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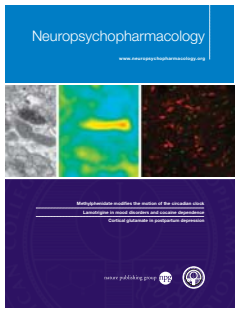

Associations between Daily Affective Instability and Connectomics in Functional Subnetworks in Remitted Patients with Recurrent Major Depressive Disorder

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TITLE Associations between daily affective instability and connectomics in functional subnetworks in remitted patients with recurrent major depressive disorder

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Abstract

Remitted patients with major depressive disorder (rMDD) often report more fluctuations in mood as residual symptomatology. It is unclear how this affective instability is associated with information processing related to the default mode (DMS), salience/reward (SRS) and fronto-parietal (FPS) subnetworks in rMDD patients at high risk of recurrence (rrMDD). Sixty-two unipolar, drug-free rrMDD patients (≥2 MDD-episodes) and 41 HC (HC) were recruited. We used Experience Sampling Methodology (ESM) to monitor mood/cognitions (10 times a day for 6 days) and calculated affective instability using the mean adjusted absolute successive difference. Subsequently, we collected resting-state functional Magnetic Resonance Imaging data and performed graph theory to obtain network metrics of integration within (local efficiency) the DMS, SRS and FPS, and between (participation coefficient) these subnetworks and others. In rrMDD patients compared to HC, we found that affective instability was increased in most negative mood/cognition variables and that the DMS had less connections with other subnetworks. Furthermore, we found that rrMDD patients, who showed more instability in feeling down and irritated, had less connections between the SRS and other subnetworks and higher local efficiency coefficients in the FPS, respectively. In conclusion, rrMDD patients, compared to HC, are less stable in their negative mood and these dynamics are related to differences in information processing within and between specific functional subnetworks. These results are a first step to gain a better understanding of how mood fluctuations in real-life are represented in the brain and provide insights in the vulnerability profile of MDD.

Introduction

Generally, major depressive disorder (MDD) has an intermittent course in which remission and recovery are often followed by relapse and recurrence, respectively (Steinert *et al*, 2014). The most important predictors of relapse/recurrence are the number of previous episodes and the presence of residual symptomatology after recovery from MDD (Hardeveld *et al*, 2010). To gain insight in vulnerability for MDD, it is important to investigate residual symptomatology (Fava and Visani, 2008) and its associated neurobiological correlates (De Raedt and Koster, 2010; Marchetti *et al*, 2012) in remitted patients with recurrent MDD as they are at high risk for another episode.

Residual symptoms, often reported by rMDD patients, are alterations in mood (aan het Rot *et al*, 2012; Fava and Visani, 2008). These can be monitored on a daily basis using the *experience sampling method* (ESM). In ESM, individuals fill out short self-report questionnaires on affect, physical status and context several times a day (Trull *et al*, 2008). Prior ESM studies showed that rMDD patients report on average higher negative mood and lower positive mood compared to healthy controls (HC) (Knowles *et al*, 2007; van Winkel *et al*, 2015). Furthermore, rMDD patients showed higher reactivity in mood to daily (Husky *et al*, 2009; O'Hara *et al*, 2014) and social (van Winkel *et al*, 2015) stressors compared to HC. These latter results may point to more affective instability (higher variability and lower temporal dependency in mood ratings; Trull *et al*, 2008) in rMDD patients, since reactivity of negative affect to negative external events has been positively related to instability of negative affect (Thompson *et al*, 2012). An indication for the latter in rMDD patients has been observed by Thompson *et al.* (2011), who showed increased affective instability in these patients compared to individuals without a lifetime history of MDD using the borderline personality disorder module of the Personality Disorder Interview-IV (PDI-

IV; Widiger *et al*, 1995). Furthermore, a recent meta-analysis has shown an association between lower psychological well-being and higher affective instability (specifically negative emotions) in MDD patients (Houben *et al*, 2015). In the current study, using ESM, we investigated whether high affective instability is characteristic of *remitted recurrent* MDD (rrMDD) patients, who are at high risk of relapse/recurrence.

