PGC-PTSD Secondary Analysis Proposal *(revised 05-2019, kck)*

Version Date	***9/23/19***
Title	***Data-driven approach to dynamic resting state functional connectivity in Posttraumatic Stress Disorder***

NOTE: EVERY SECONDARY ANALYSIS PROPOSAL MUST GO THROUGH THE FOLLOWING PROCESS: A) BE SUBMITTED TO THE ENTIRE LIST SERVE FOR COMMENT (1 WEEK PERIOD) AND THEN B) BE DISCUSSED ON A WORKING GROUP CALL (EMAIL KARESTAN TO SCHEDULE KKOENEN@HSPH.HARVARD.EDU AFTER THE ONE WEEK COMMENT PERIOD).

Date Submitted to list serve for comment	
Date presented to working group	
Date approved by working group	

Investigative Team. Underline the a) PGC-PTSD PI taking responsibility for all aspects of this proposal and b) the person primarily responsible for performing the analysis

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What data from the PGC PTSD working groups will your proposal require? Please indicate the DATE of permission from each group.

Data access requested. No permission required for published results (only pre-publication)

Group	Individual data	Summary results	Date of Permission from group?	Version (e.g. cross-sectional; longitudinal)
GWAS				
EWAS				
Gene expression				
Imaging	*X*			*Cross sectional*
CNV				
Physical health	*X*			*Cross sectional*
Psychophysiology				
Microbiome				
Systems Biology				

Will your proposal require data from working groups other than PGC-PTSD? If so indicate this in the table below. Please indicate the DATE of permission from each group.

Data access requested. No permission required for published results (only pre-publication)

Group	Individual genotypes	Summary results	Date of Permission from group?	Version (e.g., MDD2, SCZ3)
ADHD				
AN				
AUT				
BIP				
Drug/alcohol				
MDD				
OCD/TS				
SCZ				

A. Research Question, Goal, or Specific Aims

Provide a brief description (e.g., 1 paragraph) describing the aims of the proposal and the research questions to be addressed.

Posttraumatic stress disorder (PTSD) is a psychological disorder that is common following exposure to a traumatic event. Clinical presentation of the disorder is heterogenous as symptoms span several cognitive and affective domains. The leading theory on brain network dysfunction in PTSD suggests disruptions in an amygdala-hippocampal-frontal network may underlie impaired extinction learning, a prevailing theoretical model of PTSD (Shin & Liberzon, 2010; Rauch, Shin & Phelps., 2006; Spadoni et al., 2018). Thus, the bulk of imaging research to-date has focused on these regions with respect to major symptoms of the disorder (i.e. amygdala and hyperarousal; hippocampus and memory deficits; frontal cortices and impaired extinction learning) (Shin & Liberzon; Rauch, Shin, & Phelps, 2006). This approach, however, ignores the contribution of other, larger network disturbances that may also be important for the pathophysiology of PTSD (Negreira & Abdallah, 2019).

For instance, a growing body of literature also reports large-scale disruptions in resting state canonical networks [i.e. default mode (DMN), central executive (CEN), salience network (SN)] in those with PTSD (reviewed in Akiki, Averill & Abdallah, 2017 and Menon 2011). The DMN is a network thought to involve introspective processes of which core regions include the medial temporal lobe including the hippocampus, posterior cingulate cortex, and ventromedial prefrontal cortex (Menon 2011). The CEN is a network active during tasks requiring cognitive control and consists of dorsolateral prefrontal cortex, middle frontal gyri, precuneus, and premotor cortices (Menon 2011). Finally, the SN is a network involved in the detection of stimuli and primarily consists of the amygdala, insula, and dorsal anterior cingulate cortex (Menon 2011). Although differential alterations in these networks have been associated with specific deficits in PTSD (Akiki et al., 2017; Spadoni et al., 2018), there are common regions implicated in more than one network, while many of the network "hubs" are regions previously identified in ROI-based approaches (e.g., hippocampus, amygdala, frontal cortex). In contrast, and of interest to the current study, we know relatively little about disruptions in spatially diffuse networks with regions outside of the amygdala-hippocampal-frontal network in those with PTSD. Furthermore, given the heterogenous nature of PTSD, it is unlikely dysfunction can be explained by a single region or even by a canonical network consisting of a few regions.

