

Compromised Hippocampus-Striatum Pathway as a Potential Imaging Biomarker of Mild-Traumatic Brain Injury and Posttraumatic Stress Disorder

D. Rangaprakash ^{1,2}, Gopikrishna Deshpande,^{1,3,4*} Thomas A. Daniel,^{3,5}
Adam M. Goodman,^{3,6} Jennifer L. Robinson,^{1,3,4} Nouha Salibi,^{1,7}
Jeffrey S. Katz,^{1,3,4} Thomas S. Denney, Jr.,^{1,3,4} and Michael N. Dretsch^{8,9*}

¹AU MRI Research Center, Department of Electrical and Computer Engineering, Auburn University, Auburn, Alabama

²Department of Psychiatry and Biobehavioral Sciences, University of California Los Angeles, Los Angeles, California

³Department of Psychology, Auburn University, Auburn, Alabama

⁴Alabama Advanced Imaging Consortium, Auburn University and University of Alabama at Birmingham, Birmingham, Alabama

⁵Department of Psychology, Westfield State University, Westfield, Massachusetts

⁶Department of Psychology, University of Alabama Birmingham, Birmingham, Alabama

⁷MR R&D, Siemens Healthcare, Malvern, Pennsylvania

⁸U.S. Army Aeromedical Research Laboratory, Fort Rucker, Alabama

⁹Human Dimension Division, HQ TRADOC, Fort Eustis, Virginia



Abstract: *Objectives:* Military service members risk acquiring posttraumatic stress disorder (PTSD) and mild-traumatic brain injury (mTBI), with high comorbidity. Owing to overlapping symptomatology in chronic mTBI or postconcussion syndrome (PCS) and PTSD, it is difficult to assess the etiology of a patient's condition without objective measures. Using resting-state functional MRI in a novel framework, we tested the hypothesis that their neural signatures are characterized by functionally hyperconnected brain regions which are less variable over time. Additionally, we predicted that such connectivities possessed the highest ability in predicting the diagnostic membership of a novel subject (top-predictors) in addition to being statistically significant. *Methods:* U.S. Army Soldiers ($N = 87$) with PTSD and comorbid PCS + PTSD were recruited along with combat controls. Static and dynamic functional connectivities were evaluated. Group differences were obtained in accordance with our hypothesis. Machine learning classification (MLC) was employed to determine top predictors. *Results:* From whole-brain connectivity, we identified the hippocampus-striatum connectivity to be significantly altered in accordance with our hypothesis. Diffusion tractography revealed compromised white-matter integrity between aforementioned regions only in the PCS + PTSD group, suggesting a structural etiology for the PCS + PTSD group rather than being an extreme subset of PTSD. Employing MLC, connectivities provided worst-case accuracy of 84% (9% more than psychological measures). Additionally, the

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*Correspondence to: Gopikrishna Deshpande; E-mail: gopi@auburn.edu and Michael N. Dretsch; E-mail: michael.n.dretsch.mil@mail.mil

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hippocampus-striatum connectivities were found to be top predictors and thus a potential biomarker of PTSD/mTBI. *Conclusions:* PTSD/mTBI are associated with hippocampal-striatal hyperconnectivity from which it is difficult to disengage, leading to a habit-like response toward episodic traumatic memories, which fits well with behavioral manifestations of combat-related PTSD/mTBI. *Hum Brain Mapp* 38:2843–2864, 2017. © 2017 Wiley Periodicals, Inc.

Key words: functional magnetic resonance imaging; functional connectivity; dynamic connectivity; posttraumatic stress disorder; mild traumatic brain injury; network dynamics; hippocampus

INTRODUCTION

About 20% of military service members develop post-traumatic stress disorder (PTSD) (Hayes et al., 2012). PTSD is characterized by high anxiety, re-experiencing traumatic memories, hypervigilance, and hyperarousal. In combat veterans, PTSD has high comorbidity with mild-traumatic brain injury (mTBI) (Hoge et al., 2008, 2009) due to the risk of being exposed to improvised explosive devices (IEDs) and nonblast events. A significant percentage of those who sustain mTBI suffer from chronic symptoms (postconcussion syndrome [PCS] (Cicerone and Kalmar, 1995)). With current diagnostic procedures and treatments centering on subjective assessments, a thorough understanding of the mechanistic basis for PTSD and PCS is essential for accurate diagnosis, targeted treatment and for making return-to-duty decisions. Owing to largely overlapping symptomatology between PTSD and PCS (Eierud et al., 2014), it is necessary to identify and validate objective biomarkers of the respective neurologic and neuropsychiatric conditions to improve clinical evaluation and, ultimately, treatment outcomes.

We employed resting-state functional MRI (rs-fMRI), which avoids task dependency and subsequent performance differences. We performed connectivity analysis on rs-fMRI data, without *a priori* assumptions concerning regions of interest (ROIs). Functional connectivity (FC) refers to measures of instantaneous correlation between a pair of fMRI time series obtained from different brain regions. There have been several recent fMRI FC studies with PTSD (Hayes et al., 2012; Simmons and Matthews, 2012; Spielberg et al., 2015) and PCS (Costanzo et al., 2014; Eierud et al., 2014; Hoge et al., 2008). However, very little work has been done on comorbid PTSD and PCS, even though comorbidity is the norm rather than the exception in military populations (Spielberg et al., 2015). Existing findings have been mixed (Simmons and Matthews, 2012).

Hyperconnectivity is seen as a response to neurological disruption (Hillary et al., 2015) and is observed in individuals with PTSD (Cisler et al., 2014; Hayes et al., 2012; Simmons and Matthews, 2012). Most studies employ only static FC (SFC) and ignore dynamic variation of connectivity over time, known as dynamic FC (DFC). Recent studies have highlighted the enormous importance of dynamics in deciphering brain functioning (Hansen et al., 2015). Several

studies show that DFC signatures in subjects with mental disorders are different from those in healthy subjects (Deshpande et al., 2006; Keilholz et al., 2013; Li et al., 2014; Majeed et al., 2011). DFC is also related to real world cognitive behaviors (Thompson et al., 2013), which may make it a good tool for studying disorders such as PTSD and PCS where cognitive functioning is compromised. SFC and DFC provide different types of information regarding connectivity between two brain regions (Hutchison et al., 2013). Reduced temporal variance in DFC is associated with psychiatric disorders as well as compromised behavioral performance in healthy individuals (Jia et al., 2014; Sakoğlu et al., 2010). This reduction is associated with compromised ability to dynamically adjust (e.g., behavior, thoughts, etc.) to changing conditions. This phenomenon is well recognized in other biological systems such as reduced heart rate variability being a risk factor of cardiovascular disease (Greiser et al., 2009). As external influences and internal body states are continually changing, a healthy biological system varies its activity in real-time to accommodate these changes. In these terms, “frozen” connectivity reflects compromised brain health. This study uses these principles to identify functional connectivities in the brains of soldiers with PTSD and PCS which are in a “frozen” hyperconnected state compared to healthy soldiers.

Active duty, U.S. Army soldiers who screened positive for PTSD, both PCS and PTSD (PCS + PTSD), and healthy combat controls were recruited. We tested an overarching hypothesis that PTSD with and without PCS is associated with higher connectivity strength (SFC) but lower connectivity variance (variance of DFC [vDFC] calculated over time, Fig. 4) as compared to healthy controls (Fig. 1). Furthermore, we hypothesized that the connectivities would be more extreme (i.e., higher SFC and lower vDFC) in PCS + PTSD subjects compared to PTSD subjects, indicative of greater symptom severity. We notably tested the hypothesis on whole-brain connectivity data without imposition of any priors or assumptions. Figure 1 provides an illustration of our hypothesis.

In addition to the primary hypothesis, there were multiple corollary hypotheses addressed in this study. First, if the connectivities were indeed more extreme (i.e., higher SFC and lower vDFC) in PCS + PTSD subjects compared to PTSD subjects, it raises the question as to whether the PCS + PTSD group’s condition is being driven by PTSD.

Alternatively, is this comorbid group's state unique, potentially due to the addition of mTBI sequelae? We attempt to address this question by investigating structural alterations of white-matter tracts in all the three groups with the hypothesis that the changes in axonal integrity must be exclusive to the PCS + PTSD group, likely attributed to the mTBI suffered by these subjects. MRI diffusion tensor imaging (DTI) tractography provides meaningful information concerning diffusion of water molecules in white matter as a measure of tract trajectory, integrity, and directionality. White-matter neuropathology can result in increased diffusivity, for example, with inflammation from demyelination (Harsan et al., 2006; Henry et al., 2003). In a recent study involving veterans from the Iraq and Afghanistan wars who were diagnosed with PCS (Morey et al., 2013), DTI showed differences in white-matter diffusivity associated with the regions that also had abnormal functional connectivity. In line with this finding, we predicted that there would be congruently greater diffusivity in the tracts connecting regions with altered functional connectivity; therefore, supporting the argument that the PCS + PTSD group is etiologically different from the PTSD group.

Second, if we are successful in finding functional connectivities in the brain which satisfy our overarching hypothesis, it will be important to determine their relevance to behavior and clinical diagnostics. Our subjects are traditionally assigned diagnostic groups based on clinical observation and symptom reporting. While self-report symptom scores provide subjective assessments of severity of the disorders (i.e., psychopathology), neuroimaging data provide mechanistic characterization of underlying pathophysiology. Hence, as our secondary hypothesis, we hypothesized that grouping of subjects based on significant connectivity values would be superior (in terms of how the groups map to behavioral clusters) than conventional diagnostic grouping. Indeed, this approach has been actively promoted by the National Institute of Mental Health (NIMH) in the United States by publication of "Research Domain Criteria" (RDoC, <http://www.nimh.nih.gov/research-priorities/rdoc/nimh-research-domain-criteria-rdoc.shtml>). RDoC is agnostic about current disorder categories, and the intent is to generate classifications in a data-driven way. The "core unit of analysis" advanced by RDoC is the "measurements of particular circuits as studied by neuroimaging techniques." In line with this ideology, a recent report demonstrates how data-driven definition of groups in psychiatric spectrum disorders can identify new groups which map better onto behavioral clusters (Brodersen et al., 2013). Our regrouping strategy is inspired by these recent developments.

To address our secondary hypothesis, behavioral measures obtained from a neurocognitive battery were separately grouped using both conventional grouping and the proposed imaging-based grouping methods. Next, the statistical separation between the groups was compared for both grouping methods. This comparison was done to test



Figure 1.

Illustration of our primary hypothesis: increasing font size of SFC implies increasing connectivity strength from the control group to the PTSD group to the PCS + PTSD group. Decreasing font size of DFC implies decreasing variance of connectivity from the control group to the PTSD group to the PCS + PTSD group. [Color figure can be viewed at wileyonlinelibrary.com]

the hypothesis that the imaging-based grouping, based on underlying neurobiology (as inferred from connectivity), will map better onto neurobehavior than conventional grouping, based on symptom severity scores.