Moreover, neuroimaging studies have demonstrated alterations in neural correlates related to emotion processing in MDD patients (Groenewold *et al*, 2013). A recently adopted perspective, wherein the brain is viewed as a complex network supporting the integration and segregation of information processing, has provided further insights in the large-scale abnormalities in topological network organization in these patients (Gong and He, 2015). Using *graph theory*, the brain is defined as a graph consisting of nodes (i.e. brain regions, voxels) and edges (i.e. connections between nodes) on which global/local (e.g. efficiency) and nodal (e.g. degree) metrics can be calculated (Rubinov and Sporns, 2010). To date, two white-matter structural connectomic studies have been performed in rMDD patients, showing alterations in metrics calculated on nodes that are part of the default mode (DMS; self-reflection), salience (SS; negative attention bias/reward) and fronto-parietal (FPS; cognitive control) subnetwork (Bai *et al*, 2012; Qin *et al*, 2015). Alterations in these subnetworks have been related to symptoms in (r)MDD patients (Hamilton *et al*, 2013; Jacobs *et al*, 2014; Mulders *et al*, 2015). Studies on the *functional* network organization have not yet been performed in rMDD. However, this would allow researchers to investigate whether disturbances in the integration and segregation of information processing in abovementioned subnetworks - as observed in MDD patients (Gong and He, 2015) – are characteristic of rrMDD patients, who are at high risk of relapse/recurrence.

An interesting property of resting-state functional magnetic resonance imaging (rs-fMRI) data, on which graph theory is performed, is that it exhibits experience-dependent changes over

time (Sporns, 2012). During rest, the connectome seems to rehearse mental states via spontaneous neural activity by reactivating genetically and experientially modified pathways (Sporns, 2012). Hence, it is of specific interest to combine rs-fMRI with ESM, which has not yet been done. This would allow us to investigate whether alterations in affective instability are associated with alterations in connectivity within (i.e. segregation of information processing) and between (i.e. integration of information processing) specific functional subnetworks. In the current study, we hypothesized to find, in rrMDD patients compared to HC, i) increased affective instability in mood/cognition variables, specifically variables related to negative affect, ii) alterations in network metrics capturing connectivity within (i.e. local efficiency) the DMS, SS and FPS, and between (i.e. participation coefficient) these subnetworks and others (Servaas *et al*, 2015), iii) increased affective instability in mood/cognition variables to be associated with alterations in abovementioned network metrics.

Materials and methods

Participants

This study was part of a larger project on the vulnerability for new episodes in recurrent MDD (see Mocking *et al. (*2016) for the project description). Inclusion criteria for rrMDD patients (n=62) were: 1) ≥2 MDD episodes according to the Structured Clinical Interview for DSM-IV Disorders (SCID), 2) current state of stable remission, defined as i) a score of ≤7 on the 17-item Hamilton Depression Rating Scale (HDRS) for ≥8 weeks and ii) no current depressive episode according to the SCID, 3) age between 35-65 years (to include a homogeneous age group and preclude conversion to bipolar disorder due to later experience of (hypo)manic episodes), 4) Dutch or English proficiency. Exclusion criteria for rrMDD patients were: 1) current diagnosis of alcohol/drug dependence, psychotic or bipolar disorder, a predominant anxiety disorder or severe personality disorder according to the SCID, 2) MRI incompatible implants or tattoos, 3) claustrophobia, 4) electroconvulsive therapy within 2 months prior to scanning, 5) history of seizure or head injury, 6) neurological disorder, 7) current severe physical illness, 8) use of psychoactive drugs/medication <4 weeks before assessments. Incidental benzodiazepine use was allowed, but had to be terminated >2 days (≥5 half-lives) before assessments. HC (n=41) were included when i) they (according to the SCID) or their first degree relatives did not have a lifetime diagnosis of psychiatric disorders and ii) they met inclusion criteria 3 and 4 and did not meet exclusion criteria 2, 3 and 5-8. The samples were matched for sex, age, educational level, socio-economic status, ethnicity and handedness. Participants were recruited from previous studies and primary and secondary mental health care institutes and through advertisements in online and house-to-house papers and posters in public places. Written informed consent was

obtained and the study was approved by the Medical Ethical Committee of the Academic Medical Center.

ESM

Participants received an ESM palmtop before the scanning session (median=13 days; interquartile range=14 days) and were asked to fill out a short self-report questionnaire on affect, physical status and context ten times a day for six days. Beeps fell randomly in ten 90-minute time blocks between 7:30-22:30h. Participants were instructed to complete the questionnaire within 15 minutes. For the current study, we selected all variables related to mood and cognition (see Mocking *et al. (*2016) for the complete ESM protocol, including –among others- items on company and daily events), including four positive mood items (enthusiastic, cheerful, relaxed, satisfied), seven negative mood items (agitated, anxious, down, irritated, lonely, guilty, restless), two positive cognition items (empowered, selflike) and four negative cognition items (ashamed, self-doubt, suspicious, worry) (see S1, Table S1 for the content of the variables). Items were rated on a 7-point Likert scale ranging from 1 = 'not at all' to 7 = 'very'. Ninety-seven participants completed the ESM protocol (57 rrMDD patients; 40 HC). Next, we cleaned the data (see S2 for details on the cleaning steps) and removed 903 observations from a total of 4466 observations, thereby excluding 28 participants (14 rrMDD patients; 14 HC; see S3 for differences in sample characteristics between included and excluded participants per group). This left a total of 3563 observations and 69 participants (43 rrMDD patients; 26 HC) for ESM analyses. Subsequently, we checked whether variables contained substantial variation based on the visual inspection of boxplots. Three variables did not show substantial variation across participants: mood item 'anxious', mood item 'guilty' and cognition item 'suspicious' (S4, Figure S1) and were excluded from further analyses. In order to capture affective instability, we calculated the Mean Adjusted