Another potentially limiting factor in this line of research is the use of static or stationary functional connections among regions. Stationary functional connectivity does not necessarily account for changes in connectivity among regions over time, whereas dynamic rs-fMRI, a relatively newer analysis method in fMRI, allows for the observation of engagement and disengagement of regions in the brain over time (reviewed in Hutchison et al., 2013). Dynamic rs-fMRI was developed over the last decade or so based on the observation that in the absence of a given task brain regions show time invariant fluctuations in activity throughout the duration of a resting state scan (Heitmann & Breakspear, 2018; Allen et al., 2014; Deco, Jirsa, & McIntosh, 2011; Hutchison et al., 2013). By breaking up the duration of a longer rs-fMRI the scan into smaller "windows" of time and examining the strength and direction of functional connectivity amongst brain regions within each window, we isolate network changes over time (Hutchison et al., 2013). This method may be a more sensitive way of understanding network dysfunction in PTSD in particular. For instance, in a sample of earthquake survivors, dynamic functional connectivity of a network consisting of 190 regions was a better predictor of PTSD than the more "traditional" static functional connectivity of the same network (Jin et al., 2017). Therefore, dynamic functional connectivity may be a more ecologically valid approach to understanding the properties of brain network dysfunction in PTSD (Yu et al., 2016; Fornito & Bullmore, 2012).

Very few studies have examined dynamic rs-fMRI in those with PTSD, and those that have are likely underpowered. Further, the majority of dynamic rs-fMRI studies in PTSD rely on ROI-based or

canonical network approaches to characterize network changes over time which has limitations discussed previously (Negreira & Abdallah, 2019; Zhang et al., 2016). Therefore, a data driven approach to dynamic rs-fMRI may illuminate under-studied neuronal processes that underlie PTSD (Akiki et al., 2017). The ENIGMA PCG-PTSD Neuroimaging group provides an unmatched opportunity to conduct an adequately powered investigation into data driven dynamic rs-fMRI in a PTSD population.

This proposal will complement other proposals under consideration. Sun et al. has proposed to examine dynamic functional connectivity (FC) in a seed-to-voxel manner (seeds: amygdala, hippocampus, ACC, and mPFC), while King et al. has proposed using graph theory metrics of whole session resting state in large-scale pre-defined networks, specifically within the Default Mode Network and Salience Networks. We propose to look at dynamic FC in a data driven manner (independent of *a priori* seed or canonical ICN) to characterize "connectivity states" in those with PTSD.

Specific Aims:

Aim 1: Use data driven approach in dynamic resting state functional connectivity to characterize changes in brain networks in those with and without PTSD (within groups analysis).

Aim 2: Identify dynamic functional connectivity states that differentiate those with or without PTSD (between groups analysis).

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B. Analytic Plan

Provide a brief description of the analyses to be performed to address the research questions described above. Include relevant details e.g. phenotype definition, QC, analysis, plans to address population stratification and other confounders, power.

Participants: We will use the resting state fMRI scans, demographic and clinical data collected by the ENIGMA-PGC-PTSD consortium workgroup. Approximately 3000 participants data will be used (~1500 mixed trauma with current PTSD, ~1500 trauma exposed controls). Where available, covariates of interest for this proposal include scanner site, age, sex, substance or medication use, childhood and adult trauma exposure, and comorbid psychopathology (especially major depressive disorder and anxiety disorders).

MRI pre-processing: For consistency across resting state analyses within the PGC-PTSD work group, and to conserve computational resources, we propose to use resting state data that has already undergone preprocessing using the standardized ENIGMA pipeline (Adhikari et al., 2018).

