Focal Activity Differences at Rest in Posttraumatic Stress Disorder

Proposed Analysis for ENIGMA/PGC-PTSD Working Group

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Abstract
Determining the pathophysiology of posttraumatic stress disorder (PTSD) is a critical step toward reducing its debilitating impact. A key step in that process is identifying regions in the brain where changes in resting-state activity may be associated with PTSD or PTSD symptom severity. Identification of such regions of interest (ROIs) would not only shed light on the neural processes underlying PTSD, but could also serve as markers of relevant networks, seeds for functional connectivity analysis, or targets for clinical intervention (e.g. neuromodulation). Focal activity can be measured at rest using various neuroimaging techniques, including regional homogeneity (ReHo) and fractional amplitude of low-frequency fluctuation (fALFF), each of which are commonly used as proxies for resting-state activity at the voxel-level. The primary goal of the current study is to identify regional differences in resting-state activity (measured using ReHo and fALFF) between PTSD cases and trauma-exposed controls (TECs). A secondary analysis will use linear regression to find ROIs where focal activity is linked to symptom severity. ROIs will then be made available for other research efforts within and outside of the consortium. By modeling the neurobiological correlates of PTSD, we can increase our understanding of this debilitating disorder and guide the development of future clinical innovations.

Background

Patterns of resting-state neural activity without explicit perceptual engagement with environmental stimuli are theorized to reflect consolidation of past experiences and preparation for future events (Buckner & Vincent, 2007). PTSD is a debilitating disorder characterized by physiological reactivity to cues and memories related to past psychological traumas (Orr, McNally, Rosen, & Shalev, 2004). The maladaptive defensive arousal evident in individuals with the disorder suggests the presence of enduring neural abnormalities independent of external stimuli. Indeed, functional connectivity brain networks have been closely linked with PTSD symptomatology (Patel, Spreng, Shin, & Girard, 2012; Sylvester et al., 2012) suggesting focal differences in resting neural activity (also known as spontaneous neural activity) can shed light on localized neural abnormalities that underlie PTSD (Yan et al., 2013). Differences in focal activity are theorized to moderate sensory-, cognitive-, and motor-driven neural responses (Mantini, Perrucci, Del Gratta, Romani, & Corbetta, 2007). Furthermore, assessing for areas of differential activity can empirically identify promising regions of interest (ROIs) for subsequent research (e.g. functional connectivity or network-based analyses Ke et al., 2016)) or treatment (e.g. neuromodulation).

There are multiple approaches that are widely-used for identifying focal activity differences from resting-state fMRI data. Among the most common approaches are regional homogeneity (ReHo) and amplitude of low-frequency fluctuation (ALFF). ReHo is a voxel-based measure of focal connectivity that evaluates the synchronicity of the time series between a given voxel and each of its 26 neighboring voxels (Y. Zang, Jiang, Lu, He, & Tian, 2004). ReHo is widely used as a proxy for ongoing activity, including in the Default Mode Network, where ReHo increases at rest and decreases during task completion (Long et al., 2008; Y. Zang et al., 2004). Whereas ReHo measures connectivity between a given voxel and its neighbors in the time domain, ALFF measures signal variability of a single voxel in the frequency domain. Specifically, ALFF quantifies the amplitude of low-frequency BOLD signal in a given voxel (between 0.01-0.08 Hz), which is used as a proxy for the overall rate of neural activity during the resting period (Y.-F. Zang et al., 2007). Fractional ALFF (fALFF) is an adaptation of ALFF that calculates the ratio of low-frequency signal (0.01-0.08 Hz) to the total frequency range (0-0.25 Hz for studies with TR <= 2; Zou et al., 2008). Though both ALFF and fALFF have been shown to be viable biomarkers for various psychiatric conditions, including schizophrenia and ADHD (Xu, Zhuo, Qin, Zhu, & Yu, 2015; Y.-F. Zang et al., 2007), fALFF in particular is designed to reduce the sensitivity to physiological noise, thus making it more robust for comparing results across multiple sites. Critically, neither ReHo nor ALFF/fALFF require *a priori* definition of ROIs, allowing for a more empirical, data-driven identification of key regional differences.