Absolute Successive Difference (MAASD) per ESM variable and subject (Jahng *et al*, 2008, see S5 and S6, Table S2 for details on the method). Differences in MAASD between the rrMDD and HC group were calculated using an independent samples Mann-Whitney U test. Results with Bonferroni-corrected p-values of ≤0.004 (0.05/14 variables) were considered significant.

Image preprocessing

For details on the image acquisition parameters, preprocessing and postprocessing steps and scrubbing, see S8-10. After image processing, 13 participants (9 rrMDD patients; 4 HC) were excluded because of anatomical abnormalities (n=5), excessive scrubbing (viz. removal of ≥1/3 of the volumes) (n=6) or preprocessing failures (n=2). This left a total of 90 participants (53 rrMDD patients; 37 HC) for fMRI analyses.

Graph theory

Network construction

As previously described in (Servaas *et al*, 2015), nodes were built by creating a sphere of 5 mm radius around 270 coordinates (Power *et al*, 2011), including bilateral amygdala, hippocampus and caudate. The coordinates for these latter regions were determined using the Harvard-Oxford Subcortical Structural Atlas (80% probability). No overlap was observed between the additional ROIs and the ROIs of Power *et al.* (2011). Next, a whole-brain group mask was built based on the EPI images to locate the parts of the brain, which are free from susceptibility artifacts in all participants. Subsequently, the overlap was calculated voxel-wise between all nodes and the group mask. When a node overlapped <50% with the group mask, it was excluded from further analysis. This was the case for 45 nodes. Next, we constructed a connectivity matrix per participant by extracting the regional mean time series for each of the

remaining 225 nodes and calculated Pearson correlations between all pairs. Furthermore, to prevent biases due to shared non-biological signal between adjacent nodes, correlations were set to zero when the distance was less than 20 mm between the centers of two nodes (Power *et al*, 2011). In addition, correlations on the diagonal of the connectivity matrix and negative correlations were set to zero as well.

Thresholding and decomposition

We applied a range of proportional thresholds to each correlation matrix per participant to separate relevant from irrelevant edges. The threshold values ranged from 1%-30% in increments of 1%. Network measures were calculated on weighted graphs across the selected range of threshold values. Subnetworks were derived from the whole-brain graph by applying the algorithm of Blondel *et al.* (2008) and the modularity fine-tuning algorithm of Sun *et al.* (2009). For this procedure, we selected a single optimal threshold by using the method of Geerligs *et al.* (2015). The optimal threshold in the current study was 1.35%. In total, six subnetworks were derived with a maximum number of within-group edges and a minimum number of between-group edges (Rubinov and Sporns, 2010). These included the affective, DMS, FPS, somatosensory-motor, salience/reward (SRS) and visual subnetworks (S11, Figure S4). For the analysis, we focused on the DMS, FPS and SRS (Figure 1). The reason that we limited the results to these three subnetworks, as indicated in the introduction, was that they have most consistently been related to depression (Hamilton *et al*, 2013; Mulders *et al*, 2015). Furthermore, we sought to limit the number of tests in the statistical analyses.