Dynamic rs-fMRI analysis: Following the analysis example of Yu et al. (2016), first, group ICA will be performed on the whole resting state time series to identify components (nodes) across the sample using GIFT (http://mialab.mrn.org/software/gift/) (Calhoun et al., 2001). A two-step data reduction will be performed first at the individual subject level then at the group level. Subject-specific principal components will be chosen whereby standard economy-size decomposition results in components that retain 99% of the variance. Subject-specific PCs will then be decomposed into 100 aggregate components in an approach similar to other published methods (Yu et al., 2016; Allen et al., 2014). To ensure the reliability of the group estimation the Infomax ICA algorithm will be repeated 10 times in ICASSO. Based on the group PCs, subject specific components and time courses will then be back-reconstructed using the GICA algorithm. Final independent components will be chosen if peak activations are in grey matter and their time courses are predominantly low-frequency fluctuations (evaluated using power spectral analysis). These components will undergo postprocessing involving detrending, despiking, and regression of motion parameters.

Once the nodes of the network have been identified, pairwise correlations among components from the whole timeseries will be calculated to establish a matrix of stationary connectivity among nodes. Then to see how the dynamics of nodes within the network change over time, a sliding window of 20 TRs (~40sec) will be used to segment the time series of the network correlations (Shirer et al., 2012; Li et al., 2013). Correlations between any pair of nodes will be calculated for each time window. Correlation coefficients will then be converted to a signed similarity measure, (equation 1 in Yu et al., 2016), which is used to distinguish between positive and negative correlations (r=-1 has a similarity of s=0, r=0 has a similarity of s=0.5). Using the Brain Connectivity Toolbox (http://www.brain- connectivity-toolbox.net/) various graph theory metrics (discussed below) will be calculated and averaged within each time window so they can be correlated across time windows (Rubinov & Sporns, 2010). The graph theory metrics of interest include degree, clustering coefficient, path length, and global and local efficiency as these measures have been demonstrated as highly reproducible (Telesford et al., 2013).

Connectivity state analysis: To identify "connectivity states" we will use the method developed by Yu et al., 2016. Connectivity states can be described as functional connectivity patterns that reoccur over time. First, correlations between each pair of the dynamic rs-fMRI time windows (described above) will be calculated to construct a matrix of "modules" of connectivity. Time windows that show higher correlations of functional connectivity are considered modular as they may reflect structured patterns of activity that ebb and flow over time (Yu et al., 2016). Based on these modularity results, time windows will be reordered so that the time courses of functional connectivity from each window can be

combined and averaged within each module. The averaged time course of connectivity within each module is then considered a "connectivity state" for which graph theory metrics (same as stated above) can be calculated.

At the first-level analysis, this method will allow us to quantify the number of connectivity states for each individual to be subsequently quantitatively compared across diagnostic group. This method will allow us to identify qualitative differences in connectivity states at the group level to identify unique connectivity states by disorder.

Pilot data results: The aforementioned methodology was tested using pilot data from an ongoing study in the lab of Dr. Larson (University of Wisconsin—Milwaukee). A sample of 16 trauma exposed participants (4 PTSD+ per CAPS-5 criteria) 8-minute resting state data were run through the pipeline. Forty-seven of the 100 independent components generated in the group ICA step survived evaluation using the criteria that peak activations were in grey matter and their time courses were predominantly low-frequency fluctuations (evaluated using power spectral analysis). Static or stationary functional connectivity was calculated by correlating each pairwise connection of the 47 components. As described above, these correlations were then converted to similarity indices (Figure 1). See Figure 2 for schematic of static connectivity patterns by group. These 47 components were then carried over into the dynamic functional connectivity step.