Third, both our primary hypothesis and corollary hypotheses are based on an analysis framework which relies on statistical separation between the groups. However, statistical separation of between-group connectivities does not necessarily imply that they have predictive diagnostic ability (Craddock et al., 2009; Deshpande et al., 2010); that is, they may not be able to predict group membership at an individual level with reasonable accuracy. Consequently, those connectivities which are statistically significant as well as possess the discriminative power to classify subjects with high accuracy are more powerful. Several studies report that machine learning classifiers can be successfully used on fMRI data for diagnostic prediction, including, but not limited to, major depressive disorder (Deshpande et al., 2009), Parkinson's disease (Marquand et al., 2013), PTSD (Liu et al., 2015), dementia (Chen et al., 2011), autism (Deshpande et al., 2013), and prenatal cocaine exposure syndrome (Deshpande et al., 2010). However, to the best of our knowledge, there are no studies which have used connectivity markers in the classification of both PTSD and PCS subjects. For neuropsychiatric disorders such as PTSD and PCS, which are currently diagnosed solely through clinical observation, classification using neuroimaging signatures could be applied to obtain more accurate diagnoses in these highly comorbid conditions. We thus employed a machine learning technique which, in a data-driven way, recursively eliminates unimportant features from whole-brain connectivity data to identify those connectivities (i.e., top predictors) which predict the diagnostic membership of a novel subject with high accuracy. We specifically investigated whether there was an overlap between connectivity paths satisfying the overarching hypothesis and those identified as having high predictive ability. We hypothesize that these paths (i) will better predict the diagnostic membership of a novel subject than the available non-imaging measures and (ii) will predict the group membership of a novel subject with significantly better accuracy for the proposed imaging-based grouping (as elucidated in the previous paragraph), as compared to the conventional grouping.

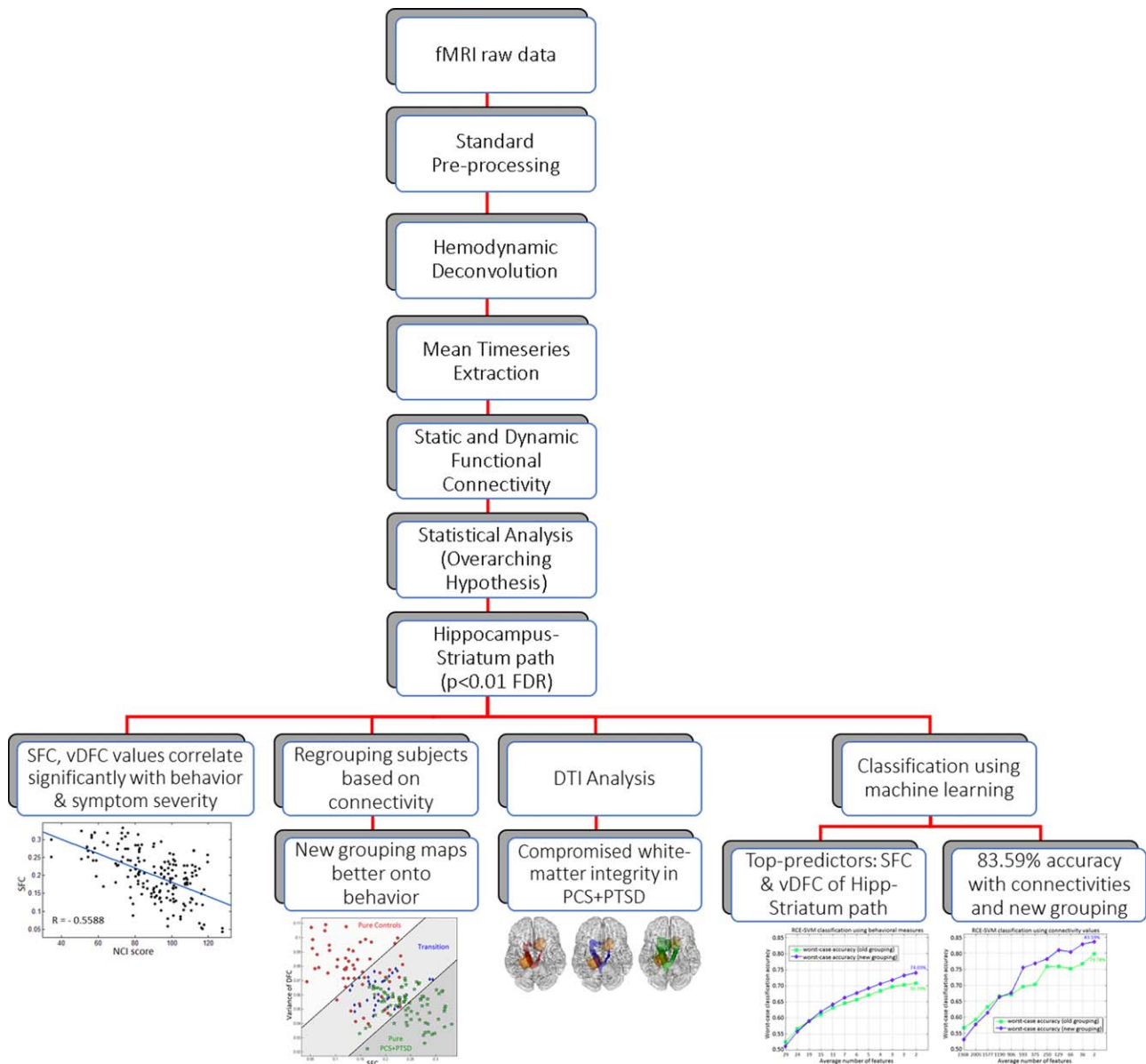


Figure 2.

Flowchart illustrating the analysis pipeline. [Color figure can be viewed at wileyonlinelibrary.com]

Figure 2 illustrates the complete analysis pipeline with a hierarchical flowchart (outcomes are discussed in the Results section).

METHODS

Recruitment

Active-duty soldiers between the ages of 18 and 50 years were recruited from Fort Rucker, AL, USA and Fort Benning, GA, USA to voluntarily participate in this study.

Recruitment utilized posters and flyers distributed and posted at local facilities including the TBI Clinic and Behavioral Health Clinics. Soldiers who were being treated for PTSD and/or PCS were referred to the study by clinicians if believed to meet eligibility criteria. Interested soldiers called the provided phone number whereupon they were prescreened and sent the consent form via post or email to be signed and returned. Upon receipt of the returned consent, potentially eligible participants were called to schedule their testing session at Auburn University's MRI Research Center.

The study was carried out in accordance with the latest version of the Declaration of Helsinki and the protocol and procedures were approved by Auburn University Institutional Review Board (IRB) and the Headquarters U.S. Army Medical Research and Materiel Command, IRB (HQ USAMRMC IRB).

Participants

Eighty-seven male, active-duty soldiers, 17 with PTSD, 42 with both PCS and PTSD (PCS + PTSD), and 28 controls (with all three groups matched in age, race and education), all having combat experience in Iraq (Operation Iraqi Freedom, OIF) and/or Afghanistan (Operation Enduring Freedom, OEF), were enrolled in the study.

Subjects were grouped based on PTSD symptom severity using the PTSD Checklist-5 (PCL5) score, clinician referral, postconcussive symptoms using the Neurobehavioral Symptom Inventory (NSI) score and medical history. (i) Subjects with no history of mTBI in the last 5 years, a total score ≥ 38 on the PCL5 were grouped as posttraumatic stress group (PTSD group). (ii) Subjects with a history of medically documented mTBI, postconcussive symptoms, and scores ≥ 38 on the PCL5 were grouped as the comorbid PCS + PTSD group. (iii) Subjects with a score < 38 on the PCL5, no DSM-IV-TR or DSM-V diagnosis of a psychotic disorder (e.g., schizophrenia), no mTBI within the last 5 years, and no history of a moderate-to-severe TBI were grouped as combat controls. All subjects were screened for MRI contraindications. All participants reported having deployed to a combat environment. The PCL5 scores were significantly different ($P = 3.64 \times 10^{-44}$) between the control group and the PTSD and PCS + PTSD groups combined. The reason for such a comparison was that PTSD is the common factor between the PTSD and the PCS + PTSD groups, and PCL5 score reflects only PTSD symptom severity. Similarly, postconcussive symptom (NSI) scores were significantly different ($P = 1.32 \times 10^{-29}$) between the PCS + PTSD group and the PTSD and control groups combined.

Measures

PTSD Checklist-5 (PCL5 (Dickstein et al., 2015)). The PCL5 is a 20 item self-report measure that assesses DSM-5 symptoms of PTSD. The PCL5 has a variety of purposes, including screening individuals for PTSD, making PTSD diagnoses, and monitoring symptom change during and after treatment. Items are rated using a 5-point Likert scale; 1 = "Not at all" to 5 = "Extremely." A total symptom severity score (range: 20–100) can be obtained by summing the scores for each of the 20 items with a cut score of 38 for a precursory diagnosis of PTSD (Weathers et al., 2015).

Neurobehavioral Symptom Inventory (NSI (Cicerone and Kalmar, 1995)). The NSI is a 22-item self-report questionnaire designed to assess postconcussive symptoms in individuals who have sustained a TBI (Cicerone and Kalmar,

1995). Participants rate the severity of each symptom within the past month on a 5-point Likert scale ranging from 0 (none) to 4 (very severe). A total symptom severity score (range: 0–88) can be obtained by summing the scores of the 22 items (Cicerone and Kalmar, 1995).

CNS-Vital Signs[®] (CNS-VS (Gualtieri and Johnson, 2006)). CNS-VS is a computerized neurocognitive assessment battery (Gualtieri and Johnson, 2006). This study used five CNS-VS sub-tests (verbal memory, symbol digit coding, Stroop test, continuous performance test, and shifting attention test). The following CNS-VS domain scores were calculated: verbal memory (VM), complex attention (CA), reaction time (RT), processing speed (PS), cognitive flexibility (CF), and executive functioning (EF). Domain scores have a mean of 100 and standard deviation of 15. Domain scores were averaged to form a single score or neurocognitive composite index (NCI) (Gualtieri and Johnson, 2006).

Procedures

When participants arrived at Auburn University's MRI Research Center for their scheduled testing appointment, they were rescreened for eligibility, thoroughly screened for MRI contraindications, and re-consented to ensure full comprehension of the study's procedures, benefits, and their rights.

fMRI

Participants were scanned in a 3 T MAGNETOM Verio scanner (Siemens Healthcare, Erlangen, Germany) using T2*-weighted multiband echo planar imaging (EPI) sequence in resting state (the participants were asked to keep their eyes open and fixated on a white cross displayed with dark background on the screen using an Avotec projection system and not think of anything specific), with TR = 600 ms, TE = 30 ms, FA = 55°, multiband factor = 2, slice gap = 1 mm, anterior to posterior phase encoding direction, voxel size = $3 \times 3 \times 4 \text{ mm}^3$, and 1000 volumes. For the anatomical scan (MPRAGE), we used TR = 1900 ms, TE = 2.5 ms, FA = 9°, and voxel size = $1 \times 1 \times 1 \text{ mm}^3$. Brain coverage was limited to the cerebral cortex, subcortical structures, midbrain, and pons (the cerebellum was excluded). For each subject, two identical but separate scans were performed, thus providing us 174 sessions of resting-state fMRI data for the 87 subjects. The two sessions of data were processed independently. Mathematically, this boosted the statistical power for our analysis beyond that which would have been available from single scans from the 87 subjects, because statistics were performed with connectivity values which were double in number (per connectivity path) compared to the number of subjects in the groups.