Calculation of network measures and statistical analysis

Network measures were calculated on weighted graphs using functions implemented in the Brain Connectivity Toolbox (Rubinov and Sporns, 2010). Averaged across nodes, we calculated local efficiency and the participation coefficient per subnetwork. Local efficiency is calculated as the average inverse shortest path length between the neighbors of a specific node and the participation coefficient is calculated as the ratio of intra- versus intermodular connections per node (for an explanation of these measures, see Rubinov and Sporns (2010); the selection of these measures was based on Power *et al*. (2011), wherein the authors investigated the compartmentalization (i.e. segregation) and diversity (i.e. integration) of relationships of subnetworks by using local efficiency and the participation coefficient, and we applied this method in the following previous papers Geerligs *et al*. (2015); Servaas *et al*. (2015, 2016) to specifically investigate information processing within and between subnetworks; for a graphical explanation of the measures see S12, Figure S5). In other words, local efficiency is a measure of integration among the neighbors of a node. High local efficiency means that a node is part of a highly connected environment. Low local efficiency means that the node is part of a sparsely connected environment. The participation coefficient measures the extent to which a node connects to other nodes that are part of a different subnetwork. High participation coefficients mean that a node is mostly connected to nodes of a variety of other subnetworks. Low participation coefficients mean that a node is mostly connected to nodes of the same subnetwork (Power *et al*, 2011). Across the selected range of threshold values, we calculated i) mean differences between the rrMDD and HC group per network measure for the DMS, FPS and SRS, ii) Pearson correlations between the MAASD of ESM variables, that showed significant group differences, and network measures for the DMS, FPS and SRS in the rrMDD group only (n=39). The latter was not calculated in the HC group, since the MAASD of the ESM variables did not show substantial variation (S13, Figure S6). In order to obtain summarized scalars that are

independent of single threshold selection, we applied the area under the curve (AUC) and threshold-free cluster enhancement (TFCE; Smith and Nichols, 2009) method across threshold values per network measure. The AUC gives an overall measure of significance across threshold values, while the TFCE method gives a measure of significance per threshold value (corrected for the number of threshold values). Next, non-parametric permutation testing was applied on the AUC and TFCE per network measure to assess whether results could have occurred by chance. To this end, group membership/MAASD values was/were permuted randomly and both difference measures were recalculated. This procedure was repeated 5000 times and a two-tailed test of the null hypothesis ($p<0.05$) was performed.

Results

Sample characteristics

No significant differences were observed between rrMDD patients and HC, except for residual symptomatology as measured by the HDRS (rrMDD patients>HC, Table 1). When differences were recalculated for the selected samples used in the ESM and fMRI analyses, no significant differences were observed between rrMDD patients and HC, except for employment status in the fMRI sample (employment status: rrMDD patients<HC; $\chi^2_{(2,N=90)} = 6.13$, p=0.047) and residual symptomatology in both samples (residual symptomatology: rrMDD patients>HC; ESM: U=312.00, p=0.002; fMRI: U=540.00, p<0.0001).

Main effect of group on affective instability (MAASD)

The MAASD of the negative mood/cognition variables agitate, down, irritate, restless and worry showed significant differences between rrMDD patients and HC (rrMDD patients>HC, Figure 2 and Table 2). No significant differences in MAASD were observed between the two groups for positive mood/cognition variables and the other negative mood/cognition variables. Although the focus of the current study is on affective instability, it is notable that significant differences were found for the median of the positive mood/cognition variables enthusiastic, cheerful, relaxed, satisfied and empowered (rrMDD patients<HC, see S14, Table S3)

Results on network measures

Main effect of group

We found that the participation coefficient was decreased in the DMS in rrMDD patients compared to HC. No significant group differences were observed for local efficiency or the

participation coefficient calculated for the SRS and FPS (Table 3).

Association between affective instability and network measures in rrMDD patients

For $MAASD_{mood\ down}$, a significant negative correlation was found with the participation coefficient in the SRS. For $MAASD_{mood\ irritate}$, a significant positive correlation was found with local efficiency in the FPS (S15, Figure S7 and S8). No significant correlations were observed for the mood variables agitate and restless, and the cognition variable worry (Table 4).

Reanalyzing the data using binary graphs led to the same conclusions (S16, Table S4 and S17, Table S5). Furthermore, bootstrapping performed on the correlation slopes showed that the associations between affective instability and networks measures are fairly stable (S18, Figure S9 and S10). Moreover, a factor analysis on the MAASD data was performed to investigate the association between composite scores of the ESM data (instability in negative and positive affect) and network measures in rrMDD patients (see S19, Table S6 and S7 and Figure S11). No significant associations were found, only a trend significant positive correlation between local efficiency in the DSM and Factor 1 Instability in negative affect ($p=0.080$).

Discussion

We investigated associations between affective instability and connectomics in functional subnetworks in rrMDD patients. For the ESM analysis, we found increased affective instability in most negative mood/cognition variables in rrMDD patients compared to HC. For the graph analysis, we found that the DMS has less connections with other subnetworks in rrMDD patients compared to HC. For the ESM-fMRI analysis, we observed highly specific associations between affective instability and network measures. rrMDD patients, who showed more instability in feeling down, had less connections between the SRS and other subnetworks. Furthermore, rrMDD patients, who showed more instability in feeling irritated, had higher local efficiency coefficients in the FPS.

