ENIGMA Resting State Pipeline:

Resting state functional connectivity using seed-based and dual regression analyses applied to PTSD

Sanne J.H. van Rooij[a]
Stephanie DeCross[b]
Kate McLaughlin[b]
Neda Jahanshad[c]
Peter Kochunov[d]

Rajendra A. Morey[e,f,g] on Behalf of ENIGMA-PTSD

[a] Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA,
[b] Department of Psychology, University of Washington, Seattle, WA
[c] Imaging Genetics Center, Stevens Neuroimaging & Informatics Institute, Keck School of Medicine of USC, Marina del Rey, CA
[d] Maryland Psychiatry Research Center, Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD
[e] Duke-UNC Brain Imaging and Analysis Center, Duke University, Durham, NC
[f] Department of Veteran Affairs (VA) Mid-Atlantic Mental Illness Research, Education and Clinical Center, Durham, NC
[g] Department of Psychiatry and Behavioral Sciences, Duke University, Durham, NC

ABSTRACT

While there are studies that have investigated resting state functional connectivity (RSFC) of the default mode and salience network in PTSD, study samples were small and findings not always consistent. Furthermore, not much research in PTSD has focused on other brain networks. Therefore, a large PTSD study investigating within and between network resting state functional connectivity is needed to better understand the role of these networks in the disorder, which may eventually contribute to the development new interventions or treatments for PTSD. Here we propose to investigate resting state functional connectivity in a large sample of PTSD patients (~1500) and controls (~1500) recruited through ENIGMA-PGC PTSD. A standardized seed-based and dual regression approach will be used, which allows future cross-disorder analyses

BACKGROUND

Posttraumatic stress disorder (PTSD) is a debilitating psychiatric disorder that one can develop after experiencing a traumatic event. In the last two decades, research has started to identify brain regions that are implicated in this disorder. Studies have revealed structural abnormalities, such as a smaller hippocampus (Logue *et al*, 2018), as well as altered brain activation during task-based fMRI. As it has become clear that brain structures operate in networks, neuroimaging studies have started to investigate brain functional connectivity, mainly during resting state. Most neuroimaging studies in psychiatric disorders have focused on one of three functional networks, the default mode network (DMN), the salience network (SN) and the executive control network (ECN), as it is suggested that abnormal organization, functioning, and interaction of these networks characterize many psychiatric and neurological disorders (Menon, 2011).

Resting state functional connectivity (RSFC) studies in PTSD have shown reduced functional coupling between different nodes of the DMN, i.e., between precuneus/posterior cingulate cortex (PCC) and the medial prefrontal cortex (mPFC), middle temporal gyrus (Bluhm *et al*, 2009) and posterior hippocampus (Chen and Etkin, 2013), and between the vmPFC and hippocampus (Sripada *et al*, 2012b). Several studies have also demonstrated increased functional connectivity between nodes of the SN, mainly the amygdala and insula (Rabinak *et al*, 2011; Sripada *et al*, 2012a; Sripada *et al*, 2012b). Results for amygdala-dorsal anterior cingulate (dACC) connectivity studies were mixed, with one study showing no difference with controls (Rabinak *et al*, 2011), whereas other studies showed increased (Brown *et al*, 2014) or decreased (Sripada *et al*, 2012a) dACC-amygdala connectivity in PTSD. It was suggested that psychiatric disorders are not (only) associated with alterations within these networks, but often characterized by abnormal interaction between networks. Resting state studies in PTSD demonstrated increased coupling between the salience and default mode networks (Jin *et al*, 2013; Sripada *et al*, 2012b). Altered functional connectivity is thought to result in an imbalance between salience detection and internally focused thought in PTSD patients: more intra-network connectivity and increased activation of the nodes of the SN and reduced connectivity and activation of nodes of the DMN (and CEN). Increased processing of stimuli that are considered salient by the individual (SN), while self-referential processing (DMN) is decreased, is thought to result in attentional bias to and increased processing of external threat stimuli. Furthermore, one study investigated connectivity between networks related to treatment outcome, and showed that increased connectivity between nodes of the CEN and DMN, i.e., the DLFPC and PCC, was related to a better treatment response to mindfulness-based exposure therapy (King *et al*, 2016).