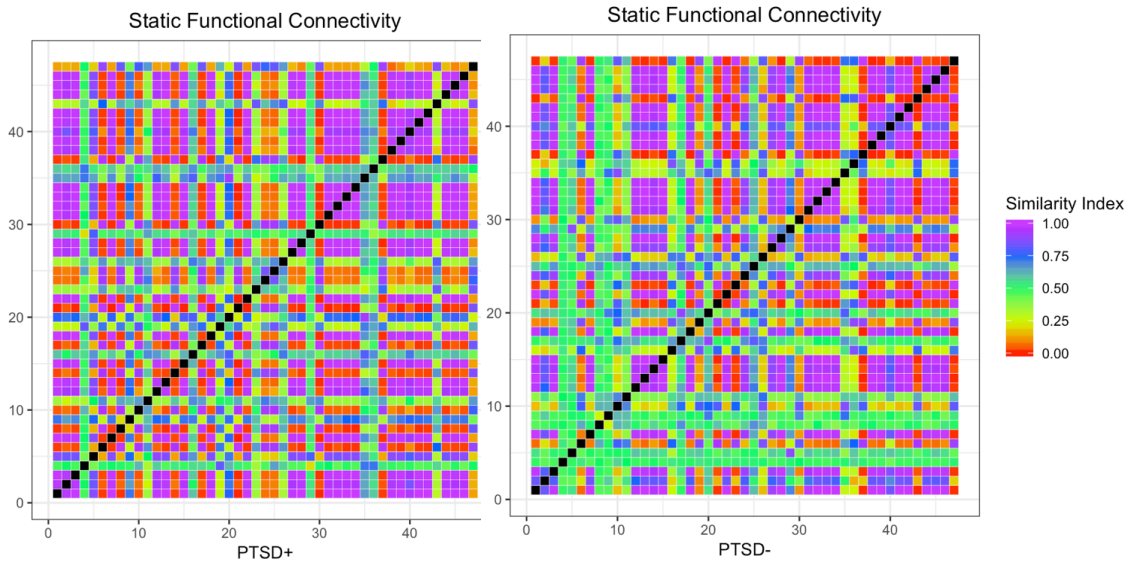

Figure 1. Static functional connectivity (similarity S matrix) between 46 nodes in PTSD+ and PTSD- groups.

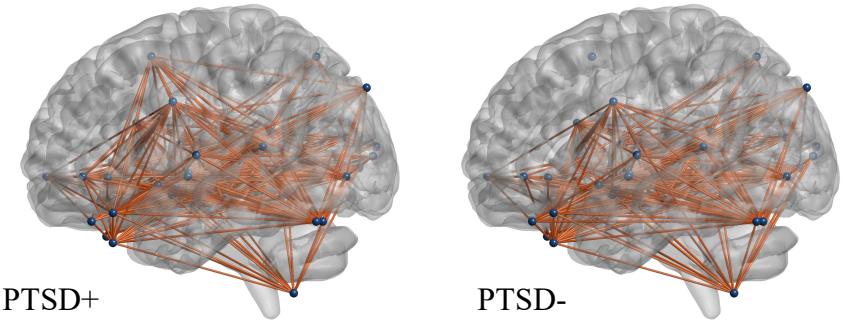

Figure 2. Schematic of static connectivity patterns by group. Nodes reflect location of peak activation within each ICA component, edges reflect connectivity strength between nodes (threshold=0.65).

For this sample, a sliding window of 20 TRs (TR=2 sec) resulted in 219-time windows. For each participant, the time series for each pairwise connection of the 47 components was correlated for each of the 219-time windows. These correlation matrices were then converted to similarity matrices (described above). The similarity matrices were then used to calculate the graph theory metrics of interest using the Brain Connectivity Toolbox. Metrics including global and local efficiency, clustering coefficient, path length, and connectivity strength were averaged within each participant's 219-time windows. Then, Wilcoxon rank sum tests were used to assess the equality of variances of these graph theory metrics between PTSD+ and PTSD- groups. These results showed there were no group differences in any of the graph theory metrics (Figure 3). Time courses of the graph metrics by time window and group are shown in Figure 4.

