Vulnerability and emotional processing in depression

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Chapter 3

The neural correlates of self-reflection in depression: activation and network connectivity

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Abstract

It has been proposed that depression involves an imbalance in default-mode and control networks, which may be associated with facilitated self-reflective processing. Activation abnormalities during self-reflection have been reported in the medial prefrontal cortex of depressed patients, which is an important default-mode area. However, the involvement of intrinsic connectivity networks has not yet been investigated. Activation during a self-reflection task was compared between 24 depressed and 24 matched non-depressed participants, both in voxel-wise and network-level analyses. Independent component analysis was used to delineate intrinsic connectivity networks, in order to investigate group differences in connectivity within and between networks during self-reflection task performance. The networks of interest were the default mode, salience and frontoparietal control networks. Depressed participants showed higher activation in the dorsomedial prefrontal cortex compared to non-depressed participants during reflective processing (cluster-level $pFWE<0.05$ after small-volume correction). In the whole sample, the anterior default-mode network was deactivated during semantic but not reflective processing, and showed positive connectivity with the task-positive control network. No network-level group differences in activation or connectivity emerged. Increased activation in the dorsomedial prefrontal cortex during self-reflection might be associated with impairments in self-evaluation and decision-making in depression. However, contrary to expectations, depressed patients recruited networks of interest in a similar way as non-depressed participants during self-reflection task performance. The results suggest that node-level activation abnormalities during self-reflection may be more characteristic for depression than network-level abnormalities.

3.1. Introduction

Increased self-focused attention is an important characteristic of depression. A meta-analysis has demonstrated that the experience of negative affect is consistently associated with an increase in self-focused attention (Mor and Winquist, 2002). Sustained self-focused attention in a negative context can contribute to the onset and maintenance of depressed mood and other depressive symptoms such as feelings of guilt and a negative self-image (Pyszczynski and Greenberg, 1987). Reciprocally, the continuous experience of low mood may lead to an inability to escape the self-evaluation process. In line with this reasoning, depressed patients appear to be characterized by facilitated processing of self-related information (Northoff, 2007; Phillipi and Koenigs, 2014).

At the level of brain functioning, self-related processing is often investigated with a self-reflection task, in which participants judge the self-relevance of presented stimuli. A meta-analysis has demonstrated cortical midline structures to be key regions that are activated during self-reflection (Van der Meer et al., 2010). Specifically, the medial prefrontal cortex is more activated by self- than other-reflection (Van der Meer et al., 2010; Murray et al., 2012). Previous studies have shown that, during self-reflection, depressed patients may show subtle activation abnormalities in the medial prefrontal cortex (Grimm et al., 2009; Johnson et al., 2009; Sarsam et al., 2013). The direction of effects may be dependent on the reference condition. Although self-reflection was not contrasted with reflection on close others, depressed patients did show more dorsal activation for self compared to semantic processing, yet less ventral activation for self compared to passive processing of stimuli. Lemogne and colleagues (2012) have proposed that these conflicting findings are due to impaired deactivation of default mode areas.

The default mode network is a unique collection of brain areas that are typically more active at rest than during task performance (Gusnard et al., 2001). These areas are functionally connected during rest, which means they show correlated time-courses of activation. Subsequent connectivity analysis of task data has demonstrated that the default mode network is still functionally connected during task execution, despite the absolute decrease in activation. Therefore, it has been defined as an intrinsic connectivity network of the brain (Smith et al., 2009). Partial spatial overlap has been demonstrated between the cortical midline structures (active during self-reflection) and the default mode

network (active during rest) in a meta-analysis of healthy subjects (Qin and Northoff, 2011). It is remarkable that self-reflection elicits activation in these areas, because the default mode network is generally deactivated during task execution. This suggests that the default mode network may be deactivated to a lesser extent during self-reflection than during other tasks.

The frontoparietal control and salience networks are other intrinsic connectivity networks that may be relevant for self-reflection. Both networks are functionally connected during both rest and task execution, yet are more active during task execution and less active during rest (Fox et al., 2006). The salience network is thought to be sensitive to salient and emotional stimuli (Seeley et al., 2008). Sometimes it is classified as a control network (eg. Allen et al., 2011), although it is also thought to modulate switching between dominance of the default mode and frontoparietal control networks (Sridharan et al., 2008).Their complex interplay may be involved in the distribution of attention between internal (i.e. self) and external (i.e. environmental) stimuli (Buckner et al., 2008; Vanhaudenhuyse et al., 2011). The "dorsal nexus" is a hub node that connects the default mode, salience, and control networks together, and shows increased connectivity during rest in depressed patients (Sheline et al., 2010). The activation abnormalities observed in the dorsomedial prefrontal cortex during self-reflection in depressed patients is close to the dorsal nexus. Therefore, the interplay between these three networks may be relevant for self-reflection abnormalities in depression (Sheline et al., 2009, Marchetti et al., 2012).