DTI

Participants were scanned in the same 3T MAGNETOM Verio scanner (Siemens Healthcare, Erlangen, Germany)

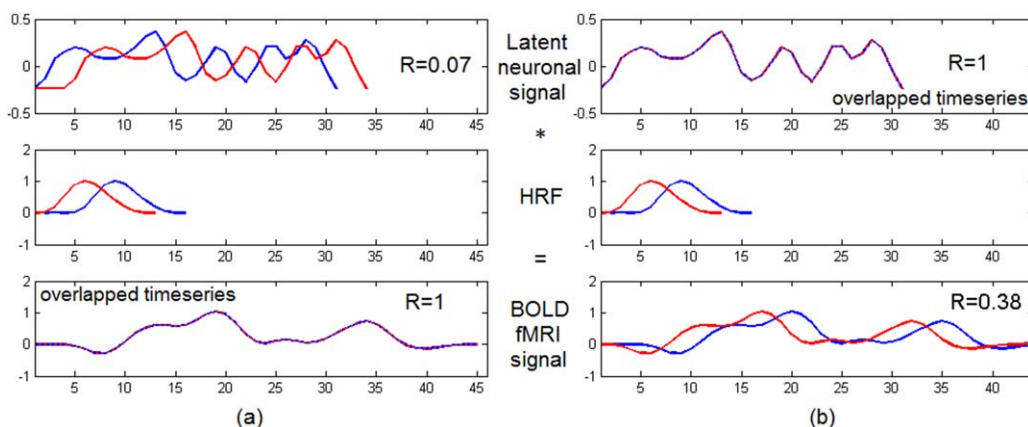


Figure 3.

Illustrative example showing the importance of performing hemodynamic deconvolution. Inter-subject and spatial variability of the HRF could potentially give rise to a scenario wherein (a) the BOLD fMRI time series are synchronized while the underlying neural variables are not, thus giving high correlation while

the true correlation is low and (b) the underlying neuronal variables are synchronized while the BOLD fMRI time series are not, thus giving low correlation while the true correlation is high. In both examples, a lag of 2 TRs was used. [Color figure can be viewed at wileyonlinelibrary.com]

using diffusion weighted multiband EPI sequence, with TR = 3600 ms, TE = 95 ms, FA = 90°, voxel size = $1.8 \times 1.8 \times 3 \text{ mm}^3$, $b = 0$, 1000, 25 slices acquired parallel to the AC-PC plane, matrix = 128×128 , field of view (FOV) = 230 mm and number of diffusion directions = 20. Participants with partial brain coverage (defined as coverage that did not inferiorly cover $z = -12$; $n = 3$) or excessive motion (via visual inspection for artifact; $n = 2$) were excluded from analyses.

Data Analysis

Non-imaging measures

Mean, median, standard deviation, and range were calculated for the self-report and neurocognitive measures. Ordinal data were analyzed using Kendall's Tau B (τ_b) test. Separate one-way analyses of variance (one-way ANOVA) with Dunnett's C correction for multiple comparisons were run when comparing continuous variables between groups.

fMRI data preprocessing

Standard preprocessing of resting-state fMRI data was performed including realignment, normalization to MNI space, elimination of temporal linear trends, and regressing out nuisance covariates (six head motion parameters, white matter (WM) signal, and cerebrospinal fluid (CSF) signal). The data were temporally band-pass filtered (0.01–0.1 Hz). Maximum allowed head motion was half the voxel size, that is, 1.5 mm. The groups did not exhibit any significant difference in subject head motion ($P > 0.05$). An additional preprocessing pipeline was executed which

was identical to the one described above, but with the added step of global mean signal regression (GSR) to examine its effects given conflicting reports about its utility (Power et al., 2015; Saad et al., 2012). Preprocessing was performed using Data Processing Assistant for Resting-State fMRI (DPARSF v1.7) (Chao-Gan and Yu-Feng, 2010), which is based on Statistical Parametric Mapping (SPM8) (Friston et al., 2007) and Resting-State fMRI Data Analysis Toolkit (Song et al., 2011).

The time series data were then deconvolved to obtain latent neuronal variables using a recently reported method (Wu et al., 2013). The deconvolution is blind because there is no external input in case of resting-state fMRI data and consequently, both the hemodynamic response function (HRF) and the underlying neuronal latent variables must be simultaneously estimated from observed fMRI data, making this an ill-posed estimation problem. Briefly, the approach relies on modeling resting-state fMRI data as event-related data with randomly occurring events using point processes (Power et al., 2015; Saad et al., 2012) and then estimating voxel-specific HRFs using Weiner deconvolution. The deconvolution was performed because inter-subject and spatial variability of the HRF (Handwerker et al., 2004) could potentially give rise to a scenario wherein fMRI time series are synchronized while the underlying neuronal variables are not and vice versa (Please refer to Fig. 3 for an illustration). Given the high dimensionality of whole-brain data, mean deconvolved (as well as non-deconvolved) fMRI time series were obtained from 125 functionally homogeneous regions identified using spectral clustering (known as the cc200 template (Craddock et al., 2012)). Further connectivity analysis (performed on the Matlab® platform) utilized these 125 time series from each

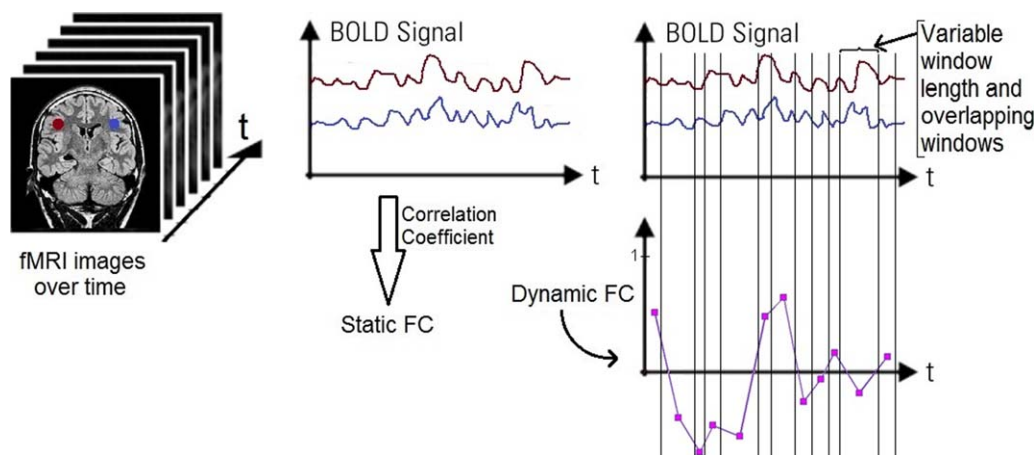


Figure 4.

Illustration of the evaluation of static and dynamic functional connectivities. [Color figure can be viewed at wileyonlinelibrary.com]

subject for each of the four preprocessing pipelines (with and without GSR, with and without deconvolution).

Connectivity analysis

Most studies investigate functional connectivity (FC), a metric of synchronicity of activity in disparate brain regions, assuming connectivity to be temporally stationary. Dynamic fluctuations of connectivity are not captured when using static connectivity. It has been shown that dynamic changes in FC are relevant to neuropathology (Sakoğlu et al., 2010) as well as behavioral performance in different domains (alertness, cognition, emotion, and personality traits) in healthy individuals (Jia et al., 2014). For a comprehensive overview of DFC of resting-state fMRI, see Hutchison et al. (2013).

Previous studies have not enumerated the utility of dynamic information in connectivity fluctuations, over and above the information obtained from conventional static connectivity, in clinical applications. In this study, we have used static as well as dynamic functional connectivity measures. SFC and DFC values were obtained between all pairs of 125 brain regions. For SFC, Pearson's correlation calculated from the entire time series was used. For DFC, we employed sliding windowed Pearson's correlation with variable window length, which was determined adaptively by assessing time series stationarity through the augmented Dickey-Fuller test (ADF test), as in our recent study (Jia et al., 2014). This procedure searches for the optimal window length within a specified range using the stationarity of the time series as the criteria for optimization. We have used a liberal range of 20–140 data points. The justification for using this range for resting-state fMRI data is provided in Jia et al. (2014). Figure 4 illustrates the concept underlying SFC and DFC.

SFC and DFC were obtained between all pairs of 125 regions, thus obtaining a 125×125 SFC matrix and a $1000 \times 125 \times 125$ DFC matrix per subject (1000 being the number of time points). Variance of DFC (vDFC) values over time was evaluated to obtain a 125×125 DFC variance matrix per subject. Significant group differences in SFC (and vDFC) were obtained individually for each connectivity path with whole-brain connectivity data ($P < 0.01$ FDR corrected). A Multivariate N -way ANOVA (MANCOVAN) statistical test was used. Significant group differences were controlled for age, gender, race, education, and head motion (using mean framewise displacement obtained across all brain voxels for each subject as defined by Power et al. (2012)). As mentioned before, we investigated the existence of significant connectivity paths which had higher SFC but lower vDFC in disease compared to controls, with connectivities being more extreme in PCS + PTSD compared to PTSD. For the paths which fit our hypothesis, their connectivity values were also correlated with neurocognitive scores (NCI and subtests) and symptom severity in PTSD (PCL5 score) and in PCS (NSI score).

Regrouping Subjects Based on Connectivities

Connectivities from significant paths which fit our overarching hypothesis were used to regroup the subjects, yielding two distinct groups (pure control and pure PCS + PTSD) and an intermediate group. In line with our hypothesis, we postulated the following: (i) new diagnostic groups created based on the separation of significant connectivity values would better map onto behavior, as compared to commonly used symptom severity scores (PCL5 and NSI) and (ii) PTSD and PCS are spectrum disorders wherein individuals are likely to lie on a continuum

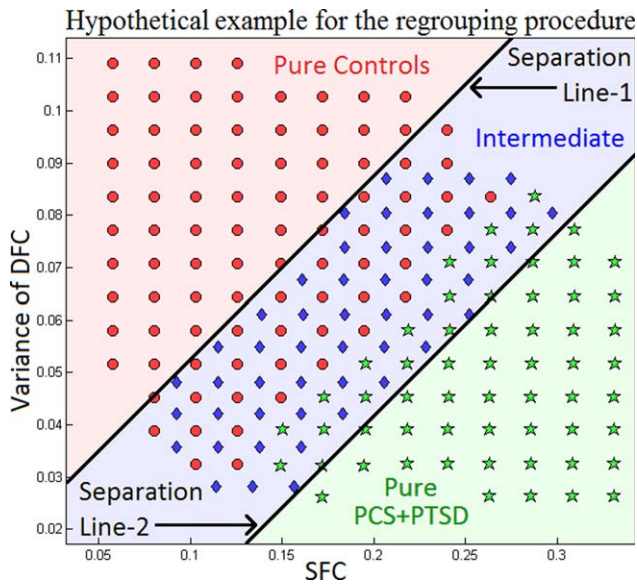


Figure 5.

Hypothetical example using simulated data, illustrating the proposed regrouping procedure. [Color figure can be viewed at wileyonlinelibrary.com]

ranging from healthy controls to comorbid PCS + PTSD, rather than form distinct clusters; hence forming pure healthy and comorbid groups (with diagnostic confidence being very high in the pure groups) and an intermediate group (low diagnostic confidence) may be clinically useful. All the control subjects also had combat experience, and hence a percentage of them might possess borderline neural and behavioral alterations associated with PTSD and PCS, or both. Furthermore, subjects that fall within the PCS + PTSD group might exhibit anywhere from mild to moderate neurocognitive decrements, making it indistinguishable from the PTSD group. With all these factors in place, it may be desirable to develop objective clinical classifications using imaging rather than symptom reporting. Hence, we propose a practical approach wherein the subjects are grouped into two extreme pure groups and an intermediate group based on imaging measures such as SFC and vDFC. This paradigm is still compatible with the fact that the groups may have significantly different mean values, but a large standard deviation so that they overlap. Therefore, we devised a method wherein the subjects are regrouped into the following three groups: (1) pure control, (2) intermediate group, and (3) pure PCS + PTSD.