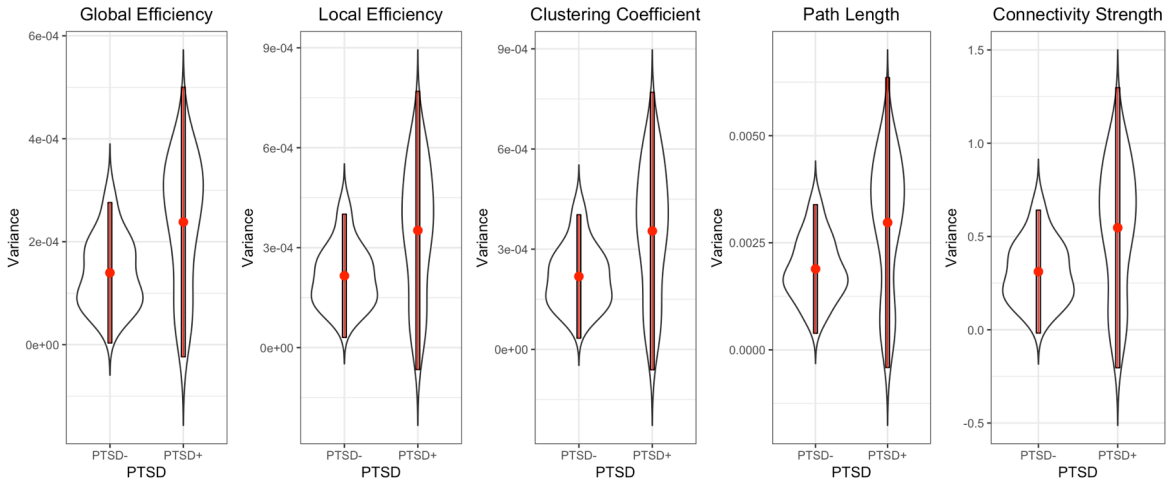

Figure 3. Variances of the graph metrics over 219-time windows. The mean confidence interval is shown in red within smoothed density histograms. Wilcoxon tests revealed no group differences in any of the 5 metrics (all *p's*>0.05).