While there are several studies that have investigated resting state functional connectivity (RSFC) of the DMN and SN in PTSD, study samples were small and findings not always consistent. Furthermore, not much research in PTSD has focused on the nodes of the ECN or the connectivity between the ECN nodes, and studies investigating functional connectivity of other networks, such as the auditory, visual, sensory motor, fronto-parietal or attention networks are lacking. Therefore, a large PTSD study

investigating RSFC is needed to better understand the role of these networks in the disorder, which may eventually contribute to the development new interventions or treatments for PTSD.

OBJECTIVE

Investigate RSFC in a large sample of PTSD patients and controls using a standardized seed-based and dual regression approach. Within-network RSFC of 8 networks will be compared between PTSD patients and controls using FDR-correction for multiple comparisons. We expect real findings to survive this strict correction as the study is well powered. Exploratory analyses comparing connectivity between whole networks will be performed. We will use standardized RSFC analyses (Adhikari *et al*, 2018), which will allow future cross-disorder analyses within the ENIGMA consortium.

RESEARCH PLAN

Participants: Resting state scans from ~1500 PTSD patients and ~1500 controls will be collected through the ENIGMA-PGC PTSD consortium. Written informed consent should be available for all participants, and only participants with no contraindication for MRI scans are included in the studies. PTSD is assessed with the clinician administered PTSD scale (CAPS), the PTSD symptom scale (PSS) or equivalent. Controls with past or current Axis I disorders will be excluded from the analyses. PTSD patients with a comorbid psychotic disorders or current substance disorder will be excluded from the analyses.

Resting state fMRI

Preprocessing

Resting state data from the different cohorts will be analyzed centrally using the ENIGMA resting-state pipeline (Figure 1; (Adhikari *et al*, 2018)). First, Marchenko-Pastur distribution-based principal components analysis (MP-PCA) will be applied for denoising (Veraart *et al*, 2016). This will improve signal-to noise ratio (SNR) and temporal SNR (tSNR) properties of the time series data without loss of spatial resolution of the image and the introduction of additional partial volume effects. Then spatial distortions associated with long-TE gradient echo imaging will be corrected using the gradient- echo 'fieldmap' or the reversed-gradient approach. Subsequently, the time of

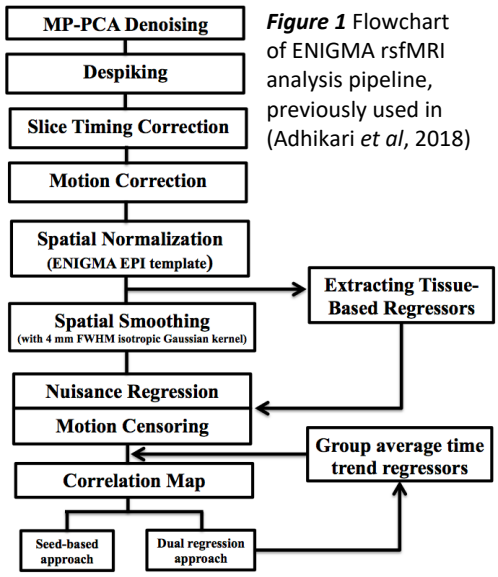


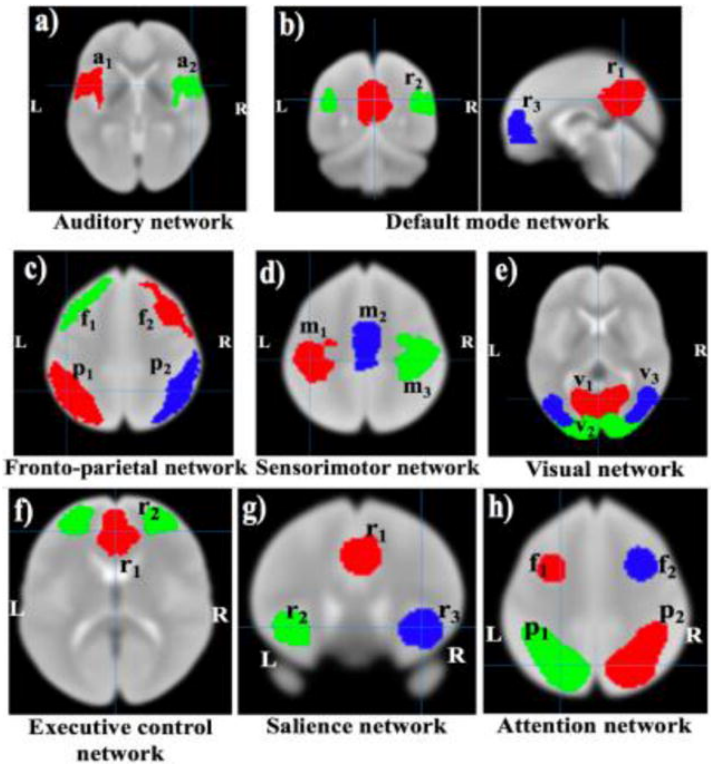
Figure 1 Flowchart of ENIGMA rsfMRI analysis pipeline, previously used in (Adhikari *et al*, 2018)

