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14. ABSTRACT

It is estimated that 10% to 20% of warfighters who have served in Iraq and Afghanistan have PTSD 1-4. An important limitation of these estimates is the reliance on self-report screening measures and clinical interviews to make the diagnosis of PTSD. These methods are subject to a number of biases, including underreporting of PTSD symptoms because of stigma of mental illness and concerns about adverse effects on careers, and exaggeration of symptoms in those seeking compensation for service- connected disability. Development of biomarkers of PTSD is critical for DOD and VA as objective indicators of PTSD for use in post-deployment medical screening, treatment selection, treatment outcome monitoring, disability evaluations, and for informing novel targets for treatment development.

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Year 5
Submitted 7/03/2014

INTRODUCTION:

It is estimated that 10% to 20% of warfighters who have served in Iraq and Afghanistan have PTSD [1-4]. An important limitation of these estimates is the reliance on self-report screening measures and clinical interviews to make the diagnosis of PTSD. These methods are subject to a number of biases, including underreporting of PTSD symptoms because of stigma of mental illness and concerns about adverse effects on careers, and exaggeration of symptoms in those seeking compensation for service- connected disability[5]. Development of biomarkers of PTSD is critical for DOD and VA as objective indicators of PTSD for use in post-deployment medical screening, treatment selection, treatment outcome monitoring, disability evaluations, and for informing novel targets for treatment development. Additionally, biomarkers hold great potential for explaining and mitigating the associations between war zone-related PTSD and physical health problems, including cardiovascular and metabolic disorders [6-10]. In order to address this critical gap we will perform a pilot study to determine feasibility for larger scale biomarker identification and biomarker informed intervention studies by carefully examining 200 OIF/OEF warfighters through an extensive biological protocol. The first phase will pilot the integration of methods across five leading research laboratories and identify the most promising biomarkers in preparation for larger scale studies. Given the sample size for the pilot and large number of biomarkers of interest, we will specify a limited set of biomarkers for hypothesis testing. It is predicted that compared with controls the PTSD group will have smaller dentate/CA3 hippocampal subfield volumes, lower ambient cortisol levels, and greater cortisol suppression following dexamethasone administration. It is also predicted that lower neuropeptide Y levels will be associated with smaller Dentate/CA3 volumes, and that APO E4 polymorphisms will be associated with smaller Dentate/CA3 volumes.

Goal:

The major goals of this study are:

(1) Advance the diagnosis of PTSD by developing objective biological indicators of the disorder that are not subject to the under and over reporting biases associated with self-report and clinical interview measures;
(2) Advance the understanding of the pathogenesis of PTSD, for example identifying susceptibility genes, neuroendocrine and neurochemical markers and the underlying neurocircuitry of PTSD;
(3) Identify novel biomarker correlates of risk and resilience factors that explain individual differences in the onset, severity, course and impact on functioning of war zone PTSD; and
(4) Identify novel panel of sensitive and specific biomarkers for PTSD and conduct validation and replication studies on these markers.

PROJECT ACCOMPLISHMENTS:

The Biomarkers for PTSD study is in the implementation phase. In year 5 of the grant we accomplished several milestones and goals. Our success in meeting these goals is detailed below.

1. Regulatory Approvals:

Approval for annual continuation from NYU Institutional Review Board (IRB) and all IRBs of collaborating sites (Bronx VA, MSSM, Emory and UCSF) was obtained. The study also received approval for continuation from the Army's Human Research Protection Office (HRPO).

The study protocol is approved and renewed until October 14, 2014 at NYU. We submitted an Application to Human Research Protection Office (HRPO) for the annual continuation review and

received approval until 14 October 2014. We also received annual continuation renewal with The Clinical and Translational Science Institute at NYU (CTSI) until 11/15/2014.

2. Communication & Reporting

The team continued to engage in bi-weekly communication meetings via teleconference to ensure the successful and timely execution of the Implementation Phase. Calls took place between the PIs and investigators at each site (SF VAMC, UCSF, Mt Sinai, Bronx VA, Emory University and NYU). Meetings addressed safety issues, clinical questions, strategies for improving subject recruitment and enrollment, strategies for maximizing participation in Visits 2-4, and ensuring that participants moved through all stages of the study quickly and efficiently (in order to avoid attrition).

The clinical team, under the supervision of Dr. Henn-Haase, conducted weekly calibration meetings across sites to establish clinical consensus in scoring the frequency and intensity of symptoms on the CAPS and clinical assessment. Each discrepancy from the evaluation of participants was resolved by group consensus during these meetings.
PIs participated in the Systems of Biology quarterly meetings and provided preliminary findings for an age, gender, and ethnicity matched group of 52 PTSD positive and 52 PTSD negative participants and a cross-sectional validation sample of 20 PTSD positive and 20 PTSD negative participants. These findings were investigated from data from diagnostic clinical evaluations, self-reports and neurocognitive data, as well as, biomarkers data on neuroimaging, genetics, endocrine and metabolic biomarkers. These presentations were held on 7/2/13, 11/22/13, 2/6/14, and 5/2/14.

Dr. Marmar also presented at the Systems Biology in Progress Reporting (IPR) meeting on February 26th, 2014 at Fort Detrick, Maryland. The data included project progress including the Male and Female Biomarkers for PTSD studies. The report included an overview of demographics of the recruited sample, data from the diagnostic clinical evaluation, self-reports and neurocognitive data and areas of statistical significance. Dr. Marmar also presented biomarkers findings on neuroimaging markers, genetic markers, multi-omics markers, endocrine and metabolic markers.

On April 28, 2014, the Systems Biology Investigators presented the most up-to-date findings for an American Institute of Biological Sciences (AIBS) Review at Fort Detrick, MD; in order to determine eligibility for study advancement into the Advanced Development category. In preparation for this meeting the group collaborated to create a comprehensive report of all findings to date. All core PIs presented these findings at the meeting, as well as their goals for the future of the study and future research.

On May 2, 2014, the Systems Biology Investigators Quarterly meeting was held at NYU with all core PIs and key study personnel in attendance. The focus of this meeting was a debriefing on the AIBS review. Collaborators spent the meeting addressing reviewer's comments for suggestions that would strengthen future projects from the study. During the meeting, the PIs identified four panel domains, each of which includes 10-20 individual targets. These four panel domains are: mRNA transcripts, DNA methylation targets, miRNA + plasma protein combined targets, and Biochemical and cellular targets. They also established a timeline for selection of these panel targets and a Biomarker identification pipeline to down-select for the key biomarkers candidates.

3. Recruitment and Enrollment

Our outreach staff participated in multiple events and meetings to recruit participants for this research study. Below is a list of partner organizations that were contacted by the study outreach staff:

Outreach to Partner Organizations for Research Participant Recruitment:

We have developed an extensive network of 76 partner organizations for research participant recruitment.

American Veterans for Equal Rights
Art Therapy Outreach Center NY
Black Veterans for Social Justice
Bronx Vet Center
Brooklyn Vet Center
Dutchess County Division of Veterans Services
Ed Thompson Veterans Center
Fort Hamilton
Hope for the Warriors
Institute for Community Living
Institute for Family Health
Integral Yoga Institute
Iraq and Afghanistan Veterans of America
Iyengar Yoga Association
JBFCS Home Again
Maimonides Sleep Disorder Clinic
Mayor's Office of Veteran's Affairs
Montford Point Marines Association, Inc.
New Era Veterans
New Jersey War related Illness and Injury Study Center (WRIISC)
New York Institute of Technology
New York Public Library/Single Stop USA Veteran Hub
Project Renewal
Project TORCH
PROVE (Project for Return and Opportunity in Veterans Education)
Queens Vet Center
Rutgers Anxiety Disorders Clinic Veteran PTSD Support Group
Samaritan Village Veterans Program
Staten Island Vet Center
The Doe Fund
Times Square Church Military Ministry
VA NY Harbor HCS
Veteran Stand Down
Veterans Writing Workshop
Warrior Writers
Workforce 1
Yoga Warriors
Baruch College
Bergen County Community College
Berkeley College
Borough of Manhattan Community College
Bronx Community College
Brooklyn College Student Veterans Club
Brooklyn College
College of Staten Island
Columbia University Counseling and Psychological Services
Columbia University MilVets
Fairleigh Dickinson University
Fairleigh Dickinson University Center for Psychological Services
Hostos Community College

Hudson County Community College
Hunter College
Hunter College Student Veterans Club
John Jay Armed Forces/Veterans Association
Kingsborough Community College
LaGuardia Community College
Lehman College
Medgar Evers Community College
Mercer County Community College
Mercy College
Middlesex County College
Middlesex County College Student Veterans Club
Nassau Community College
New Jersey City University
Norwalk Community College
Pace University Student Veterans Club
Passaic County Community College
Queens College
Queensborough Community College
Rutgers Student Veterans Club
Rutgers University
The City College of New York
The City College of New York Veterans Club
Touro College
NAMI Family-to-Family 12-Week Course for Veterans

4. Research Participant Recruitment and Enrollment

We have continued recruitment on this project. To date we have clinically assessed 506 male veterans. 187 met full eligibility criteria; 88 met criteria for combat related PTSD and 99 were found negative for combat related PTSD (See figure 1). For the 187 study participants, we completed clinical assessment measures, entered and managed their data, conducted biomarker acquisition procedures (imaging, genetics, endocrinology, metabolism, and proteomics), and delivered materials to cores for analysis.

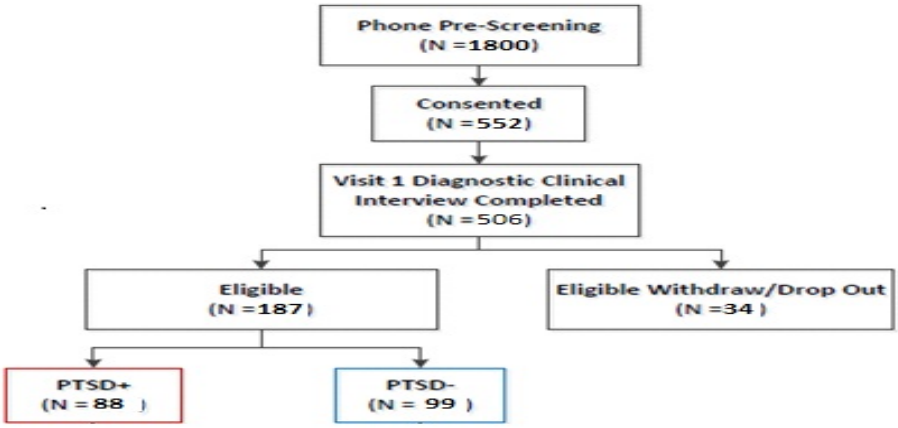


Figure 1: Study Recruitment

5. Data Management

All Clinical Assessment data from the baseline interview, self-report and neurocognitive measures for all study participants were completed and entered as digital data directly into the study secure SQL database server. Data from all cores is also shared with NYU and saved into a single database. The data was saved on the NYU server and shared with all the cores. Each core is able to access to the data via VPN log in. A variable description and dictionary was also developed and shared with all the cores.

