



RESEARCH ARTICLE

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# Altered functional connectivity in posttraumatic stress disorder with versus without comorbid major depressive disorder: a resting state fMRI study [v2; ref status: indexed, <http://f1000r.es/398>]

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**Abstract**

Posttraumatic stress disorder (PTSD) is an anxiety disorder that is often diagnosed with comorbid depressive disorder. Therefore, neuroimaging studies investigating PTSD typically include both patients with and without comorbid depression. Differences in activity of the anterior cingulate cortex (ACC) and insula have been shown to differentiate PTSD patients with and without major depressive disorder (MDD). Whether or not comorbid MDD affects resting state functional connectivity of PTSD patients has not been investigated to our knowledge. Here, resting state functional connectivity of PTSD patients with (PTSD+MDD; n=27) and without (PTSD-MDD; n=23) comorbid MDD was investigated. The subgenual ACC and insula were investigated as seed regions. Connectivity between the subgenual ACC and perigenual parts of the ACC was increased in PTSD+MDD versus PTSD-MDD, which may reflect the presence of depressive specific symptoms such as rumination. Functional connectivity of the subgenual ACC with the thalamus was reduced, potentially related to more severe deficits in executive functioning in the PTSD+MDD group versus the PTSD-MDD group. In addition, the PTSD+MDD group showed reduced functional connectivity of the insula with the hippocampus compared to the PTSD-MDD group. However, this cluster was no longer significantly different when PTSD patients that were using medication were excluded from analyses. Thus, resting state functional connectivity of the subgenual ACC can distinguish PTSD+MDD from PTSD-MDD, and this may therefore be used as a neurobiological marker for comorbid MDD in the presence of PTSD. As PTSD+MDD are more treatment resistant, these findings can also guide treatment development, for example by targeting the subgenual ACC network with treatment.

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Post-hoc analyses on a subsample of medication free PTSD patients were performed and added. In order to investigate confounding effects of general PTSD severity, correlation analyses with PTSD severity scores (total CAPS scores) and peak voxels of functional connectivity differences were performed (as opposed to an ANCOVA). In addition, correlation plots were added representing the relation between PTSD symptom clusters and the peak voxels of functional connectivity differences. Finally, we elaborated on the interpretation of our findings in the abstract and discussion.

**See referee reports**

**Introduction**

Posttraumatic stress disorder (PTSD) is an anxiety disorder that can develop after a traumatic event. It is characterized by re-experiencing the traumatic event, avoidance of trauma reminders and emotional numbing symptoms, and increased arousal<sup>1</sup>. PTSD frequently co-occurs with other Axis I psychiatric disorders, such as major depressive disorder (MDD<sup>2</sup>). Patients with both PTSD and depression were found to have more psychological distress and are also more treatment resistant than patients with PTSD or depression alone<sup>3-5</sup>. Studies have demonstrated that comorbidity between mood and anxiety disorders increases risk for cardiovascular disease, autoimmune diseases and mortality<sup>6,7</sup>. In addition, depressive symptom severity and comorbidity of MDD are related to poorer executive functioning in PTSD<sup>8,9</sup>. In order to better prevent, diagnose or treat these disorders it is of importance to determine biological overlap and differences between mood and anxiety disorders, and also the effect of comorbidity. About 48% of PTSD patients were found to have comorbid MDD in a large national survey in the United States<sup>2</sup>. Therefore, studies investigating the neurobiology of PTSD often comprise patients with and without comorbid MDD. Neuroimaging studies have demonstrated dysfunction of similar brain regions in both PTSD and MDD. That is, PTSD and MDD are both associated with alterations in structure and function of the medial prefrontal cortex (mPFC), amygdala, insula, and anterior cingulate cortex (ACC<sup>10-12</sup>). To what extent comorbid MDD contributes to the reported neurobiological alterations of PTSD is yet to be determined.

Thus far, two neuroimaging studies have directly investigated differences in PTSD patients with and without comorbid MDD. First, reduced activity of the mPFC and amygdala was found in PTSD patients with comorbid MDD versus PTSD patients without MDD, when fearful faces were presented<sup>13</sup>. Second, during a symptom provocation paradigm PTSD patients with comorbid MDD had decreased activity in the insula, and increased ACC and posterior cingulate cortex (PCC) activation versus PTSD patients without MDD<sup>14</sup>. In addition, decreased insula activation remained significant after controlling for PTSD severity. One other study has investigated the effects of depressive symptoms in PTSD patients. A positive correlation between depressive symptoms and (para) hippocampal and ventral ACC activity during an emotional memory task was observed in PTSD patients. A fourth fMRI study involving PTSD patients versus both controls and MDD patients found increased activity in several brain areas of PTSD patients including the insula when emotional pictures were presented<sup>15</sup>.

The four studies discussed above were limited by small sample sizes (8 PTSD-MDD, 8 PTSD+MDD<sup>13</sup>, 11 PTSD-MDD and 15 PTSD+MDD<sup>14</sup>, 21 PTSD+MDD and 12 PTSD-MDD<sup>16</sup>, 16 PTSD and 16 MDD<sup>15</sup>). In addition, these studies investigated neurobiological alterations during emotional tasks, potentially inducing PTSD (and/or depressive) symptoms. It is expected that PTSD (and/or MDD) symptom provocation induces an altered state in PTSD with or without MDD, which is reflected by alterations in brain activity. Whether regular functioning of the brain in the absence of symptom-inducing stimuli deviates in PTSD with versus without comorbid MDD remains unclear. To our knowledge, functioning of the brain during resting state, without presenting stimuli or requiring task performance, has not been investigated in PTSD patients with and without comorbid MDD. Thus, the effect of comorbid MDD on brain functioning at baseline of PTSD patients deserves further investigation.

Here, we investigate the effects of comorbid MDD on resting state functional connectivity in PTSD patients. Since the studies described above indicated that functioning of the ACC distinguishes PTSD with and without MDD during emotional tasks<sup>13,14,16</sup>, this brain area was chosen as a region of interest. MDD has been associated with alterations in structure<sup>17</sup>, function<sup>18</sup>, structural connectivity<sup>19</sup>, and reduced resting state functional connectivity<sup>20-22</sup> of the subgenual ACC in particular, which is a subdivision of the ventral ACC. In addition, subgenual ACC activation and cortical thickness have been associated with symptom improvement in PTSD<sup>23,24</sup>. Therefore, the subgenual ACC was selected as a more specific region of interest. Second, alterations in activation of the insula also differed between PTSD patients with and without PTSD, even when controlling for PTSD severity<sup>14</sup>. Furthermore, insula activation distinguished PTSD patients from MDD patients<sup>15</sup>. Alterations in structure<sup>10,25</sup>, function<sup>10,26</sup>, and resting state functional connectivity<sup>27,28</sup> have been reported in PTSD patients and MDD patients respectively. Thus, the insula was chosen as a second region of interest. As increased ACC activity was found in PTSD with comorbid MDD, as well as a positive correlation of ACC activity with depressive symptoms, we hypothesize that functional connectivity of the subgenual ACC is increased in PTSD with versus without comorbid MDD. Since insula activity is increased in PTSD versus MDD and insula activity was reduced in PTSD with comorbid MDD versus PTSD without MDD, we expected to find lower insula functional connectivity in PTSD with MDD as compared to PTSD without MDD. In summary, in order to provide more insights into the potential effects of MDD on the neurobiology of PTSD, the present study examined the effects of comorbid MDD on subgenual ACC and insula resting state functional connectivity in PTSD patients.

