Specific alterations of resting-state functional connectivity in the triple network related to comorbid anxiety in major depressive disorder

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Abstract

The brain’s default mode network (DMN) and the executive control network (ECN) switch engagement influenced by the ventral attention network (VAN). Alterations in resting-state functional connectivity (RSFC) within this so-called triple network have been demonstrated in patients with major depressive disorder (MDD) or anxiety disorders (AD). This study investigated alterations in the RSFC in patients with comorbid MDD and ADs to better understand the pathophysiology of this prevalent group of patients. Sixty-eight participants (52.9 % male, mean age 35.25 years), consisting of 25 patients with comorbid MDD and ADs (MDD+AD), 20 patients with MDD only (MDD-AD) and 23 healthy controls (HC) were investigated clinically and with 3T resting-state fMRI. RSFC utilizing a seed-based approach within the three networks belonging to the triple network was compared between the groups. Compared to HC, MDD+AD showed significantly reduced RSFC between the ECN and the VAN, the DMN and the VAN and within the ECN. No differences could be found for the MDD group compared to both other groups. Furthermore, symptom severity and medication status did not affect RSFC values. The results of this study show a distinct set of alterations of RSFC for patients with comorbid MDD and AD compared to healthy controls. This set of dysfunctions might be related to less adequate switching between the DMN and the ECN as well as poorer functioning of the ECN. This might contribute to additional difficulties engaging and utilizing consciously controlled emotional regulation strategies.

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0 Abstract

The brain’s default mode network (DMN) and the executive control network (ECN) switch engagement influenced by the ventral attention network (VAN). Alterations in resting-state functional connectivity (RSFC) within this so-called triple network have been demonstrated in patients with major depressive disorder (MDD) or anxiety disorders (AD). This study investigated alterations in the RSFC in patients with comorbid MDD and ADs to better understand the pathophysiology of this prevalent group of patients. Sixty-eight participants (52.9 % male, mean age 35.25 years), consisting of 25 patients with comorbid MDD and ADs (MDD+AD), 20 patients with MDD only (MDD-AD) and 23 healthy controls (HC) were investigated clinically and with 3T resting-state fMRI. RSFC utilizing a seed-based approach within the three networks belonging to the triple network was compared between the groups. Compared to HC, MDD+AD showed significantly reduced RSFC between the ECN and the VAN, the DMN and the VAN and within the ECN. No differences could be found for the MDD group compared to both other groups. Furthermore, symptom severity and medication status did not affect RSFC values. The results of this study show a distinct set of dysfunctions might be related to less adequate switching between the DMN and the ECN as well as poorer functioning of the ECN. This might contribute to additional difficulties engaging and utilizing consciously controlled emotional regulation strategies.

1 Introduction

Major depressive disorder (MDD) and anxiety disorders (AD), one of its most common comorbid groups of disorders (Fava et al., 2000), are each among the most common psychiatric disorders worldwide, (Malhi & Mann, 2018; van Tol et al., 2021) with devastating effects on virtually all areas of a patient’s life as well as contributing significantly to the overall burden of disease (James et al., 2018; Kessler & Greenberg, 2002; Silverstone & Studnitz, 2003). Comorbid MDD and AD are shown to have significantly less favourable treatment outcome, more severe symptom presentation and higher impact on quality of life (van Tol et al., 2021).

Despite extensive research, both disorders are still lacking reliable biomarkers for diagnosis, leading to uncertainty both in diagnosis and treatment (Mulders et al., 2015) and high rates of patients failing to achieve remission (Mulders et al., 2015; Trivedi et al., 2008). In particular, linking differences in the engagement of individual brain areas to psychiatric disorders has been shown to be insufficient in explaining the full symptomatic complexity of these disorders (Menon, 2019; Menon, 2011). One of the most consistent findings has been a hyperactivation of the amygdala as a common core mechanism of anxiety disorders (Shin & Liberzon, 2010). However, this finding could not account for the dynamics and individual heterogeneity
of symptoms associated with anxiety disorders (Menon, 2011; Sylvester et al., 2012). Therefore, in recent years, the focus has shifted to alterations in neural networks. One important tool for quantifying the activity of neural circuits has been resting-state functional connectivity (RSFC), which measures the correlation of spontaneous fluctuations of the blood oxygen level-dependent (BOLD) signal between brain regions at rest (Cullen et al., 2014; Greicius et al., 2007; Kelly et al., 2012; Ogawa et al., 1990; van den Heuvel & Hulshoff Pol, 2010). RSFC has proven to be an advantageous and reliable method to examine psychiatric disorders, with disruptions of brain networks being consistently reported in both MDD (Frodl et al., 2010; Javaheripour et al., 2021; Kaiser et al., 2015; Schirmer et al., 2023; Sharpley & Bitsika, 2013) and AD (Northoff, 2020; Peterson et al., 2014; Sylvester et al., 2012; Xu et al., 2019).