In resting state studies, depressed patients have shown stronger connectivity in anterior parts of the default mode network (Zhu et al., 2012; Li et al., 2013) and posterior parts of the salience network (Veer et al., 2010; Manoliu et al., 2014), and weaker connectivity in the frontoparietal control network (Zhu et al., 2012; Veer et al., 2010). Moreover, stronger connectivity between the default mode and salience networks, and weaker connectivity between the control and salience networks in rest have been found (Hamilton et al., 2013, Tahmasian et al., 2013, Manoliu et al., 2014). Seed-based connectivity studies on self-reflection in depressed participants have found deviances that are spatially consistent with these networks. However, connectivity was mostly increased in depressed patients for a range of brain areas, including control areas (Lemogne et al., 2009, Yoshimura et al., 2010). Therefore, it is still uncertain whether the alterations in

connectivity that are observed during rest also occur during self-reflection task performance.

Intrinsic connectivity networks offer a modular perspective to functional brain organization, that is conceptually parsimonious and reduces the multiple testing problem. Alterations in intrinsic connectivity networks may explain the distributed pattern of activation abnormalities observed in a range of psychopathological conditions, including depression (Barrett and Satpute, 2013). The advocated network approach has not yet been used to directly test the involvement of default mode, salience and frontoparietal control networks in self-reflection in general, or to investigate network level abnormalities during self-reflection in depressed participants in particular.

Therefore, the current study aims to replicate results from previous studies using a voxel-wise approach to test activation abnormalities during self-reflection in depression and extend these findings by 1) examining average activation levels of the default mode, salience and frontoparietal control networks during self-reflection, other-reflection and semantic processing, and 2) examining whether depressed patients have abnormal activation and/or connectivity within and connectivity between these networks during self-reflection.

3.2. Materials and methods

3.2.1. *Participants and study procedure*

Data were derived from the study "Depression in the Picture", which aimed to investigate the neural correlates of depression by comparing depressed outpatients (N=24) to control participants (N=24). Depressed outpatients were recruited from specialized mental health care institutions in Groningen, the Netherlands (Lentis N=11 and University Center of Psychiatry N=7). Clinicians were asked to screen their caseloads for depressed patients that fulfilled the major in- and exclusion criteria of the study. Clinicians provided eligible patients with written information about the study. Five additional depressed participants responded to advertisements at the participating institutions, and one depressed participant responded to an advertisement at a depression research website and received treatment at a different mental health care institution. Control participants were recruited by means of advertisements at public places and in local newspapers. The researchers

provided these participants with written information about the study. A subsample of control participants also served as controls for a study in renal patients.

General exclusion criteria were: age younger than 18 years, neurological problems that may influence task performance, medication use that may interfere with task performance, inadequate comprehension of Dutch language, concrete suicidal plans, and general MRI-contraindications such as metal implants or pregnancy. For the current research groups, end-stage renal disease, cerebro-, and cardiovascular disease (consistent with the substudy on renal disease patients) and psychotropic medication use other than SSRI/SNRI/TCA or infrequent benzodiazepine were additional exclusion criteria. Moreover, participants were excluded when meeting criteria for any other psychiatric diagnosis than depressive or anxiety disorder in the patient group, and any other than current nicotine dependence or history of substance dependence/abuse in both groups. The healthy control participants were matched with the depressed participants on sex, age, education level and self-reported handedness.

Initial screening was performed with the second edition of the Beck Depression Inventory (BDI-II; Beck et al., 1996). Depressed participants were included when reporting at least mild depressive symptoms (BDI-II score >13). The control group was selected on low depressive symptom levels (BDI-II score <9). A telephonic interview was conducted to screen for lifetime psychiatric comorbidity, somatic health and current medication use. Adapted screening questions from the shortened version of the Schedules for Clinical Assessment in Neuropsychiatry (mini-SCAN; Nienhuis et al., 2010) were used to screen for psychiatric diagnoses and the information was cross-checked with medical records when necessary. The study protocol was approved by the local Medical Ethics Committee and all participants gave written informed consent before entering the study. The first session consisted of establishing current (past-month) psychiatric diagnosis by a full mini-SCAN interview, several neuropsychological tasks and questionnaires. The second session consisted of instructions, an MRI protocol that comprised several structural and functional scans (duration: 75 minutes, task order counterbalanced between subjects) and questionnaires.