The lead biostatistician at NYU analyzed and scored all the Clinical and Self Report measures on a cohort of 52 PTSD positive and 52 controls who are matched on age, gender and ethnicity. In addition, we selected and additional cohort of 20 PTSD positive and 20 control participants who were matched on age, gender, and ethnicity as a cross-sectional validation sample. Furthermore, an ROC curve analysis was done to identify the strongest candidate markers from the current findings. The data was saved on the NYU server and shared with all the cores. Each core is able to access to the data via VPN log in. A variable description and dictionary was also developed and shared with all the cores.

6. Acquisition of Study procedures

All study procedures including blood draw procedures and MRI imaging are completely operational. See summary of study recruitment for the study in Figure 1 and Study procedures completed for eligible participants to date in Table 1.

Table 1: Procedures Completed by Eligible Participants

Biomarkers For PTSD	
Procedure: Baseline Clinical Interview (BCI)	
Clinical Assessment	506
Eligible by Evaluation	187
PTSD+	88
PTSD-	99
Procedure: Cognitive Testing	
Completed	166
PTSD+	75
PTSD-	91
Procedure: Self-Report Questionnaire	
Completed	173
PTSD+	79
PTSD-	94
Procedure: Blood Draw (1)	
Completed	170
PTSD+	82
PTSD-	88
Procedure: Blood Draw (2)	
Completed	163
PTSD+	78
PTSD-	85

Procedure: MRI	
Completed	165
PTSD+	74
PTSD-	91

7. Shipment of Material to cores

Shipments of Blood samples were transferred to all collaborating sites, Integrative Systems Biology Laboratory: Mouse Models of PTSD (Principal Investigator Dr. Jett), and Institute for Systems Biology (ISB): Genetics, Metabolomics (Principal Investigator Dr. Hood), Genetics Core at Emory University (PI: Dr. Kerry Ressler), and to the Metabolism Core at UCSF (PI: Dr. Owen Wolkowitz).

Data transfer from NYU to the imaging core at UCSF is running smoothly and Q & A procedures indicate high quality of data collection. All scans were processed through freeSurfer v5.1. Manual Hippocampal Subfield Marking on scans was completed on 106 subjects.

8. Data Sharing Agreements:

NYU and Bronx VA teams worked with Privacy Officers and Offices of Industrial Liaison/Technology Transfer (OIL) to execute Material Transfer Agreements (MTAs) with Dr. Ressler's laboratory at Emory University that will conduct analysis on assays for the genetics core.

9. Publication and Dissemination Committee

The group developed a publication and dissemination committee. The committee developed guidelines for reviewing proposals and manuscripts before they are published. These guidelines were shared with the investigators on this study. All investigators will submit a manuscript pre-proposal form to be reviewed by the publication committee before the manuscript is written.
List of Members of the Publication Policy:

1) Charles Marmar (Chair)
2) Marti Jett
3) Rachel Yehuda
4) Mike Weiner
5) Owen Wolkowitz
6) Frank Doyle

Members of the publication committee will not contribute to the writing or be authors merely by being members of the committee, although any member of the committee may participate in any writing team.

10. Publications & Dissemination of Data:

Manuscript Preparation:

All cores continued drafting manuscripts to disseminate preliminary data on their findings. Investigators submitted a manuscript pre-proposal form to be reviewed by the publication committee before the manuscript is written.

Publications:

12 papers have been published and 3 papers have been submitted for review from both the animal and the human studies.

Almli LM, Duncan R, Feng H, Ghosh D, Binder EB, Bradley B, Ressler KJ, Conneely K, Epstein MP (2014) Correcting Systematic Inflation in Genetic Association Tests That Consider Interaction Effects: Application to a Genome-Wide Association Study of Post-Traumatic Stress Disorder. *JAMA Psychiatry, in press.*

Almli LM, Fani N, Smith AK, Ressler KJ. (2014) Genetic approaches to understanding post-traumatic stress disorder. *Int J Neuropsychopharmacol*. 2014 Feb;17(2):355-70. PubMed PMID:24103155.

Almli LM, Srivastava A, Fani N, Kerley K, Mercer KB, Feng H, Bradley B, Ressler KJ. (2014) Follow-up and Extension of a Prior Genome-wide Association Study of Posttraumatic Stress Disorder: Gene × Environment Associations and Structural Magnetic Resonance Imaging in a Highly Traumatized African-American Civilian Population. *Biol Psychiatry*. 2014 Jan 28. [Epub ahead of print] PubMed PMID: 24576688.

Cho JH, Lee I, Hammamieh R, Wang K, Baxter D, Scherler K, Etheridge A, Kulchenko A, Gautam A, Muhie S, Chakraborty N, Galas DJ, Jett M, Hood L. Molecular evidence of stress-induced acute heart injury in a mouse model simulating posttraumatic stress disorder. Proc Natl Acad Sci U S A. 2014 Feb 10. [Epub ahead of print]

Hammamieh R, Chakraborty N, De Lima TC, Meyerhoff J, Gautam A, Muhie S, D'Arpa P, Lumley L, Carroll E, Jett M. Murine model of repeated exposures to conspecific trained aggressors simulates features of post-traumatic stress disorder. Behavioural Brain Research. 235(1):55-66 (2012). DOI 10.1016/j.bbr.2012.07.022

Koenen KC, Duncan LE, Liberzon I, Ressler KJ. (2014) From candidate genes to genome-wide association: the challenges and promise of posttraumatic stress disorder genetic studies. *Biol Psychiatry*. 2013 Nov 1;74(9):634-6. PubMed PMID: 24120289.

Lindqvist D, Wolkowitz OM, Mellon S, Yehuda R, Flory JD, Henn-Haase C, Bierer LM, Abu-Amara D, Coy M, Neylan TC, Makotkine I, Reus VI, Yan X, Taylor NM, Marmar CR, Dhabhar FS. Proinflammatory milieu in combat-related PTSD is independent of depression and early life stress. Brain, Behavior, and Immunity. DOI: 10.1016/j.bbi.2014.06.003 (In press).

Muhie S, Hammamieh R, Cummings C, Yang D, Jett M. Transcriptome characterization of immune suppression from battlefield-like stress. 2013, Genes Immun 14(1): 19-34.
Sriram K, Rodriguez-Fernandez M, Doyle FJ 3rd. A detailed modular analysis of heat-shock protein dynamics under acute and chronic stress and its implication in anxiety disorders. PLoS One. 7(8):e42958 (2012). DOI 10.1371/journal.pone.0042958

Sriram K, Rodriguez-Fernandez M, Doyle FJ 3rd. Modeling cortisol dynamics in the neuro-endocrine axis distinguishes normal, depression, and post-traumatic stress disorder (PTSD) in humans. PLoS Comput Biol. 8(2):e1002379 (2012). DOI 10.1371/journal.pcbi.1002379

Yan X, Brown AD, Lazar M, Cressman VL, Henn-Haase C, Neylan TC, Shalev A, Wolkowitz OM, Hamilton SP, Yehuda R, Sodickson DK, Weiner MW, Marmar CR. Spontaneous brain activity in combat related PTSD. *Neurosci Lett 547*: 1-5 (2013).

Yang R, Daigle BJ Jr, Muhie SY, Hammamieh R, Jett M, Petzold L, Doyle FJ 3rd. Core modular blood and brain biomarkers in social defeat mouse model for post traumatic stress disorder. BMC Systems Biology. 7(1):1-12 (2013). DOI 10.1186/1752-0509-7-80
Yang R, Daigle BJ, Petzold LR, Doyle FJ 3rd. Core module biomarker identification with network exploration for breast cancer metastasis. BMC Bioinformatics. 13(1):12 (2012). DOI 10.1186/1471-2105-13-12

Yehuda R, Flory J, Bierer L, Henn-Haase C, Lehrner A, Desarnaud F, Makotkine I, Daskalakis NP, Marmar CR, Meaney MJ. Lower methylation of glucocorticoid receptor gene promoter 1_F in peripheral

blood of veterans suffering from post-traumatic stress disorder. Biological Psychiatry. DOI: 10.1016/j.biopsych.2014.02.006.

In submission:

Lindqvist D, Wolkowitz OM, Mellon S, Yehuda R, Henn-Haase C, Abu-Amara D, Reus V, Coy M, Dhabhar S, Marmar CR. High pro-inflammatory cytokine levels in male combat veterans with post-traumatic stress disorder. *Under review.*

Mueller SG, Ng P, Neylan T, Mackin RS, Wolkowitz O, Mellon S, Yan X, Flory J, Yehuda R, Marmar CR, Weiner MW. Evidence for Disrupted Structural Connectivity Due to Cortical Thinning and Gray Matter Loss in Posttraumatic Stress Disorder. *submitted to Human Brain Mapping.*

Xiaodan Yan, Mariana Lazar, Arieh Y Shalev, Thomas C. Neylan, Owen M. Wolkowitz, Adam D. Brown, Clare Henn-Haase, Rachel Yehuda, Janine D. Flory, Duna Abu- Amara, Fernando Boada, Daniel K. Sodickson, and Charles R. Marmar. "Precuneal and amygdala functional connectivity in warzone-related PTSD". Psychiatry Research – Neuroimaging. *Under Review.*

Conferences:

Dr. Marmar and all core PIs presented a 3-hour symposium at the annual American Psychiatric Association meeting on May 3, 2014 in New York. The presentation included an overview of the study, data from the clinical and self-report data, data from the following cores:

1. Neurocognitive
2. Endocrine
3. Metabolism
4. Genetics
5. Multi-omics

11. Summary of Study Results from Each Core

Results of the Baseline Clinical Assessment

For initial sample analysis, an original test sample of 52 PTSD positive cases and 52 PTSD negative controls was selected from the group of eligible participants. These subjects were matched on the basis of age, gender, and ethnicity and completeness of study procedures. Demographics for this test sample are summarized in Table 2.