Of particular interest in this area has been the so-called triple network, encompassing the default mode network (DMN), the ventral attention network (VAN) and the executive control network (ECN). According to Menon’s (2011) model, the VAN, with key nodes in the insula and dorsal anterior cingulate cortex (Sridharan et al., 2008), associated with filtering and integrating information (Fan et al., 2017) and assigning saliency to stimuli, operates as a switch between the task-negative DMN and the task-positive ECN (Menon & Uddin, 2010). The DMN, with key nodes located in the ventromedial prefrontal cortex (VMPFC) and posterior cingulate cortex (PCC) (Buckner et al., 2008; Manoliu et al., 2013; Raichle et al., 2001), is activated during times of cognitive rest and associated with self-referential thoughts (Buckner et al., 2008; Raichle, 2015) and internal attention (Li et al., 2021). The ECN on the other hand includes areas of the dorsolateral prefrontal cortex (dlPFC) and posterior parietal cortex (PPC) (Fan et al., 2017; Jiang et al., 2017) and is associated with working memory, decision-making, and cognitive control (Ernst et al., 2019; Lerman et al., 2014).

A failure to adequately switch between the DMN and the ECN could lead to “deficits in engagement and disengagement” of these networks (Menon, 2011). Aberrant functional connectivity between these networks consistently found for both MDD (Kaiser et al., 2015; Mulders et al., 2015; Zheng et al., 2015) and AD (Etkin & Wager, 2007; Massullo et al., 2020; Sylvester et al., 2012; Williams, 2016) could therefore be related to a set of various core symptoms in these disorders, such as rumination, cognitive deficits or emotional dysregulation (Hamilton et al., 2013; Menon, 2011).

While MDD and AD have both separately been extensively studied in regards to alterations in RSFC, research examining comorbid MDD and AD has been scarce, with only three studies covering the topic to the authors knowledge at this time: Pannekoek et al. (2015) examined patients with comorbid MDD and one or more anxiety disorder compared to those with only MDD or anxiety disorders alone and healthy controls. For the comorbid group, they found significantly increased RSFC between the limbic network and a cluster containing the bilateral precuneus, intracalcarine cortex, lingual gyrus, and posterior cingulate, and with a cluster including the right precentral gyrus, inferior frontal gyrus, and middle frontal gyrus. No difference was found for the other groups. From the same cohort study, Nawjin et al. (2022) on the other hand, found no significant differences when comparing patients with comorbid MDD and anxiety disorders with patients with MDD and healthy controls. Oathes et al. (2015) did not find any significant results within the triple network.

While the posterior cingulate region found to be significant in Pannekoek et al. (2015) is considered part of the DMN, no study has yet examined the triple network specifically in the context of comorbid MDD and AD. For this reason, we investigated the RSFC between the three core networks belonging to the triple network, DMN, VAN and ECN, comparing patients with MDD, patients with comorbid MDD and AD and healthy controls, using a seed-based approach utilizing commonly used seed-regions of all three networks. The RSFC was compared both within and between all three networks studied and its relationship to symptom severity and medication status was explored.
2 Methods

2.1 Participants

The group of 68 participants consisted of 23 healthy controls with no history of psychiatric disease (HC), 20 patients with a current diagnosis of MDD (MDD), and 25 patients with a current comorbid diagnosis of MDD and at least one anxiety disorder (10 patients with agoraphobia, 6 with social phobia, 13 with panic disorder and 7 with generalized anxiety disorder; 8 patients exhibited more than one AD at the time of testing) (MDD+AD). Patients were recruited from the Clinic of Psychiatry and Psychotherapy at the University Hospital Magdeburg and affiliated mental health services in the area. Healthy controls were recruited from the local community and matched for age and sex. Table 1 shows participant characteristics split by participant group. The mean age of the participants was 35.25 (12.4) years. 52.9% of the participants were male and the mean years of education was 15.5 (3.4). Of the patient groups, 73.4% were currently receiving psychopharmacotherapy and the mean duration of illness was 57.14 months.