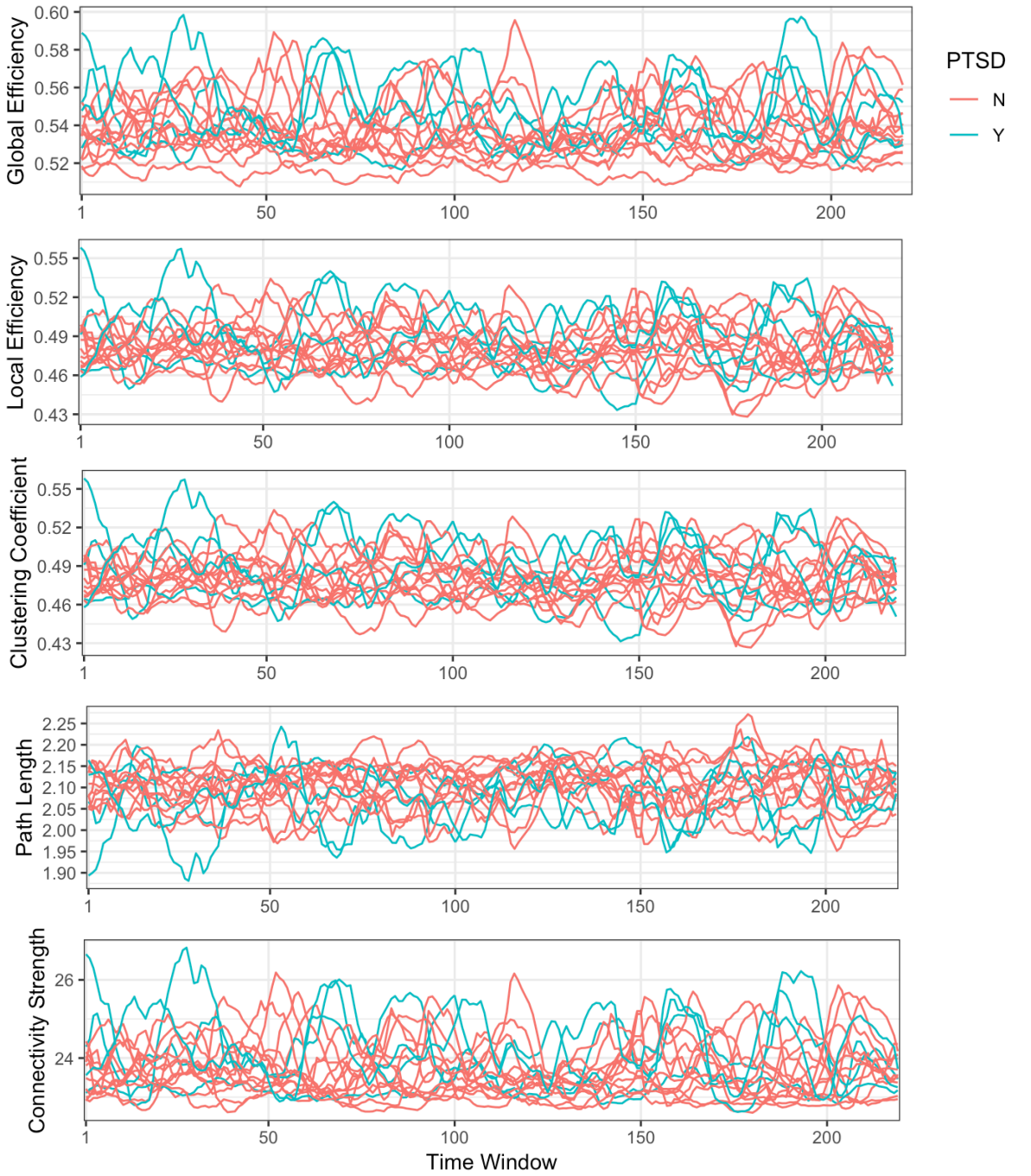

Figure 4. Time courses of the graph metrics over 219-time windows.

Next, to identify connectivity states, connectivity strength of each node within each time window was calculated for each subject. Then, for each subject, the modularity of these nodal connectivity strengths across time windows was calculated using the Brain Connectivity Toolbox. Once modules were identified, the time windows were reordered according to module so that graph metrics could be calculated within each module. A range of 2 to 4 connectivity states were identified per subject (40 total connectivity states, 9 PTSD +, 31 in PTSD-, Figure 5). Figure 6 shows a schematic of the similarity indices across modules for PTSD+ and PTSD- groups. Wilcoxon rank sum tests yielded no

differences in graph properties between states for those with PTSD and those without (all p's>0.05, Figure 7).

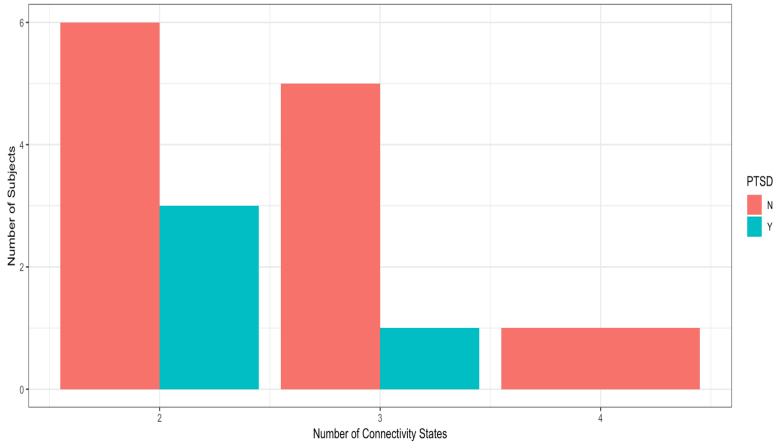

Figure 5. Histogram of first level connectivity states (modules) counts.