Table 2: Demographics for 52/52 Test Sample

	PTSD Positive (n=52)	PTSD Negative (n=52)	P Value
Age in Yrs [Mean(SD)]	34 (8.7)	33.6 (9.0)	0.69
Education in Yrs [Mean(SD)]	14.1 (1.7)	15.5 (2.1)	0.001
Gender [Frequency(%)]			
Male	52 (100 %)	52 (100%)	
Race/Ethnicity [Frequency(%)]			
Hispanic	26 (50%)	20 (38.46%)	
Non-Hispanic African American	12 (23.08%)	13 (25%)	0.46
Non-Hispanic Caucasian	13 (25%)	15 (28.85%)	
Non-Hispanic Other	1 (1.92%)	4 (7.69%)	

Results of the comparison of clinical symptoms data between the PTSD+ versus PTSD- are summarized in Table 3:

Table 3: Clinical results for 52/52 Test Sample

Measure	Positive (n=52) Mean (SD)	Negative (n=52) Mean (SD)	P value
CAPS Re-experiencing Lifetime	27.00 (6.63)	2.92 (3.74)	<.0001
CAPS Re-experiencing Current	16.71 (7.08)	0.48 (1.09)	<.0001
CAPS Avoidance Lifetime	34.48 (7.32)	2.44 (3.75)	<.0001
CAPS Avoidance Current	26.69 (7.13)	1.06 (2.73)	<.0001
CAPS Hyperarousal Lifetime	29.38 (6.37)	3.42 (3.63)	<.0001
CAPS Hyperarousal Current	24.62 (6.93)	1.44 (2.48)	<.0001
CAPS Total Score Lifetime	90.87 (15.47)	8.79 (7.82)	<.0001
CAPS Total Score Current	68.02 (16.80)	2.98 (4.24)	<.0001
CAPS Partial PTSD Lifetime	1.00 (0.00)	0.10 (0.30)	<.0001
CAPS Partial PTSD Current	1.00 (0.00)	0.00 (0.00)	<.0001
Peritraumatic Dissoc. Exper. Q-RV	1.81 (0.51)	1.24 (0.24)	<.0001

In order to cross-sectionally validate the findings of the test sample, we selected an additional sample of 20 PTSD+ and 20 PTSD- participants were matched on the basis of age, gender and ethnicity and completeness of study procedures. Demographics for the Validation Sample is summarized in Table 4.

Table 4: Demographics of 20/20 Cross Validation Sample.

	PTSD Positive (n=20)	PTSD Negative (n=20)	P Value
Age in Yrs [Mean(SD)]	31.3 (6.9)	30.8 (6.2)	0.74
Education in Yrs [Mean(SD)]	14.5 (2.2)	15.3 (2.5)	0.39
Gender [Frequency(%)]			
Male	20 (100%)	20 (100%)	
Race/Ethnicity [Frequency(%)]			
Hispanic	6 (30%)	3 (15%)	0.58
Non-Hispanic African American	8 (40%)	7 (35%)	
Non-Hispanic Caucasian	4 (20%)	6 (30%)	
Non-Hispanic Other	2 (10%)	4 (20%)	

Results of the comparison of clinical symptoms data between the PTSD+ versus PTSD- are summarized in Table 5.

Table 5: Clinical results for 20/20 Cross Validation Sample

	Positive (n=20)	**Negative (n=20)**	
Measure	**Mean (SD)**	**Mean (SD)**	**P value**
CAPS Re-experiencing Lifetime	29.25 (5.68)	2.30 (3.29)	<.0001
CAPS Re-experiencing Current	16.95 (7.13)	0.00 (0.00)	<.0001
CAPS Avoidance Lifetime	35.95 (6.73)	3.80 (5.03)	<.0001
CAPS Avoidance Current	28.75 (6.83)	2.20 (3.65)	<.0001
CAPS Hyperarousal Lifetime	28.90 (6.94)	5.90 (4.80)	<.0001
CAPS Hyperarousal Current	24.30 (5.94)	5.20 (4.88)	<.0001
CAPS Total Score Lifetime	94.10 (15.52)	12.00 (9.84)	<.0001
CAPS Total Score Current	70.00 (16.10)	7.40 (7.50)	<.0001
CAPS Partial PTSD Lifetime	1.00 (0.00)	0.00 (0.00)	<.0001
CAPS Partial PTSD Current	1.00 (0.00)	0.10 (0.31)	<.0001
Peritraumatic Dissoc. Exper. Q-RV	2.42 (0.74)	1.39 (0.77)	<.0001

The 52/52 test sample showed significant difference on all measures between the PTSD+ and PTSD- groups. The results of the clinical data obtained on the 20/20 cross validation sample were consistent with the test sample, thereby validating the original test sample results.

Neurocognitive Core

New York University Langone Medical Center

Many veterans experience cognitive deficits related to prolonged war-zone exposure leading to symptoms of posttraumatic stress disorder (PTSD). Research has focused on key cognitive domains related to PTSD: intellectual functioning, memory or working memory, attention, learning, executive functioning, and visuospatial functioning. The neurocognitive findings for PTSD point to cognitive impairments; however, few studies have identified neurocognitive markers associated with biological markers for PTSD. We view cognitive impairment as potentially important in endophenotyping PTSD, therefore the aim of the neurocognitive core within a study of Biomarkers for PTSD is to determine whether or not neurocognitive functioning on tests of intellectual performance, working memory and attention, executive functioning, verbal memory, and verbal fluency are impaired and may be precursors and/or predictors of PTSD in a sample of OEF and OIF veterans with and without PTSD. The second aim of this study is to determine if neurocognitive markers for PTSD may be associated with markers across cores including genetics, endocrine, decreased hippocampal volume in imaging, and increased allostatic load.
This study examined differences in neurocognitive functioning between OEF/OIF male service members and veterans with and without PTSD in a Test Sample matched on age and ethnicity (N=52 PTSD+ and 52 PTSD-.). A Validation Sample (N=20 PTSD+ and 20 PTSD-) meeting the same criteria as the Test Sample was used to validate the test sample, and finally an examination of the combined Test and Validation Samples (Combined Sample) was examined to identify group differences on measures of neurocognitive functioning. The participants included active military service members and veterans returning from the Iraq and Afghanistan war who were exposed to warzone trauma. After completing a structured diagnostic assessment using the SCID-IV and CAPS-IV and meeting all eligibility criteria, participants were identified as either PTSD positive or PTSD negative. The administration of the

neurocognitive testing battery was conducted at one of the two sites conducting recruitment and diagnostic assessment, NYU Medical Center (NYUMC) and Mount Sinai School of Medicine (MSSM)/James J Peters VAMC (JJPVAMC). The battery of tests focused on obtaining measures aligned with the core neurocognitive domains: IQ, working memory (auditory/visual), processing speed, verbal memory (immediate and delayed), executive functioning, and verbal fluency.

The results using the model with and without covariates of language and education identify significant group differences for several hypotheses: The estimate of IQ is lower in the PTSD positive group compared to the PTSD negative group in the test sample and combined sample with a similar pattern for the validation sample. Similarly, performance on tests of auditory and visual Working Memory were significantly different between groups with the PTSD positive group performing more poorly on memory tasks in the test sample and combined samples with a similar pattern in the validation sample. A deficit in processing speed was found to be significant in the PTSD positive group compared to the PTSD negative group in the combined sample with a similar pattern in the validation sample. The results did not support a significant difference between groups on tests of immediate and delayed verbal memory. Significant group differences support the hypothesis that verbal fluency will be impaired in the PTSD positive group compared to the PTSD negative group. This finding held up in the test sample and combined samples with a similar pattern in the validation sample. Executive functioning and cognitive flexibility was found to be impaired in the validation sample; however no significant group differences were found in the test sample or combined sample.

The test sample was well validated by a smaller validation sample and the majority of significant findings were maintained when the test sample and validation sample was combined. Comparison tests found the PTSD positive and PTSD negative groups to be equivalent on all measures with the exception of coding. It is unclear why the groups differed on this subtest, however the validation sample is limited by the small sample size.

In summary, the findings indicate key neurocognitive deficits associated with PTSD including IQ as a possible premorbid precursor for the development of PTSD, deficits in both auditory and visual working memory which requires an individual to temporarily store and manipulate information for later use, slower ability to process information which can affect learning, and deficits in verbal fluency or the impaired ability for successful retrieval of information that requires executive control over cognitive processes such as selective attention, mental set shifting, internal response generation, and self-monitoring.

Structural Imaging Core

University of California, San Francisco

1. Background: Brain Structures Involved In Stress

Posttraumatic stress disorder (PTSD) is a complex reaction to life threatening or otherwise extremely stressful events that is characterized by re-experiencing symptoms in form of nightmares or flashbacks, states of hyperarousal or numbing and avoidance of trauma related situations and is typically accompanied by poor concentration and difficulty of recalling the details of the traumatic event. Evidence from functional imaging suggests that these symptoms are associated with an impaired interaction between brain regions belonging to the prefrontal-limbic network that is involved in experiencing fear, anxiety and negative emotions. This network encompasses cortical regions particularly mesial and dorsolateral prefrontal and orbitofrontal regions and the insular cortex but also subcortical structures, most importantly amygdala and hippocampus but also thalamus and nucleus (ncl) accumbens regions[11,12]. PTSD related abnormalities however are not purely functional. Structural abnormalities, e.g., gray matter volume losses or thinning have also been described and are most commonly found in the hippocampus but also in the mesial prefrontal cortex, particularly anterior cingulate, but occasionally also in the dorsolateral prefrontal and orbitofrontal and insular cortices[13-20.] The hippocampus is not a homogeneous structure but consists of functionally and histologically specialized subfields. Animal studies suggest that stress and PTSD related volume losses are more pronounced in hippocampal subfields with a high density

of cortisol receptors (CA1) and/or capability for live long neurogenesis (dentate gyrus). A study done in our lab that used high resolution images of the hippocampal formation obtained on a 4T magnet and a manual parcellation strategy to subdivide the hippocampus into subfields was the first to demonstrate a regional selectivity of PTSD in human patients who had the most prominent volume loss in the CA3&dentate gyrus label [11]. In contrast to its prominent role in functional studies, structural abnormalities in the amygdala are only rarely described[6,12]. On the one hand this might simply reflect the difficulty of an accurate manual or automated amygdala parcellation. On the other hand it could indicate that functional abnormalities are not necessarily associated with structural abnormalities and thus that the amygdala volume is either preserved or highly variable. The latter assumption is supported by the observation that amygdala lesions or small amygdala volumes reduce the likelihood to develop PTSD while larger volumes predispose to behavioral problems[13,14], Finally, PTSD related abnormalities might not necessarily be permanent, since there is evidence from animal and human studies that recovery from PTSD is associated with a normalization of at least some of the mesial prefrontal functional and the hippocampal functional and structural abnormalities[15,16].