Exclusion criteria were: (1) age under 18 or over 65, (2) a history of other comorbid psychiatric disorders, substance abuse or dependence, (3) other severe medical illness, head injury or neurological disorders, (4) MRI contraindications. Demographic variables and inclusion/exclusion criteria were assessed using a standardized questionnaire and a structured interview conducted by trained medical staff. Diagnoses were confirmed according to DSM-IV (American Psychiatric Association, 2013) criteria using the Structured Clinical Interview for DSM-IV (SCID I and II) (First et al., 1996). In addition, the Beck Depression Inventory II (BDI-II) (Beck et al., 1996) and the Beck Anxiety Inventory (BAI) (Beck et al., 1988) were filled out by each participant as a measure of symptom severity. Neurological disorders were examined via a neuropsychological test battery.

All participants received detailed oral and written information about the study and gave written informed consent prior to participation. Ethical approval for the study was granted by the Ethics Committee of the Otto-von-Guericke University. The study was conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

2.2 MRI data acquisition

Magnetic resonance images were obtained using a 3 Tesla Siemens MRI scanner (MAGNETOM Prisma Syngo MR D13D; Siemens, Erlangen, Germany) with a 64-channel phased array head coil. All subjects received earplugs to protect them from the noise of the head coil. Structural images were recorded using a magnetization-prepared rapid gradient echo (MPRAGE) sequence. High-resolution T1-weighted three-dimensional anatomical scans of 192 sagittal slices with a voxel size of 1.0 x 1.0 x 1.0 mm³ of the whole brain were obtained (repetition time (TR): 2500 ms, echo time (TE): 2.82 ms, inversion time (TI): 1100 ms, field of view (FOV) from foot to head (FH): 256 mm, anterior to posterior (AP): 256 mm and right to left (RL): 192 mm, flip angle of 7°, matrix size = 256 x 256). The scan time required for structural acquisition was 9 min 20 s.

T2-weighted functional images were collected in single runs using echo-planar imaging (TE = 30 ms; TR = 2000 ms; field of view = 216 mm; flip angle = 90°, voxel size 3 × 3 × 3 mm, EPI-Factor 72) using blood oxygenation level-dependent contrast. Covering the whole brain, 36 contiguous 3-mm-thick slices were acquired sequentially in ascending order parallel to the anterior–posterior-commissure. Within a total scan time of 10:06 min, 306 volumes were acquired continuously. Participants were instructed to stay awake with their eyes closed during the resting-state scans.

2.3 MRI data pre-processing

Functional data were preprocessed using Data Processing Assistant for Resting-State fMRI (DPARSF 4.3) (Chao-Gan & Yu-Feng, 2010). Imaging preprocessing included: interleaved slice time correction, realignment to temporal mean image, motion regression, normalization to Montreal Neurological Institute (MNI) reference...
space combined with reslicing to an isotropic resolution of 3-mm, temporal band-pass filtering (0.01 < f < 0.10 Hz) and smoothing using a full-width-at-half-maximum Gaussian-kernel of 4 mm. Spurious variance was removed by nuisance regression including covariates for head motion using the Friston 24-parameter model as well as averaged signals from global brain signal, cerebrospinal fluid and white matter signal.

To compute RSFC within and between the three networks, we proceeded as follows. First, for each network, we utilized established a priori defined, literature-based seed regions (Table 2) belonging to the DMN, VAN and ECN to compute seed-to-whole-brain connectivity maps by calculating the Pearson correlation coefficient of the average time series of each seed region to the rest of the brain. Then, we converted the correlation coefficient maps using Fisher’s z-transformation to improve the Gaussianity of their distribution.