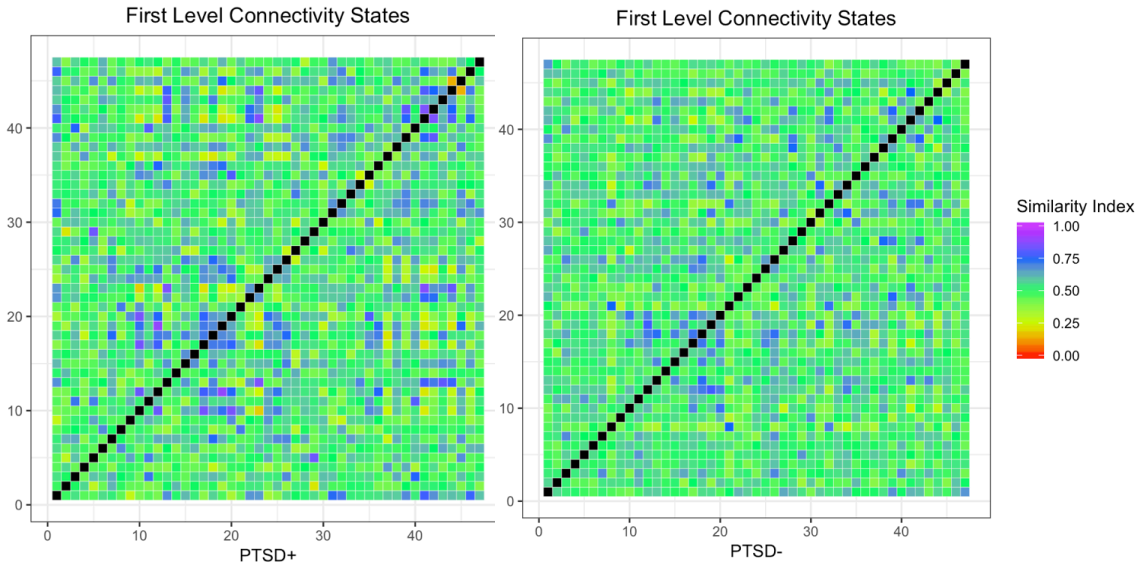

Figure 6. Connectivity pattern structure of first level connectivity states (modules) for PTSD+ and PTSD- groups.

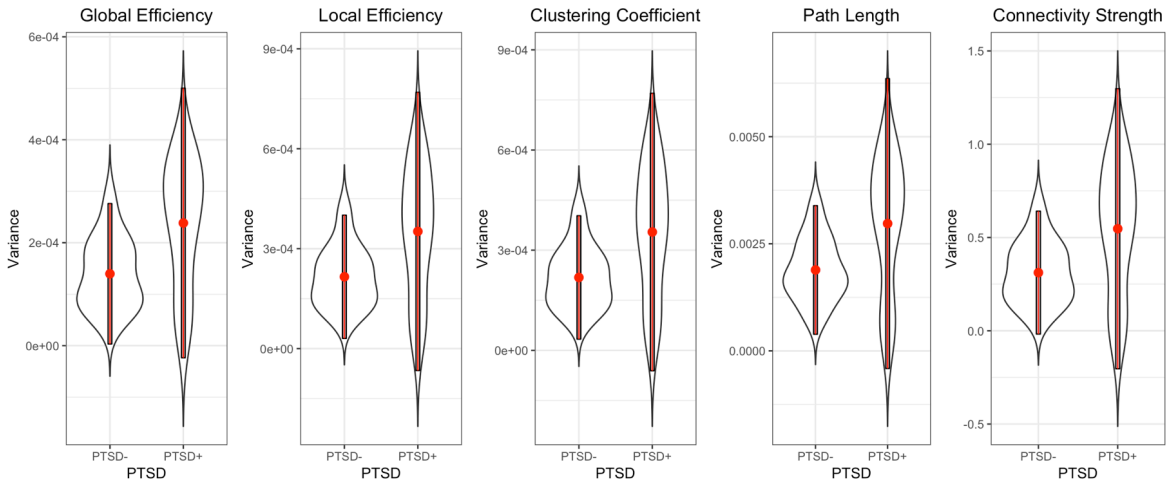

Figure 7. Distributions of graph metrics over first level connectivity states (40 total connectivity states, 9 PTSD +, 31 in PTSD-). The mean confidence interval is shown in red within smoothed density histograms. Wilcoxon tests revealed no group differences in any of the 5 metrics (all *p's*>0.05).

