eligibility will be made by the study staff in collaboration with the MRI technologist who may refuse participation if additional information is revealed after consenting is obtained.

Prior to the MRI session, participants will meet with a study staff to complete the Informed Consent Form and HIPAA authorization form. At this time, participants will be able to ask study staff questions about their participation and about the MRI environment. Participants will be asked if they agree to the following:

* Be contacted for the follow-up visits described above (~12 months and ~5 years after the initial visit).
* If necessary, have an X-ray taken to assess for the potential presence of metallic or foreign bodies that could exclude them from the study
* To have a credential investigator observe the scan for training purposes
* To have the results from their scans shared with their clinical provider

***Summary of the neuroimaging procedures and innovative aspects of the proposed research.*** We will perform a detailed analysis of structural and functional imaging parameters acquired from MRI including measures of brain morphometry, white matter tissue structure from diffusion-weighted imaging (DWI), and resting-state functional brain networks (fMRI). To our knowledge, no prior study has examined mild TBI with this array of tools, or collected such data simultaneously during an imaging session. We will employ analysis procedures developed at the Athinoula A. Martinos Center for Biomedical Imaging as well as procedures developed by our colleagues at Oxford University for inter-participant averaging of cortical, diffusion, and functional data. Other sites including the Martinos Center will also process values obtained from the VA scanner. Scanner data will be physically transferred to and from other sites such as MGH via a FIPS certified Aegis Padlock Fortress Encrypted USB Hard Drive or VA issued software encrypted 2 Gb Flash Drives. Scanner data will be virtually transferred to and from other sites including MGH through Secure Copy Protocol, or SCP. Any and all data transferred to other sites including MGH will be subject to VA Records Management protocol and will be managed accordingly. All imaging will occur on a 3-Tesla Siemens Prisma scanner. This whole-body scanner has the advantage of high signal-to-noise, yet has been optimized for minimal gradient distortions typical of high field imaging.

Once at the scanner suite, participants will be asked to review the clearance form with the MRI technologist on staff. They will be asked to change into MRI safe clothes provided on site (i.e., hospital gowns, MRI safe pants and socks). They will then be escorted to the MRI scanner by the MRI technologist and study staff member. Throughout the MRI session, participants will be able to communicate with the research staff via intercom. Participants will be regularly instructed as to the length of each acquisition and as to required behavior (e.g., keeping eyes opened or to stay still). In case of excessive movement from the participants, they may be asked to re-do a sequence. In case this happens, other acquisitions may be sacrificed to respect time limit. The approximate length of the scan is 90 minutes. Some sequences may be prioritized for collection purposes. It is not scientifically necessary to complete all sequences of the TRACTS protocol to collect viable data.

We will also seek to examine whether Veterans presenting with conditions such as mTBI and PTSD have accelerated aging in comparison to Veterans without these disorders in our TRACTS cohort. This project will utilize publicly available and coded MRI and supplementary data from open data initiatives, such as ADNI and the Human Connectome Project to create algorithms of the conditions that can also be applied to those in our already collected and coded dataset of veterans (i.e., the TRACTS cohort). Datasets from these open data projects are available only to internal credentialed investigators, and kept on password-protected lab workstations behind the VA firewall.

**Risks to Subjects.** The main risk involved in the MRI scanning is related to the presence of metal in the body of participants. Metallic implants can move or be distorted due to the magnetic field. Therefore participants will be asked by study staff and the MRI technologist to describe the presence of any foreign material in the body. If presence of an unsafe implant or other body is suspected, participants will be asked to provide information necessary to evaluate the risk. This evaluation will be conducted by the MRI technologists. If safety cannot be ascertained, participants will be excluded. This may happen during recruitment, consenting, or even at the MRI suite. The MRI scanner is also a small environment that may trigger claustrophobic reactions in some participants. In our experience, there are no clear predictors of who may be susceptible to such reactions. In case of anxious reactions, participants will be taken out of the MRI scanner as soon as possible and may be excluded from further participation.

**MR Data Acquisition.** The following is a brief description of some scans acquired. The complete protocol may include additional sequences similar to the ones described below.