3.2.2. Measures

Depression severity was measured with the BDI-II. The BDI-II consists of 21 questions addressing cognitive and somatic-affective symptoms of depression that were present in the past two weeks (Beck et al., 1996). The questions are scored by the participant on a 4-point rating scale (range total score: 0-84). Higher ratings reflect higher frequency and severity of the experienced symptoms. The BDI-II is well-validated and has a high internal consistency (Cronbach's $\alpha \approx 0.90$; Beck et al., 1996). The BDI-II was used at the initial screening procedure and was administered again after the MRI scan. As an additional characteristic, self-esteem was measured with the Rosenberg Self-Esteem Scale (RSES, Rosenberg, 1965) containing 10 items scored on a 4-point rating scale, summed up to a total score with range 0-30.

Psychiatric diagnoses were established with the MINI-SCAN according to criteria from the Diagnostic and Statistical Manual of mental disorders, fourth edition, text revision (DSM-IV-TR; APA, 2004) during a face to face interview. The MINI-SCAN is a semi-structured interview that was developed as a more practical and shorter version of the SCAN. The MINI-SCAN has demonstrated good psychometric properties and validity has been confirmed by comparisons with the longer version of the SCAN (Nienhuis et al., 2010). In the current study, two trained interviewers conducted the interviews with depressed patients (NG and MM).

The self-reflection task that was performed during the MRI-scan was adapted from Modinos and colleagues (2009). Prior to the scan, participants received elaborate task instructions and chose the name of a close-other that would be presented to them in the other-condition of the task. The close-other was restricted to be a relatively less-familiar other (not a partner or first-degree family). The restriction was chosen to guarantee similar episodic memory retrieval processes in the self- and other-conditions of the task, whilst optimizing the difference in self-relatedness between the self- and other-conditions (for a discussion of self-relatedness see Murray et al., 2012). The task contained three main conditions: self-reflection ("I am trustworthy"), other-reflection ("Other is friendly"), and semantic ("Snow is black") that consisted of 60 sentences each. Stimuli in the self and other conditions comprised traits balanced on valence and quality (mental vs physical), whereas stimuli in the semantic condition were balanced on verity, as reported by Van der Meer and colleagues (2013).The stimuli were presented in E-

prime 2 (Psychology Software Tools Inc., Pittsburgh, PA). For each sentence, participants were asked to indicate the level of agreement on a 4-point rating scale, from 1-fully disagree to 4-fully agree, with an MRI-compatible button box. Stimuli were presented in blocks of five sentences per main condition. The order of conditions was initially randomized and thereafter fixed for each participant. Sentence presentation took 4000ms followed by a 500ms fixation cross. Condition blocks were interspersed with 7 blocks of 20s fixation cross.

3.2.3. Image acquisition

Images were acquired with a 3.0 Tesla whole body scanner and a 32-channel coil (Philips Intera, Best, NL). Head position was fixed with foam cushions on each side of the head and participants were instructed to lie still during image recording. Functional images consisted of 475 T2*-weighted echo planar images that were acquired for each participant. Images were tilted to approximately a 25-30° angle from the anterior commissure - poster commissure (AC-PC) plane. Additional local shimming was performed in the preparation phase to minimize artefacts in the orbitofrontal cortex (Weiskopf et al., 2007). Each image consisted of 37 descending axial slices (slice gap = 0 mm; TR = 2.00s; TE = 20ms; FOV = 224, 224, 130 mm; 64×61 matrix of 3.5×3.5×3.5 mm voxels). A T1-weighted 3D fast-field echo (FFE) image was acquired parallel to the AC-PC plane (170 slices; TR = 9ms; TE = 3.6ms; FOV = 256, 232, 170 mm; 256x231 matrix of 1×1×1 mm voxels) for anatomical reference.