Though no significant findings resulted from this analysis, the sample analyzed was quite underpowered to detect any group differences. Nevertheless, this pilot data allowed us to finalize the fMRI processing and analysis pipeline to demonstrate feasibility. Repeating this analysis in a larger sample available within the ENIGMA PCG-PTSD Neuroimaging group may prove more insightful into the brain network dynamics of those with and without PTSD.

C. Analytic Personnel

Indicate who will be responsible for performing the analyses.

Carissa Weis will be responsible for performing the analysis. Carissa Weis, Jacklynn Fitzgerald, and Christine Larson will work together to present results and interpretations. We'd also like to include Dr. Raj Morey and Dr. Delin Sun as collaborators given their experience with similar analytic techniques.

D. Resources Needed

Describe the resources needed to achieve the aims of the analysis, including variables needed, analytic support, and any other issues that may affect the feasibility of the plan.

We'll need the preprocessed resting state fMRI scans from PTSD+ and trauma exposed PTSD- individuals. We'd also like basic demographics and where available, the following covariates of interest scanner site, age, sex, substance or medication use, childhood and adult trauma exposure, and comorbid psychopathology (especially major depressive disorder and anxiety disorders).

F. Timeline

Estimate time required to complete the plan and write a paper (should be ≤ 6 months).

The longest part of the analysis process will likely be data organization on the front end. The dynamic resting state functional connectivity analysis shouldn't take more than a week to run once the data has been organized. Final write up of the paper I'd anticipate would take a month. However, to allow for troubleshooting I'd anticipate the entire project to take 6 months.

F. Collaboration

The following is the standard PGC policy about secondary analyses. Any deviation from this policy needs to be described and justified, and could negatively impact the proposal.

PGC investigators who are not named on this proposal but who wish to substantively contribute to the analysis and manuscript may contact the proposing group to discuss joining the proposal.

G. Authorship

Please note that Caroline Nievergelt and Adam Maihofer, due to their exceptional efforts both in terms of securing funding and analyzing data, are automatically included as named co-authors on every manuscript unless they explicitly opt-out. Please indicate that you acknowledge this and have read the PGC PTSD working group authorship policy and will comply with that policy in all authorship decision related to any presentations or manuscripts resulting from your proposal by checking the box below and signing your name:

☑ _____

This is an extremely important part of this proposal. Describe how authorship will be handled in the manuscript resulting from this analysis. To avoid a revision, first review the authorship policy of the group(s) whose data you wish to analyze.

Points to consider:

(a) are you following the authorship policies of the groups involved?
(b) will there be a writing group and if so, who will be included?
(c) what groups or individuals will be listed as authors in addition to Caroline and Adam?
(d) will PGC members not listed as named authors be listed at the end of the manuscript?
(e) will PGC members or groups be listed as "collaborators" on the PubMed abstract page?

We will follow the authorship policy of the PGC-PTSD found at https://pgc-ptsd.com/wp-content/uploads/2017/06/Authorship-Guidelines-PGC-PTSD.pdf

1. *Are you following the authorship policies of the groups involved?*

   Yes

2. *Will there be a writing group and if so, who will be included?*

   The writing group will be comprised of the investigative team listed above.

3. *What groups or individuals will be listed as authors?*

   Authors will include the writing group plus individual and group contributors of data and analysis from each site (generally 2-3 co-authors from each site).

4. *Will PGC members not listed as named authors be listed at the end of the manuscript?*

   All individuals who meet the criteria established in the PGC-PTSD authorship policy will be co-authors. Other PGC members will not be listed at the end of the manuscript.

5. *Will PGC members or groups be listed as "collaborators" on the PubMed abstract page?*

   All individuals and groups who meet the authorship criteria of the PGC-PTSD authorship policy will be listed as collaborators on the PubMed abstract page. No other individuals or groups will be listed.

6. *How will funding sources be handled or acknowledged?*

   All funding sources that supported data collection and analysis will be listed in the manuscript.

H. Funding

Please indicate your understanding that all of the PGC and PGC-PTSD working group funders including Cohen Veteran's Bioscience, NIMH R01MH106595 and NIMH U01MH109539, One Mind, *and the Stanley Center need to be acknowledged in your manuscript by checking the box below and signing your name:*