**Methods****Participants**

In total, 30 male veterans with PTSD with comorbid MDD (PTSD+MDD, mean age  $34.2 \pm 8.5$ ), and 25 male veterans with PTSD without comorbid MDD (PTSD-MDD, mean age  $37.4 \pm 10.1$ ) were included in this study. All patients were recruited from the Military Mental Health Care Center, the Netherlands. Patients were included after a clinician (psychologist or psychiatrist) diagnosed PTSD with or without MDD. PTSD and MDD diagnoses were confirmed using the Clinician Administered PTSD scale (CAPS<sup>29</sup>) and

the Structural Clinical interview for DSM-IV (SCID<sup>30</sup>). A clinician, a trained PhD student or a trained research assistant administered the interviews. Training included a CAPS training, and additionally observing at least five interviews, and performing at least five interviews under supervision of an experienced clinician. Several patients were medication naive (PTSD+MDD; n=15, PTSD-MDD; n=13), some patients were currently taking antidepressants (e.g. selective serotonin reuptake inhibitors; PTSD+MDD; n=4, PTSD-MDD; n=5), and some patients used benzodiazepines (PTSD+MDD; n=4, PTSD-MDD; n=1), or both antidepressants and benzodiazepines (PTSD+MDD; n=2, PTSD-MDD; n=2). One patient from the PTSD+MDD group used both antipsychotics and antidepressants. Most of the veterans had been deployed to Afghanistan (n=28) and to the former Yugoslavia (n=10). After receiving a complete written and verbal description of the study, all participants gave informed consent. Participants received financial compensation of €250 for their participation. The Medical Ethical Committee of the UMC Utrecht approved the study (protocol number NL29550.041.09), and the study was performed in accordance with the Declaration of Helsinki<sup>31</sup>.

### Data acquisition

Functional and structural images were obtained using a 3.0 Tesla magnetic resonance imaging scanner (Philips Medical System, Best, the Netherlands). Before the resting state scan, a ten minute T1-weighted high-resolution image (TR = 10 ms TE = 4.6 ms flip angle 8, 200 slices sagittal orientation, FOV 240 × 240 × 160, 304 × 299 matrix) was acquired. This image was utilized for co-registration and segmentation purposes and also allowed the participants to adapt to the scanner environment. During the nine minute resting state scan participants were asked to relax, to let their mind wander and to focus on a fixation cross. Three hundred and twenty T2\* echoplanar interleaved images were collected (TR = 1600 ms, TE = 23 ms, flip angle = 72.5°, 30 transverse slices, FOV 256 × 208 × 120, 64 × 51 matrix).

### Image analyses

Pre-processing was conducted with SPM5 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm5/>), which included slice-timing correction, realignment, co-registration with the anatomical scan, normalization, and spatial smoothing (8 mm FWHM). Five participants (2 PTSD+MDD, 3 PTSD-MDD) were excluded due to excessive motion (more than 2 mm displacement in any direction (x, y or z) or 2 degrees rotation (pitch, roll or yaw)).

The Data Processing Assistant for Resting-State fMRI (DPARSF) was utilized for further analyses (restfmri.net<sup>32</sup>), which is based on MRICroN (<http://www.mricro.com>), SPM5 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm5/>), and the Resting-State fMRI Data Analysis Toolkit<sup>32</sup>. Resting state images were band-pass filtered (0.08–0.01 Hz) to reduce low-frequency drift and high-frequency noise, and detrended to correct for general signal drift. In order to correct for physiological processes and motion, the motion parameters from the realignment step, mean global signal, white matter signal, and cerebral spinal fluid signal were included as covariates in the analysis. In addition, motion scrubbing was applied to scans that surrounded a minimum of 0.5 mm frame displacement (one scan before displacement, two scans after displacement), using

nearest neighbour interpolation<sup>33</sup>. A minimum of approximately 5 minutes of resting state (183 unscrubbed resting state images) was set as a required threshold for correct scrubbing. One participant was excluded due to excessive scrubbing, resulting in the following groups: 27 PTSD+MDD, and 22 PTSD-MDD.

### Functional connectivity analysis

For the subgenual ACC two spherical seeds (left and right, 3.5 mm radius) were created around two seed point coordinates, as previously described by Kelly *et al.* (2009)<sup>34</sup>. The anterior insula seed was created from two distinct anterior insula subdivisions that were described as the insula regions involved in emotion and cognition, as reported by Kelly *et al.* (2012)<sup>35</sup>. The mean time series for each of those seeds was extracted for all individuals and correlated with the time series of every voxel in the brain in order to create functional connectivity maps. These correlation maps were normalized using Fishers z-transform, resulting in a z-map for each ACC network per participant. The individual z-maps were used for second-level group analysis (full factorial design, SPM). A general effect of group (F-test) was investigated to determine group differences within the positive and negative network of the seed pairs.

Cluster-level multiple comparison correction was applied according to Gaussian Random Field theory<sup>36</sup>. A height threshold of  $p < 0.001$  was applied and combined with a cluster threshold extent that corresponds to a corrected  $p < 0.05$  (as determined with 1000 Monte Carlo simulations using AlphaSim, implemented in the REST toolbox).

In addition, functional connectivity values (z-values) were extracted from the peak voxels of clusters of significant differences in order to perform post-hoc correlations with PTSD and MDD symptom severity. Post-hoc correlation analyses were performed including the total CAPS score and the signal extracted from the peaks of clusters of significant connectivity differences, in order to assess whether the results are related to PTSD severity. In addition, the relation of positive affect (PA) score from the mood and anxiety questionnaire (MASQ<sup>37</sup>), which has been reported to reflect a core feature of MDD<sup>38</sup>, to the functional connectivity of the peak of the clusters of significant difference was assessed. Subsequently, correlations between whole brain functional connectivity and CAPS and inverse PA scores were calculated respectively. Finally, we performed a post-hoc analysis on a subsample of medication naive patients and patients that occasionally used benzodiazepines, but had not taken benzodiazepines at least 48 hours prior to scanning.