3.2.4. Statistical analysis: Behavioral data

Behavioral data from the self-reflection task were analyzed in SPSS package version 20. To ensure reliability of the data, reaction times (RT) and associated responses were considered missing if RT < 500ms and the previous sentence showed no response. Responses were dichotomized into agree and disagree responses. Repeated measures analysis of variance (ANOVA) was used to examine main and interaction effects of group, condition, and valence separately for RT and percentage agreed responses.

3.2.5. Statistical analysis: Preprocessing

After converting images from Philips PAR to NIFTI format, data were preprocessed in the Statistical Parametric Mapping package (SPM8; www.fil.ion.ucl.ac.uk), run in Matlab7 (The MathWorks Inc., Natick, MA). First, anatomical images were manually aligned and functional images were reoriented towards the AC-PC plane. Functional images were realigned and then coregistered to the anatomical image. After visually checking and manually correcting coregistrations, images were spatially normalized using a template combining data from all participants generated with Diffeomorphic Anatomical Registration Exponentiated Lie algebra (DARTEL) towards MNI space, resampled into a 3x3x3 mm grid and smoothed with a 10mm full-width/half-maximum Gaussian kernel.

3.2.6. Statistical analysis: Voxel-wise general linear model

For first level analyses, a canonical hemodynamic response function was applied to the task condition regressors in a general linear model. Onset and duration of task condition blocks were modeled at the voxel level. When realignment parameters indicated the presence of motion spikes, high-motion data points (Framewise Displacement>0.5, in accordance with Power et al., 2012) were censored by adding these volumes as regressors to the model. Hereafter, contrast maps were created for self compared to semantic and other compared to semantic blocks.

For voxel-wise second level analyses, the contrast maps were entered into a 2-by-2 analysis of variance (ANOVA) model with group and valence as factors. Considering the anticipated strong task effects, within the ANOVA model T-tests were created for self versus semantic, other versus semantic, and self versus other comparisons at voxel-wise pFWE<0.05 and k>10 for task validation. Then, the interaction of group (between-subjects: depressed versus controls) and condition (within-subjects: self>semantic versus other>semantic) was tested, followed by the main effect of group. These results were evaluated against a threshold of pFWE<0.05 at cluster level, with an initial voxel-wise threshold of p<0.001 uncorrected. Small-volume correction was applied to the medial prefrontal cortex (region-of-interest derived from the automated anatomic labeling scheme (AAL; Tzourio-Mazoyer et al., 2002) implemented in the Wake Forest University Pickatlas toolbox: superior medial frontal gyrus and medial orbitofrontal cortex). The

ANOVA model was estimated with and without censoring of high-motion data points in order to visualize the impact of motion on the main outcomes.

3.2.7. Statistical analysis: Group independent component analysis

Next, preprocessed functional data were decomposed into functional networks with group-level spatial independent component analysis (ICA; Calhoun et al., 2001) using the GIFT toolbox (v3.0a, available at http://mialab.mrn.org/software/gift). The number of estimated components was based on the mean minimum description length (MDL; Li et al., 2007). Components were selected to visually resemble the default mode, frontoparietal control, and salience network as described by Laird and colleagues (2011). Visual inspection of the spatial maps was performed by two independent researchers (NG and EO) to exclude potential artifacts, based upon localization predominantly in grey-matter and low spatial overlap with known artifacts. The robustness of the decomposition was confirmed by means of 10 consistent repetitions of the algorithm with random starting points using the ICASSO toolbox (Himberg et al., 2004) in GIFT. Next, GICA3 back-reconstruction (Erhardt et al., 2011) was applied to the components in order to generate subject-specific spatial maps and time-courses, scaled in Z-scores to facilitate interpretation of the outcomes. Finally, the previously identified high-motion data points were removed from the time-course and interpolated using a spline function.To determine group differences in connectivity within the networks of interest, subject-specific spatial maps generated by independent component analysis were compared in SPM8. An F-test was used ($p_{FWE}<0.05$) to create spatial masks for the components of interest, which were subsequently used for small-volume correction. Group differences were tested with a two-sample t procedure and results were tested against a cluster-level $p_{FWE}<0.05$, with an initial threshold of $p<0.001$ uncorrected.

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In order to relate time-courses of the components to the task conditions, temporal regression was performed in GIFT with the design matrices from uncensored first-level analyses. First, beta weights for the self, other and semantic conditions were compared against the fixation cross resting baseline. Then, group differences in task relatedness were tested. For temporal regression analyses, thresholds for significance were adjusted (α=0.05 / K components of interest) to take the number of multiple comparisons into account. Both original and interpolated time-courses were analyzed to visualize the impact of motion.