2.4 Statistical MRI data analysis

Following preprocessing of the data, using Statistical Parametric Mapping (SPM 12) (Friston, 2007) functional connectivity maps were compared using analyses of variance, where age and sex were included as covariates. For each network-pair, we then computed group-wise comparisons between all the three groups using post-hoc t-tests for independent samples. All clusters were formed using a cluster-forming-threshold of \( p < .001 \) and controlled for Family-Wise-Error (\( p_{\text{FWE-corr}} < .05 \)). Additionally, all clusters were subsequently corrected for multiple comparisons using Bonferroni-correction and considered significant at \( p_{\text{corr}} < .05 \). The atlas of Yeo et al. (2011) was used to assign the resulting clusters to one of the three networks. For all 5 seeds, the within-network (seed to other structures within the network the seed belonged to) and the between-network connectivity (seed to structures belonging to the other networks included in this study) was examined. For post-hoc analysis using SPSS 27.0 (IBM Corp., 2022), we extracted the beta values of these clusters indicating the degree of connectivity from our first level models using the REX toolbox (Gabrieli Lab). Automated anatomical labelling atlas (AAL 3) (Rolls et al., 2020) was used to identify and label clusters with aberrant RSFC. Connectivities were visualized using Brain Net Viewer (2013).

SPSS (IBM Corp., 2022) software was used for comparison of group characteristics as well as correlation and regression analysis. All results were considered to be significant at \( p_{\text{corr}} < .05 \) after correcting for multiple comparisons using Bonferroni-correction. Characteristics of the participant groups were summarized using mean and standard deviation for continuous variables and percentage for categorical variables. Variables were tested for normal distribution using Shapiro-Wilk-Test. Age and clinical scores were compared between all groups using ANOVA. Between patient groups duration of illness and clinical scores were compared using Mann-Whitney- \( U \) -test. Differences in the distribution of sex and medication were assessed using \( \chi^2 \)-tests.

To further investigate the nature of the relationship of RSFC-values and symptom severity, we performed regression analyses using linear models, for all clusters found to be significant within the patient group (MDD and MDD+AD). Mean beta values were used as dependent variables and severity of symptoms as measured by BDI-II and BAI-scores as independent variables. Additionally, since an effect of pharmacotherapy on RFSC has commonly been found (Rolls et al., 2019), we performed an independent-sample-t-test within the patient group, testing for a difference in RSFC-values between medication groups. Bonferroni-correction was used to correct for multiple comparisons.

3 Results

3.1 Participant characteristics

Demographic and clinical characteristics of all participant groups are summarized in Table 1. The groups did not differ in age, sex and education. Healthy controls and patients showed significant differences in clinical scores. Comparing both patient groups, we found significant differences in medication status. Both patient groups did not differ in duration of illness and BDI-II- and BAI scores.
3.2 Resting state functional connectivity

Table 3 summarizes the network aberrations found for the three examined networks. Figure 1 illustrates the clusters found in their respective networks. Comparing MDD+AD and HC, MDD+AD showed a significantly reduced RSFC for the VAN with both the ECN and the DMN. No alteration in RSFC of the ECN and DMN could be found.

Between the DMN and the VAN, the right posterior cingulate cortex seed of the DMN showed a significantly decreased RSFC with regions in the VAN (RPCC to: right middle cingulate and paracingulate gyrus, \( t = 4.64, p_{\text{corr}} = .015 \); right middle cingulate and paracingulate gyrus, \( t = 5.86, p_{\text{corr}} = .011 \)) and the left and right dorsal anterior insula of the VAN showed significantly decreased RSFC with regions in the DMN (RdIns to: right anterior cingulate cortex, pregenual parts, \( t = 5.21, p_{\text{corr}} = < .001 \); LdIns to: left anterior cingulate cortex, pregenual parts, \( t = 5.80, p_{\text{corr}} = < .001 \)).

Between the ECN and the VAN, the left dorsomedial prefrontal cortex and the left dorsolateral prefrontal cortex of the ECN showed significantly decreased RSFC with the VAN (LdmPFC to: left middle cingulate and paracingulate gyri, \( t = 5.06, p_{\text{corr}} = .002 \); LdlPFC to: right middle cingulate and paracingulate gyri, \( t = 4.53, p_{\text{corr}} = .001 \)). The left dorsal anterior insula of the VAN showed significantly reduced RSFC with the ECN (LdIns to: right superior frontal gyrus, dorsolateral parts, \( t = 4.36, p_{\text{corr}} = .024 \)).

The ECN showed significantly reduced RSFC on the within-network level (RdmPFC to: left middle frontal gyrus, \( t = 4.84, p_{\text{corr}} = .006 \)).

We could not find any significant differences for the group comparisons including the MDD group (MDD – HC and MDD – MDD+AD).

3.3 Post-hoc regression analysis

Outcomes of regression analysis for the relationship of severity of symptoms as measured by BDI-II and BAI total scores and RSFC values of the found clusters within the patient group (MDD and MDD+AD) are shown in Table 3. No cluster showed a significant connection.