acquisition of each slice will be corrected. Furthermore, each functional volume will be registered to the volume with the minimum outlier fraction to correct for head motion, where each transformation is concatenated with the transformation to standard space to avoid unnecessary interpolation. Images will be spatially normalized to the ENIGMA EPI template in Montreal Neurological Institute (MNI) standard space for group analysis, and then smoothed with a 4-mm kernel. The effects of nuisance variables such as the linear trend, 6 motion parameters (3 rotational and 3 translational directions), their 6 temporal derivatives (rate of change in rotational and translational motion) and time courses from the local white matter and cerebrospinal fluid (CSF) from lateral ventricles will be regressed out. Finally, the time points with excessive motion (> 0.2 mm) estimated as the magnitude of displacement from one time point to the next, including neighboring time points and outlier voxels fraction (>0.1), will be censored from statistical analysis. Outlier images will be created based on the time points with excessive motion, and entered into the individual-level general linear model to remove the influence of these time points on estimates of functional connectivity while maintaining the temporal structure of the data. For more details (Adhikari *et al*, 2018).

First level analyses

First level analyses will also be performed using the ENIGMA resting state pipeline (Adhikari *et al*, 2018). Following Adhikari *et al.,* (2018), resting state network template ROIs will be based on the BrainMap activation database and resting fMRI dataset (Figure 2). In prior resting state studies different templates and ROIs have been used and sometimes other regions have been included in certain networks, but we will use the same template for consistency across ENIGMA studies. Mean time series will be extracted from the seed regions of each network and correlated with time series of the other regions. To get a normal distribution, fisher's *r*-to-*z* transformations will be applied. Seed-based functional connectivity values will be calculated between seed regions within each network and between networks. Second, dual regression analyses will be performed for the different network template ROIs and functional connectivity measures will be calculated. For each subject, average single time series will be computed from the preprocessed data, and averaged across all participants to calculate an average time series representing the group average time trend. Before calculating the functional connectivity values for each subject, this group average time trend will be regressed out from the data to measure how individual subjects differ within the group.

Figure 2 Resting state network template ROIs.



The resting state network template ROI are the same as in Adhikari *et al*., 2018. The *brain regions in the different networks are: a) a1, left primary auditory cortex, a2, right primary auditory cortex b) r1, posterior cingulate cortex, r2, angular gyrus 3, medial prefrontal cortex c) f1, left lateral prefrontal cortex, f2, right lateral prefrontal cortex, p1, left posterior parietal cortex, p2, right posterior parietal cortex d) m1, left postcentral gyrus, m2, supplementary motor area, m3, right postcentral gyrus e) v1, v2, v3, visual areas f) r1, anterior cingulate cortex, r2, dorsolateral prefrontal cortex g) r1, dorsal anterior cingulate cortex, r2, left insula, r3, right insula h) f1, left middle/inferior frontal gyrus, f2, right middle/inferior frontal gyrus, p1, left inferior parietal lobe, p2, right inferior parietal lobe*

Commented [Sv1]: not sure if all are correct. Especially: sensorimotor and visual networks

Network	Regions	PTSD vs. Controls	p-value
a. AN	a1-a2		
	a2-a1		
b. DMN	r1-r2		
	r2-r3		
	r3-r1		
	r2-r1		
	r3-r2		
	r1-r3		
c. FPN	f1-p1		
	p1-f1		
	f2-p2		
	p2-f2		
d. SMN	m1-m2		
	m2-m3		
	m3-m1		
	m2-m1		
	m3-m2		
	m1-m3		
e. VN	v1-v2		
	v2-v3		
	v3-v1		
	v2-v1		
	v3-v2		
	v1-v3		
f. ECN	r1-r2		
	r2-r1		
g. SN	r1-r2		
	r2-r3		
	r3-r1		
	r2-r1		
	r3-r2		
	r1-r3		

h. AttN	f1-p1		
	p1-f1		
	f2-p2		
	p2-f2		

Second level analyses

For the group analyses, functional connectivity measures within networks will be compared between the PTSD and control groups. Age, sex, depression (yes/no), comorbid anxiety disorder (yes/no), and scanner site will be used as covariates of no interest. For group-level effects to be considered significant a height-threshold of $p<0.001$ and a cluster-level false discovery rate (FDR) correction of $p<0.05$ will be used.