Functional connectivity between the networks was analyzed with the MANCOVAN toolbox in GIFT (Allen et al., 2011). Because "spikes" in the time-course greatly affect connectivity measures, and the interpolation of high-motion data points did not remove all spikes from the data, subject-specific timecourses were preprocessed with the default detrending, filtering and despiking steps implemented in GIFT. The components of interest were pairwise correlated, and group was added as a predictor. Overall connectivity was determined and group differences in connectivity were tested against a false-discovery rate (FDR) corrected $p<0.05$, which is the MANCOVAN default. Results were presented for the original and interpolated time-courses.

3.2.8. Statistical analysis: Post-hoc connectivity analysis based on activation results

To examine whether focal activation differences in depressed patients were accompanied by altered connectivity, connectivity analysis was performed between the timecourses of the activation foci and timecourses of the components of interest. First, timecourses were extracted from activation foci from the voxel-wise GLM analysis, and preprocessed (motion-corrected, detrended, and filtered – similar to the GIFT procedure). Pairwise correlations with the component timecourses were calculated for each condition for each individual. A Fisher r-to-z transformation was applied to the data and the data were analyzed with repeated measures ANOVA (group, condition, group-by-condition) in SPSS.

3.3. Results

3.3.1. Descriptives

In total, 24 depressive en 24 healthy control participants were included for analysis. The groups were matched on age, sex, education level and handedness (Table 1). Depressed patients reported lower self-esteem than controls (T=-11.4, p<0.001). Three participants fulfilled the criteria for depression not otherwise specified rather than major depressive episode in the month before the diagnostic interview, but still received treatment for depression in mental health care. On average, depression severity was moderate at the time of scanning. Ten depressed participants used antidepressant medication.

Table 1. Demographic and clinical characteristics for depressed and healthy control groups.

Variable	Depressed (N=24)	Healthy Control (N=24)
Demographics		
Age (M,sd)	44.3 (13.8)	43.8 (14.9)
Female sex (N, %)	18 (75.0)	17 (70.8)
Left handedness (N, %)	5 (20.8)	5 (20.8)
Education (N, %)		
Low	0 (0.0)	1 (4.2)
Middle	8 (33.3)	7 (29.2)
High	16 (66.7)	16 (66.7)
Clinical characteristics		
Severity (BDI-II:M,sd)	24.5 (9.9)	1.4 (1.8)
Diagnosis (N, %)		
Depression NOS	3 (12.5)	-
First MDE	6 (25.0)	-
Recurrent MDE	15 (62.5)	-
Comorbid anxiety* (N, %)	6 (25.0)	-
Antidepressant use (N, %)		
SSRI	6 (25.0)	-
SNRI	2 (8.3)	-
TCA	2 (8.3)	-

*Comorbid anxiety in past month: 4x generalized anxiety disorder, 1x social phobia, 1x agoraphobia. *Abbreviations*: BDI-II=Beck Depression Inventory-2nd edition, NOS=not otherwise specified, MDE=major depressive episode.

3.3.2. *Task performance*

The repeated measures ANOVA for reaction times revealed a group by condition interaction ($F(2,45) = 5.30$, $p=0.01$), without group or condition main effects ($p>0.05$). Post-hoc group comparisons showed that depressed patients responded slower than control participants in the self-condition ($T=-2.2$, $p=0.03$), but not in the other ($T=-1.7$, $p=0.1$) and semantic ($T=-0.3$, $p=0.8$) conditions.

Since valence was not included in the semantic condition, valence effects were explored solely in the self and other conditions with another repeated measures ANOVA. There was a main effect of valence ($F(1,46) = 6.16$, $p=0.02$) for RT, but no interactions ($p>0.05$). Participants responded more slowly to positive than to negative statements in both groups (Table 2).

Finally, percentage of items agreed showed a valence by group by condition interaction ($F(1,46) = 37.54$, $p<0.01$). Participants in the control group more often agreed with positive statements than with negative statements ($F(1,23) = 375.74$, $p<0.01$), to a similar extent for themselves and others (valence by condition: $p=0.87$). In contrast, depressed participants evaluated themselves more negatively compared to others (valence by condition: $F(1,23) = 59.49$, $p<0.01$).

Table 2. Behavioral results - ratings and reaction times for the depressed and healthy control groups.