Furthermore, we did not find a significant effect of medication status on RSFC-values for the clusters that were found to be significant in group-wise analysis.

4 Discussion

The present study examined RSFC network differences between patients with a diagnosis of MDD, patients with comorbid MDD and at least one anxiety disorder and healthy controls within the context of the triple network model. Comparing MDD+AD to HC, we found significantly reduced RSFC for the VAN with both the ECN and the DMN, whereas there were no alterations between the ECN and the DMN. This finding confirms the triple network model by Menon et al. (2010). In addition, the ECN showed significantly reduced within-network-connectivity. These effects were independent of severity of symptoms and medication status. No effects were found when comparing MDD with HC and MDD with MDD+AD.

Although we did not find any differences between patients with MDD and HC, aberrations of RSFC within the triple network are well documented (Kaiser et al., 2015; Mulders et al., 2015; Zheng et al., 2015). However, results in the RSFC-literature are generally heterogenous (Kaiser et al., 2016; Lydon-Staley et al., 2019) and with the relatively small sample size of this study and rigorous correction for multiple testing (Bonferroni correction is regarded as a comparatively conservative correction method (Chen et al., 2017)), smaller effects could have gone undetected. Therefore, we cannot exclude, that the MDD group does show aberrant RSFC compared to HC, in particular since this group does not differ to MDD+AD.
In research concerning MDD and the triple network model, the introspective qualities of the DMN and the more outwards oriented qualities associated with the ECN have been central to the discussion of the symptomatic correlates of the commonly found aberrations (Jiang et al., 2017; Li et al., 2021). Here, alterations in DMN connectivity have been found to be associated with higher levels of rumination (Brakowski et al., 2017; Kühn et al., 2012) and negative self-referential thoughts (Cullen et al., 2014). The ECN, on the other hand, shows a more outward-directed and stimulus-driven set of tasks, being involved in decision making and cognitive control (Li et al., 2021; Manoliu et al., 2013). Within the triple network model, alterations in the RSFC between these two networks and the VAN as a switch between them are thought to impair the engagement of the ECN and the disengagement of the DMN (Menon & Uddin, 2010), leading to maladaptive rumination and impaired cognitive abilities (Schimmelpfennig et al., 2023).

Similar to the MDD literature, in the much more sparse research regarding anxiety, the VAN is thought of as an important intermediary between the DMN and the ECN as well (Nawijn et al., 2022; Pannekoek et al., 2015). Here, however, other functions of the DMN and ECN are considered central to the symptomology, namely their role in the regulation of emotion and fear response (Sylvestre et al., 2012). The DMN has been shown to be linked to emotion regulation (Macêdo et al., 2022), with a focus on emotion perception (Kim & Yoon, 2018), reinforcement expectancy (Blair, 2007) and fear extinction (Sylvestre et al., 2012). The ECN on the other hand has been associated with more conscious and control oriented emotional regulation strategies, such as redirection of attention towards non-emotional stimuli and suppression of amygdala responses when attention is engaged with a non-emotional stimulus (Bishop et al., 2004; Sylvester et al., 2012). Impaired switching between the DMN and the ECN by the VAN is thought to be associated with less adequate control over fear responses and emotional regulation (Sylvestre et al., 2012).

In summary, alterations within the triple network in the context of MDD are interpreted in terms of an inadequate switch between internally (DMN) and externally (ECN) oriented attention and stimulus processing. In contrast, in the context of AD, alterations in the triple network are interpreted as an imbalance between a more automated and unconscious processing of emotions (DMN) and conscious, cognitively controlled emotion regulation (ECN).

In this context, studying patients with comorbid MDD and AD serves to identify those neurological alterations within the triple network, that distinguish this important group of patients from those with MDD alone. We found reduced connectivity between the DMN and the VAN with clusters in the right middle and anterior cingulate and paracingulate cortex and the left cingulate cortex, between the ECN and the VAN, with clusters in the left and right middle cingulate and paracingulate gyrus and the right superior frontal gyrus, and within the ECN with a cluster in the left middle frontal gyrus. In the context of the interpretation of alterations discussed above, the alterations we found could lead to additional difficulties switching between the DMN and the ECN and in turn contribute to the specific symptomology of ADs by impairing the switch from unconscious, automated modes of emotional regulation to more conscious, cognitively controlled strategies (Fan et al., 2017). This could manifest in the high degree of internally oriented attention characteristic for AD (Fan et al., 2017). Additionally, the hypoconnectivity found within the ECN may be associated with a higher difficulty in using and applying these strategies effectively (Sylvestre et al., 2012).