## Results

### Participants

Groups did not differ significantly in age, handedness, the number of times they were deployed, the time since their last deployment, and educational level as measured with the international standard classification of education (ISCED<sup>39</sup>). The PTSD+MDD group differed from the PTSD-MDD group in total PTSD severity (CAPS score;  $p = 0.008$ ), which appeared to be largely driven by differences in avoidance and emotional numbing symptom scores (cluster C;  $p = 0.001$ ). In addition, the PTSD+MDD group had lower PA scores versus the PTSD-MDD group ( $p = 0.012$ ), while negative affect and somatic anxiety did not differ between groups. In the PTSD+MDD group 10 patients were diagnosed with a comorbid anxiety disorder

(n=10), and one patient had a comorbid somatoform disorder. In the PTSD-MDD group seven patients met the current diagnostic criteria for a comorbid anxiety disorder, one patient had a somatoform

disorder only, and one patient was diagnosed with both a comorbid anxiety and somatoform disorder. An overview of demographical and clinical data is presented in [Table 1](#).

**Table 1. Demographic and clinical characteristics of the PTSD+MDD and the PTSD-MDD group.**

Measure	PTSD + MDD (mean ± SD)	PTSD - MDD (mean ± SD)	df	Sig. (two-tailed)
N	27	22		
Age (range 21–57)	37.41 (±10.12)	33.87 (±8.43)	47	0.239
Education (ISCED level)	4.00 (±1.20)	3.65 (±1.23)	46	0.311
Handedness (Right/Left/Ambidexter)	(21/4/2)	(20/0/2)	2	0.169
Number of times deployed (range 1–15)	2.16 (±1.43)	3.18 (±4.22)	45	0.898
Time since last deployment (years)	8.00 (±8.537)	7.05 (±8.72)	45	0.706
Country of last deployment				
Afghanistan	13	17		
Former Yugoslavia	6	3		
Other	8	5		
CAPS total score	75.15 (±12.45)	65.09 (±12.87)	47	0.008*
Cluster B	22.67 (±5.61)	22.64 (±5.43)	47	0.985
Cluster C	27.48 (±8.76)	18.59 (±8.30)	47	0.001*
Cluster D	25.00 (±4.47)	23.86 (±4.97)	47	0.404
Negative Affect (MASQ)	52.12 (±14.91)	46.00 (±10.50)	42	0.130
Positive Affect (MASQ)	40.87 (±15.80)	51.70 (±10.50)	42	0.012*
Somatic Anxiety (MASQ)	44.75 (±13.52)	41.50 (±10.91)	42	0.392
Current comorbid disorder (SCID)				
Major depressive disorder	27	-		
Anxiety disorder	10	7		
Anxiety disorder & somatoform disorder	-	1		
Somatoform disorder	1	1		

\*Significant differences between groups;  $p < 0.05$

## Functional connectivity

**Spatial connectivity maps.** [Figure 1](#) shows the positive and negative networks for the bilateral insula and the bilateral subgenual ACC. Positive functional connectivity of the subgenual ACC was found with the ventromedial PFC, temporal regions (including the hippocampus) and a posterior cluster comprising the PCC/precuneus. Positive functional connectivity of the insula was found around the insular lobe, extending into the temporal and parietal lobe. A medial cluster around the dorsal ACC showed positive functional connectivity with the insula.

## Group differences

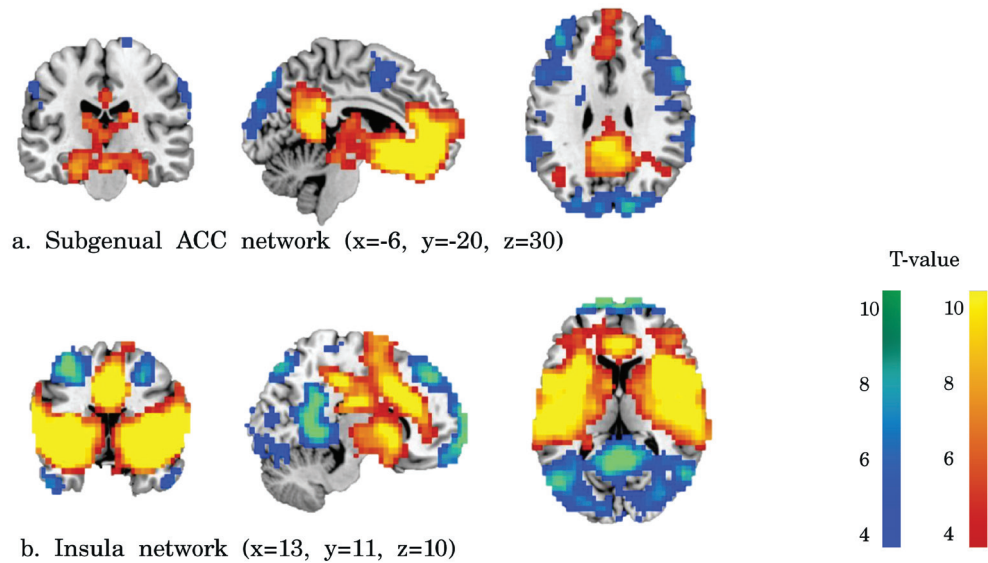
**Subgenual ACC.** Reduced functional connectivity of the PTSD+MDD group versus the PTSD-MDD group was found in functional connectivity of the subgenual ACC with the bilateral thalamus (Left thalamus; 29 voxels; peak value  $F=25.71$ ; peak MNI-coordinates  $x=-12, y=-16, z=4$ . Right thalamus; 16 voxels; peak value  $F=34.37$ ; peak MNI-coordinates  $x=20, y=-12, z=4$ ). Increased functional

connectivity was found between the subgenual ACC and perigenual regions of the ACC (peak in left perigenual ACC; 100 voxels; peak value  $F=25.71$ ; peak MNI-coordinates  $x=-12, y=40, z=-4$ ) in the PTSD+MDD group versus the PTSD-MDD group (see [Figure 2](#), [Figure 3](#) and [Table 2](#)).

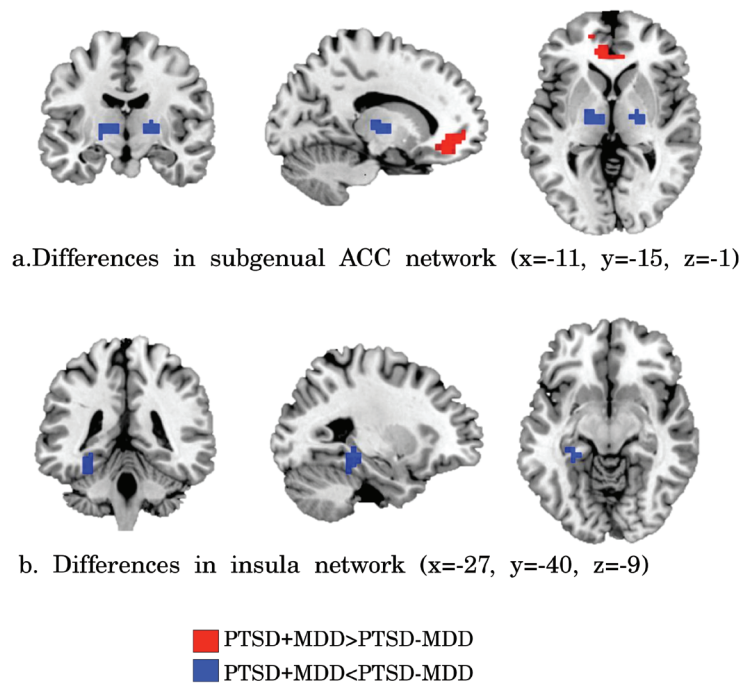
**Insula.** Functional connectivity of the bilateral insula with the left hippocampus (17 voxels; peak value  $F=19.05$ ; peak MNI-coordinates  $x=-28, y=-32, z=-8$ ) was reduced in the PTSD+MDD group as compared to the PTSD-MDD group, which showed no functional connectivity between these regions (see [Figure 2](#), [Figure 3](#), and [Table 2](#)).