2. Specific Aims

The overall goal of the Structural Imaging Core is to replicate the previous finding of selective PTSD effect on the dentate gyrus and to investigate how PTSD related MR structural abnormalities in the hippocampus but also in other mesial-limbic-prefrontal structures can be used as a biomarker to diagnose PTSD. To that purpose the total population that will be recruited for this project will be divided into two equal sized groups (each consisting of about 100 subjects, 50 with and 50 without PTSD). One of the groups will serve as trainings/development population (development group) in whom different strategies can be developed and tested while the second group (test group) will be used to validate these strategies. In order to make a potential imaging biomarker widely accessible and cost effective, clinical MR systems and automated standard image processing approaches, e.g. Freesurfer to obtain regional cortical and subcortical volumetric measures, tissue segmentation by SPM etc, will be used for this project or developed if they do not already exist, e.g. automated hippocampal subfield labeling. These techniques will be used to address the following specific aims.

Specific Aim 1: Confirmation of the regional selective effect of PTSD on the hippocampus with volume loss in dentate gyrus/CA3 subfield that has been shown in the pilot study. This will be accomplished by A. Adaption of the 4T high resolution T2 weighted sequence employed for hippocampal subfield volumetry in the pilot study for the use on a 3T clinical system. B. Obtaining subfield specific volumetric data using the same manual subfield labeling strategy that was employed in the pilot study to test the hypothesis that PTSD is associated with volume loss in the CA3&DG region that is independent from aging or other factors influencing the CA3&DG volume. C. Optimization of an semi-automated approach for hippocampal subfield volumetry (ASHS) that has been developed together with collaborators at the University of Pennsylvania (Yushkevich Lab[17]) and comparing its performance with that of the manual labeling strategy.

Specific Aim 2: Characterization of PTSD related extrahippocampal structural abnormalities and investigation of the impact of those abnormalities on networks associated with PTSD. This will be accomplished using the Freesurfer software suite that provides automated thickness measurements for cortical regions and volume information for subcortical structures. Based on the findings reported in the literature, we expect to find cortical thinning, volume loss in the following structures belonging to the mesial-limbic-prefrontal network: entorhinal cortex, hippocampus, amygdala, thalamus, ncl. accumbens, rostral and caudal anterior cingulate cortices, superior frontal lobe cortex, rostral and caudal dorsolateral frontal cortices, lateral and medial orbitofrontal cortices and insula. The impact of structural abnormalities on networks associated with PTSD and other brain regions will be assessed using graph analysis.

Specific Aim 3. Development of classifier that uses different features extracted from the structural images (T1 weighted whole brain image alone or in combination with DTI) to distinguish between PTSD positive and PTSD negative subjects. Three different approaches that have been used at CIND will be evaluated. A. Bayesian networks[18]. B. Support Vector machine. C. regional texture/complexity measures combined with Random Forrest data mining algorithm[19]. The performance of the three approaches will be assessed

by calculating sensitivity and specificity and ROC curves. The features identified by the method with the best performance (highest accuracy in the development and test population) and will be chosen and used in the validation study.
Additional Aims: In addition to the specific aims listed above, all imaging data will be made available to the collaborators of the other cores to investigate the associations between clinical, genetic, endocrine, multi-omic abnormalities and brain structural abnormalities in PTSD and to identify which combination of imaging, clinical and multi-omics measures differentiates best between PTSD positive and negative combat exposed veterans.

3. Work Accomplished by February 2014

3.1. Image Processing

The following section provides an overview over the image processing procedures routinely employed for the biomarker project and s summary of the data processed so far.

1. Raw data visual quality check and parameter checks to ensure the data is complete and usable.
2. Freesurfer (http://surfer.nmr.harvard.edu) : brain cortical reconstruction and volumetric segmentation. All Freesurfer data is checked for quality and brain segmentations are edited if necessary and then rated on Pass/Partial/Fail criteria.
3. T2 Intracranial Volume: An automated T2 mask is edited manually to achieve an accurate skull-stripped mask containing only white matter, gray matter, and CSF. Brain volume is generated by voxel count.
4. SPM8 Segmentation (www.fil.ion.ucl.ac.uk/spm/) : a T1-weighted image is segmented into probabilistic gray mater, white matter, and CSF masks. Data is then rated on Pass/Fail criteria, and re-run on different parameters if failed.
5. Diffusion Tensor Imaging: a visualization of the water movement in the brain which allows us to assess the condition of an individual's white matter tracts. The data is processed through an in-house pipeline, with multiple quality checks in between, to generate Functional Anisotropy (FA) Maps. Data is rejected if distortion corrections failed or the FA map does not appear to follow the anatomy of the brain.
6. Manual Hippocampal Subfield Segmentation: T2-weighted high resolution images of the hippocampus are manually segmented to create volumetric measures of the following subfields: CA1, CA1-2 transition, CA3/Dentate Gyrus, Subiculum, and the Entorhinal cortex. This was done for the development population only as the overall goal is ultimately to use the automated method.
7. Automatic Segmentation of Hippocampal Subfields (ASHS, https://www.nitrc.org): ASHS uses the same T2 weighted high resolution images used for manual labeling. It employs an automated multi-atlas segmentation approach combined with similarity-weighted voting to label new images. To improve the performance of ASHS a project specific atlas was generated by selecting 30 representative PTSD pos and PTSD neg cases (regarding image quality but also reflecting the anatomical variance of the hippocampal formation observed during the manual labeling of the trainings/development population) and having an expert rater manually editing the labels that were generated by ASHS using its generic atlas.

Process	Image used	Data Acquired	Data Processed	# Pass	# Partial	# Fail
Freesurfer	3D-T1	188	188	170	15	3
T2 Intracranial Volume	3D-T2	188	188	188	0	0
SPM8 Segmentation	3D-T1	188	186	146	0	0
Diffusion Tensor Imaging	DWI	182	106	7	96	3
Manual Hippo Segmentation	High Res T2	188	106	102	n/a	4
ASHS	High Res T2	188	186	-	-	-

3.2. Results: Summary

All analyses were restricted to trainings group that consisted of 100 subjects (50 PTSD positive and 50 PTSD negative veterans, matched regarding age).

Specific Aim 1. Confirmation of regional selective effect of PTSD on hippocampal subfield and optimization of the automated subfield processing. 1. B. The study population consisted of a subset of the 85 PTSD positive (n=40, mean age 32.7(3.7) range:23 – 53) and negative subjects (n=45, mean age:33.6 (8.4) range : 22 - 58). The remaining 15 had to be excluded because of insufficient imaging data quality/image processing problems. Multiple linear regression analyses with subfield volume as dependent and group (PTSD pos, PTSD neg), age and ethnicity as independent variables were used to test for PTSD effects on subfield volumes. Table 1 summarizes the findings regarding hippocampal subfield measurements using the manual parcellation approach. In contrast to our expectations, there were no significant differences between the two groups in any of the subfields although CA3&DG and total hippocampal volumes (derived from Freesurfer) were smaller in PTSD positive subjects. Including additional factors that could have an influence on hippocampal volumes, e.g. depression or Apo E4 carrier state etc., into the statistical model did not change this finding. Based on these findings we conclude that that in contrast to the pilot study, there was no PTSD associated global or regional (subfield) hippocampal volume loss. There are several possible explanations, e.g., age (current population younger than pilot population), combat experience (all subjects in this study compared to about 60% of the previous study), unaccounted genetic or metabolic factors etc. In a next step the influence of genetic and metabolic factors on hippocampal subfield volumes in this population will be further investigated . 1. C. Subfield measurements using both the manual parcellation strategy and ASHS with the customized template/atlas were obtained in 92 subjects of the development population. The numbers generated by the two approaches are not directly comparable because ASHS labels a larger section of the hippocampus than the manual approach that is restricted to the anterior third of the hippocampal body. Nonetheless, the findings of the two methods were comparable regarding the power to detect a difference between PTSD pos and PTSD neg which for the subfields of interest (CA1, CA3&DG) were 0.58 and 0.16 or ASHS and 0.05 and 0.07 for the manual labeling approach. In a similar methods comparison that compared subjects with and without mild cognitive impairment due to incipient Alzheimer's Disease, both methods identified CA1 as the affected subfield and had a power of over 0.9 to detect this volume loss that is typical for AD at this stage. Based on these results, it was concluded that the automated method is sufficiently mature to be used throughout this project.