A hypothetical example of regrouping is shown in Figure 5. Plotting the connectivity values in the two-dimensional space of SFC (x -axis) and vDFC (y -axis), we can expect to find connectivities of control subjects and PCS + PTSD subjects at opposite ends. Furthermore, we can expect to see an intermediate region between these two extremes where there would be a combination of subjects that are borderline healthy, those with PTSD

symptoms, and comorbid postconcussive and PTSD symptoms. In contrast, pure controls and pure PCS + PTSD groups can be defined as those which have no nongroup members included in them. In Figure 5, this corresponds to the subjects who fall outside the intermediate group on either side. In a mathematical sense, the objectives of the regrouping procedure, like in a typical optimization problem, were to minimize the heterogeneity of the two “pure” groups, while simultaneously maximizing the number of subjects in the two “pure” groups.

Due to computational feasibility, a grid search was used to achieve subject grouping (more on this aspect in the *results* section). Assuming that N paths satisfy our overarching hypothesis (i.e., significantly stronger SFC with lower vDFC in disease compared to healthy), the connectivity values of all the subjects were embedded in the $2N$ -dimensional connectivity space (each path is associated with an SFC and a vDFC value, hence $2N$). To explain this procedure intuitively, we consider the two-dimensional example shown in Figure 5. Subjects in the control group were tagged as “1,” the PTSD group as “2,” and the PCS + PTSD group as “3.” We used the variance of tagged group values as a measure of heterogeneity. For example, the pure control group would ideally have only control subjects (tag = 1), hence the variance of the tags for the group would be zero. The intermediate group would have a mixture of all three groups (1, 2, and 3); hence, it would have higher nonzero variance.

Two separation hyperplanes of dimension $2N-1$ (lines in the two-dimensional example being considered) were arbitrarily initiated. In regard to our specific 2D example, the equation of a line in two dimensions is given by $y = mx + c$, where y and x are the variables on the y -axis and x -axis, respectively, m is the slope and c is the intercept. For a given range of x and y values, the position of the line is determined by its angle (m) and shift (c). Within the given range of SFC ($=x$) and vDFC ($=y$) values, we generated all possible pairs of lines using all possible values of angle, shift, and separation between the lines. The feature space between the two separation lines was identified as the intermediate group, and the feature space outside them as the two pure groups. We evaluated the heterogeneity of each of the three groups (using variance as explained earlier) for all possible separation lines generated. We then searched for that pair of nearest separation hyperplanes (or lines in our 2D example), which resulted in highest variance in the intermediate group and zero variance in the two pure groups (to maintain “purity” of the groups). These two were chosen as the final separation hyperplanes (or lines in 2D case), which were used to create the new groups based on connectivity: pure controls, intermediate, and pure PCS + PTSD.

If the new imaging-based grouping maps better onto behavior than the conventional grouping (which was based on symptom screening and clinician referral), it can be postulated that the imaging-based grouping can predict

PTSD and PCS sequelae better than conventional methods. To test this, we statistically compared the subjects on neurocognitive performance measures grouped using both the conventional grouping as well as the new imaging-based grouping. Corresponding mean and standard deviation (SD) values of individual groups were obtained along with the statistical significance of the differences between the groups.

It is notable that the regrouping technique proposed by us can be applied to any arbitrary set of features. If an application identifies multiple measures as relevant (connectivity, behaviors, etc.), then the same regrouping technique can be applied on those multiple measures (say R_N different measures, some connectivities, and some behaviors). From this, one could obtain an R_N -dimensional neurobiologically informed feature space which has multiple decision boundaries, using which a diagnostic decision similar to ours can be made.

Classification Using Support Vector Machine

Statistical separation between neural signatures (e.g., *t*-test) does not necessarily guarantee generalizability or predictive capacity of those signatures for diagnosis. A statistically significant connectivity path need not have high predictive ability and vice versa (Craddock et al., 2009; Deshpande et al., 2010). Consequently, those connectivity paths which are both statistically significant (according to our hypothesis) and top classifiers (high predictive ability) assume more power and, therefore, relevance. Hence, we have used machine learning methods to identify those connectivity paths (or features) which can accurately classify individuals between PTSD, PCS + PTSD, and controls. A Recursive Cluster Elimination based Support Vector Machine (RCE-SVM) classifier (Deshpande et al., 2010) was used to classify the subjects based on whole-brain SFC and vDFC values. Notably, results from prior analysis were not used to bias the machine learning method.

First, significant group differences were found for all the three comparisons (control vs PTSD, control vs PCS + PTSD, and PTSD vs PCS + PTSD), using a threshold of $P < 0.05$ (controlled for age, race, education, and head motion), for both SFC and vDFC. We used an uncorrected $P < 0.05$ threshold as we wanted to be liberal about which features serve as input to the classifier, and let the classifier choose the most predictive features. Next, we found overlapping paths between the three comparisons. The resulting SFC and vDFC features were combined to provide the input features to the classifier. This initial filtering enhances the quality of classification (Craddock et al., 2009), and ensures that non-discriminatory features are not fed into the classifier.

Our choice of support vector machine (SVM) (Vapnik, 1995) for classification was motivated by its wide acceptance and applicability for classification in several fields, including neuroimaging (Wang, 2005). Previous studies have shown that using discriminatory features enhances

classification performance of SVMs (Craddock et al., 2009; Deshpande et al., 2010). Therefore, we employed recursive cluster elimination (RCE), a wrapper method which iteratively eliminates features to minimize the prediction error, where feature selection and classification steps are embedded together. The RCE-SVM classification technique involves the clustering step, the SVM scoring step and the RCE step. The features that were initially input into the classifier were divided into training and testing data sets. The classifier was trained using the training data set, while the testing data set was totally kept blind to the classifier. Once training was complete, the testing data were input into the classifier and classification accuracy was obtained. This method ensures generalizability of the results.

In the clustering step, *k*-means algorithm was used to cluster the training data into "*n*" clusters. The number of clusters was initially set to the number of features, and then was iteratively decreased by one until no empty clusters were left. The "*n*" obtained by this iteration served as the initial "*n*" for the RCE-SVM loop. In the SVM-scoring step, each cluster was scored based on its capacity to differentiate between the two groups by using linear SVM. To assess the performance of the clusters, the training data were randomly partitioned into 6 non-overlapping subsets of equal sizes (six folds). Using 5 subsets, the SVM was trained and performance (accuracy) was computed using the remaining subset. All possible partitions were generated by repeating the clustering and cross-validation procedures 100 times. For each of these 100 repetitions, the classification accuracy was obtained using the testing data.

Using the outcome of 100 repetitions and six folds for each repetition, the average value of the accuracies was assigned as the cluster's score. The bottom 20% of low scoring clusters was eliminated in the RCE step. Remaining features were merged and the value of "*n*" was reduced by 20%. This ensures that only certain top classifying features qualify for the next iteration. The clustering step, the SVM-scoring step and the RCE step were repeated again iteratively. After each iteration, performance of the classifier was obtained using the reduced number of features compared to the earlier iterations. Once the number of clusters reached two, the procedure was stopped. Figure 6 illustrates the RCE-SVM procedure using a flow-chart. Complete separation of testing and training data sets in this procedure eliminates bias in the computation of classification accuracy (Kriegeskorte et al., 2009). Further, the features in the final two clusters are those with highest discriminative ability and hence carry predictive value for diagnosis. Complete details about the RCE-SVM algorithm can be obtained from previous reports (Deshpande et al., 2010; Yousef et al., 2007).

In this work, we made the following parameter choices in classification. Eighty percent of the subjects were chosen in the training set, while 20% were reserved as the testing set. With *k*-means clustering of features, we started with 40 clusters in the first RCE step, and bottom 20% clusters

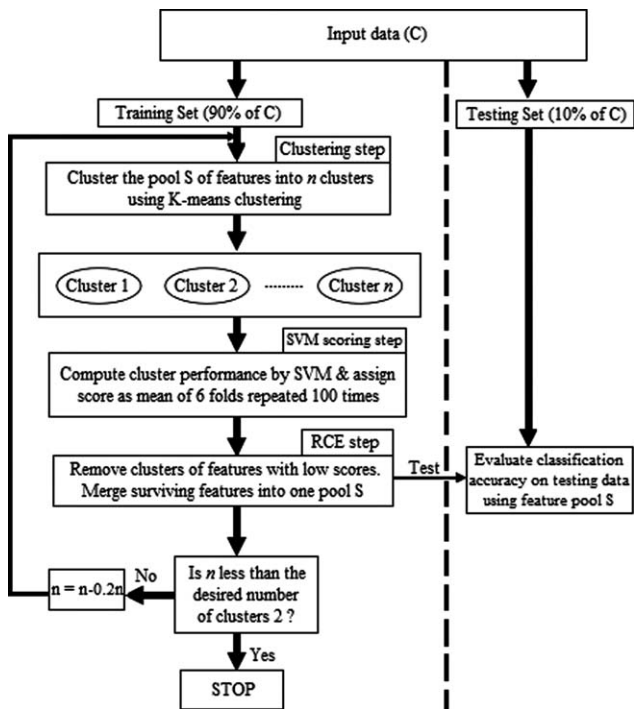


Figure 6.

Flowchart illustrating the RCE-SVM classification procedure.

(based on performance) were eliminated in each subsequent RCE step. The final RCE step involved two clusters, which contained the top-predictive features. Sixfold cross-validation was performed over 100 random iterations, giving a total of 600 iterations over the execution.

Classification was performed separately for the three comparisons in the conventional as well as the proposed imaging-based groupings (i.e., control vs PTSD, control vs PCS + PTSD, and PTSD vs PCS + PTSD in the conventional grouping; and pure control vs intermediate, pure control vs pure PCS + PTSD, and intermediate vs pure PCS + PTSD in the imaging-based grouping). For both groupings, outcome measures such as accuracy and final set of discriminative features were obtained by intersecting the results obtained by each of three individual classifiers. To be conservative, we obtained the worst-case classification accuracy by considering the minimum accuracy value obtained from the test data set among all 600 iterations (100 repetitions \times 6 folds). The statistical significance of accuracies was obtained by estimating the P values using a binomial null distribution $B(\eta, \rho)$, ρ being the probability of accurate classification and η being the number of participants, as in previous studies (Pereira et al., 2009). Only those accuracies whose P values were <0.05 (Bonferroni corrected) were considered as statistically significant for further inference.

We repeated the above procedure and performed classification independently using 32 nonimaging measures (NIMs)

as input features instead of SFC and vDFC connectivities. The 32 measures were (i) behavioral measures: all CNS-VS measures including the NCI score; (ii) psychological health measures: Perceived Stress Scale, Pittsburgh Sleep Quality Index, Epworth Sleepiness Scale, Zung Anxiety Scale, and Zung Depression Scale; (iii) exposure/injury descriptives: Combat Exposure Scale, lifetime concussions, and Life Events Checklist. Worst-case classification accuracies and top classifying features were obtained, as before, and these results were compared with the results obtained using connectivity values.