## Post-hoc analyses

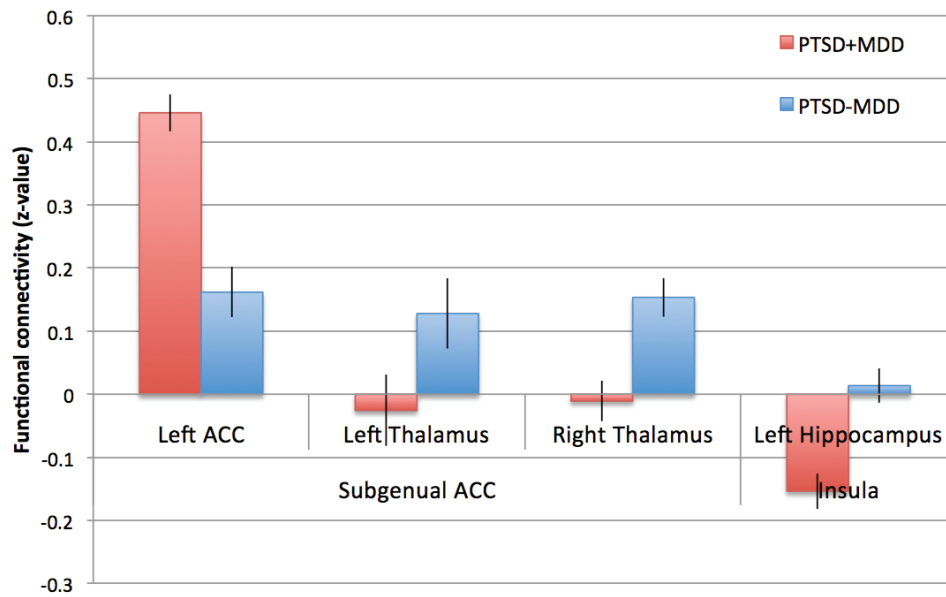
Post-hoc correlation analyses of the peak voxels of significant functional connectivity difference with CAPS total, CAPS symptom cluster, and inverse PA scores were performed within both groups separately. No significant correlations were found between the



**Figure 1.** Functional connectivity of the subgenual ACC (a), and insula (b) seeds. Positive connectivity is represented in red-yellow and negative connectivity in blue-green. The effects were FDR corrected  $p < 0.001$  for illustrative purposes.



**Figure 2.** Clusters of significant different functional connectivity of the insula (a) and subgenual ACC (b) seeds. Increased functional connectivity in PTSD+MDD versus PTSD-MDD is shown in red and reduced connectivity in blue (FDR corrected  $p < 0.05$ ).



**Figure 3.** Functional connectivity of peak voxels of significant differences for the subgenual ACC and insula network. Z-values of the peak voxels for the PTSD-MDD group (red) and the PTSD+MDD (blue) group are presented. Error bars represent the standard error of the mean. Z-values of the peak voxels for the PTSD-MDD group (red) and the PTSD+MDD (blue) group are presented.

**Table 2.** Peak voxels of significant differences between PTSD+MDD and PTSD-MDD for the subgenual ACC and insula.

Network	Number of voxels (k)	Peak value (F)	MNI coordinates			Brain area
			x	y	z	
Subgenual ACC	100	25.71	-12	40	-4	Left Anterior cingulate cortex
	29	23.79	-12	-16	4	Left thalamus
	16	34.37	20	-12	4	Right thalamus
Insula	17	19.05	-28	-32	-8	Left hippocampus

peak voxels and total CAPS score and inverse PA scores. Correlations with symptom clusters revealed two significant correlations and these correlations are also represented over all participants for illustrative purposes (Figure 4). Within the PTSD+MDD group CAPS cluster B scores correlated negatively with connectivity of the subgenual ACC with the peak voxel of significant difference in the perigenual ACC ( $r = -0.396$ ,  $p=0.041$ ; Figure 4a). CAPS cluster C scores correlated negatively with connectivity of the subgenual ACC with the peak voxel of significant difference in the left thalamus ( $r = -0.523$ ,  $p=0.012$ ) within the PTSD-MDD group (Figure 4b). No correlations were found between CAPS cluster D scores or inverse PA scores and the peak voxels of difference in connectivity.

Exploring the relation of whole brain subgenual ACC connectivity with CAPS and inverse PA scores revealed a negative correlation of CAPS and inverse PA scores with subgenual ACC-PCC/precuneus