The overall goal of our proposed analysis will be to identify focal differences in resting-state activity, measured using ReHo and fALFF, in fMRI data collected through the Psychiatric Genomic Consortium (PGC)-Enhancing Neuroimaging Genetics Through Meta-Analysis (ENIGMA) PTSD Working Group. We expect to address *the following specific aims*:

Aim 1: Identify regions with differential resting activity between PTSD cases and trauma-exposed controls using ReHo and fALFF. Regions identified in case-control analysis can then be used in follow-up analyses as ROIs to facilitate machine learning/classification techniques.

Aim 2: Using dimensional indices of PTSD (e.g. PCL score), identify regions where activity may have a continuous relationship with symptom severity. Considering that case-control analyses generally exclude individuals with sub-threshold PTSD, which has been linked to significantly greater functional impairment compared to controls (Cukor, Wyka, Jayasinghe, & Difede, 2010), it is necessary to use both categorical and dimensional operationalizations of PTSD to more fully understand the neural impact of this devastating disorder.

Preliminary Work: We have recently published an activation likelihood estimation (ALE) meta-analysis of the existing literature on focal activity differences at rest in PTSD, composed of 457

PTSD cases, 292 trauma-exposed controls (TECs), and 293 non- traumatized controls (NTCs) across 22 published studies (Disner, Marquardt, Mueller, Burton, & Sponheim, 2017). When comparing PTSD cases to TECs (as is being proposed), two ROIs withstood correction for multiple comparison: focal hyperactivity in the left inferior parietal lobule (IPL) and focal hypoactivity in the right lingual gyrus (see Figure 1).