Furthermore, while anxiety symptoms accompanying depression are often associated with higher depression severity (Gaspersz et al., 2017), this was not the case in the current study as both groups showed similar severity of depression symptoms. Additionally, the severity of anxiety symptoms measured with the BAI was also not significantly different between groups. For this reason, our results may provide important indications of specific network signatures of comorbid MDD and anxiety disorders as opposed to greater overall symptom severity in the patient groups. Another result supporting this notion was our finding that the strength of the RSFC was not associated with symptom severity within the patient groups. This is consistent with previous findings (Pannekoek et al., 2015). Pannekoek et al. (2015) suggested that this may be due to the changes in RSFC being a result of trait rather than state characteristics or alternatively, the relatively mild overall symptom severity of their patient groups. However, as our patients were recruited from an inpatient setting, typically shortly after admission, our findings may further reinforce the qualitative nature of these
alterations.

5 Limitations

Several limitations must be considered when interpreting the results of the current study.

First, this study only compared the comorbid patient group (MDD+AD) with patients with MDD alone. Future research comparing comorbid patients with patients with only anxiety disorders and no MDD would be essential to gain further insight into the specific pathophysiology of this group of disorders.

In addition, the close connection of the VAN to the amygdala plays a key role in the interpretation of RSFC changes in patients with anxiety disorders. Several studies have shown altered RSFC between the VAN and the amygdala (Pisoni et al., 2021; Williams, 2016). The amygdala is known to play a central role in the development and maintenance of anxiety disorders (Rauch et al., 2003). One possibility from a network perspective is that the amygdala functions by signalling saliency to the insula, a key node of the VAN (Menon, 2011; Paulus & Stein, 2006). Thus, disruptions in amygdala function and its connectivity to the VAN are thought to alter the ability of the VAN to adequately switch between the DMN and the ECN (Paulus & Stein, 2006). Because we utilized the networks as defined by Yeo et al. (2011), the amygdala was not included in our analysis. However, several authors, including Menon (2011), consider the amygdala as part of the salience network, commonly equalized with or seen as a part of the VAN (Yeo et al., 2011). Therefore, we suggest this as an important issue for further research.

Medication was not found to have a significant effect on the results. However, there are findings that antidepressant medication influences RSFCs (Rolls et al., 2019) and in our study the effect of medication may not have been detected since only 25 % of patients were unmedicated at the time of the study. Thus, future studies should focus on antidepressant-free patients.

6 Conclusion

To the best knowledge of the authors, this study is the first to examine the triple network model in comorbid depression and anxiety disorders against depression alone. Using the well examined triple network model, we were able to find a distinct set of alterations in RSFC within the triple network, contributing to the search for a distinct neuropathology of this highly prevalent patient population. The clusters found within the cingulate and paracingulate and frontal areas may play an important role in the specific symptomatology of comorbid MDD and AD, contributing to the less favourable treatment outlook and higher impact on quality of life. The present study therefore contributes to a better understanding of this patient group, which is essential for targeted pharmacotherapy and more effective interventions, as underlined by several studies showing an effect of different types of psychotherapy on RSFC alterations both in MDD (Dunlop et al., 2023; Katayama et al., 2023; Pantazatos et al., 2020) and AD (Gonsalves et al., 2022; Yuan et al., 2016).