*T1 acquisition.* We may collect 2 high resolution T1-weighted Magnetization-Prepared Rapid Gradient Echo (MP-RAGE) scans on each participant for the morphological analysis of cortical thickness and volume, and for anatomical co-registration with other datasets including but not limited to diffusion data. The MP-RAGE scans have been empirically optimized by our group for high contrast and will be motion corrected and averaged to create a single high signal, high contrast volume. The algorithms for the reconstruction of brain surfaces, measuring cortical thickness, and segmentation of volumetric structures, have all been developed and validated using similar imaging protocols and we have extensively examined the test-retest reliability of these procedures.

*T2 acquisition.*  We may collect a T2-weighted Fluid-Attenuated Inversion Recovery (FLAIR) scan and T2-weighted saggital scan. These scans are optimal to characterize and quantify white matter lesions, are used in the registration of fMRI and diffusion scans, and may be additionally used for clinical review of cases. Algorithms for the use of these acquisitions in the processing of functional MRI data have been validated in previous studies.

*DWI acquisition.* Diffusion scans will use single shot echo planar imaging, and a twice-refocused spin echo pulse sequence, optimized to minimize eddy current-induced image distortions. Sixty-four slices will be acquired in the AC-PC plane. The 60 diffusion-weighted directions may be obtained using the electrostatic shell method. The diffusion tensor will be calculated on a voxel-by-voxel basis using conventional reconstruction methods as previously described. To our knowledge, this would be the first study of TBI using such acquisition procedures.

*fMRI acquisition.* fMRI data for assessing resting functional brain networks may be collected and analyzed as described in TRACTS publications [1], but optimized for higher-resolution acquisition (current standard voxel resolutions of 3mm3 will be utilized to allow for whole brain coverage in a reasonable amount of acquisition time). Whole brain EPI acquisition will be used for functional imaging. Data will be collected in resting conditions with the participant asked to fixate on a visual point and to keep their eyes open, similar to published work. If task-based fMRI sequences are used, subjects may be asked to complete tasks during the scan. Completing these tasks may require motor responses (such as button pressing, or completing simple motor actions such as finger tapping); viewing visual or hearing auditory stimuli; and completing cognitive, attention, or memory tasks. No task paradigm, on its own, would constitute any risk to the subject greater than that encountered in daily life.
*Breath control paradigms.* While in the scanner participants may be asked to engage in a paradigm in which they will be asked to actively control their breathing. This will consist of five 15-second intervals of breath holding separated by 30-second breaks. We may also ask participants to alter their normal breathing rate from .25 Hz to .30 Hz and .20 Hz. While engaging in a breath control paradigm, we may use MR-compatible hardware to present visual stimuli and/or monitor the participant’s behavior and physiology.

**3. DATA ANALYSIS.**

Below are descriptions of some techniques that may be used to analyze the data collected. Because of advances in techniques and computation, this list cannot be exhaustive and may be changed if better, more reliable or more innovative techniques are made available.

*Computerized reconstruction of the cortical surface and measuring cortical thickness.* Cortical reconstruction will subsequently be used for three purposes: a) measuring cortical thickness in individual participants; [2] spatial normalization for optimal interparticipant averaging and comparison using a spherical averaging procedure, and c) visualization of thickness data, diffusion data, and statistical maps. The distance between the gray matter/white matter boundary and the outer cortical surface is used to calculate cortical thickness at each point across the cortical mantle. This software is freely available through the internet, along with documentation, tutorials, and example data. The cortical reconstruction procedure is practically fully automated when analyzing high quality data, allowing high analysis throughout.

*Automated volumetric measurement of the hippocampus and other subcortical gray matter.* We will use an automated procedure for the measurement of hippocampal, amygdala, and other structural volumes [3]. The automated hippocampal segmentation technique is comparable in accuracy and reliability to manual labeling. Volumetric measurements are automatically calculated from a variety of volumetric neural structures with this procedure, yet the proposed studies will focus on hippocampal and amygdalar volumes to achieve the Specific Aims. We will monitor the segmentation of other structures that have been noted to be affected by mild TBI for secondary analyses including the thalamus. This labeling will provide volumes of each structure and will also allow for the calculation of the volume of infarction or other types of damage common to TBI within each structure.