Table 1. ICV corrected A Priori Mean (SD) Subfield and Subcortical Volumes

	PTSD negative	PTSD positive
ERC	128.5 (21.4)	132.1 (25.6)
Sub	105.1 (17.1)	110.3 (18.9)
CA1	384.0 (41.5)	384.0 (42.1)
CA1-2 transition	20.9 (24.3)	17.5 (29.5)
CA3&DG	266.0 (43.6)	255.2 (37.9)
Hippocampus	8704.9(641.6)	8562.7 (647.1)

(SD) standard deviation, ERC entorhinal cortex
CA, cornu amonis sectors;DG, dentate gyrus;Ncl, nucleus

Specific Aim 2. Characterization of extrahippocampal structural abnormalities and their impact on PTSD related networks. The study population consisted of the same subjects as in the previous paragraph. Multiple linear regression analyses with volume/mean thickness as dependent and group (PTSD pos, PTSD neg), age and ethnicity as independent variables were used to test for PTSD effects on cortical and subcortical structures belonging to the mesial limbic prefrontal network (a priori regions) and the rest of the brain (non a-priori regions). False discovery rate (FDR) $p<0.05$ was used to correct for multiple comparisons

Table 2. Mean (SD) Volume Cortical Thickness Measurements of A Priori Regions

	PTSD negative	PTSD positive
Amygdala	3507.4 (312.0)	3523.0 (37
Ncl. accumbens	1271.9 (155.5)	1217.6 (178.9)
Thalamus	16340.7 (1148.3)	16424.5(1188.4)
Rostral Anterior Cingulate	5.76 (0.32)	5.60 (0.37)*
Caudal Anterior Cingulate	5.12 (0.38)	5.03 (0.35)
Superior Frontal	5.51 (0.21)	5.45 (0.18)
Rostral Mid Frontal	4.74 (0.19)	4.74 (0.16)
Caudal Mid Frontal	5.20 (0.19)	5.13 (0.20)
Medial Orbitofrontal	4.87 (0.20)	4.83 (0.25)
Lateral Orbitofrontal	5.30 (0.22)	5.20 (0.29)
Insula	6.2 (0.24)	6.10 (0.32)*

Left and right side are combined, thickness in mm
(SD) standard deviation
*p<0.05 compared to PTSD neg

in the non a priori regions. Tables 2 summarize the findings in the a priori regions. PSTD associated cortical thinning was found in the posterior cingulate, precentral and transverse temporal region in the post hoc analyses but these differences were no longer significant after FDR correction. However, as has been shown by previous studies PTSD was associated with cortical thinning in the rostral anterior cingulate and the insula. Structural white matter abnormalities were assessed by comparing the fractional anisotropy maps of PTSD and PTSD neg at the voxel level. PTSD pos had clusters of voxels with decreased FA in the region of the posterior corona radiata. However, these abnormalities became non-significant after applying a correction for multiple comparisons ($p<0.05$, FDR). In conclusion, The graph analysis was done using the Graph-Analysis-Toolbox 1.3.2. (GAT) (http://ncnl.stanford.edu/tools.html) running under Matlab 2012b. GAT uses the routines in the Brain Connectivity Toolbox (BCT, https://sites.google.com/site/bctnet/) to extract network measures from the binary adjacency matrices at a global and regional level. The raw (not corrected for head size) left and right subiculum, CA1, CA3&dentate gyrus volumes from the high resolution image, and the volumes from the left and right amygdala, accumbens, thalamus and all regional cortical thickness measures from FreeSurfer (total 80 regions or nodes) were used to calculate a Pearson correlation matrix for each of the two groups. These matrices were thresholded at minimal density (density below which the networks are no longer fully connected) and converted into binary adjacency maps. The network properties were assessed at the whole brain level with all nodes (80 regions, 'whole brain network') and in a network limited to the a priori regions (28 regions, 'prefrontal-limbic network'). Prefrontal-limbic network: The minimal density threshold was 0.3992 for PTSD neg and 0.4426 for PTSD pos. The findings within the a priori network are summarized in Table 3. Whole brain network: The minimal density threshold was 0.4223 for PTSD neg and 0.4893 for PTSD pos in the whole brain analysis. A higher nodal betweeness in the left precuneus in PTSD pos compared to PTSD neg (546.26 vs 49.22) was the only significant finding in non a priori region after FDR correction. There was a trend for a shorter mean characteristic path length in PTSD neg compared to PTSD pos (1.898 vs 2.220, $p=0.061$) in the global analysis. The small world index sigma was significantly higher in PTSD neg compared to PTSD pos (1.298 vs 0.875, $p=0.011$). In conclusion, the PTSD associated changes throughout the prefrontal-limbic network are consistent with an increased connectivity that negatively affected its integration with the rest of the brain and resulting loss of the economical small world topology found in healthy brains. These findings are also consistent with those reported by the functional imaging group.

Table 3. Summary of Significant Regional Graph Analytical findings

Network	Measure	Region	PTSD negative	PTSD positive
prefrontal-limbic	NodalDegree	R Medial Orbitofrontal	2	9
		R Insula	6	12
	Cluster Coeff	L Medial Orbitofrontal	0.6	1
	NodalBetw	L Lateral Orbitofrontal	4.434	155.778
		R MedialOrbitofrontal	0.000	49.216
		R Insula	2.994	100.712
whole brain	Nodal Degree	L Medial Orbitofrontal	30	10
		L Rostral AnteriorCingulate	18	3
		R Caudal Anterior Cingulate	17	4
	Cluster Coeff	L Thalamus	1	0
		R Thalamus	1	0
	NodalBetw	L Insula	0.070	415.790
		R Medial Orbitofrontal	0.000	173.3
		L Precuneus	0.000	546.264

Cluster Coeff, cluster coefficient; Nod Betw, nodal betweeness; *italics, not included in a priori selection*

Specific Aim 3. Development of classifier that uses different features extracted from the structural images to distinguish between PTSD positive and PTSD negative subjects. This work is still in progress, only preliminary findings are reported

Two-level Bayesian Classifier (Dr. Mueller): Background: Even though there were significant group differences, the descriptive statistics of the volumetric data showed a large overlap of these measures between PTSD pos and PTSD neg. This data spread could not be explained by accounting for differences in PTSD severity, depression or history of head trauma etc. Based on this it was assumed that this could indicate the existence of different PTSD subtypes that are associated with different degrees of brain atrophy. Methods: To test the hypothesis that spatially normalized gray matter maps were generated from the whole brain T1 weighted images and whole brain z-scores calculated by comparing them to spatially normalized gray matter maps of a population of healthy civilian control subjects. A hierarchical cluster analysis was used to identify three subgroups (mild, moderate and severe gray matter volume losses) in PTSD pos and PTSD neg subjects based on this whole brain z-score. Each individual's gray matter map was then compared to the mean of the gray matter maps of the PTSD neg group with mild gray matter losses ("super" PTSD neg) and voxels with volume losses surpassing a predefined amount of volume loss identified to generate individual binary "abnormality maps". Graphical-Model-based Morphometric Analysis (GAMMA) was used to compare these "abnormality maps" of each subgroup with those from each other subgroup. GAMMA uses a Bayesian network and a contextual clustering method to produce feature maps that provide the maximal distinction between two groups and calculates a probability distribution and a group assignment based on this. Figure 1 shows the feature maps generated by GAMMA when comparing the three PTSD pos groups with the three PTSD neg groups. Each subjects "abnormality map" was compared to the resulting 18 different feature maps to determine to what degree the abnormality pattern matched the feature map. The information was combined in a second level Bayesian network (cf. Figure 2) and the probability of each subject to belong to one of the 6 groups calculated. This approach allowed for a correct classification of 71.3% (Sensitivity 75.5%, Specificity 67.3%) of the subjects in the development population. In the next step it is planned to further improve the accuracy of this classifier by integrating the information from the DTI.

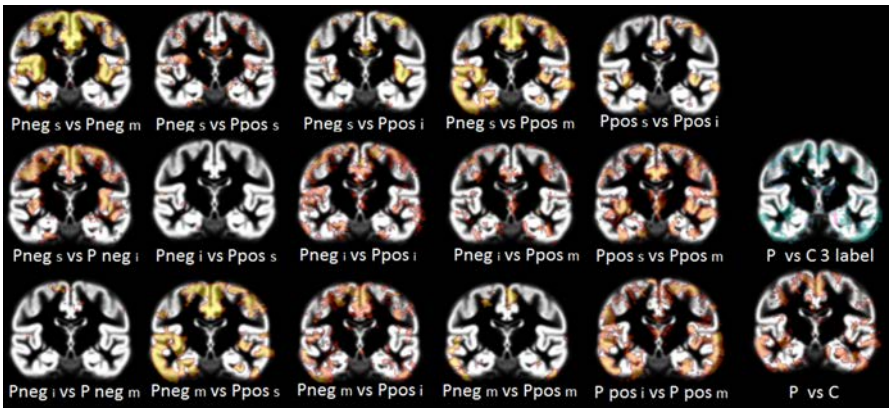


Figure 1.

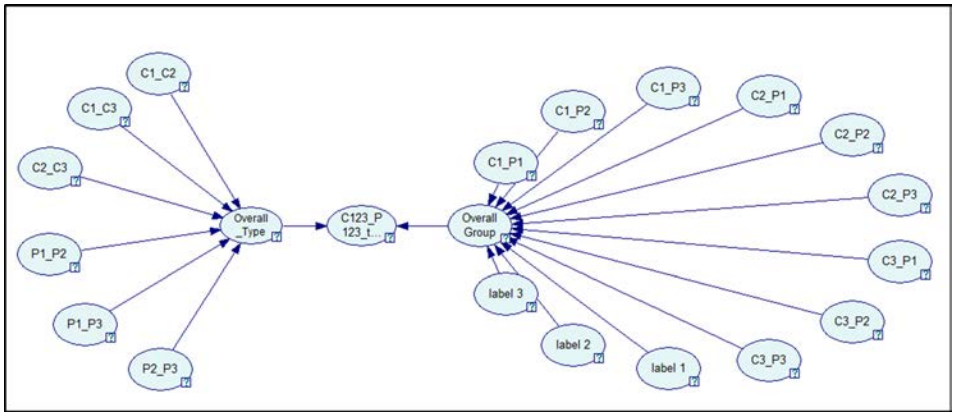


Figure 2

Multivariate approach to PTSD diagnosis using regional texture/complexity (Dr. Schuff)
Goal: We aim to use regional texture and complexity-based measures as features for classifying PTSD from structural magnetic resonance images of the brain. We have previously shown that texture/complexity can outperform conventional volume and thickness measures (20-22). The cohort consists of 99 age-matched subjects of which 47 are PTSD+ and 52 are healthy controls.

Processing Approach 1A: T1-weighted magnetic resonance images are segmented into probabilistic white matter, grey matter, and cerebrospinal fluid (CSF) maps, where each in-brain voxel is assigned a probability of belonging to one of the three groups. The probabilistic tissue maps are then spatially normalized to a conventional template space (MNI) and anatomically labeled. The texture of each anatomical region is then evaluated by the co-occurrences of how many white matter, grey matter, and CSF "neighbors" each voxel within the region has at different scales, ranging from fine (e.g. nearest neighbors at a distance of 3 voxels) to a coarse (e.g. neighbors at a distance of 12 voxels). Statistical measures of textural complexity (20,21) are then used to summarize the co-occurrences in each region for each subject.