References


**REX toolbox** [Computer software]. Gabrieli Lab, Massachusetts Institute of Technology. Cambridge, Massachusetts, USA.


**IBM SPSS Statistics** (Version 29.0) [Computer software]. (2022). IBM Corp. Armonk, NY.


**Table 1.** Participant characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>MDD-A (n = 25)</th>
<th>MDD (n = 20)</th>
<th>HC (n = 23)</th>
<th>(p-value)</th>
<th>(p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Demographic</em></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Age, years (s.d.)</td>
<td>33.3 (10.9)</td>
<td>39.40 (14.3)</td>
<td>33.8 (11.7)</td>
<td>0.164</td>
<td>0.203</td>
</tr>
<tr>
<td>Sex, male, n (%)</td>
<td>12 (48)</td>
<td>11 (55)</td>
<td>13 (56.5)</td>
<td>0.164</td>
<td>0.203</td>
</tr>
<tr>
<td>Pharmacotherapy, yes, n (%)</td>
<td>16 (64)</td>
<td>17 (85)</td>
<td>0 (0)</td>
<td>0.40</td>
<td>0.820</td>
</tr>
<tr>
<td>Duration of illness, months (s.d.)</td>
<td>72.07 (105.68)</td>
<td>38.49 (47.83)</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Clinical scores</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>BDI-II, mean (s.d.)</td>
<td>25.8 (12.5)</td>
<td>30.8 (11.5)</td>
<td>1.00 (1.4)</td>
<td>0.645</td>
<td>0.213</td>
</tr>
<tr>
<td>BAI, mean (s.d.)</td>
<td>17.8 (10.9)</td>
<td>22.1 (13.7)</td>
<td>3.0 (4.8)</td>
<td>0.645</td>
<td>0.213</td>
</tr>
</tbody>
</table>

**Table 2.** Seed-Literature.

<table>
<thead>
<tr>
<th>Seed</th>
<th>Literature</th>
</tr>
</thead>
</table>
Seed Literature

PCC
ECN
dmPFC
dIPFC (Seeley et al., 2007; Uddin et al., 2019; Vincent et al., 2008)

VAN* daIns dmPFC
dlPFC (Seeley et al., 2007; Uddin et al., 2019; Vincent et al., 2008)

VAN* daIns dACC

*According to Yeo et al. (2011) the VAN “is likely an aggregate of (or closely adjacent to) multiple networks in the literature variably referred to as the salience […] and cingulo-opercular networks […]”. For this reason, findings concerning all three network models will be included in further considerations.

Abbreviations: dACC, dorsal anterior cingulate cortex; daIns, dorsal anterior insula; DMN, default mode network; dIPFC, dorsolateral prefrontal cortex; dmPFC, dorsomedial prefrontal cortex; ECN, executive control network; PCC, posterior cingulate cortex; VAN, ventral attention network

Table 3. Network pairs with aberrant RSFC for the comparison of MDD+AD and HC with results of regression analysis for severity of symptoms for BDI and BAI.