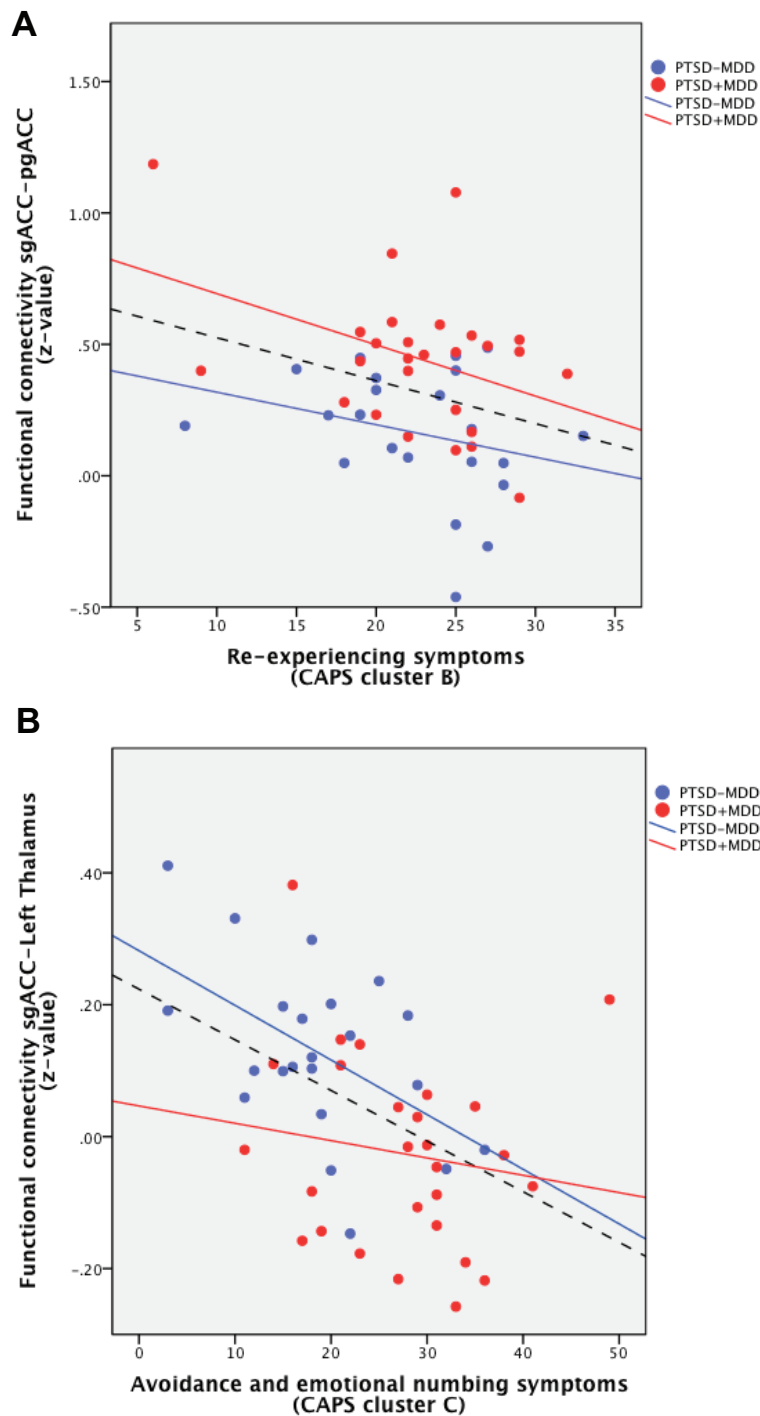
connectivity, amongst other regions (see Supplementary Figure S1). In addition, a negative correlation was found between CAPS and inverse PA scores and negative functional connectivity of the insula with the PCC/precuneus (see Supplementary Figure S1).

Finally, when PTSD patients that were taking medication were excluded from analyses (PTSD+MDD  $n = 20$ , PTSD-MDD  $n = 15$ ) similar clusters of significant differences for the subgenual ACC network were found. The cluster of significant differences in functional connectivity between the hippocampus and insula was no longer significant in this subsample.

**fMRI data of PTSD patients with and without comorbid Major Depressive Disorder**

1 Data File

<http://dx.doi.org/10.6084/m9.figshare.882837>



**Figure 4.** Correlations between CAPS symptom cluster scores and resting state functional connectivity of peak voxels of the significant different clusters within the PTSD+MDD group (red) and the PTSD-MDD group (blue). A correlation line for the whole group is also represented for illustrative purposes (dashed black line). Connectivity of the subgenual ACC with the perigenual ACC correlated with cluster B symptoms (re-experiencing; **4a**). Connectivity of the subgenual ACC with the left thalamus correlated with cluster C symptoms (avoidance and emotional numbing; **4b**). Abbreviations: sgACC: subgenual ACC, pgACC: perigenual ACC.



## Discussion

This study compared PTSD patients with and without comorbid depressive disorder and revealed differences in resting state functional connectivity of the subgenual ACC with the perigenual ACC and thalamus, and of the insula with left hippocampus. This study complements previous task-based studies<sup>13,14</sup> by showing that differences in the subgenual ACC and insula between PTSD patients with and without comorbid MDD are already apparent during resting state functional connectivity, in the absence of symptom-inducing stimuli or task performance. Based on these findings, it can be hypothesised that MDD comorbidity in the context of PTSD is related to general alterations in subgenual ACC and insula functioning.

Increased subgenual ACC connectivity with the perigenual ACC was found in PTSD+MDD versus PTSD-MDD, which is in line with neuroimaging studies that have found increased resting state functional connectivity between the subgenual ACC and perigenual ACC in MDD versus controls<sup>20–22</sup>. Reduced functional connectivity of ACC regions has been shown in PTSD patients versus controls<sup>40</sup>. Thus, the current finding of increased connectivity of the subgenual ACC with the perigenual ACC may indeed be a marker of the presence of MDD in the context of PTSD. The perigenual ACC, which is part of the medial PFC, has been related to self-referential processing<sup>41</sup>, which underlies depressive symptoms such as helplessness, self-reproach and (guilt) rumination<sup>21,42</sup>. Increased resting state functional connectivity in the medial PFC (including the perigenual ACC) has been directly related to rumination in MDD<sup>43</sup>, while decreased functional connectivity with the medial PFC has been related to autobiographical memory recall in PTSD versus controls<sup>44</sup>. Altered functioning of the medial PFC during self-referential processing tasks has also been found in MDD patients versus controls<sup>42,45,46</sup> (reduced medial PFC deactivation), and in PTSD versus controls<sup>47</sup> (reduced medial PFC activation). Increased subgenual-perigenual ACC connectivity in the PTSD+MDD group versus the PTSD-MDD group could thus reflect a difference in self-referential processing, and potentially reflects symptoms such as rumination. However, this was not directly investigated here, and is subject to further investigation.

A negative correlation between re-experiencing symptoms and functional connectivity of the subgenual ACC and perigenual ACC was found within the PTSD+MDD group (and across all patients). The same pattern was visible in the PTSD-MDD group, although this correlation was not significant. These correlations indicate that stronger functional connectivity between the subgenual ACC and perigenual ACC is related to lower (PTSD-specific) re-experiencing symptoms. This is in line with a previous study describing reduced connectivity in midline structures during autobiographical memory recall in PTSD versus controls<sup>44</sup>, indicating that the medial PFC can indeed be involved in re-experiencing autobiographical traumatic events. Thus, stronger functional connectivity between the subgenual ACC and perigenual ACC may reflect the presence of MDD, and is also negatively related to (PTSD specific) re-experiencing symptoms.

Connectivity between the thalamus and subgenual ACC was reduced in PTSD+MDD versus PTSD-MDD, which was also reported in previous studies in both depression<sup>48</sup> and PTSD<sup>49</sup> versus healthy

controls. The thalamus is the relay station of the brain<sup>50</sup>, and can modulate attention and arousal<sup>51</sup>. Therefore, reduced thalamus-subgenual ACC connectivity may explain the more severe problems with executive function that are prevalent in PTSD with comorbid MDD<sup>8,9</sup>. Functional connectivity between the subgenual ACC and thalamus was negatively correlated with avoidance and emotional numbing symptoms in the PTSD-MDD group (and across all participants). Emotional numbing is a shared PTSD and MDD symptom. A weaker connection between the subgenual ACC and thalamus, that was found in PTSD+MDD versus PTSD-MDD, may therefore reflect the presence of depression-related symptoms. Thus, reduced thalamus-subgenual ACC connectivity is a marker for comorbid MDD in the context of PTSD, and also relates to avoidance and emotional numbing symptoms.