Affective instability

We found that rrMDD patients, compared to HC, were temporally less stable in worrying and feeling down, agitated, irritated and restless. Our findings are in line with ESM research showing i) increased reactivity in negative affect to social stress (van Winkel *et al,* 2015) and stressful events (Husky *et al,* 2009), and ii) increased negative affect on stressful days (O'Hara *et al,* 2014) in rMDD patients compared to HC. The current study is the first ESM study in rrMDD patients, since patients in abovementioned studies did not suffer from multiple episodes or this was unspecified. Although a positive relationship has been found between reactivity of negative affect to negative external events and instability in negative affect in ESM studies, caution is warranted because i) reactivity does not fully explain the level of instability, ii) we only focused on mood in the current study, leaving out daily events and iii) results with regard to reactivity in MDD in naturalistic settings have been inconsistent (Thompson *et al,* 2012). However, a recent

meta-analysis has shown an association between lower psychological well-being and increased affective instability in MDD patients (Houben et al., 2015). The fact that we found increased affective instability in rrMDD patients may indicate that this represents a trait effect rather than a state effect. Though, more research in rrMDD patients is needed to confirm the latter proposition.

The experience of positive affect was as temporally stable in rrMDD patients as in HC, but was overall lower in rrMDD patients. In line with this, Knowles *et al.* (2007) did not find differences in the degree of fluctuations in positive affect between rMDD patients and HC, though neither for negative affect. Furthermore, van Winkel *et al.* (2015) did not find significant differences in reactivity in positive affect to various stressors between rMDD patients and HC, however O'Hara *et al.* (2014) found lower positive affect on stressful days between these two groups. It is also in line with research in MDD wherein no differences in instability of positive affect were found in patients compared to HC, only lower levels of positive affect (Peeters *et al,* 2006; Thompson *et al,* 2012). Notably, different methods were used in abovementioned studies, which should be kept in mind when interpreting their findings. O'Hara *et al.* (2014) and van Winkel *et al.* (2015) used multilevel models to predict mood state/reactivity from the group x stress interaction. Knowles *et al.* (2007) used within-participant standard deviations. Notably, the MAASD is a more comprehensive measure to capture fluctuations in affect than the standard deviation, since temporal dependency (besides variability) is taken into account. This may explain the null findings in the study of Knowles *et al.* (2007).

Several possible explanations have been proposed in the literature for increased affective instability in MDD patients, which may also play a role in r(r)MDD patients, including interpersonal impairment, difficulties in emotion regulation and increased cognitive reactivity (O'Hara *et al,* 2014; Thompson *et al,* 2011, 2012). Furthermore, decreased positive affect may weaken personal resources and adaptive coping, since positive affect acts as a resilience factor

(O'Hara *et al,* 2014). It would be of particular interest to investigate whether affective instability in negative affect predicts recurrence in MDD in future research. If this is the case, stabilizing negative affective responses to stress and increasing positive affect may represent clinical objectives to prevent relapse and guide decision-making for the implementation of recurrence preventive strategies.

Connectomics

We found that the DMS has less connections with other subnetworks in rrMDD patients compared to HC, possibly leading to a more isolated position of this subnetwork within the network organization. The DMS has been reported to be more active during conditions of rest and is postulated to subserve functions, such as autobiographical memory, self-reflection, introspection and emotion regulation (Buckner *et al,* 2008). Furthermore, numerous studies have shown the involvement of the DMS in rumination; an important feature in depressive states and an established risk factor for recurrence of MDD (Hamilton *et al,* 2011; Marchetti *et al,* 2012). Markedly, a theoretical model of the underlying neural mechanisms of rrMDD was constructed based on the DMS, postulating that most of its dysregulations observed in the acute phase are still present during remission (Marchetti *et al,* 2012). Specifically, it is proposed that ineffective switching between the DMS and task positive subnetwork - involved in external attention/cognition - increases the risk for recurrence (Marchetti *et al,* 2012). Thus far, connectomic studies in MDD have primarily been focused on within-subnetwork integration and showed increased regional connectivity in the DMS in patients compared to HC (Gong and He, 2015). In our sample of rrMDD patients, we did not find increased local efficiency in the DMS, only in relation to $MAASD_{mood\ down}$ (positive, p=0.053). Furthermore, we did not find significant

differences in the network metrics for the SRS and FPS. It is possible that, during remission, these measures are normalized or influence mood and behavior in a more complex way. Future research should address which brain areas have altered connectivity with the DMS, and whether recurrence can be prevented by normalizing these abnormalities. For instance, a study, in which transcranial magnetic stimulation (TMS) is used, showed a reduction of depression-related hyperconnectivity in the DMS and an induction of anticorrelated connectivity between the DMS and FPS after five weeks of treatment (Liston *et al*, 2014).