<table>
<thead>
<tr>
<th>Network pair Seed-Target Network</th>
<th>Peak region</th>
<th>p-value corrected</th>
<th>Peak t-value</th>
<th>MNI coordinates (peak voxel)</th>
<th>Cluster size</th>
<th>Association RSFC – BDI-II score</th>
<th>Association RSFC – BAI score</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDD+AD &lt; HC DMN – VAN</td>
<td>MDD+AD &lt; HC</td>
<td>MDD+AD &lt; HC</td>
<td>MDD+AD &lt; HC</td>
<td>MDD+AD &lt; HC 9, -36, 48 22</td>
<td>11.33, 1.00, R² = .05, F(1,43) = 2.21, p = .144.</td>
<td>1.00, R² = .00, F(1,43) = 1.00, p = .323</td>
<td>1.00, R² = .00, F(1,43) = 1.00, p = .688.</td>
</tr>
<tr>
<td>1. DMN/RPCC VAN</td>
<td>Right middle cingulate and paracingular gyri</td>
<td>.015* 4.64</td>
<td>9, -36, 48</td>
<td>22</td>
<td>b = 11.33, t(42) = 1.00, R² = .05, F(1,43) = 2.21, p = .144.</td>
<td>b = 1.60, t(42) = .02, p = .898.</td>
<td>b = 6.78, t(42) = .41, R² = .16, F(1,43) = .144.</td>
</tr>
<tr>
<td>2. DMN/RPCC VAN</td>
<td>Right middle cingulate and paracingular gyri</td>
<td>.011* 5.86</td>
<td>6, -3, 39</td>
<td>23</td>
<td>b = 22.25, t(42) = 1.49, R² = .41, F(1,43) = 2.21, p = .144.</td>
<td>b = 1.00, R² = .00, F(1,43) = 1.00, p = .323</td>
<td>b = 6.78, t(42) = .41, R² = .16, F(1,43) = .144.</td>
</tr>
<tr>
<td>Network pair Seed-Network/Seed</td>
<td>Target-Network</td>
<td>Peak region</td>
<td>p-value</td>
<td>Peak t-value</td>
<td>MNI coordinates (peak voxel)</td>
<td>Cluster size</td>
<td>Association RSFC – BDI-II score</td>
</tr>
<tr>
<td>-------------------------------</td>
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<tr>
<td>3 VAN/RdaIns DMN</td>
<td>Right anterior cingulate cortex, pregenual</td>
<td>&lt;0.001*** 5.21</td>
<td>3, 42, 3</td>
<td>150</td>
<td>b = 4.94, t(42) = .38, ( R^2 = .00, F(1,43) = .14, p = .709, R^2 = .00, F(1,43) = .28, p = .589.</td>
<td>b = 7.92, t(42) = .53, ( R^2 = .00, F(1,43) = .28, p = .589.</td>
<td></td>
</tr>
<tr>
<td>4 VAN/LdaIns DMN</td>
<td>Left anterior cingulate cortex, pregenual</td>
<td>&lt;0.001*** 5.80</td>
<td>-9, 39, -3</td>
<td>89</td>
<td>b = 17.73, t(42) = 1.39, ( R^2 = .04, F(1,43) = .04, p = .536, R^2 = .00, F(1,43) = .30, p = .590.</td>
<td>b = 7.53, t(42) = .54, ( R^2 = .00, F(1,43) = .30, p = .590.</td>
<td></td>
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<tr>
<td>ECN – VAN ECN/LdmPFC</td>
<td>Left middle cingulate and paracingulate gyri</td>
<td>.002** 5.06</td>
<td>3, 3, 42</td>
<td>28</td>
<td>b = 15.01, t(42) = 1.63, ( R^2 = .06, F(1,43) = .26, p = .110, R^2 = .00, F(1,43) = .358, p = .356.</td>
<td>b = 10.24, t(42) = ( R^2 = .06, F(1,43) = .26, p = .110, R^2 = .00, F(1,43) = .358, p = .356.</td>
<td></td>
</tr>
<tr>
<td>6 VAN ECN/LdlPFC</td>
<td>Right middle cingulate and paracingulate gyri</td>
<td>.001** 4.53</td>
<td>3, 0, 39</td>
<td>29</td>
<td>b = 9.00, t(42) = .94, ( R^2 = .02, F(1,43) = .88, p = .354, R^2 = .00, F(1,43) = .652, p = .652.</td>
<td>b = 5.37, t(42) = ( R^2 = .02, F(1,43) = .88, p = .354, R^2 = .00, F(1,43) = .652, p = .652.</td>
<td></td>
</tr>
<tr>
<td>7 ECN VAN/LdaIns</td>
<td>Right superior frontal gyrus, dorsolateral</td>
<td>.024* 4.36</td>
<td>27, 54, 15</td>
<td>21</td>
<td>b = .07, t(42) = .01, ( R^2 = .00, F(1,43) = .00, p = .994, R^2 = .00, F(1,43) = .88, p = .355.</td>
<td>b = .07, t(42) = ( R^2 = .00, F(1,43) = .00, p = .994, R^2 = .00, F(1,43) = .88, p = .355.</td>
<td></td>
</tr>
</tbody>
</table>

**ECN – ECN**
<table>
<thead>
<tr>
<th>Network pair Seed-Target-Network</th>
<th>Peak region</th>
<th>p-value corrected</th>
<th>Peak t-value</th>
<th>MNI coordinates (peak voxel)</th>
<th>Cluster size</th>
<th>Association RSFC – BDI-II score</th>
<th>Association RSFC – BAI score</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECN/RdmPFC</td>
<td>Left middle frontal gyrus</td>
<td>.006**</td>
<td>4.84</td>
<td>-39, 39, 15</td>
<td>24</td>
<td>$b = 5.68, \quad t(42) = .56, \quad R^2 = .01, \quad F(1,43) = .32, \quad p = .577.$</td>
<td>$b = 13.51, \quad t(42) = 1.33, \quad R^2 = .04, \quad F(1,43) = 1.76, \quad p = .192.$</td>
</tr>
</tbody>
</table>

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Figure 1. Clusters with significant hypoconnectivities for the MDD+AD group compared to HC and their connections to their respective seed regions. Small nodes: seed-regions; large nodes: clusters. Colours: orange: nodes belonging to the DMN; green: nodes belonging to the ECN; blue: nodes belonging to the VAN. Letters: a: PCC, b: dmPFC, c: dlPFC, d: daIns: Numbers of clusters correspond with numbers in Table 3.