Statistics: We then apply statistical learning algorithms, such as naïve Bayes (23), to individually classify a patient or control from the respective image's complexity measures. The classification is augmented by ten-fold cross validation and pre-selection of a subset of attributes (based on the degree of information gain) to reduce the dimensionality of the classification problem. Classification accuracy is then evaluated in terms of the confusion matrix (see Table 4), which provides the frequency of false positives, false negatives, and correct classifications, using the clinical diagnosis as ground truth.

```
a  b   <-- classified as
39 13 |  a = PTSD-
18 29 |  b = PTSD+
```

Table. 4: Confusion matrix

Results 1A: The best classification yielded 69% correct to 31% incorrect accuracy, corresponding to 62% sensitivity and 75% specificity; the algorithm is more likely to correctly identify controls than patients. Table 5 lists regions and scales which contributed most to the classification.

Attribute	Fine Scale (3-5mm)	Medium Scale (6-9mm)	Coarse Scale (10-12mm)
Entropy	Postcentral_Left Precuneus_Left	Medial_Frontal_Orbital_Left	
Statistical Complexity	ParaHippocampal_Left	Medial_Frontal_Orbital_Left	Medial_Frontal_Orbital_Left Middle_Temporal_Right
Correlation		Postcentral_Right Postcentral_Left Occipital_Right Thalamus_Left	Postcentral_Left Lateral_Frontal_Orbital_Left
Contrast		Occipital_Right Lateral_Frontal_Orbital_Left Precuneus_Left Lingual_Left	Occipital_Right Occipital_Left Lateral_Frontal_Orbital_Left Lateral_Frontal_Orbital_Right

Table 5: Anatomical regions and texture/complexity scales that contribute most prominently to the classification.

Processing Approach 1B: Since the last progress report, we have slightly modified the complexity procedures by a) consolidating several anatomical regions to cover a larger scale and b) by using ensemble classifiers, i.e. random forest to potentially improve classification performance. Furthermore, to reduce complications from the dimensionality of possible imaging features, we based the analysis on features that capture the degree of structural uniformity, i.e. entropy, and regional variability, i.e. statistical complexity, of brain structures. As before, the classification accuracy is then evaluated in terms of the confusion matrix (see table 6), which provides the frequency of the false positives, false negatives, and correct classifications, using the clinical diagnosis as ground truth.

```
=== Confusion Matrix ===
 a   b  <-- classified as
30  17 |  a = PTSD+
24  28 |  b = PTSD-
```

Table 6: Confusion Matrix

Results 1B: The best classification using approach 1B yielded 58.6% correct and 41.4% incorrect accuracy, demonstrating that our hypothesis regarding the size of the regions may need further exploration. The algorithm classifies patients better than it classifies controls.

Conclusion: The best classification achieved with this method falls short of clinically-acceptable specificity and sensitivity, but nonetheless was significantly better than a classification by chance. We suspect that a "hidden" heterogeneity in the PTSD cohort may limit the accuracy of texture/complexity to mirror the clinical diagnosis. Texture/complexity of structural MRI may be a method to differentiate potential PTSD subgroups but classification will likely benefit incorporating complexity of other imaging modalities, e.g. functional and diffusion MRI. An identical approach was taken to analyze subtypes in Frontotemporal Dementia, and the results were very promising in terms of a differential diagnosis of dementia subtypes, providing hope that use of PTSD subtype information from other cores may lead to a more accurate classification.

Future directions for complexity: We plan to refine the texture/complexity approach by using more powerful learning algorithms, such as local linear embedding5. Furthermore, we would like to incorporate functional MRI measures into statistical learning to test whether a combination of structural, functional, and diffusion imaging can improve classification.

Additional Aims: Collaboration with other cores to investigate associations between structural imaging and metabolic/genetic/endocrine abnormalities.
The exchange of data between different cores has only recently been initiated. One of the first joint analyses was to investigate the relationship between peripheral blood monocytic cells (PBMC) telomere length (TL) and telomerase activity (TA) with cortical thickness. Given the fact that TA decrease and TL shortening are interpreted as evidence for an accelerated aging process, it was expected to find associations between TA/TL and brain regions typically affected by aging, i.e., frontal lobe, temporal lobe and parietal lobe. Table 7 summarizes the major findings in the subset of the 52/52 sample who had both measurements. The combined PTSD pos&neg and PTSD neg groups showed positive associations between telomerase activity in PBMCs and cortical thickness measures associated with aging. These associations were lost in PTSD pos subjects indicating that additional influence cortical thickness in this group.

PTSD pos&neg	35	Pars Orbitalis	telomerase	0.04	0.0037
	35	Pars Opercuaris	telomerase	0.016	0.046
	35	Temporal Pole	telomerase	0.063	0.001
PTSD neg	20	Insula	telomerase	0.021	0.0461
	20	Pars Orbitalis	telomerase	0.034	0.0047
	20	Superior Frontal	telomerase	0.191	0.0367
	20	Temporal Pole	telomerase	0.059	0.0005

Table 7: Linear regression model: cortical thickness adjusted for age * telomere length.

4. Future Directions

1. Integration of the imaging findings with the findings of other cores: In the next phase it will be important to get access to all the clinical data to be able to properly account for the variability in the data, e.g. CAPS sub-scores etc. In addition to that, the integration of the imaging data with the other biomarker data should be initiated, .i.e., discussions about how the imaging data can be implemented, e.g. numerical outputs, abnormalities maps etc., identification of appropriate a priori hypotheses and exploratory, data-mining like approaches etc.
2. Confirmation of the findings/classifiers developed based on the 52/52 population in the remaining 100 subjects and in the "validation" cohort

Functional Imaging Core
New York University Langone Medical Center

There have been neuroimaging studies about PTSD, which suggested abnormal brain structure as well as abnormal responsivity to fearful stimuli. The resting state fMRI studies aim to investigate the brain functionality at a spontaneous state. Hypotheses include higher spontaneous brain activity at amygdala in PTSD compared to controls, and lower spontaneous brain activity at the default mode network, as well as altered functional connectivity in the default mode network and fear circuitry. Resting state fMRI was acquired from both groups of subjects with and without PTSD, and analyzed to observe any group differences on the magnitudes of spontaneous brain activity as well as functional connectivity. Results showed that PTSD group compared to the control group, had increased spontaneous brain activity at the amygdala, insula, orbital frontal cortex and anterior cingulate cortex, with decreased spontaneous brain activity at the precuneus, thalamus, and dorsal lateral prefrontal cortex. The PTSD group also showed altered functional connectivity compared to the control group in the default mode network, the fear circuitry, and the reward circuitry. Future research should include more subjective reporting and physiological recording of anxiety levels during the scanning. Neural correlates of cognitive, metabolic, endocrine and genetic markers in PTSD will be investigated by collaborating with other cores. This research significantly increased our knowledge about the spontaneous brain activity and functional connectivity in PTSD and its relevance with PTSD symptoms. The neural parameters obtained from this study could be potential biomarkers to assist differential diagnosis of PTSD and identifying subtypes of PTSD.

Metabolism, Inflammation & Cell Aging Core
University of California, San Francisco

PTSD is associated with increased physical morbidity and early mortality. In particular, individuals with PTSD are more likely to develop illnesses more commonly seen in the elderly, e.g., cardiovascular disease, diabetes, metabolic syndrome, arthritis, immune disorders and dementia. This raises the possibility that PTSD accelerates the normal aging process. Discovering the nature of PTSD effects on cellular physiology may yield new biomarkers for PTSD, may help stage the progression of the illness and responses to treatment and may yield new treatment targets. In this study of 55 combat-exposed veterans with PTSD and 55 matched, combat-exposed veterans without PTSD, we explored multiple inter-related cellular/ physiological markers related to overall health and to rates of biological aging. We found notable differences in such markers, many of which have not previously been described, between those with and without PTSD. The results, as summarized below, cumulatively support an acceleration of the normal aging process, across multiple dimensions, in individuals with PTSD. This may identify new aspects of the pathophysiology of PTSD. Several of the identified markers have been shown, in other populations, to predict risk of serious medical illness and premature mortality. Certain of these markers, particularly those involving changes in metabolic pathways, distinguished PTSD(+) and PTSD(-) individuals with reasonably high sensitivity and specificity, suggesting they may contribute to a biomarker panel to diagnose PTSD.

Latest Findings
(Note that most of these findings remained significant after co-varying for the effects of comorbid depression, past history of early childhood adversity, concurrent medical diseases and concurrent prescription medications. This more strongly implicates PTSD per se, rather than concomitant features, in the abnormalities observed. Further, many of these findings were directly related to the duration or severity of PTSD symptoms, suggesting a “dose-response” relationship between PTSD and these biological perturbations.)

1. Individuals with PTSD have a significantly higher Metabolic Syndrome Total Score ($p=0.008$).
2. Individuals with PTSD have a significantly increased degree of pro-inflammatory, relative to anti-inflammatory, cytokines ($p=0.001$).
3. Analyses so far have failed to reveal significant differences in oxidative stress measures, but this may be due to a modest effect size and a limited sample size. We plan to combine a larger number of subjects to further study this possibility.
4. Individuals with PTSD show increased serum BDNF levels ($p<0.03$). However, those individuals with more chronic PTSD showed lowered BDNF levels compared to controls. This raises the novel possibility that elevated BDNF levels in more acute PTSD are involved in over-consolidation of the traumatic memories, whereas BDNF levels gradually diminish with more chronic illness, perhaps contributing to the intransigence of the stress-related memories and the difficulty overlaying them with new, non-stress-related learning.
5. Assay of neurosteroids remains pending due to difficulties operationalizing this assay, which requires extremely high sensitivity, Refinement of the assay continues.
6. Individuals with PTSD show trends toward immune cell changes suggestive of an accelerated rate of cellular aging. In most cases, this was proportional to the severity of lifetime and current PTSD symptoms and/or the length of time since the index traumatic event. Specific findings, at the statistically significant level or at the trend level include: shortened leukocyte telomere length ($p=0.13$), decreased peripheral blood mononuclear cell telomerase activity in proportion to the severity of PTSD symptoms ($p<0.04$) increased CD4:CD8 ratio, increased CD4+CD28- and CD8+CD28- T cells (immunosenescent cells) with a concomitant decrease in CD4+CD28+ and CD8+CD28+ T cells (young or naïve cells), and increased CD16+CD56- ("dim") NK cells ($p<0.05$) (senescent, dysfunctional, highly cytotoxic cells) with a concomitant decrease in CD16-CD56+ ("bright") ($p<0.06$) NK cells (younger cells, more efficient at cytokine production, less susceptible to oxidation and apoptosis).
7. Metabolomics analysis revealed 47 biochemicals that differentiated PTSD(+) from PTSD(-) subjects. Some of these differences implicated stress hormones, fatty acids and other lipids, and the gut microbiome, but the most consistent differences involved impairments in mitochondrial function, such as deficiencies in oxidative phosphorylation and energy utilization, including abnormalities in the Kreb's and urea cycles. These alterations were proportional to the severity of PTSD symptoms ($p<0.03$). This finding is, to our knowledge, the first demonstration of this in living individuals with PTSD but is consistent with data from animal models of PTSD as well as post-mortem studies of PTSD. This implicates mitochondrial dysfunction and inefficient intracellular energy generation, including a shift toward anaerobic respiration in PTSD, consistent with preclinical models of PTSD.