Insula connectivity with the hippocampus was reduced in the PTSD+MDD group versus PTSD-MDD. The hippocampus is a brain region that is often associated with PTSD<sup>11,52,53</sup> and is involved in memory<sup>54</sup>. Therefore, differences found in connectivity between the insula and hippocampus can be related to more severe difficulties in executive functioning that are prevalent in PTSD+MDD versus PTSD-MDD<sup>8,9</sup>. However, the cluster was no longer significant when patients that were taking medication were excluded from analyses. Thus, hippocampus-insula connectivity differences may have been induced by medication use.

In our whole brain post-hoc correlation analysis negative correlations were found between symptom severity scores and subgenual ACC connectivity with the PCC/precuneus (see [Supplementary Figure S1a](#)). Specific correlations between CAPS scores and subgenual ACC-PCC/precuneus connectivity were also present, while controlling for inverse PA scores. The medial PFC (including ACC regions) and PCC/precuneus are regions of the default mode network (DMN), which is the network that is active during rest and deactivated during task performance<sup>55</sup>. DMN functional connectivity has been negatively correlated with general symptom severity in PTSD in previous studies, even when correcting for depression diagnosis<sup>56</sup> and depression severity<sup>57</sup>, which is in line with our results. In addition, a negative correlation was found between symptom severity scores and negative functional connectivity (anticorrelation) between the insula and PCC/precuneus (see [Supplementary Figure S1d](#)). Alterations in anticorrelation between the insula network and the DMN has been described in PTSD and depression<sup>27,28,58</sup>. In healthy subjects the insula-PCC/precuneus anticorrelation represents a dynamic equilibrium between engagement of networks during different circumstances, and dysfunctional anticorrelation is thought to underlie attentional problems<sup>59</sup>. Thus, the negative correlation between symptom severity and anticorrelation between DMN and insula may reflect a disequilibrium between networks and can potentially be related to attentional problems in PTSD patients (with or without comorbid depression).

Unravelling the neurobiological features of MDD and PTSD during rest can provide insights into which specific brain areas could be targeted for effective treatments. For example, tasks, psychotherapy, or brain stimulation methods that alter functional connectivity between the regions with dysfunctional connectivity may be effective<sup>60,61</sup>. Future studies should investigate long-term effects of

training, transcranial magnetic stimulation, transcranial direct current stimulation, or deep brain stimulation on functional connectivity. In addition, in severe treatment resistant PTSD+MDD surgical treatment may be considered, targeting the regions with altered functional connectivity. The thalamus for example has already been implicated as a target for deep brain stimulation of severe MDD<sup>62</sup> and can therefore be a candidate for treatment in PTSD+MDD as well. This is particularly relevant for treatment of PTSD patients with comorbid MDD, since patients with this combination of psychiatric disorders tend to be more treatment resistant.

### Limitations

This study has some limitations. First, no MDD only group or control group was included for analyses in the current study. Thus, this study does not show whether subgenual ACC and insula connectivity differs from patients with MDD only nor does it show if the patients deviate from controls. The current results only give insight in the effects of comorbid MDD in the context of PTSD, and not on general effects of PTSD or MDD. Inclusion of more control groups in future research can provide more insight in the specific effects of PTSD, MDD, and their neurobiological overlap or differences. Second, no validated measure of the severity of all MDD symptoms was included in the study. If MDD severity was measured, it would have been possible to determine common and distinct factors of PTSD symptom severity and MDD symptom severity by including both measures in a single model (as attempted in the [Supplementary Figure S1](#)). Here, MDD diagnosis was determined with the SCID, and depressive symptom severity was approximated with the positive affect scale of the MASQ, which is only representative of a subset of symptoms (reduced positive affect). Future studies should investigate the specific effect of MDD symptom severity in the presence of comorbid PTSD, measured with more sensitive and comprehensive instruments.

### Conclusion

This study revealed differences between PTSD+MDD and PTSD-MDD in resting state functional connectivity of the subgenual ACC with the perigenual ACC and bilateral thalamus. Reduced connectivity of the perigenual ACC with the subgenual ACC may be related to specific depressive symptoms, such as rumination. A negative relation was found with PTSD-specific re-experiencing

symptoms, indicating that reduced subgenual ACC connectivity with the perigenual ACC is a marker of MDD and negatively related to PTSD-specific symptoms. Increased thalamus connectivity with the subgenual ACC can potentially be related to deficits in executive functioning in PTSD+MDD versus PTSD-MDD. Differences in connectivity of the insula and hippocampus were also found, but may have been induced by confounding effects of medication. The current study shows the potential of resting state analyses to differentiate between PTSD patients with versus without MDD and provides more insight in the neurobiological differences between these subgroups. These findings provide neurobiological markers for the presence of comorbid MDD in the context of PTSD and may potentially be targeted with treatment.

### Data availability

figshare: fMRI data of PTSD patients with and without comorbid Major Depressive Disorder, <http://dx.doi.org/10.6084/m9.figshare.88283762>

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### Author contributions

EG and AR have made a substantial contribution to the conception and design of the study. MK and SvR have made a substantial contribution to the acquisition of data. MK performed the analyses and prepared the first draft of the manuscript. EG, AR, SvR and RK were involved in the interpretation of the data, and critically reviewing the article. All authors have agreed to the final content of the article.

### Competing interests

No competing interests were disclosed.