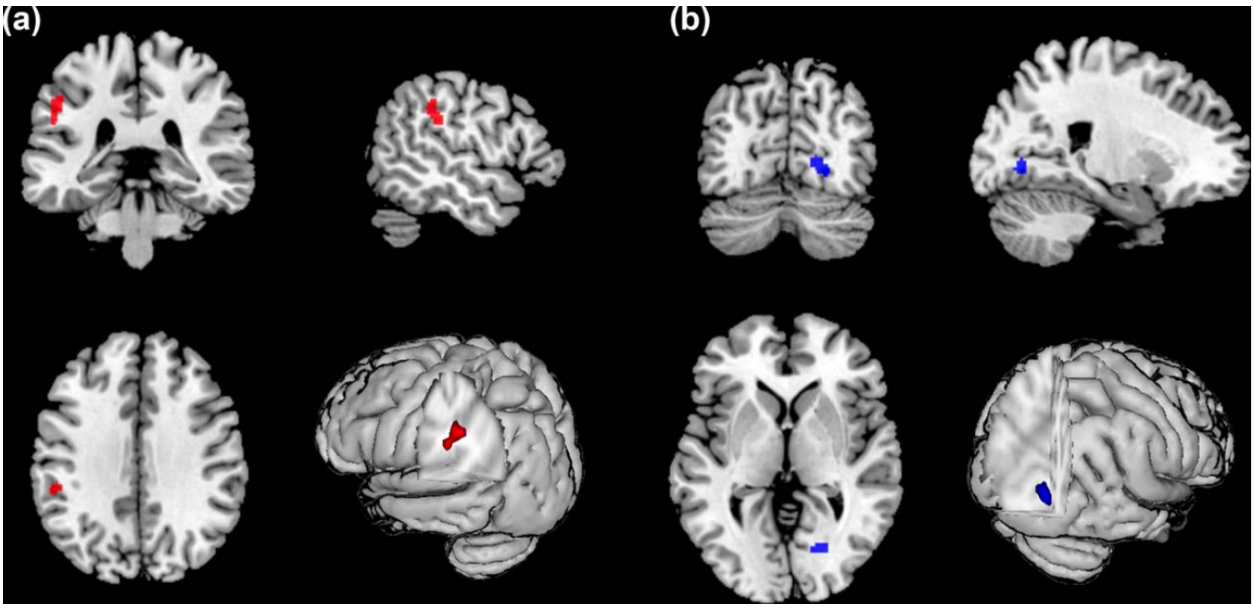


Figure 1 – *Meta-analysis-identified differences in PTSD cases compared to TECs*. A) Cluster of increased activity centered in the left IPL (BA 40), modestly overlapping with the left supramarginal gyrus and insula (BA 13). B) Cluster of decreased activity centered in the right lingual gyrus (BA 18), prominently overlapping with BA 19.

We sought to validate these ROIs by calculating ReHo and ALFF values in our local sample (N=205 US military veterans, who are included in the ENIGMA/PGC dataset). We then used multivariate multiple regression to determine if focal activity at rest was linearly associated with PTSD symptom severity. A separate secondary analysis replaced the total PTSD symptom severity predictor with four PTSD symptom groupings (re-experiencing, avoidance, dysphoria, and hyperarousal).

The left IPL was the only cluster to show an association between focal activity and PTSD total symptom severity while withstanding FDR correction (ReHo partial r=0.188, p=0.007, q*=0.01; ALFF partial r=0.202, p=0.004, q*=0.005). Greater left IPL activity was associated with increased PTSD symptom severity (Table 1 and Figure 2). There was no significant association between PTSD symptom severity and ReHo or ALFF in the lingual gyrus ROI, nor did any of the ROIs show any significant association with the four PTSD symptom groupings in the separate statistical models.

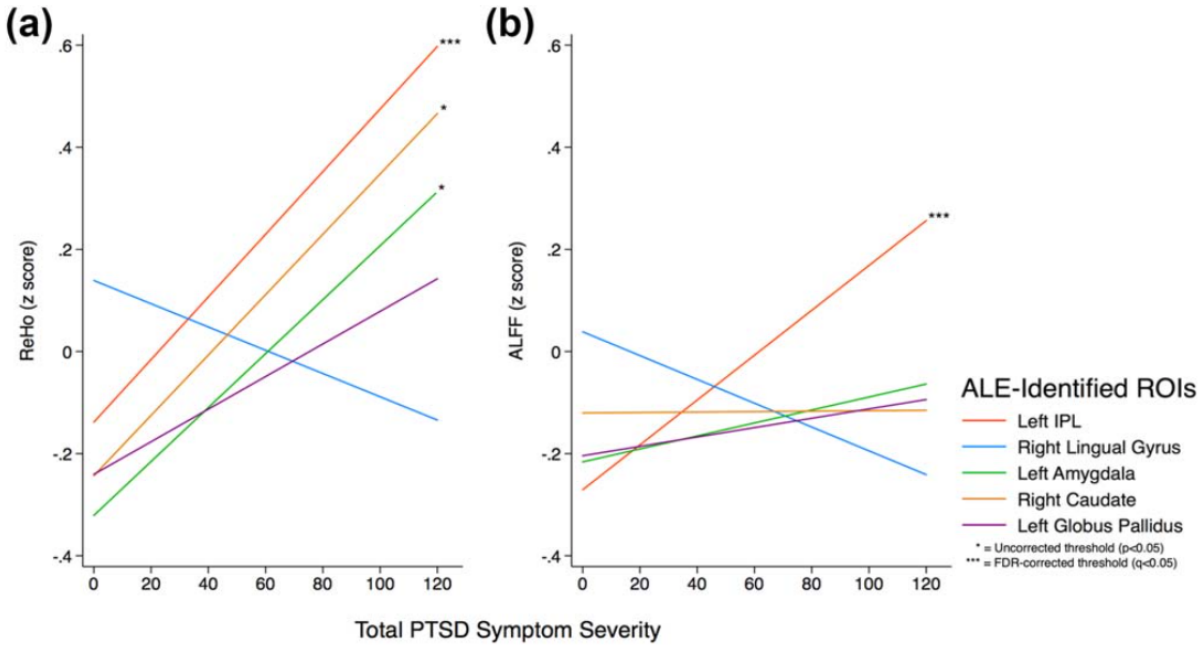
In addition to being the largest-to-date meta-analysis of focal resting-state activity in PTSD, these results helped to identify a plausible ROI for subsequent seed-based analysis (left IPL) that is not generally considered amongst the most-common PTSD-related seeds. A similar approach using the case-control status (Aim 1) and the dimensional PTSD severity (Aim 2) could further support *a priori* ROIs (e.g. amygdala, hippocampus, ACC) and/or facilitate discovery of novel ROIs that might shed light on relevant networks.