Cumulatively, these preliminary findings help define and explain the increased health risks associated with chronic PTSD and they implicate specific molecular, cellular and systemic targets that may be amenable to novel therapeutics. Continued research with these markers will determine the possible diagnostic, prognostic and treatment implications of these findings and will replicate them in a larger sample of individuals, including a larger number of females.

Progress to Date

Biomarkers	Subject samples assayed	Biomarkers analyzed:
Allostatic Battery	103*	completed
Cytokines	103*	completed
F2-Isoprostanes	103*	completed
8-OH-dG	103*	completed
Glutathione Peroxidase	103*	completed
GSH/GSSG	103*	completed
Vitamin C & E	103*	completed

BDNF	103*	completed
Telomere Length	103*	completed
Telomere Activity	103*	completed
Telomere Induced Foci	---	---
Flow cytometry: Lymphocytes	approx. 185**	103*
Flow cytometry: EPC	approx. 185**	103*
Neurosteroids	---	---
Metabolomics	103*	completed

* One subject in the match group did not complete blood draw
** Fresh blood is sent for assaying each week

Proteomics Core

Institute for Systems Biology

Post-Traumatic Stress Disorder (PTSD) has been attributed as a psychological disorder caused by pathologies in central nervous system. PTSD poses a challenging problem for contemporary medicine in that its primary effects are mediated through the brain. Current medicine has limited tools to assess the progression and mechanistic basis of this traumatic disease because the brain is one of the most inaccessible human organs for analysis. Apart from several modes of imaging and the analysis of cerebral spinal fluid, the assessment methods for PTSD are largely based on psychological, sensory and motor tests—that is, high-level phenotypic measurements with significant uncertainty. Therefore there is an urgent need to develop clinically relevant molecular measurement for accurate PTSD diagnosis. We adapted systems approaches to undertake a comprehensive, serial analysis of various tissues and peripheral blood components to identify PTSD associated molecular changes and blood biomarkers using a mouse model initially and then apply these insights to human disease.

A better understanding of the underlying molecular pathogenesis for PTSD is essential to identify informative biomarkers and effective therapeutic targets. To gain such knowledge, we conducted a comprehensive analysis on a social defeat mouse PTSD model developed in Dr. Jett's laboratory at the USACHER. To our surprise, we (ISB and USACHER) observed acute heart injury among stressed animals and uncovered several affected molecular processes with key regulatory molecules including transcription factors and microRNAs. This finding fits to prior reports regarding higher risk of cardiovascular diseases for people suffered with PTSD based on epidemiological studies. We also observed similar molecular changes in other major organs. These findings suggest PTSD is not just a neurological disorder, but a systemic condition that affects different organs in the body.

The blood is likely the most information-rich organ (or fluid) in that it bathes all of the tissues in the body, and it is easily accessible for diagnostic procedures. To discover blood biomarker candidates, we conducted comprehensive analyses on blood proteins and circulating RNAs from samples provided by Dr. Marmar's laboratory at NYU. We characterized the circulating microRNA by qPCR and microarray methods. Two different but complementary proteomics approaches, isobaric tag for relative and absolute quantitation (iTRAQ), a global protein identification and quantitation method, and a selected reaction monitoring (SRM), a targeted proteomics method were adapted to assess the changes of blood proteome in PTSD. Several microRNA and protein biomarker candidates have been identified. Molecular classifiers have also been built to generate diagnosis model to detect PTSD. We also conducted next generation sequencing platform to attain comprehensive profile of circulating RNA on limited number of samples and demonstrated the possibility of using RNAseq to gain a global view of RNA in plasma samples so that more informative RNA based biomarkers can be identified.

Molecular Aspects for Systems Biology Studies of PTSD

US Army CEHR, University of California, Santa Barbara and Institute for Systems Biology

Post-Traumatic Stress Disorder (PTSD) has been attributed as a psychological disorder caused by pathologies in central nervous system. PTSD poses a challenging problem for contemporary medicine in that its primary effects are mediated through the brain. Current medicine has limited tools to assess the progression and mechanistic basis of this traumatic disease because the brain is one of the most inaccessible human organs for analysis. Apart from several modes of imaging and the analysis of cerebral spinal fluid, the assessment methods for PTSD are largely based on psychological, sensory and motor tests—that is, higher-level phenotypic measurement with significant uncertainty. Therefore there is an urgent need to develop clinically relevant molecular biomarkers for PTSD.

Global multi-omic studies were carried out and include transcriptome and epigenomic studies. These data types measure the genome-wide amounts of and changes to DNA sequence, mRNA expression, DNA methylation/histone modification, metabolite production, and protein expression, respectively. Commonly used experimental techniques for collecting these data include high-throughput sequencing methods, array-based hybridization assays (e.g. microarrays), liquid and gas chromatography, and mass spectrometry. Data from all of the above experimental modalities are polluted with noise. Our studies have utilized classic means and newly developed methods to extract signals, signatures and features to enable a comprehensive study of host response alterations from repeated trauma exposures in military personnel.

Neuroendocrine Core

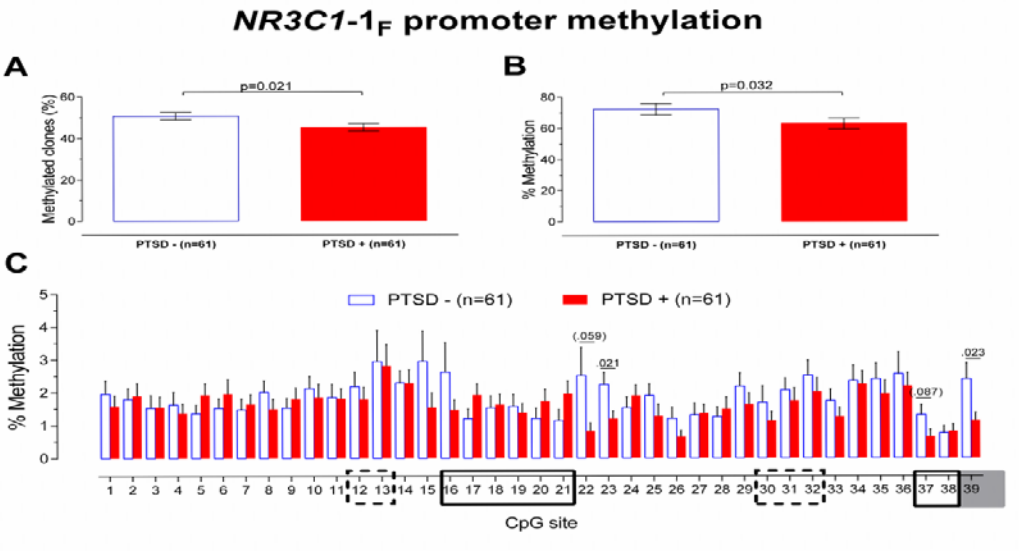
James J. Peters Bronx VAMC

The Neuroendocrine Core is co-located at the James J Peters VAMC (JJPVA) and Icahn School of Medicine at Mount Sinai (ISMMS), directed by Rachel Yehuda, Ph.D. There are three functions of the core. The first function is to recruit, determine eligibility and assess (i.e., diagnostic evaluation, cognitive assessment) half of the study cohort. As of April, 2013, the JJP VAMC/ISMMS site has enrolled 220 veterans, with 133 meeting the eligibility criteria and 99 participants (86 men and 13 women) successfully completing the protocol. The second function of the core is to collect blood samples and process into several blood elements; collect urine samples; and store, inventory and ship samples to other cores and investigators. One hundred and ninety-seven people have completed blood draws and samples have been shipped to Ft. Detrick, Emory University and University of California, San Francisco.

Finally, the third function of the core is to neuroendocrine and neuroendocrine-related molecular markers. The focus of the initial analyses was on an epigenetic marker - cytosine methylation of the NR3C1-1F gene (glucocorticoid receptor (GR)), which has previously been associated with early childhood trauma. Results showed that a significantly lower percentage of methylated clones ($F_{1,120}= 5.46$, $p=.021$) was observed in the NR3C1-1F promoter across the 39 CpG sites in the PTSD+ compared to the PTSD- group. This difference persisted when controlling for PBMC ratio ($F_{1,117}= 6.28$, $p=.014$), when using the untransformed (raw) data ($F_{1,120}= 4.91$, $p=.029$); Fig. 1A), when controlling for use of psychotropic medications ($F_{1,119} = 4.10$, $p=.045$) and when the data were expressed as the sum percent methylation across the 39 sites ($F_{1,120}=4.70$, $p=.032$; Fig. 1B). Fig. 1C illustrates percent methylation at each of the 39 CpG sites of exon 1F. The PTSD+ group also showed greater evidence of GR sensitivity in PBMCs as reflected by the lysozyme stimulation test, compared to the PTSD- group (lysozyme IC50-DEX: $F_{1,116}=5.13$, $p=.025$). Similarly, the PTSD+ group showed a greater decline in cortisol in response to 0.50 mg DEX ($F_{1,112}=7.95$, $p=.006$;), a finding that persisted when the analysis was repeated using plasma levels of DEX as a covariate ($F_{1,111}=7.68$, $p=.007$), and when additionally co-varying for use of any psychotropic medication ($F_{1,110}=4.80$, $p=.031$). Results of 24-hr urinary cortisol excretion showed a significantly lower urinary cortisol excretion ($F_{1,112}=3.97$, $p=.049$) in association with PTSD, an effect that was no longer below the significance threshold when covaried for use of psychotropic medication ($F_{1,111}=3.65$, $p=.057$), or when raw, rather than log-transformed, values were used in the analysis

(F1,112=2.98, p=.087). Finally, both percent methylated clones (r=-.220, p=.019, n=114) and sum percent methylation (r=-.198, p=.034, n=114) were inversely correlated with cortisol decline in response to DEX. The results reported above were recently published in Biological Psychiatry and analyses were conducted with a sample of 122 male veterans. Results were replicated in a larger sample of males, and for the most part, also in the subsample of female veterans though the power is restricted in this smaller sample.