DTI Data Processing

One of the ways to find out whether increased severity in the PCS + PTSD group is due to an mTBI (structural damage) or the compounding effect of two disorders (PTSD and PCS) combined, is to look at structural changes using DTI. Probabilistic diffusion tractography was carried out using FSL's Diffusion Toolbox (FDT) (Behrens et al., 2003; Johansen-Berg et al., 2005). Regions of interest (ROIs) which were connected by functional paths satisfying our overarching hypothesis (i.e., significantly stronger SFC and lower vDFC in disease compared to healthy) were identified as seed and target regions. Briefly, a probability density function was created at each voxel on the principal fiber direction. White-matter connectivity probabilities were estimated between the seed and target ROIs by repeatedly sampling connected pathways through the probability distribution function. Samples were drawn from the connectivity distribution, and the proportion of those samples that passed through both ROIs was defined as the probability of the connection between the seed and the target. For each analysis, we thresholded and binarized individual subject's results to include only those voxels with a connection probability $>10\%$. These images were then combined to create group maps, which would help us find out whether there is any structural basis for increased severity in PCS + PTSD subjects. White matter tracts were subsequently identified using the JHU ICBM DTI 81 White Matter Label Atlas (http://www.loni.usc.edu/ICBM/Downloads/Downloads_DTI-81.shtml).

RESULTS

The PCS + PTSD group exhibited significantly higher PTSD and PCS symptom severities and performed significantly worse in neurocognitive tests compared to PTSD and controls. The PTSD group exhibited significantly higher PTSD symptom severity and performed significantly worse in neurocognitive tests compared to controls. Supporting Information SI-1 provides detailed findings on subject demographics, symptom severity, and neurocognitive functioning.

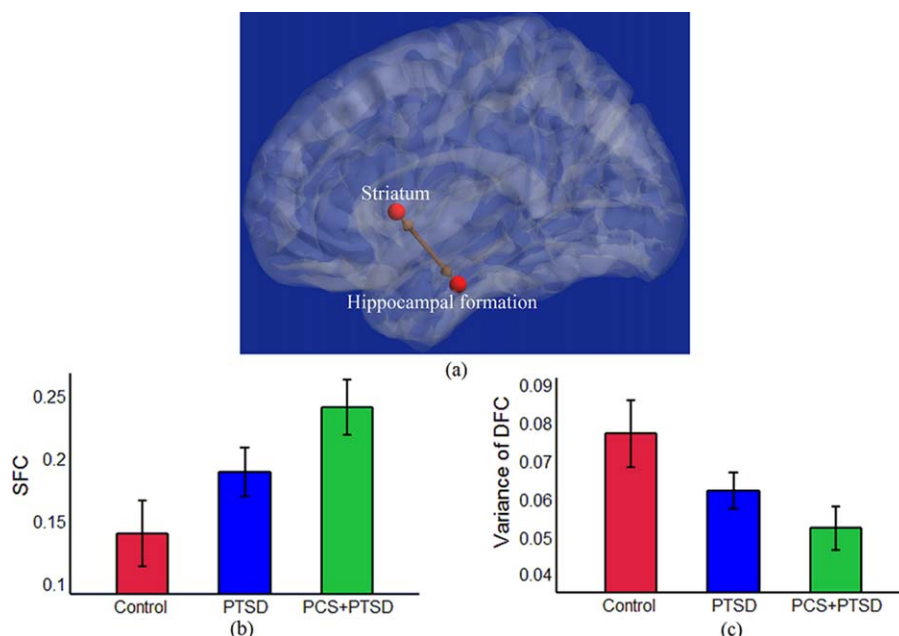


Figure 7.

(a) Sagittal view of brain showing the hippocampus-striatum path. (b) Variance of dynamicFC and (c) staticFC values for the three groups. All comparisons in (b) and (c) were statistically significant ($p < 0.05$, FDR corrected) in accordance with our hypothesis. [Color figure can be viewed at wileyonlinelibrary.com]

RS-fMRI Functional Connectivity

In accordance with our hypothesis, the connectivity path between left striatum and right hippocampal formation (Fig. 7a) showed higher connectivity strength and lower connectivity variance in the PCS + PTSD and the PTSD groups compared to the control group. This path was the only path in the whole-brain connectivity data to conform to our hypothesis. The striatal region mainly contained caudate head (MNI centroid: $-11.3, 12.2, 3.5$). The hippocampal formation contained entorhinal and perirhinal cortices, and anterior hippocampus and parahippocampal gyrus (MNI centroid: $19.4, -12.4, -25.5$).

Figure 7b,c shows the hippocampus-striatum path-weights which were significantly different between the three groups, with decreasing vDFC and increasing SFC as one moves from control to PTSD to PCS + PTSD.

This result was obtained using a threshold of $P < 0.01$ (FDR corrected) for testing statistical significance. Testing our hypothesis with a more liberal threshold of $P < 0.05$ (FDR corrected), we found two connectivity paths to be significant. In addition to the left striatum-right hippocampal formation connectivity path mentioned before, the connectivity between left striatum and left hippocampal formation was the additional connectivity path to be found significant.

It is notable that we have used deconvolved data in our analysis. Deconvolution (Wu et al., 2013) minimizes HRF

variability (Handwerker et al., 2004) in the BOLD fMRI signal and provides the estimated latent neuronal time series. Results obtained using non-deconvolved data are corrupted by HRF variability (Handwerker et al., 2004). However, we tested our hypothesis on preprocessed fMRI data without hemodynamic deconvolution as done in conventional resting-state connectivity studies. We notably did not find any significant connectivity path which fit our hypothesis, underscoring the importance of removing HRF variability from fMRI time series even for functional connectivity analyses.

Though lower connectivity variance (vDFC) has been associated with ill-health, both lower and higher connectivity strengths (SFC) have been previously associated with ill-health. Specifically, previous literature has shown both stronger and weaker connectivities (SFC) in PTSD compared to controls (Cisler et al., 2014; Hayes et al., 2012; Simmons and Matthews, 2012). Hence, we also investigated whether paths which had significantly lower connectivity strength (SFC) and lower connectivity variance (vDFC) existed in the PTSD and PCS + PTSD groups compared to the control group. However, none of the connectivity paths could fit this hypothesis using either deconvolved data or conventional non-deconvolved data.

Our results were obtained with data which had undergone global mean signal regression (GSR) while preprocessing the data. There has been debate in the scientific community about whether or not global mean signal

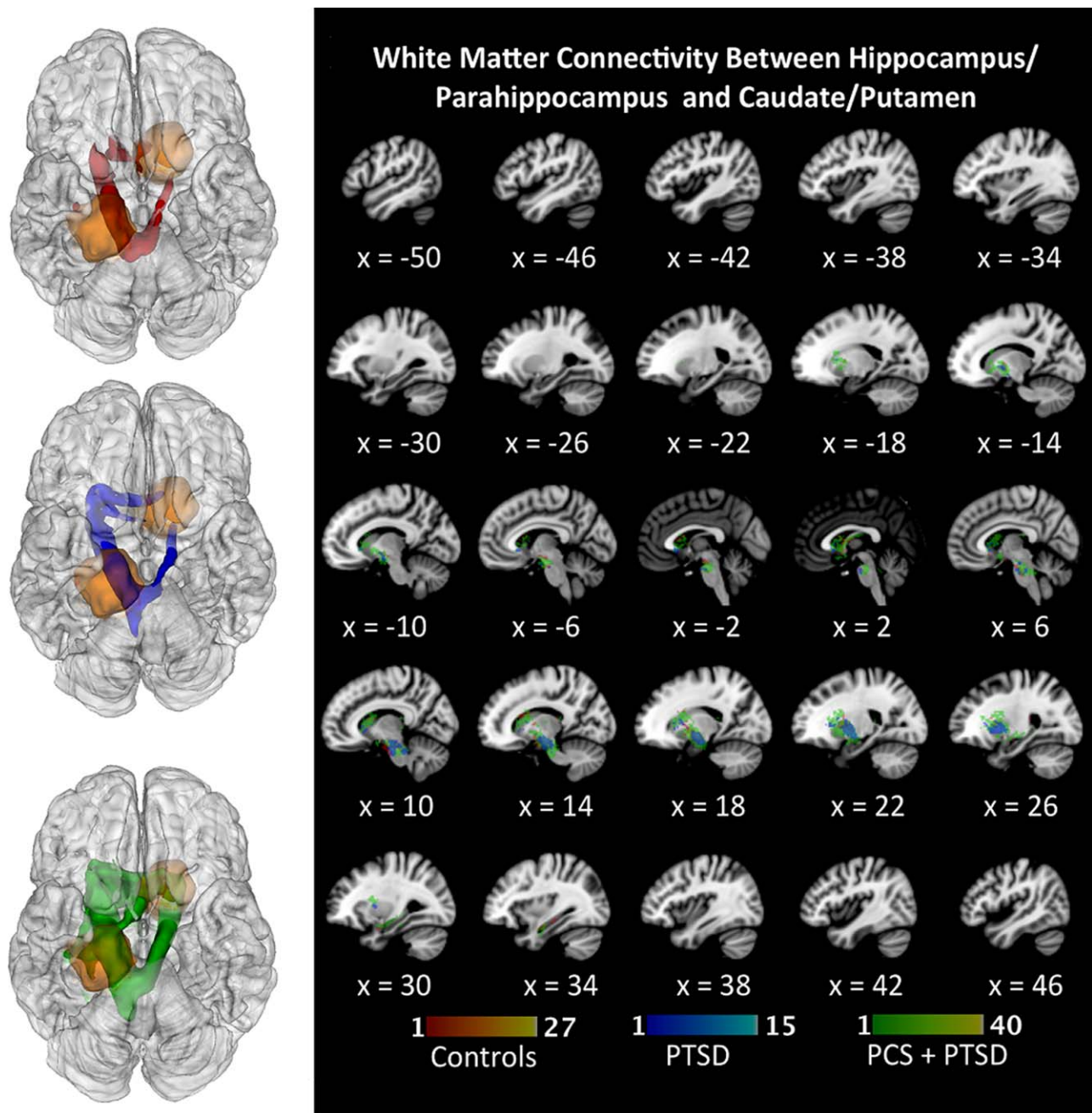


Figure 8.