Table 1 - *fMRI validation results* – Multivariate multiple regression results reveal the left IPL ROI as the only cluster significantly associated with total PTSD symptom severity (assessed using CAPS; both ReHo and ALFF as dependent variables in separate models) when correcting for multiple comparisons using the independent validation sample of previously deployed military veterans.

				fMRI Validation: Association with PTSD Total Symptom Severity			
Control Group	**Direction**	**Anatomical Region (center)**	**Hemisphere**	**Partial Correlation (ReHo)**	**p (ReHo)**	**Partial Correlation (ALFF)**	**p (ALFF)**
Combined (TEC & NTC)	Control > PTSD	Globus pallidus	L	0.101	0.150	0.054	0.443
TEC	PTSD > Control	Inferior parietal lobule (IPL; BA 40)	L	**0.188**	**0.007*** **	**0.202**	**0.004*** **
	Control > PTSD	Lingual gyrus (BA 18)	R	−0.073	0.301	−0.102	0.148
NTC	Control > PTSD	Amygdala	L	0.161	0.022*	0.071	0.311
		Caudate head	R	0.147	0.036*	0.036	0.973

*=Uncorrected threshold ($p < .05$); ***=FDR-corrected threshold ($q = 0.05$).

Figure 2- *Predicted marginal relationship between PTSD total symptom severity, measured using CAPS, and spontaneous neural activity, measured using ReHo (A) and ALFF (B).* Left IPL was the only ROI whose association withstood FDR correction using either analytic technique.



Participants: Eligible participants will have neuroimaging and PTSD clinical measures contributed to the ENIGMA-PTSD/PGC-PTSD project. This sample is estimated to include ~1500 PTSD cases and ~1500 TECs across all participating sites. Participants will be excluded due to A) history of Axis I disorders other than PTSD, MDD, or past substance abuse; B) history of significant neurological condition (e.g. stroke, epilepsy); or C) history of moderate or severe TBI. Consistent with PGC-PTSD guidelines, determination of PTSD status will be at the discretion of the PIs/research staff who oversaw the collection of MRI data. Dimensional measures of PTSD symptom severity will be used for aim 2 provided that A) symptom severity

was measured using a widely-available scale (e.g. PCL, CAPS), and B) the scale captured current PTSD symptomatology (e.g. over the past month).

Preprocessing: Preprocessing steps are consistent with recommendations by Zuo and colleagues intended to maximize reliability of functional homogeneity analyses (Zuo et al., 2013). Our existing pipeline for ReHo/fALFF analysis has been conducted using the RESTplus (version 1.2; Song et al., 2011) package for MATLAB. However, processing steps can be completed using AFNI/CONN-fMRI Functional Connectivity Toolbox to mirror ENIGMA pipeline if necessary. For functional scans intended to calculate ReHo, the preprocessing steps will be as follows. The first ten time points will be discarded for all participants. Functional images will be motion corrected using the RESTplus realignment function, co-registered to structural images, and normalized to standard MNI space using the DARTEL toolbox. Functional data will be linearly detrended to account for general signal drift. Signals from nuisance covariates will be removed via regression (six head motion parameters, white matter, and cerebrospinal fluid). For ReHo and ALFF, data will be band-pass filtered (0.01-0.08 Hz) to reduce low- and high-frequency noise, though non band-pass filtered data (0-0.25 Hz, depending on study TR) will be preserved for calculation of fALFF (see below). For functional scans intended to calculate ALFF/fALFF, the data will then undergo additional smoothing using an isotropic 6-mm FWHM Gaussian kernel.