Exploratory between network analyses will be performed assessing the interaction between networks and groups (PTSD/controls) using a whole network approach instead of seed-based analyses.

Table 1 Overview of within network comparisons

REFERENCES

Adhikari BM, Jahanshad N, Shukla D, Glahn DC, Blangero J, Reynolds RC*, et al* (2018). Heritability estimates on resting state fMRI data using ENIGMA analysis pipeline. *Pac Symp Biocomput* **23**: 307-318.

Bluhm RL, Williamson PC, Osuch EA, Frewen PA, Stevens TK, Boksman K*, et al* (2009). Alterations in default network connectivity in posttraumatic stress disorder related to early-life trauma. *Journal of Psychiatry & Neuroscience : JPN* **34**(3): 187-194.

Brown VM, LaBar KS, Haswell CC, Gold AL, Mid-Atlantic MW, McCarthy G*, et al* (2014). Altered Resting-State Functional Connectivity of Basolateral and Centromedial Amygdala Complexes in Posttraumatic Stress Disorder. *Neuropsychopharmacology* **39**(2): 361-369.

Chen AC, Etkin A (2013). Hippocampal Network Connectivity and Activation Differentiates Post-Traumatic Stress Disorder From Generalized Anxiety Disorder. *Neuropsychopharmacology* **38**(10): 1889-1898.

Jin C, Qi R, Yin Y, Hu X, Duan L, Xu Q*, et al* (2013). Abnormalities in whole-brain functional connectivity observed in treatment-naive post-traumatic stress disorder patients following an earthquake. *Psychological Medicine* **44**(9): 1927-1936.

King AP, Block SR, Sripada RK, Rauch S, Giardino N, Favorite T*, et al* (2016). ALTERED DEFAULT MODE NETWORK (DMN) RESTING STATE FUNCTIONAL CONNECTIVITY FOLLOWING A MINDFULNESS-BASED EXPOSURE THERAPY FOR POSTTRAUMATIC STRESS DISORDER (PTSD) IN COMBAT VETERANS OF AFGHANISTAN AND IRAQ. *Depression and Anxiety* **33**(4): 289-299.

Logue MW, van Rooij SJH, Dennis EL, Davis SL, Hayes JP, Stevens JS*, et al* (2018). Smaller Hippocampal Volume in Posttraumatic Stress Disorder: A Multisite ENIGMA-PGC Study: Subcortical Volumetry Results From Posttraumatic Stress Disorder Consortia. *Biological Psychiatry* **83**(3): 244-253.

Menon V (2011). Large-scale brain networks and psychopathology: a unifying triple network model. *Trends in Cognitive Sciences* **15**(10): 483-506.

Rabinak CA, Angstadt M, Welsh RC, Kenndy AE, Lyubkin M, Martis B*, et al* (2011). Altered Amygdala Resting-State Functional Connectivity in Post-Traumatic Stress Disorder. *Frontiers in Psychiatry* **2**: 62.

Sripada RK, King AP, Garfinkel SN, Wang X, Sripada CS, Welsh RC*, et al* (2012a). Altered resting-state amygdala functional connectivity in men with posttraumatic stress disorder. *Journal of Psychiatry & Neuroscience : JPN* **37**(4): 241-249.

Sripada RK, King AP, Welsh RC, Garfinkel SN, Wang X, Sripada CS*, et al* (2012b). Neural Dysregulation in Posttraumatic Stress Disorder: Evidence for Disrupted Equilibrium between Salience and Default Mode Brain Networks. *Psychosomatic medicine* **74**(9): 904-911.

Veraart J, Novikov DS, Christiaens D, Ades-Aron B, Sijbers J, Fieremans E (2016). Denoising of diffusion MRI using random matrix theory. *Neuroimage* **142**: 394-406.