White-matter connectivity between striatum and hippocampal-formation: fibers connecting the two ROIs were more diffuse in the PCS + PTSD group compared to the PTSD or control groups. [Color figure can be viewed at wileyonlinelibrary.com]

should be removed (Power et al., 2015; Saad et al., 2012). In fact, Power et al. note that the objections raised to GSR are mainly based on results from low-dimensional simulations (Saad et al., 2012), and that further work that determines the applicability of these arguments to empirical data would usefully inform decisions about using GSR as

part of denoising strategies. To account for this, we also performed the same analysis on preprocessed data without GSR. We replicated the results showing that the left striatum–right hippocampal formation connectivity path was the only significant path in accordance with our hypothesis, but with reduced statistical significance ($P < 0.05$, FDR

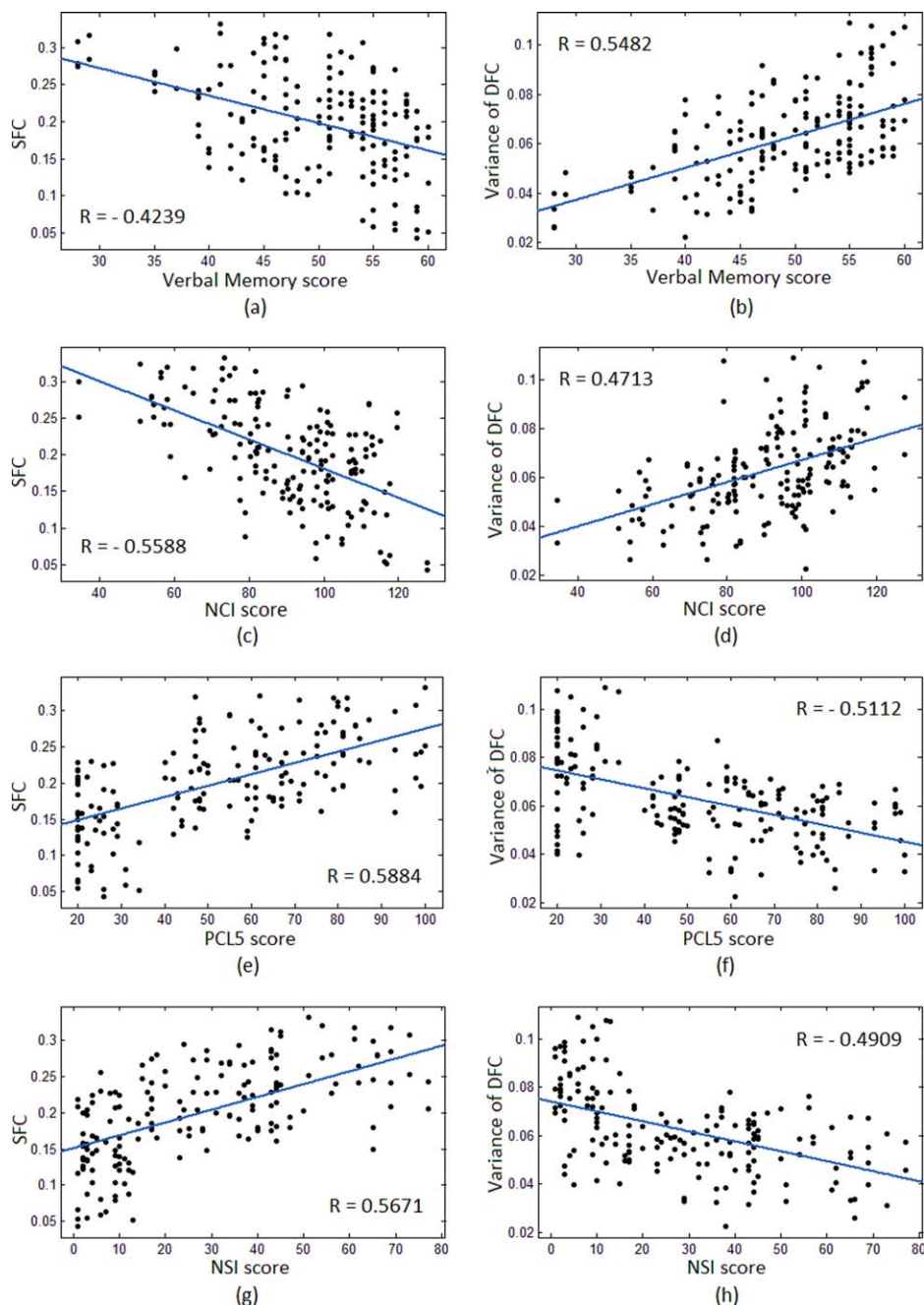


Figure 9.

Association between SFC values and (a) verbal memory, (c) neurocognitive composite index (NCI), (e) PTSD symptom severity (PCL5) and (g) PCS symptom severity (NSI); association between variance of DFC values and (b) verbal memory, (d) NCI, (f) PCL5, and (h) NSI. All correlations were statistically significant (numerical statistics are provided in Supporting Information, SI-2.2). [Color figure can be viewed at wileyonlinelibrary.com]

corrected). Importantly, the hippocampus-striatum connectivity remained significant despite concerns about confounds that global mean signal (or its regression) may

introduce. Additionally, as in the former case, without GSR, none of the connectivity paths fit our hypothesis with conventional non-deconvolved data. Also, no

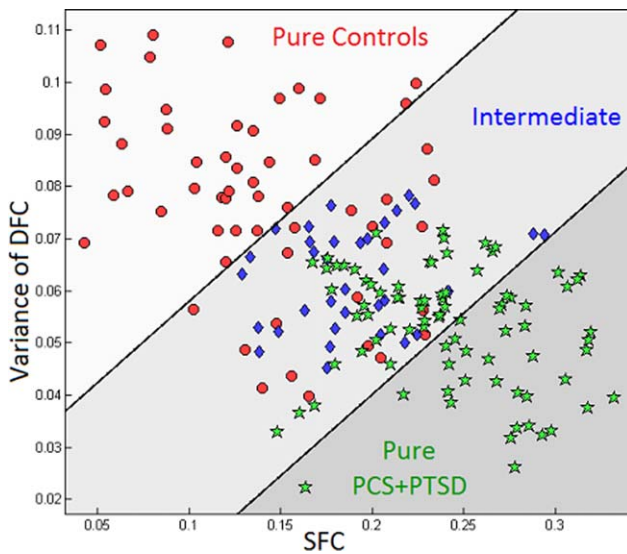


Figure 10.

SFC and vDFC values of hippocampus-striatum path with proposed imaging-based regrouping. The three new groups are shown in gray bands. Based on old grouping: red circles are subjects from the control group, blue diamonds from the PTSD group, and green stars from the PCS + PTSD group. [Color figure can be viewed at wileyonlinelibrary.com]

connectivity paths were found when we searched for lower SFC and lower vDFC values in the PTSD and PCS + PTSD groups compared to controls, with either deconvolved data or conventional non-deconvolved data. In summary, while our result was obtained with deconvolved data, preprocessed with global mean signal regression (GSR), no paths were obtained in data without deconvolution, and excluding GSR did not change the current result. All these results unequivocally support that the left striatum–right hippocampus connectivity path fits our hypothesis irrespective of several preprocessing choices debated lately in the scientific community.

DTI Results

DTI results revealed greater diversity in structural connectivity between hippocampus and striatum, with white-matter fibers connecting these two ROIs being more diffuse (implying less integrity) in the PCS + PTSD group compared to the PTSD and control groups (Fig. 8). The profile was similar in the control and PTSD groups. From this, we inferred that compromised structural integrity, greater symptom severity, and neurobehavioral impairments in individuals with PCS + PTSD could be associated with their documented mTBI, and that they are less likely just an extreme subset of PTSD. Please refer to Supporting Information SI-2.1 for details on affected white-matter pathways.

Association between fMRI Connectivities and Non-imaging Measures

Connectivity values of the hippocampus-striatum path had significant associations (Fig. 9) with neurocognitive functioning (neurocognitive composite index [NCI] and subtests), PTSD symptoms (PCL5 score), and PCS severity (NSI score), thus highlighting their relevance to the underlying neuropathology. It was notable that correlations followed the expected trend: an increase in severity and a decrease in behavioral performance corresponded with higher SFC and lower vDFC. Figure 9 also shows the correlation values. Please refer to Supporting Information SI-2.2 for detailed information.

Regrouping Subjects Based on Connectivities

In accordance with our hypothesis, we postulated the following: (i) new diagnostic groups created based on the separation of hippocampus-striatum path-weights would map better onto behavior (i.e., neurocognitive performance) as compared to the original groups based on conventional diagnostic grouping and (ii) PTSD and PCS are spectrum disorders wherein symptom severity is likely to lie on a continuum, rather than forming distinct clusters; and hence forming of high diagnostic confidence groups (pure healthy and comorbid groups) and a low diagnostic confidence group (called intermediate group) has the potential to be clinically useful (see Fig. 5 for a hypothetical example). Hence, we devised a practical approach to regroup the subjects into new diagnostic groups with the objective of maximizing heterogeneity of the intermediate

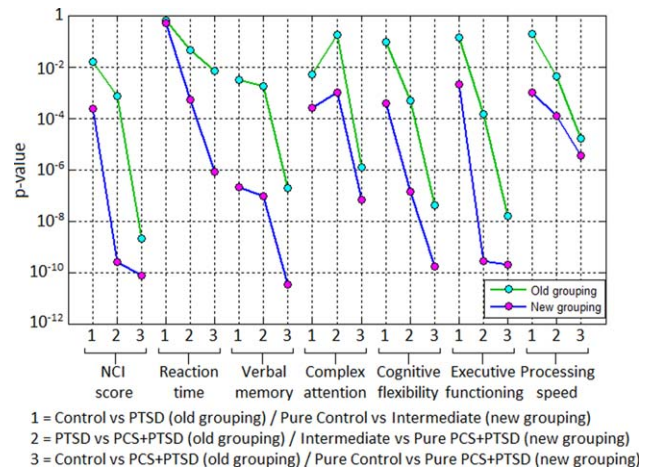


Figure 11.

Statistical significance (P value) of behavioral measures with both conventional old grouping and proposed imaging-based grouping. A logarithmic scale is used for the y -axis of P values. We observed that all behavioral measures consistently exhibited smaller P value for all comparisons with the imaging-based grouping. [Color figure can be viewed at wileyonlinelibrary.com]

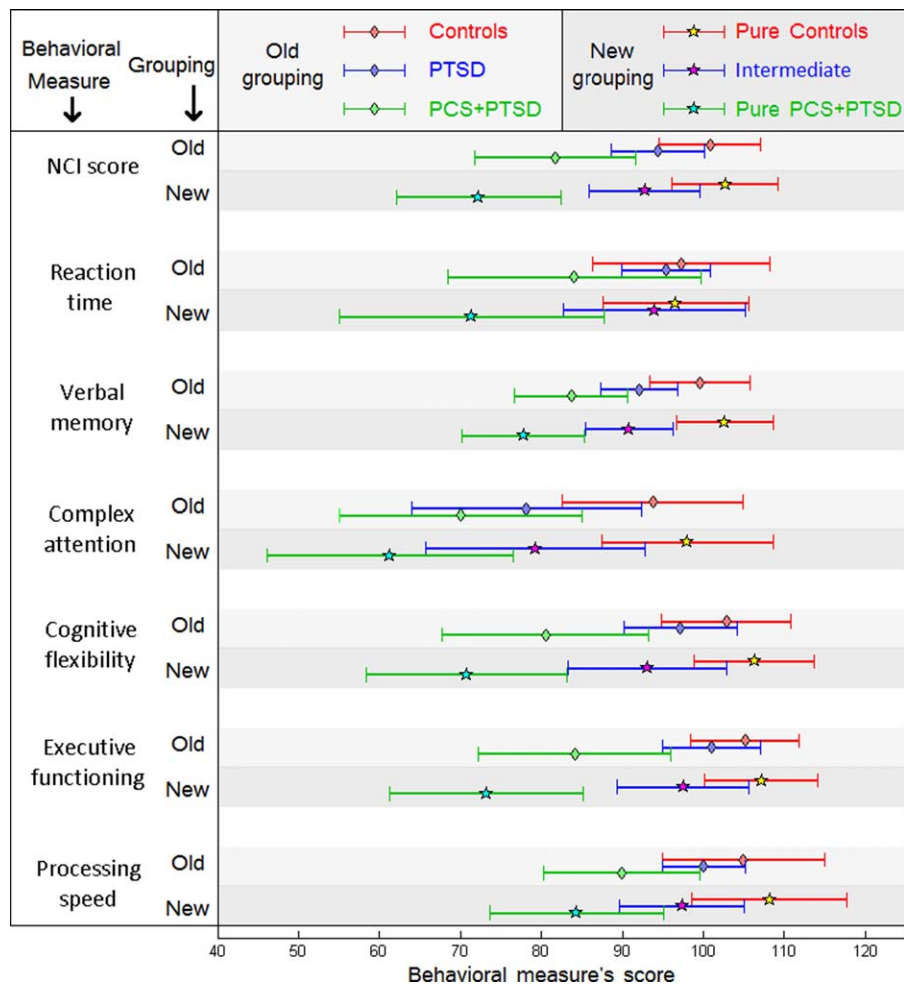


Figure 12.