Affective instability and connectomics

First, we found that rrMDD patients, who showed more instability in feeling down, had less connections between the SRS and other subnetworks. In the current study, the SRS consisted mainly of brain regions that are part of the striatum. Previous research has shown reduced striatum activation during reward processing in MDD patients compared to HC (Pizzagalli *et al*, 2009; Smoski *et al*, 2009), which has also been found in recovered MDD patients (McCabe *et al*, 2009). Furthermore, computational models of reinforcement learning have shown impairments in MDD involving –among others- the striatum (Chen *et al*, 2015). Moreover, altered fronto-striatal connectivity has been found in response to monetary gains and losses in unmedicated MDD patients compared to HC (Admon *et al*, 2015b). The authors interpreted these results as an impairment in the positive and negative feedback circuit, leading to altered saliency of positive and negative events (Admon *et al*, 2015b). In rrMDD patients compared to HC, the same authors found increased connectivity between the striatum and amygdala/hippocampus in response to mild stressors (Admon *et al*, 2015a). Thus, the results of the current study suggest that altered

integration between the SRS and other subnetworks is possibly associated with dysfunctions in depression-related processes, such as reward and stress.

Second, we found that rrMDD patients, who showed more instability in feeling irritated, had higher local efficiency coefficients in the FPS. Irritability has been shown to be prevalent in MDD and longitudinal studies in non-clinical samples have shown that irritability-related traits predict depressive and anxious psychopathology later in life (Leibenluft and Stoddard, 2013). Leibenluft (2011) proposed in her neurobiological model that ineffective frontal inhibition of emotional systems leads to feelings of irritation and frustration, when goals cannot be attained. In line with this, the FPS has been found to be involved in functions related to cognitive control (Laird *et al*, 2011). Indeed, two studies, that induced irritability by recalling autobiographical experiences, found increased prefrontal activation during irritability scripts compared to neutral scripts in HC (Cerqueira *et al*, 2010, 2014). These findings suggest that our result of higher local efficiency in the FPS may act as a compensatory mechanism to cognitively control (fluctuating) feelings of irritation and frustration. Notably, it would be interesting to investigate whether abovementioned ESM-fMRI findings are specific to rrMDD patients or can also be found in HCs when, for example, they are under more stress due to negative life events or score higher on personality traits such as neuroticism, and show more affective instability.

To prevent first or recurrent depressive episodes, the idea has been coined to strengthen network connectivity underlying resilience (a.o. DMN: ↓, SRS: ↑, FPN: ↑, DMN-FPN anticorrelations: ↑) through interventions such as mindfulness, psychotherapy and TMS. The results of our study are in line with pursuing these suggestions, but the effect of such interventions on connectomic measures/connectivity still needs to be demonstrated. With regard to TMS, multiple biomarkers have been identified that predict clinical outcome after repetitive TMS (rTMS), but there is still substantial heterogeneity in the biomarker type and effect.

Furthermore, studies are limited that investigate whether rTMS induces (lasting) plasticity changes in subnetworks. Though promising, more research on the clinical applicability of functional connectivity in depression is needed to clarify these matters (see Fischer *et al*, 2016 for a recent review).

Limitations

The results from the ESM-fMRI analysis should be considered exploratory, because of the number of statistical tests that were performed. We tried to alleviate the multiple comparison problem by calculating the AUC and a mean of the nodal network measures per subnetwork. To provide insight in the reliability of the results, we performed permutation testing and bootstrapping (S18). Notably, it is difficult to adequately correct for multiple comparisons in graph analyses, since network measures are not independent from each other. Multivariate methods would be an option, but results from these types of analysis are more difficult to interpret (Simpson *et al,* 2013). For abovementioned reasons, our results are in need of replication.

Conclusion

The aim of the current study was to investigate associations between affective instability and connectomics in functional subnetworks in rrMDD patients. We found that rrMDD patients, compared to HC, are less stable in their negative affect and that these dynamics are related to the way information is processed within and between specific functional subnetworks. The findings provide i) real-life validity to connectomics using ESM and ii) a neurobiological correlate associated with affective instability using connectomics. The findings may facilitate a better

understanding of how fluctuations in real-life mood are represented in the brain of rrMDD patients, providing insights in the vulnerability profile of MDD.