### Grant information

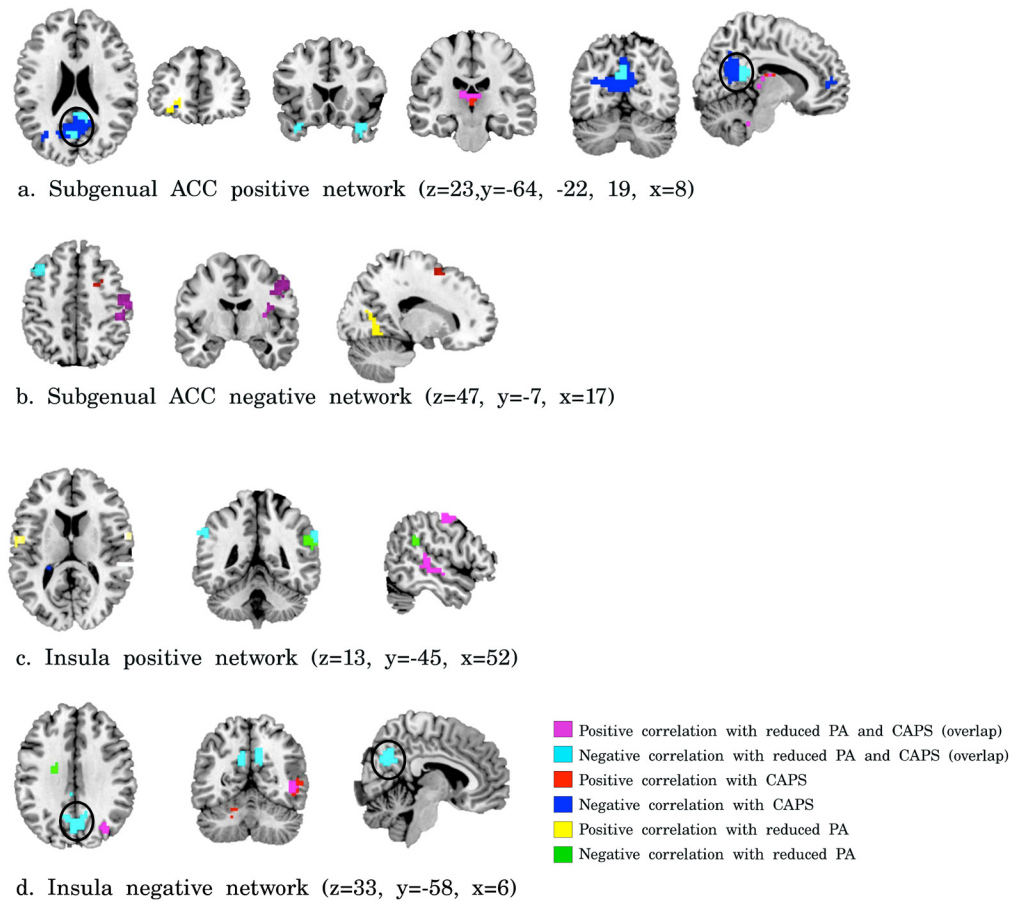
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### Acknowledgements

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## Supplementary material



**Supplementary Figure 1.** Correlations of PTSD symptom severity and reduced positive affect with subgenual ACC (**a, b**) and insula (**c, d**) functional connectivity. Violet = positive correlation with both CAPS scores and reduced PA, cyan = negative correlations with both CAPS scores and reduced PA, red = positive correlations with CAPS scores, blue = negative correlations with CAPS, yellow = positive correlations with reduced PA, and green = negative correlation with reduced PA (corrected  $p < 0.05$ ).

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# Open Peer Review

Current Referee Status:



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## Version 2

Referee Report 09 June 2014

doi:[10.5256/f1000research.4220.r4508](https://doi.org/10.5256/f1000research.4220.r4508)



**Andrew Kemp**<sup>1,2</sup>

<sup>1</sup> Centre for Clinical and Epidemiologic Research, Hospital Universitário, University of São Paulo, São Paulo, Brazil

<sup>2</sup> Discipline of Psychiatry and School of Physiology, University of Sydney, NSW, Australia

I appreciate the authors detailed responses to my earlier comments. The reiterated manuscript makes a solid and welcome contribution to the literature. Well done!

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

**Competing Interests:** No competing interests were disclosed.

Referee Report 16 May 2014

doi:[10.5256/f1000research.4220.r4792](https://doi.org/10.5256/f1000research.4220.r4792)



**Onno Meijer**

Medical Pharmacology, Leiden University, Leiden, Netherlands

I think the authors did a decent job in reviewing the paper. I have no further comments to the content of the article.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

**Competing Interests:** No competing interests were disclosed.

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## Version 1

Referee Report 26 February 2014

doi:[10.5256/f1000research.3319.r2919](https://doi.org/10.5256/f1000research.3319.r2919)



**Onno Meijer**

Medical Pharmacology, Leiden University, Leiden, Netherlands

The paper shows convincing (to the technical non-specialist) differences in resting state functional connectivity between PTSD patients with or without comorbid major depression MDD.

There are clear effects on connectivity of the subgenual anterior cingulate cortex with the left ACC (increased with MDD) and with the thalamus (decreased with MDD).

The interpretations are hampered by the design, which ideally would be a 2 X 2, i.e. +/- MDD and +/- PTSD. Therefore sentences like 'we found that connectivity ... is more reduced in PTSD +MDD than in PTSD-MDD' cannot be made.

The authors determined that effects on connectivity of insula and hippocampus are related to PTSD symptom severity - this should probably be indicated in figure 3 somehow, as this may end up being used as a graphic summary of the data.

I missed whether the authors looked at the use of medication as a factor that affected connectivity, as it may interact with the condition.

Second the authors, at correlation between connectivity (thalamus - sgACC) and symptom severity in the PTSD-MDD, group in a post hoc correlation. A similar effect is reported specifically for the PTSD+MDD group. It is unclear why the authors did not look at the correlation in all patients? What is the reason to now limit to a single group?

Interpretation: I do not see how on p.6 the authors suggest the cluster B symptoms would tilt the balance towards a PTSD only state, based on correlation, when table 1 clearly shows no differences between the groups for cluster B symptoms.

In terms of interpretation, I agree with the first reviewer that the statement in the abstract that the findings have impact on treatment development need to be substantiated. As markers? As targets?

It also seems important whether or not PTSD and MDD *interact* in terms of connectivity, or whether the association of MDD and PTSD 'just' add up. For this a 2x2 design seems necessary, but the paper may benefit from actual meaning that is given to the data. Obviously, at present describing correlates is what is done in research like this, but an explicit sense of direction would be helpful to the non-specialist reader who is nevertheless interested in the subject matter.

**Minor remarks:**

- I am not certain that (as in: I do not think that) the second, long, sentence in the discussion accurately reflects the findings, please check.
- The term *however* in the third paragraph of the intro seems needlessly negative about previous research.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

**Competing Interests:** No competing interests were disclosed.

Author Response 07 Apr 2014

**Mitzy Kennis**, University Medical Center Utrecht, Netherlands

We thank the reviewer for his valuable suggestions. See below our response to the comments.

**Design and interpretations:**

We agree with the reviewer that our study is limited by design, and lacks an MDD only group and a healthy control group. We acknowledge that we can therefore not clearly interpret the effects described here, with respect to the nature of either one of these disorders or the interaction of the two. As the reviewer suggests, we have attempted to be more cautious with our interpretations and elaborated on the implications (sense of direction, specific implications for treatment development) of the results in the discussion and abstract.

**Analyses:**

As proposed, additional analyses on a subsample of medication naive PTSD patients and PTSD patients that did not use benzodiazepines at least 48 hours prior to scanning were performed to investigate the effect of medication.

It was also questioned why we did not correlate the peak voxel of functional connectivity of the clusters of significant differences with all participants symptom scores, but only within groups. As also pointed out in our response to Andrew Kemp: a correlation analysis with the cluster of significant differences over all participants is inappropriate, since groups differ on both these values (functional connectivity cluster peak and PTSD severity (total CAPS score)) and therefore misleading correlations can be induced. To avoid this problem, within group correlations can be performed and seem more appropriate here. Finally, we have also added figures of the correlations with CAPS symptom clusters.