Figure 1



Genetics Core

Emory University

Overview: For a disorder requiring an environmental precipitant, it may seem counterintuitive to consider genetic factors in PTSD. Nevertheless, only a minority of those exposed to traumatic stressors develop PTSD, suggesting that other factors, such as heritable determinants, may influence susceptibility to PTSD in exposed persons. Patterns of PTSD in families suggest a familial component to the syndrome. From twin studies, it has been estimated that 30% to 40% of the risk for developing PTSD is heritable. Despite this recognition, the PTSD molecular genetics literature is sparse, with no genetic linkage studies in pedigrees, and a modest number of genetic association studies. Based on human and animal studies, a number of "candidate" genes in serotonergic, dopaminergic, GABAergic, and neurotrophin pathways have been studied, providing limited support. Small samples that are underpowered for the observation of small genetic effects, combined with the low prior probability of any single chosen candidate gene being truly associated with PTSD likely explain the unimpressive literature.

Recently, there have been a number of large scale genetic association studies for complex disorders like type 2 diabetes, obesity, and schizophrenia, in which large numbers of cases (>1000) are analyzed for hundreds of thousands of single nucleotide polymorphisms (SNPs), in what are termed GWAS markers study, SNP profiles of combat veterans with and without PTSD have been analyzed, in order to elucidate the specific genetic variants that may have predictive value about PTSD susceptibility, and to identify novel genetic correlates of PTSD and related phenotypes.

Candidate Genes: In the work to date, we have found that a number of a priori examined gene pathways are associated with PTSD, both from the Biomarkers dataset as well as from our collaborative studies in traumatized civilians. The primary initial finding from this analysis included 7 SNPs that are nominally significant within the Brain Derived Neurotrophic Factor (BDNF) gene. Notably, BDNF has been associated with recovery from fear by enhancing extinction learning and in decreasing risk for depression. These findings suggest that BDNF may both serve as a biomarker adding to our understanding of risk for PTSD as well as a potential target.

Genome-wide association findings for PTSD: A very exciting and new development from our core is the discovery of a novel polymorphism which is associated with a previously uncharacterized region of the genome which meets genome-wide significance in our N=148 Biomarkers cohort and which replicates at nominal significance in our N>2200 collaborative civilian cohort. This SNP is also associated with increased amygdala activation to fearful faces, a frequently used intermediate phenotype in understanding PTSD and other fear and stress-related disorders.

Using the CAPS total symptom scale as a continuous measure of PTSD symptoms within the Biomarker dataset, we found a polymorphism on chromosome 4 which was significantly associated with PTSD symptoms in an additive fashion, p=1.28x10-8, which survives genome-wide multiple testing criteria. Additionally, we see an entire cluster of SNPs that are in a cluster within this gene region that are all very strongly associated with PTSD symptoms. We next examined the same SNP within the Grady Trauma Project civilian cohort, finding a very strong association in this very different, civilian sample with the same direction of association in predicting PTSD diagnosis (N=2270, p<0.001). Finally, using functional MRI to examine brain activation, we analyzed the association of this genome-wide significant SNP and brain function in a fearful faces task. In a whole-brain analysis of the response to fearful relative to neutral pictures, the A-allele (which was most associated with PTSD) was associated with decreased activation in bilateral dorsolateral prefrontal cortex (dlPFC) and dorsomedial prefrontal cortex (dmPFC, significant clusters at a whole-brain corrected threshold of $p < .05$. N = 53 (33 GG, 16 AG, 4 AA)). While further replication and mechanistic studies are needed, these data support a very exciting potential new discovery of a pathway relevant to PTS symptomology across population cohorts.

Genome-wide association findings for other trauma-related syndromes: Excessive alcohol consumption is a common and costly public health problem and is frequently associated with stress and trauma exposure. We report results from a genome-wide association study of alcohol consumption and behavior in traumatized civilian and military cohorts as measured by the Alcohol Use Disorders Identification Test (AUDIT). The primary discovery sample was a community cohort of African American subjects from the Grady Trauma Project with the replication cohorts being the Biomarkers cohort and the Marine Resiliency Study cohorts. In a genome-wide association study using the Illumina Omni-Quad array, we found an association between total AUDIT score and rs1433375 (N=1034, p=3.67×10-8), a SNP located downstream of Sodium Channel and Clathrin Linker 1 (SCLT1) at chromosomal location 4q28. Following the initial finding within the civilian Grady Trauma Project, it replicated in a meta-analyses of the 3 independent military cohorts (N=2200, p<0.001). This SNP was also associated with SCLT1 gene expression and with cortical-cerebellar functional connectivity using fMRI. These convergent findings provide evidence for a pathway that may explain sodium channel and cerebellar effects of alcohol, shedding light on the etiology of alcohol-related disorders.

Future Directions: In the next year there are extensive plans to both further examine genetic associations with other phenotypes from the Biomarkers cohort, but also integrate the genetics data across the multiple other Biomarker cores. This will allow a much deeper level of integration across the range of biomarkers variables to further understand the mechanisms related to risk and resilience for PTS and other trauma related disorders.

KEY RESEARCH ACCOMPLISHMENTS:

- Obtained IRB continuation approvals across all sites and the DOD.
- Continued outreach efforts and networking with various veterans and community organizations. IRB approved recruitment material (brochures, flyers and advertisements) were distributed at job fairs, colleges, VA Medical Centers and veterans' organizations.
- Enrolled 187 OIF/OEF veterans and completed clinical assessments, self-report measures, and neurocognitive testing for these participants.

- Study team participated in weekly study meetings, quarterly Systems Biology meetings, and the AIBS Review.
- Entered, cleaned, and scored all data into a centralized database and ran reports for data analysis.
- Completed biomarkers study procedures for eligible participants including blood draws, MRIs and urine collection.
- Completed a number of shipments of blood samples from JJPVAMC to Integrative Systems Biology, UCSF and Emory University (Multiomics, Metabolism & Genetic cores). Neuroimaging data was transferred successfully from NYU to UCSF.
- Executed Material Transfer Agreements between JJPVAMC, NYU and Emory University.
- Analyzed 52/52 cohort and 20/20 cross validation sample.
- Participated in a 3 hour symposium at the Annual American Psychiatric Association (APA) conference.
- Presented to the AIBS review for recommendation for progressing to the Advanced Development phase. Collaborators developed a timeline/pipeline for the creation of panel tests.

REPORTABLE OUTCOMES:

- The major development during the timeframe of this annual report for this project is that the implementation phase has been established and is well underway.
- Recruitment and data collection is in progress and participants are completing all study procedures.
- Study Materials and Data are being transferred across sites and several specimen shipments were sent to the multiomics, metabolism, genetics and imaging cores.
- Data dissemination started and 12 manuscript were published and several publications are underway.
- Tasks to complete for the next annual report include: (1) Continue to recruit and enroll subjects for the study to meet our goal of 100 PTSD positive and 100 PTSD negative participants. (2) Run study participants through all procedures. (3) Continue data collection and data management. (4) Analyze demographic data for enrolled participants for the purpose of matching controls with PTSD positive participants. (5) Continue to process and ascertain biomarkers. (6) Implement Validation Study, which will allow for longitudinal follow-up with participants and additional new recruitment (7) Continue to ship samples to UCSF, Emory University, Drs. Marti Jett and Lee Hood for analysis. (8) Disseminate data via manuscripts and conferences.

CONCLUSION and FUTURE DIRECTIONS:

According to the Statement of Work, the goal of the implementation phase of this study is to:

1. Enroll and evaluate 200 OIF/OEF veterans (100 PTSD positive & 100 PTSD negative).
2. Complete all assessment measures for the enrolled participants
3. Complete Data entry for enrolled participants
4. Conduct biomarker acquisition procedures
5. Deliver Material to cores
6. Conduct preliminary analyses on sample

In year 5 of the project we exceeded the goals and milestones in the SOW by enrolling 93.5% of the required sample size, and meeting all the milestones for this project.

Given our progress to date, we have just received funding to initiate a Validating Biomarkers grant. In this grant we will validate candidate diagnostic biomarkers for PTSD in Iraq and Afghanistan Veterans from our recently initial discovery phase study. We will accomplish this aim by enrolling 35 new PTSD cases and 50 new controls. In addition, in order to assess the relationship of longitudinal changes of biomarkers with longitudinal changes in diagnosis, we will reassess 35 PTSD cases and 35 controls from our initial biomarkers discovery phase (Time1). The 35 cases and 35 controls will be reassessed 12 to 24 months after their initial assessment (Time 2). Furthermore, to ascertain biomarkers for PTSD

longitudinally, we will obtain DoD maintained Archive Sera samples drawn before and after deployment for participants enrolled in the discovery and validation phases of our study. These will be assayed for microRNAs and other protein markers to examine longitudinal changes in a larger sample. Lastly, we will evaluate biomarkers for warzone related PTSD in male and female OEF and OIF veterans to determine common vs. gender specific markers and to contribute to the understanding of gender specific pathways for developing PTSD.

Our goal for this grant is to identify four panel domains of biomarkers for PTSD each of which includes 10-20 individual targets. These four panel domains are:

- mRNA transcripts
- DNA methylation targets
- miRNA + plasma protein combined targets
- Biochemical and cellular targets

Once selected, the four panels will be further validated and tested this year. Using this process, we will then integrate the best of these constituents from each of these 4 panel domains to create a single integrative overall panel.

As we are completing these goals, we will be characterizing subtypes of PTSD which will further refine our understanding of biomarkers.

- In 2015, we will further validate our Integrated Biomarker Panel in additional available banked cohort samples, prospectively and pre- and post-deployment and enlistment, identified, across a range of trauma exposures, sex, age, comorbidity (e.g., TBI, depression, substance use, and obesity) and risk.
- To further advance and refine the bioinformatics approaches to the down selection of biomarkers, we have established a Biomarker Metrics Workgroup, chaired by Dr. Francis Doyle III, which includes experts from each of the cores.

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