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Figure legends

Figure 1 Module decomposition. Nodes could be partitioned in six functional subnetworks with a maximum number of within-group edges and a minimum number of between-group edges. In the current study, we focused on the default mode subnetwork (DMS, purple), fronto-parietal subnetwork (FPS, red) and the salience/reward subnetwork (SRS, blue). Nodes are pasted on a surface template of the human brain using BrainNet Viewer (Xia *et al*, 2013). In the panels, different views are shown: A. left lateral, B. right lateral, C. left medial, D. right medial.

Figure 2 Boxplots of the MAASD for each ESM variable per group.

Table 1 Sample characteristics

				Between-group statistics			
		rrMDD (n = 62)	**HC (n = 41)**	**χ^2**	**T**	**U**	**p**
Female	N	43	28	0.01			0.91
Age	Years; mean (SD)	53.7 (7.9)	51.8 (8.1)		1.17		0.25
Education	Levels[1]	0/0/0/4/21/23/14	0/0/0/1/16/17/7	1.49			0.69
IQ	Mean (SD)	108 (8.5)	106 (9.9)			878.5	0.14
Living situation	Levels[2]	26/0/18/14/2/0/2	10/0/16/11/4/0/0	6.23			0.18
Employment status	Levels[3]	24/23/15/0	21/16/4/0	3.70			0.16
Handedness	Levels[4]	4/50/4	2/33/4	0.44			0.80
Age of onset	Years; mean (SD)	27.18 (11.18)[5]	-				-
Episodes	Mean (SD)	8.02 (11.7) [5]	-				-
HDRS	Mean (SD) Range	2.81(2.36) 0-9	1.02(1.42) 0-5			686	<0.001

HC: healthy controls; HDRS: Hamilton Depression Rating Scale; rrMDD: remitted recurrent major depressive disorder; SD=standard deviation. [1]Level of educational attainment (Verhage, 1964). Levels range from 1 to 7 (1 = primary school not finished, 7 = pre-university/university degree); [2]Living situation: alone/living with parents/cohabiting/cohabiting with children/single living with children/other/unknown; [3]Employment status: low/middle/high/never worked; [4] Handedness: left/right/ambidexter; [5]One missing value. χ^2: chi-square test statistic; p: p-value; U: Mann-Whitney U non-parametric test statistic; T: independent-samples T test statistic.

Table 2 Main effect of group on affective instability (MAASD)

ESM variable	Between-group statistics		
	U	**z**	**p**
Positive mood			
enthusiastic	472	-1.31	0.189
cheerful	486	-1.14	0.253
relaxed	449	-1.59	0.111
satisfied	511	-0.83	0.402
Negative mood			
agitate	343.5	-2.88	0.004*
down	346.5	-2.84	0.004*
irritate	314	-3.23	0.001*
lonely	351	-2.80	0.005
restless	333.5	-3.00	0.003*
Positive cognition			
empowered	505	-0.91	0.362
self-like	510	-0.85	0.395
Negative cognition			
ashamed	490	-1.11	0.266
self-doubt	413.5	-2.03	0.043
worry	329	-3.06	0.002*

ESM: experience sampling methodology; MAASD: mean adjusted absolute successive difference; p: p-value; U: Mann-Whitney U non-parametric test statistic; z: z-score. The total sample size for the ESM analyses was n=69 (rrMDD patients: n=43; HC: n=26).
*** p-value≤(0.05/14=)0.004**

Table 3 Main effect of group on network measures

Subnetwork	Weighted		Direction
	AUC (p-value)	TFCE (threshold values)	
Local efficiency			
DMS	0.145	-	
FPS	0.363	-	
SRS	0.205	-	
Participation coefficient			
DMS	0.026**	0.2-0.30**	rrMDD<HC
FPS	0.587	-	
SRS	0.142	-	