*Fractional anisotropy (FA), axial (AD), radial (RD), and mean diffusivity (MD).* FA is a calculated measure from diffusion data that is dependent on the orientational coherence of the diffusion compartments within a voxel. The three principal eigenvalues from the diffusion tensor of the DWI data are calculated, representing the diffusion coefficients along the three principal eigenvectors (vectors of diffusion orientation). FA is computed as the variance of the three eigenvalues, normalized by the trace image. Group differences in FA values of similar anatomical regions would suggest a difference in the white matter microstructural integrity. For example, lower fiber density or decreased myelination would result in fewer tightly packed fiber bundles, and thus, water diffusion would be less restricted and FA values would be lower. In addition to the FA metric which has been a standard of diffusion studies, we will examine the axial (primary eigenvector), radial (mean of the secondary eigenvector) , and mean diffusivity (measure of the total diffusion within a voxel) components of diffusion as we have done in our recent work, as it has been suggested through animal models that these differing contrast properties provide unique information about pathologic mechanisms and may be useful in differentiating among regions with distinct and overlapping pathologies. We will utilize appropriate procedures for motion and distortion correction of diffusion data. FA analyses will employ whole brain voxel-based comparisons. FA maps will first be gently smoothed using a 2mm 3-dimensional Gaussian smoothing kernel to provide a more reliable estimate of FA at each voxel. We have recently utilized techniques for spatial normalization of diffusion data based on the creation of an ‘anisotropy skeleton’ in each individual to perform Tract-Based Spatial Statistics (TBSS), as recently introduced by our colleagues at Oxford University. Figure 1demonstrates that use of this technique to register multiple individuals in healthy older adults and patients with Alzheimer’s disease (A and B, respectively) preserves the anatomy from individual participants. Results from all voxel-based maps will be confirmed using complementary methods including manual and automated region of interest techniques as well as the examination along fiber paths for secondary analysis of the anatomical nature of the observed effects.

**Figure 1.** Mean FA in older adults (A) and in patients with AD (B). A high dimensional 3D morphing procedure was utilized to average FA data across multiple individuals in each group. Averaged data retain most of the anatomic detail of individual participants. Regional group difference can be observed in these average data (e.g., decreased FA values in AD; red areas)

***Pathway-of-interest (POI) and probabilistic tractography.*** Because of the intricate anatomical contrast of underlying white matter structure provided by DTI, it is possible to measure tissue integrity across specified large fiber bundles using diffusion tractography techniques. These procedures are highly valuable because they facilitate automated measurement of anatomically homologous white matter regions across individuals in their native space volumes and minimize a variety of confounds apparent from spatial normalization typical of voxel-based studies. Anisotropy will be sampled along various pathways expected to be affected in TBI and PTSD including pathways of frontal and temporal systems, similar to procedures utilized in our recent work. These analyses will be complementary to the voxel-based analyses which provide a picture of white matter degeneration, unconfined to a particular fiber bundle. It is of great interest to differentiate among affected systems with mild TBI. In this regard, these methods could be particularly useful towards addressing mechanistic questions about the basis of mild TBI related degeneration and cognitive decline.

***Functional brain network maps.*** Functional brain networks will be mapped using two commonly used procedures. First, functional connectivity will be utilized to examine networks of brain regions that are highly correlated utilizing dopamine-rich regions as the initial seed. Similar procedures have been utilized by our colleagues at the Martinos Center for examining memory systems associated with hippocampal activity and memory. Specifically, we will examine networks associated with activity in the hippocampus and in the amygdala. The second analysis procedure will utilize independent component analysis (ICA) such as the Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC) procedure in the FSL software suite [4]. This ‘blind’ separation of functional brain networks will allow for analyses of systems that have critical consequence to cognitive function, such as the ‘default mode’. Maps obtained from the functional connectivity and from the ICA analyses demonstrating the strength of covariance across neural structures will be compared across groups utilizing surface based general linear model procedures.

References

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