Depiction of statistical separation of neurocognitive scores for both old (conventional) grouping and new (imaging-based) grouping using population mean and SD values. New grouping provides better separation between the groups with larger difference in means and lower within-group variances. Also, the

group while simultaneously minimizing the heterogeneity of the pure control and pure PCS + PTSD groups. This novel approach deviates from traditional ways of grouping subjects based on symptom reporting and clinical judgment.

We regrouped the subjects using the SFC and vDFC values of the hippocampus-striatum path (see Fig. 10, and 3D visualization in Supporting Information, Fig. S1). Clearly, there is a relatively narrow intermediate band where all the PTSD subjects are sandwiched along with borderline controls and mild PCS + PTSD subjects. Outside this band, we find regions of the two pure groups. We observe a continuum with the pure control group merging with the narrow intermediate band, which later leads to the space of the pure PCS + PTSD group.

pure control group performed better than the original control group, and the pure PCS + PTSD group performed worse than the original PCS + PTSD group. [Color figure can be viewed at wileyonlinelibrary.com]

Mathematically defined regions for the new imaging-based groups were found to be as follows:

$$\text{Pure Control : } vDFC > 0.3122 \times SFC + 0.0268$$

$$\text{Intermediate : } 0.3122 \times SFC + 0.0268 > vDFC > 0.3122 \times SFC - 0.0223$$

$$\text{Pure PCS+PTSD : } vDFC < 0.3122 \times SFC - 0.0223$$

Further research on larger data samples is required to verify and validate these regions.

To verify whether the imaging tools used for the new grouping (viz. SFC and vDFC values) are a better classifier of PTSD and PCS than symptom scores, and to assess the quality of the imaging-based grouping, we compared the statistical differences in the neurocognitive measures using

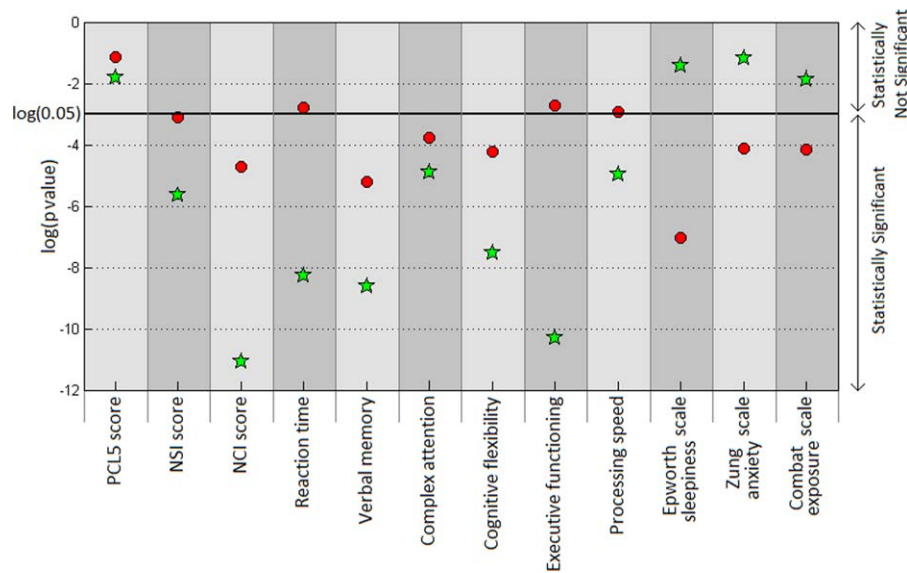


Figure 13.

Comparing symptom severity, neurocognitive functioning, and self-report measures of the original control subjects between those in the pure control group and the intermediate group (red circles), as well as the original PCS + PTSD subjects between those in the pure PCS + PTSD group and the intermediate group (green stars). The y-axis shows the P values of comparison on a logarithmic scale (log to base 10). The significance threshold ($P = 0.05$) is visible as a thick horizontal line. Control

subjects in the pure control group exhibited significantly better neurocognitive performance, less sleepiness and anxiety, and less combat exposure than those control subjects in the intermediate group (see red circles). PCS + PTSD subjects in the pure group exhibited significantly worse neurocognitive performance and higher PCS symptom severity than those PCS + PTSD subjects in the intermediate group. [Color figure can be viewed at wileyonlinelibrary.com]

the imaging-based grouping against the statistical differences obtained with the conventional grouping (Fig. 11). We observed that the P values for all behavioral measures for all groupwise comparisons were consistently smaller with the imaging-based grouping, thus supporting our secondary hypothesis (see Supporting Information SI-2.3 for tables of P values and effect sizes).

Figure 12 shows the mean and SD values of the neurocognitive scores for both the conventional old grouping and the new imaging-based grouping. This figure reiterates the findings of Figure 11, providing a different perspective of the improved separation of neurocognitive scores with the new grouping. It shows that the new imaging-based grouping resulted in these important behavioral measures being separated farther apart between the groups with lesser variance.

It is observable in Figure 12 that the pure control group has better scores on neurocognitive tests than the original control group, and the pure PCS + PTSD group has worse scores than the original PCS + PTSD group. These findings imply that the pure control group is cognitively better than the original control group, and the pure PCS + PTSD group is cognitively worse than the original PCS + PTSD group. These comparisons further add value to our defining them as “pure” groups.

Significant differences in behavioral measures were observed between control subjects in the pure control group and the intermediate group, with controls in the pure group performing significantly better than controls in the intermediate group on several neurocognitive measures (Figure 13). Those in the pure control group also had lower anxiety and sleepiness scores, and had lesser combat exposure. Similarly, PCS + PTSD subjects in the pure PCS + PTSD group performed significantly worse on neurocognitive tests and had higher symptom severity than the PCS + PTSD subjects in the intermediate group (Figure 13). Please refer to Supporting information for the list of all P values, mean, and SD values (Supporting Information, Table S8). These observations show that certain control subjects had behavioral and neurocognitive impairments, which seems to have result in them being labeled to the intermediate group with our new grouping. The PCL5 symptom severity score, however, did not diagnose them with PTSD with the conventional grouping. It is possible that the PCL5 score did not capture those impairments in these subjects. It is also possible that these subjects developed other compensatory mechanisms which rendered them healthy, thus failing to have them diagnosed with PTSD through the PCL5 score despite some degree of neurocognitive impairment (similar logic goes with PCS + PTSD subjects).

TABLE I. Worst-case classification accuracies, along with top-predictive features which displayed the ability in predicting the diagnostic membership of a novel subject with high accuracy. Comparison of the accuracies across different groupings and measures over the 600 iterations revealed high statistical significance and large effect sizes

	Conventional grouping	Proposed grouping	<i>P</i> values for column-wise comparison	Effect size (Cohen's <i>d</i>) for column-wise comparison
Non-imaging measures	70.79% Sleepiness and depression	74.03% NCI and verbal memory	5.42×10^{-11}	0.61
Connectivity values	79.78% SFC and vDFC values of hippocampus-striatum path	83.59%	1.11×10^{-13}	0.72
<i>P</i> values for row-wise comparison	7.12×10^{-26}	2.68×10^{-28}		
Effect size (Cohen's <i>d</i>) for row-wise comparison	1.69	1.81		

It is notable that the imaging-based grouping is based on the underlying neurobiology while the conventional grouping is based on screening instruments. These findings demonstrate that the imaging-based grouping maps better onto neurobehavior than the conventional grouping, indicating that the SFC and vDFC values of the hippocampus-striatum path may be a clinically significant marker of PTSD and PCS. We encourage researchers to employ our regrouping technique to realize more practical and accurate patient diagnosis.

Classification Using Support Vector Machine

Statistical significance, in simple terms, means that the difference in population mean values of the measure (e.g., connectivity) is relatively large compared to the population standard deviations (assuming Gaussianity of data distribution), implying that it is safe to assume, with certain confidence, that the two populations exhibit a difference of significance. However, success in such hypothesis testing is neither a necessary nor a sufficient condition to ensure that the measure can predict the diagnostic membership of a novel subject based on a novel measurement. It does not provide a mechanism for evaluating the predictive ability of the results, which makes it important to acknowledge what a technique like hypothesis testing can do, and cannot do.

Statistically significant neural signatures need not necessarily have generalizability or predictive ability (Craddock et al., 2009; Deshpande et al., 2010), implying that connectivities which are statistically significant (fitting our hypothesis) as well as top predictors assume higher importance. Top predictors are those connectivities which possess the highest ability among all connectivities in predicting the diagnostic membership of a novel subject. We thus used a recursive cluster elimination based support vector machine (RCE-SVM) classifier (Deshpande et al., 2010) to identify the top predictors, which recursively eliminates low-

performing connectivity features, so as to identify those connectivities which contribute most towards obtaining highest classification accuracy. Machine learning classification techniques such as RCE-SVM learn the underlying patterns in the training data, and apply the learned pattern to an untouched testing data to classify the “test” subjects into one of the groups. Given that the true membership of each “test” subject is known; the classification accuracy provides a measure of how well the classification was performed.

Classification was performed for four different paradigms: classification using 32 non-imaging measures (NIMs) with (i) conventional grouping and (ii) imaging-based grouping; classification using whole-brain connectivities with (iii) conventional grouping and (iv) imaging-based grouping. Table I summarizes the worst-case classification accuracies along with the top-predictive features (see SI-2.4: Supporting Information, Fig. S2 for accuracy in every RCE iteration and Fig. S3 for average accuracy).

We observed that RCE-SVM classification using connectivities provided significantly higher accuracy (about 9% more, $P < 0.05$ Bonferroni corrected) than classification using NIMs. This finding indicates that SFC and vDFC have better predictive ability in identifying subjects with PTSD and PCS compared to NIMs. With both NIMs and connectivities, classification with the imaging-based grouping provided higher accuracy (about 4% more, $P < 0.05$ Bonferroni corrected) than classification using the conventional grouping. This implies that the imaging-based groups derived using the hippocampus-striatum path-weights not only map onto behavior better than the PCL5 and NSI scores (as shown in Figures 11 and 12) but also have increased predictive power to determine the diagnosis of subjects irrespective of whether the features are based on connectivity or neurocognitive function.

Along with the classification accuracies, the top predictors which resulted in the highest classification accuracy are also of considerable interest. The top NIMs with the imaging-based grouping were the NCI score and the verbal memory

score, which also resulted in 4% more accuracy. It is interesting to note that the imaging-based grouping attributes the two most important neurocognitive measures in PTSD (NCI and verbal memory) with the highest predictive ability. For classification using connectivities, SFC and vDFC values of the hippocampus-striatum path were the top-predictive features. Prior to these findings, this connectivity path was attributed only with statistical significance between the groups. Statistical significance does not necessarily guarantee predictive ability of connectivity features (Pereira et al., 2009). These results show that, in addition to statistical separation, this connectivity path also has the highest predictive ability, all obtained in a data-driven way from whole-brain connectivity data. For a pictorial description of the entire pipeline and corresponding results, see Figure 2.

DISCUSSION

Evidence in Favor of Our Hypotheses

Our findings indicate perturbations in functional connectivity of the hippocampal-striatal neural network associated with PTSD with/without PCS, indicative of an increased, yet less variable drive between the regions, which supports our overarching hypothesis (Fig. 1). SFC and vDFC values also correlated significantly with neurocognitive measures and symptom severity. Furthermore, our results revealed directional concurrence in the differences in symptom severity, cognitive disruption, compromised connectivity, and diffusivity of related white-matter tracts between the groups, with the PCS + PTSD group being the most compromised, followed by the PTSD group then the combat control group.