**Competing Interests:** No competing interests were disclosed.

Referee Report 30 January 2014

doi:[10.5256/f1000research.3319.r2918](https://doi.org/10.5256/f1000research.3319.r2918)



**Andrew Kemp**<sup>1,2</sup>

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<sup>2</sup> Discipline of Psychiatry and School of Physiology, University of Sydney, NSW, Australia

Thank you for the opportunity to review this interesting study on the impact of disorder comorbidity on resting state functional MRI. This is an important and under-studied area of research. I have a number of comments that may help to improve the manuscript.

**Abstract:**

- The abstract is disappointing, dense and requires development. At present it is mostly comprised of statements relating to the findings obtained, rather than providing an interpretation of



findings. Are all the findings reported key to the bottom-lines of the article? The authors need to add a brief interpretation after each of the KEY findings. What is the take home message; what are the bottom-lines? While the findings may indeed be “*important for treatment development*”, it is unclear how. Please elaborate.

#### Introduction:

- The first paragraph would benefit from a little more context on why comorbidity is important. For instance, studies have demonstrated that comorbidity between the mood and anxiety disorders increases risk for CVD and mortality (Philips *et al.*, 2009). I would recommend the authors integrate this information to help build study rationale.
- While the rationale for focusing on subgenual ACC as a seed region in connectivity analysis is well-founded, I thought the rationale for focusing on the insula to be much weaker. I would have thought that the amygdala region would have been a more appropriate second choice, especially given the many past studies that have focused on the amygdala as a region of interest in patients with PTSD.

#### Methods:

- re Participants section: who administered the psychiatric evaluation; were they psychiatrists or research assistants? How many people conducted the evaluations? How was the reliability and validity of this measurement across assessors determined? If research assistants, how much training did they receive? What was the value of the financial compensation received by participants?
- re Data Acquisition: To what extent could this (incentivisation) have impacted on findings (i.e. group interaction) especially given the unrestricted participant instructions (i.e. relax and let the mind wander)? Was any questionnaire administered to assess mental state of participants during the resting state scan?

#### Results:

- I recommend the authors to review the article by Miller & Chapman (2001) on the problems associated with ANCOVA when participants are not randomly allocated to a group, as in the present study. The use of total CAPS scores as a covariate in post-hoc analysis of covariance is problematic.
- One potential solution to this problem that the authors could consider is post-hoc correlational analysis. Although the authors ran a series of correlational analyses, these need to be conducted on all participants regardless of group, rather than by each group individually. The authors would need to determine the unique activations associated with the PTSD, with versus without depression, a contrast that is not associated with confounding variables (i.e. CAPS, positive affect).
- Another issue the authors need to consider is the impact of medication on their findings. While the authors note in their limitations that “*future studies should investigate the effect of medication on the neurobiology of PTSD with or without MDD*”, the authors have a sufficient sample of medication naive participants on which sub-analysis could be run.
- Error bars need to be added to Figure 3.

**Discussion:**

- The authors link increases in subgenual-perigenual ACC connectivity in the PTSD+MDD group versus the PTSD-MDD group to differences in self-referential processing. This is an interesting point which I feel could be further elaborated (after of course confirming that these findings are still present after adjusting for confounds -see points I made regarding this issue under the results section). The same could be said for the discussion on the relationship between the disorders and the default mode network.
- I also feel the discussion section needs to be further developed. Currently, much of the discussion restates the findings without more detail on what the differences might mean (especially in the first paragraph of the discussion). This makes the discussion section a little underwhelming.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

**Competing Interests:** No competing interests were disclosed.

Author Response 07 Apr 2014

**Mitzy Kennis**, University Medical Center Utrecht, Netherlands

We thank the reviewer for his valuable comments. See below our response to the comments per section of the manuscript.

**Abstract and introduction:**

We have attempted to make the abstract clearer, and added more context in both the abstract and introduction. We also acknowledge that the amygdala could have been another interesting seed to investigate PTSD with and without comorbid MDD. However, since the two previous neuroimaging studies that investigated PTSD and MDD with task paradigms reported alterations in the insula ( [Lanius et al., 2007](#); [Whalley et al., 2009](#)) while differences in the amygdala between PTSD and MDD patients were only reported by [Whalley et al. \(2009\)](#). Therefore, we argued that the insula is an appropriate candidate to investigate here.

**Methods:**

In order to address the concerns on the methods section, we provide more details on the administration of the interviews and financial compensation. Reliability and validity were qualitatively assessed by comparing interview scores and diagnosis between raters during interview training.

With respect to the resting state design, we do not expect any effects of the financial incentive on the pattern of functional connectivity. Every participant received a similar financial compensation for participating in the study. We assessed mental states of participants during resting state after the scan session, by asking whether they felt relaxed during the rest scan. Most participants confirmed feeling relaxed during the resting state scan, and ten participants reported having some difficulties to relax (five in each group). Furthermore, we investigated co-activation patterns of the subgenual ACC and insula. Although mind states are of influence on brain activation it is not

expected that different mind states (e.g. individuals that tend to think of the incentive) directly alter the spatial co-activation patterns within these functional networks.

### **Results:**

We agree with the reviewer that the use of ANOVA applied here is inappropriate. However, the suggested solution, a correlation analysis with the cluster of significant differences over all participants, is also inappropriate, since groups differ on both these values (functional connectivity cluster peak and PTSD severity (total CAPS score)). Therefore misleading correlations can be induced (see Lord's Paradox; Lord, 1967).

Unfortunately, as [Miller and Chapman \(2001\)](#) have shown, there is no simple solution to "adjust" or "control" for differences between groups in non-randomized groups.

On the other hand, we agree with the reviewer that correlations can be explored in order to provide some insight in the relation between functional connectivity and symptom severity, but then *within* groups rather than over all participants (to avoid misleading correlations). Thus, we have explored correlations within PTSD+MDD and PTSD-MDD groups between symptom severity scores (total CAPS and reduced PA) and the peak voxels of significant differences. There were no significant correlations between PTSD severity and functional connectivity. This suggests that functional connectivity of the clusters of significant differences was not related to differences in total CAPS or reduced PA scores.

Additionally, for the correlations with the symptom clusters performed within groups (for the same reasons as described above), we plotted the data for all subjects and added a fitted line for the data for each group and for the whole group respectively (see Figure S2 and S3). This way, an objective overview of the data is presented and both whole group and subgroup correlations are visualized.

Finally, as requested we performed a post-hoc analysis to investigate the effect of medication by including medication naive PTSD patients and PTSD patients that did not use benzodiazepines at least 48 hours prior to scanning (PTSD+MDD n = 20, PTSD-MDD n = 15). This revealed similar results for the subgenual ACC seed, although the hippocampal cluster of difference in connectivity with the insula disappeared. This information has been added to the paper. Figure 3 now includes error bars.

### **Discussion:**

We expanded the discussion section and added more detail on the interpretation of the results. Furthermore, we have nuanced the interpretation of the correlational analyses.

**Competing Interests:** No competing interests were disclosed.