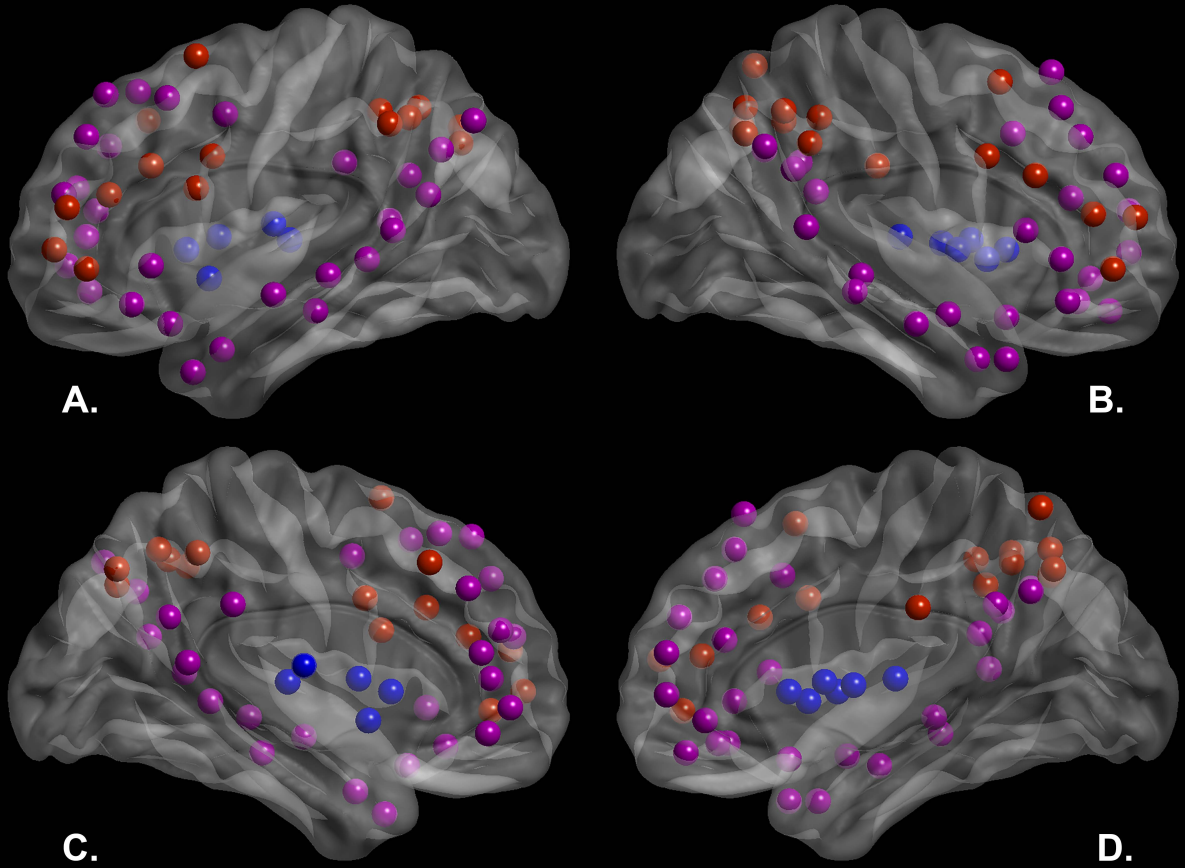
AUC: area under the curve; DMS: default mode subnetwork; FPS: frontal-parietal subnetwork; HC: healthy controls; rrMDD: remitted recurrent major depressive disorder; SRS: salience/reward subnetwork; TFCE: threshold-free cluster enhancement. The total sample size for the fMRI analyses was n=90 (rrMDD patients: n=53; HC: n=37).
**** p-value<0.05, * p-value <0.10**

Table 4 Correlation between affective instability and network measures in rrMDD patients

Subnetwork	Weighted		Direction
	AUC (p-value)	TFCE (threshold values)	
Mood agitate			
Local efficiency			
DMS	0.110	0.01-0.03**	Positive
FPS	0.199	0.01**	Positive
SRS	0.714		
Participation coefficient			
DMS	0.166	-	
FPS	0.267	-	
SRS	0.678	-	
Mood down			
Local efficiency			
DMS	0.053*	0.01-0.06, 0.09-0.11**	Positive
FPS	0.393	-	
SRS	0.754	-	
Participation coefficient			
DMS	0.161	-	
FPS	0.371	-	
SRS	0.025**	0.02-0.30**	Negative
Mood irritate			
Local efficiency			
DMS	0.250	-	
FPS	0.031**	0.01-0.30**	Positive
SRS	0.169	-	
Participation coefficient			
DMS	0.237	-	
FPS	0.066*	0.23-0.30**	Negative
SRS	0.796	-	
Mood restless			
Local efficiency			
DMS	0.792	-	

FPS	0.805	-	
SRS	0.444	-	
Participation coefficient			
DMS	0.937	-	
FPS	0.389	-	
SRS	0.599	-	
Cognition worry			
Local efficiency			
DMS	0.767	-	
FPS	0.191	-	
SRS	0.087*	0.06, 0.08-0.13**	Negative
Participation coefficient			
DMS	0.949	-	
FPS	0.477	-	
SRS	0.151	-	

AUC: area under the curve; DMS: default mode subnetwork; FPS: frontal-parietal subnetwork; rrMDD: remitted recurrent major depressive disorder; SRS: salience/reward subnetwork; TFCE: threshold-free cluster enhancement. The total sample size for the ESM-fMRI analyses was n=39 rrMDD patients.
**** p-value<0.05, * p-value <0.10**



DMS FPS SRS