Subject-level analyses

Regional Homogeneity (ReHo)

Individual ReHo maps will be generated in a gray matter mask using Kendall's coefficient of concordance (KCC)(Y. Zang et al., 2004). KCC measures the similarity of the time series for each voxel with each of its 26 contiguous voxels (i.e., the relationship between the middle voxel in a 3x3x3 cube with all of the outer voxels). ReHo for each voxel will then be divided by the subject's own global mean ReHo value within the mask for standardization. Individual ReHo maps will then be smoothed using an isotropic 6-mm FWHM Gaussian kernel to reduce the impact of anatomical variability across individuals. Unlike ALFF (or other resting state analyses), it is critical that smoothing take place after the calculation of ReHo, rather than before, as smoothing by definition increases the concordance between neighboring voxels.

Fractional Amplitude of Low Frequency Fluctuation (fALFF)

To generate individual fALFF maps, each participant's power spectrum will be derived using fast Fourier transform to convert time series data into the frequency domain. ALFF will then be computed using the square-root of power spectrum values and averaging the square rooted values across 0.01 – 0.08 Hz for each voxel (Y.-F. Zang et al., 2007). We will then calculate the ratio of ALFF to the amplitude across the entire frequency range (0-0.25 Hz) (Zou et al., 2008). fALFF values for each voxel will then be divided by the subject's own global mean fALFF value for standardization.

Note: ReHo and fALFF analyses will be calculated separately and have results reported separately. Although there does appear to be a correlation across voxels between ReHo and ALFF/fALFF (Nugent, Martinez, D'Alfonso, Zarate, & Theodore, 2015; Yuan et al., 2013), the studies comparing the two techniques have used relatively small samples. As such, we will conduct post-hoc linear regression of ReHo and fALFF values from the same ROI in order to determine the unique contributions of each technique.

Group-level analyses

General Linear Model (Mega-analysis)

Using a general linear model (GLM), ReHo and fALFF will be predicted by diagnosis (PTSD vs. TEC) with site included as a random effect and age and sex included as fixed-effect covariates (Aim 1). For Aim 2, linear regression will be used to identify the association between ReHo/fALFF and continuous measures of PTSD severity (using PGC guidelines for harmonization), again including site as a factor and age and gender as covariates. Significance thresholds will be set to $p<0.05$ FDR-corrected with an extent threshold of 10 voxels. *Note*: Mega-analysis will be conducted only on datasets where subject-level ReHo/fALFF maps are made available. Sites that can only release aggregate results can still be included in the site-level meta-analysis, described below.

Site-level Meta-analysis
In order to assess for replication of the mega-analysis within smaller subsets, we will also conduct the above described model for each site (omitting site as a factor). We will then meta-analyze statistical parametric images generated from GLM models for each sample using the anisotropic effect size seed-based *d* mapping (AES-SDM; Radua et al., 2014)) technique. If the meta-analytic effect sizes are larger than the mega-analysis effect sizes, then we have lost power by pooling the subjects across sites. If the mega-analysis effect sizes are similar or larger, the pooling across sites and including site as a factor is sufficient to account for site differences. *Note*: For sites that are not permitted to share MRI files or statistical parametric images, a list of coordinates reflecting the centers of significant clusters can be included in AES-SDM meta-analysis.

<u>Follow-up analyses</u>
Seed-based Functional Connectivity
Regions determined to have significant differences between PTSD cases and controls will be proposed for inclusion in separate seed-based FC analyses (e.g. dynamic rsFC proposal by Sun and Morey).

Machine Learning
ReHo and/or fALFF maps (including extracted values from any *a priori* identified ROIs) will be made available for use in separate classification machine learning analyses, such as support vector machine analysis (e.g. multimodal machine learning proposal by Zhu, Goldstein, Suarez-Jimenez, Wager, and Neria).

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