We also found support for three corollary hypotheses stated in the introduction. First, complementary to the connectivity findings, DTI results confirmed greater diversity (more diffuse, hence less integrity) of white-matter tracts between hippocampus and striatum in the PCS + PTSD group compared to both the control and PTSD groups, suggesting a strong structural basis for PCS. Prior work shows affected white-matter integrity with left striatum in subjects sustaining an mTBI (Yeh et al., 2015). This structural specificity implies that it is unlikely that the PCS + PTSD group is an extreme subset of PTSD. Second, we regrouped the subjects based on SFC and vDFC of the hippocampus-striatum path. We found that all neurocognitive measures separated better with our proposed imaging-based grouping compared to the conventional grouping (i.e., the P values of separation between the three new groups were smaller for all groupwise comparisons). Third, classification using imaging-based grouping provided significantly higher accuracy than conventional grouping (about 4% more). Furthermore, the accuracies obtained by imaging measures were significantly higher than non-imaging measures for both conventional and imaging-based groupings (about 9% more). SFC and vDFC of

hippocampus-striatum path were also the top-predictive features, in addition to being statistically significant.

Implications for Advancing Our Mechanistic Understanding of PTSD and PCS

Our results are interesting given that individuals with PTSD and PCS have cognitive impairments (Eierud et al., 2014; Hayes et al., 2012) that reflect habit learning or procedural memory, which when impaired, is associated with perseverative thinking. While the striatum is involved in this, the hippocampus is implicated in declarative memory (Mattfeld and Stark, 2015). Both activation and connectivity studies have previously dealt with this aspect. Goodman et al. (2012) showed that traumatic memories relatively increase the activation of striatum while decreasing the activation of hippocampus, leading to a shift from declarative to habit formation. Moreover, Packard et al. (2009) showed that this impairing effect of hippocampus-dependent memory effectively produces enhanced habit learning by reducing competitive interference between cognitive and habit memory systems, and this predominant use of habit memory is induced by stressful emotional states. In line with this finding, perseverative thoughts are also elicited by emotional states associated with stress in individuals with PTSD. Schwabe et al. (2013) showed that the striatum-based procedural memory is stress promoted, meaning that stress induces a shift from hippocampal to striatal dependent memory. Taken together, these studies suggest a neural mechanism explaining why PTSD subjects perseverate on traumatic memories, more frequently and intensely than memories of other events, which leads to habit-like responses (Hayes et al., 2012). Indeed, Spielberg et al. (2015) have shown that striatum is involved in re-experiencing issues seen in traumatized subjects. These observations could explain the involvement and interplay between striatum and hippocampus in PTSD subjects. Both hippocampus and striatum have been implicated in mTBI and PTSD across several studies (Boccia et al., 2015; Eierud et al., 2014; Hayes et al., 2012; Simmons and Matthews, 2012). We postulate that there exists a fine balance between hippocampus and striatum, implicated in the retrieval of memory, which determines the relative emphasis placed on these memories. An imbalance in this mechanism might likely increase perseveration of intrusive memories associated with these stress-related conditions (Ghiglieri et al., 2011).

Next, both structural and functional connectivity studies have investigated the role of hippocampus and striatum in studying memory alterations in PTSD. Memories of stressful negative life events, necessary for PTSD, alters the structural connectivity between striatum and hippocampus (Favaro et al., 2014), which reiterates the structural basis for our findings. In support of our findings, Cisler et al. (2014) showed that there is increased SFC between hippocampus and striatum in PTSD subjects during a “repeated exposure to traumatic memory” task. Building on this, one

of our crucial contributions is in showing that the hippocampus-striatum path has significantly lower variability of connectivity over time in the PTSD and PCS + PTSD groups. This indicates that there may be a lack of adaptability and regulation between these regions, suggesting that PTSD is associated with a hyperconnectivity state, from which it is difficult to disengage, leading to unwanted thoughts and feelings; a phenomenon which is often observed with habit formation. This mechanistic insight may be a valid explanation for clinical/behavioral manifestations in co-occurring PTSD and PCS.

In this study, we found associations between connectivities and neurocognitive scores, as well as significant group differences in neurocognitive functioning. Looking with more statistical granularity, the greatest differences were found in executive functioning (EF) and cognitive flexibility (CF) indices. The EF measures performance in rapid decision management, recognizing rules, and categories; and CF measures performance in adaptation to rapidly changing rules and information manipulation. As such, our results appear to be in accord with findings from Mattfeld and Stark (2011, 2015) which suggest functional contributions and interactions between hippocampus and striatum on tasks requiring learning of new arbitrary associations.

We have used this mechanistic understanding of altered neural circuitry to inform us about subject groupings which seem neurobiologically valid. Our regrouping strategy using connectivities has interesting implications for clinical settings wherein one could obtain fMRI connectivity values from a new subject and potentially assign a diagnostic membership to the subject based on the position of the subject's connectivities in our neurobiologically informed feature space (NIFS) (see *results* section for exact boundary equations). If the connectivities (SFC and vDFC of the hippocampus-striatum path) are within the bounds of the pure control group in the NIFS, then the subject can be diagnosed as healthy with extremely high confidence (similar logic for pure PCS + PTSD). If the connectivities are, however, within the intermediate group, then the subject's symptoms are likely due to PTSD, yet further investigation would be needed to ascertain the diagnosis. Such a classification could improve diagnostic accuracy above and beyond the traditional classification method. Future studies should focus on generalizing these results by replicating it in a large sample, so that they could be used clinically as biomarkers.

Interestingly, SFC and vDFC of the hippocampus-striatum path resulted in the highest classification accuracy. They were also identified as the top diagnostic features, which was entirely determined in a data-driven way from whole-brain connectivity data without any bias from previous results. This demonstrates that they could be a better marker of neural and behavioral characteristics of PTSD and PCS than just PCL5 and NSI scores, and have the potential as imaging biomarkers for these disorders. Our "potential biomarker" satisfies three of the four conditions

described by Woo et al. (2015) to be satisfied by a good biomarker (diagnosticity, interpretability, and deployability). In regard to the fourth condition (generalizability), based on the suggestion in Woo et al., we issue an open call for researchers possessing similar data to share with us so that the classifier can be tested on them. It is notable that our findings are also equally applicable while studying/treating PTSD alone, or even comorbid PCS and PTSD alone.

We made several methodological innovations in this work: (i) For the first time in literature, we proposed a novel unified framework which integrates static and time-varying connectivity information to provide novel characterizations of brain functioning not available earlier. Recent studies have highlighted the enormous importance of dynamics in deciphering brain functioning (Hansen et al., 2015), yet there has been no methodology in the literature which integrates the distinct information provided by static and dynamic connectivities to make a unified inference. Our methodological innovation enables this. (ii) Studies performing dynamic connectivity often do not characterize variability in connectivity. Our use of variance of DFC (vDFC) as a measure of connectivity variability, and the association of lower variability of connectivity with pathology is novel, and would contribute significantly toward understanding of psychiatric disorders and cognitive domains. Furthermore, unlike previous dynamic functional connectivity studies which have used fixed sliding windows, we have employed a method wherein the window length dynamically varies over time based on time series stationarity. (iii) We proposed a novel regrouping technique based on connectivity values and neurobiologically informed feature space. This novel technique can be used to identify objective biomarkers of mental conditions which can predict a subject's membership with complete certainty in one group while subjects in the uncertain group may require further investigation. In many cases, such an approach is more useful than traditional machine learning classifiers as they often cannot determine a subject's membership in any group with high certainty. We hope that the research community would make use of these methodological innovations for studying various mental disorders and cognitive domains.

Limitations and Future Work

We note a number of limitations and caveats which must be kept in mind while interpreting the results presented here, and simultaneously suggest how future studies may address those issues: (1) In accordance with our hypothesis, we observed that the connectivity values were more extreme in the case of PCS + PTSD compared to PTSD alone, and that they also correlated significantly with all the neurocognitive/symptom severity scores. This shows that the subjects who had the added burden of PCS along with PTSD had higher symptom severity than subjects with only PTSD, and also showed higher SFC and

lower vDFC values as compared to PTSD. Additionally, our DTI results clearly show that mTBI may lead to a more severe symptomatology due to structural changes, which might explain why the PCS + PTSD group is more extreme than the PTSD group. Although there is limited literature on comorbid imaging studies of PCS and PTSD, we speculate that (i) the burden of a prior mTBI exacerbates PTSD-related brain alterations which were potentially already prevalent in these subjects before developing PCS or (ii) subjects who sustain mTBI and concomitantly or subsequently had a traumatic experience will end up with greater functional neural alterations which correspond to higher symptom severity than subjects who experienced psychological trauma alone. Future experimental designs must aim to untangle the underlying causal mechanisms in comorbid PTSD and PCS to confirm either of the two scenarios. (2) The structural specificity for PCS in subjects who had sustained an mTBI implies that the comorbid group being an extreme subset of PTSD is unlikely. However, future studies must verify this finding in subjects with mTBI/PCS, but without PTSD. (3) Our findings were based on the results obtained from military subjects with combat exposure. Having a control population with combat exposure is a unique contribution as it provides a more representative control group. Indeed, a recent study revealed differences in resting-state fMRI connectivity patterns between healthy civilian and combat controls (Kennis et al., 2015) “potentially due to military training, deployment, and/or trauma exposure.” Therefore, further work is needed to verify whether these results are applicable to non-combat-related (or civilian) PTSD and PCS. (4) Our findings have interesting implications. When healthy individuals are subjected to stressful events and moderate emotional trauma in their day-to-day life, a temporary shift in the balance between striatal and hippocampal learning and memory might come into place, but they might be able to dynamically engage/disengage the hippocampus-striatum pathway and eventually succeed in restoring the balance to its originality. On the other hand, PTSD and PCS+PTSD are associated with impaired ability to restore this balance, and regulate thoughts and behaviors when exposed to contextually relevant stressors, which possibly has them being diagnosed as unhealthy. Future studies could test these specific hypotheses. (5) While performing RCE-SVM classification, we split the entire dataset into training (80%) and testing/validation (20%) datasets. With this, we had only about 17 subjects (20% of 87) in the testing set, which is a relatively small number for an fMRI connectivity study. (6) Only male veterans were studied, thus our findings cannot be generalized to female soldiers. To ascertain the diagnostic utility of connectivity values of the hippocampus-striatum path and apply these methods in clinical settings in the future, the results need to be replicated on a sample of much larger size, which is more representative of the target population in terms of gender, ethnicity, and so on. (7) Future

studies with the targeted populations could specifically address the habit systems and declarative systems separately, with behavioral measures involving probabilistic classification (e.g., the weather prediction task) which are sensitive to such shifts (Foerde et al., 2006; Schwabe and Wolf, 2012). (8) Time since concussion for the PCS subjects was not available. It is possible that it might correlate with SFC and vDFC values. Also, the data were acquired from the subjects only on one instance. Longitudinal studies could focus on the behavior of the connectivities of hippocampus-striatum path over the advancement, recovery, and rehabilitation phases of subjects with PTSD with and without PCS. This will be an appropriate test for validating the hippocampus-striatum path’s SFC and vDFC as a candidate imaging biomarker for PTSD and PCS + PTSD.

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The authors report no competing interests.

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