Condition	**Depressed (N=24)**		**Healthy Control (N=24)**	
	% Items Agreed	Mean RT Seconds (SD)	% Items Agreed	Mean RT Seconds (SD)
Self	-	2.30 (0.4)	-	2.07 (0.3)
Other	-	2.26 (0.4)	-	2.08 (0.3)
Semantic	-	2.17 (0.4)	-	2.14 (0.3)
Self - Negative	33	2.28 (0.4)	12	2.04 (0.3)
Self - Positive	55	2.33 (0.4)	77	2.10 (0.4)
Other - Negative	12	2.21 (0.4)	12	2.07 (0.3)
Other - Positive	76	2.31 (0.4)	77	2.10 (0.4)

3.3.3. Task activation in voxel-wise analyses: full-factorial ANOVA

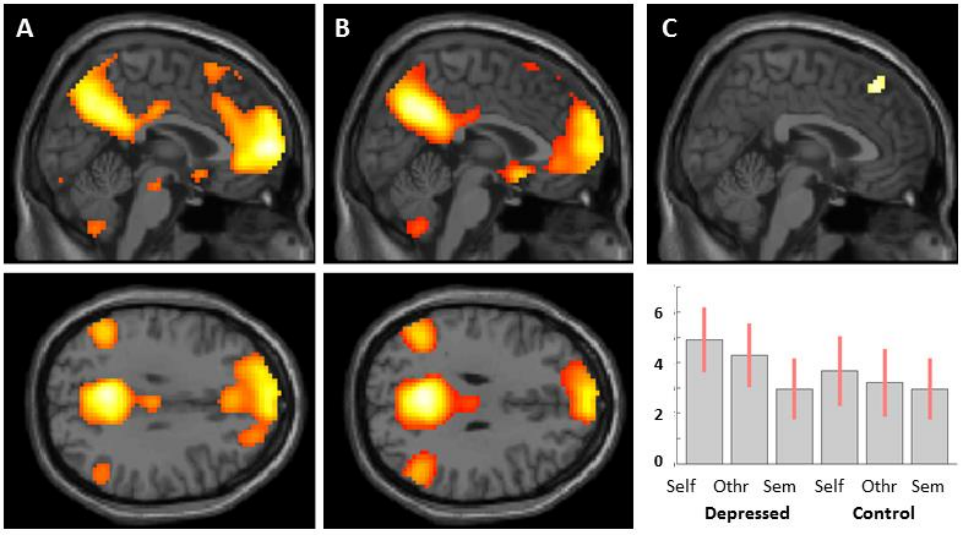
There were widespread activation differences between the self-reflection and semantic conditions, with the most prominent clusters located in the medial orbitofrontal cortex (BA10) and precuneus (BA7). These clusters were also most prominent for the other-reflection and semantic comparison. Other task activations shared by self- and other-reflection were located in the bilateral cerebellum, inferior temporal gyri, left temporoparietal junction and left fronto-insular cortex (Supplementary Tables 1-2). Visualization revealed a pattern that resembled a default-mode network (Figure 1A/B).

Several frontal areas were more active for self- compared to other-reflection, including the dorsomedial prefrontal cortex (BA6), dorsal anterior cingulate cortex (BA32) and right dorsolateral prefrontal cortex (BA46). In contrast, other-reflection more strongly activated posterior areas such as the precuneus, bilateral middle temporal gyri and temporoparietal junctions (Supplementary Table 3).

3.3.4. Group differences in task activation in voxel-wise analyses

The full factorial model did not reveal any significant group-by-condition interactions, indicating similar group differences in brain activation for the self versus semantic and other versus semantic comparisons. For the reflective conditions, depressed participants showed more activation than healthy control participants in the dorsomedial prefrontal cortex (BA8: k=57, cluster-level pFWE=0.03 after small-volume correction). The cluster contained three peaks (MNI coordinates x=-6, y=36, z=60, T=3.94; x=3, y=33, z=51, T=3.51; x=-6, y=33, z=42, T=3.22). Post-hoc comparisons with the fixation cross baseline (rest) showed that depressed participants displayed more task activation in this area compared to rest, with strongest effects observed for the self-reflection condition (Figure 1C). Furthermore, there was a subthreshold trend towards more activation in depressed patients in the left inferior frontal gyrus (BA44: k=40, peak MNI coordinates x=-33, y=21, z=21, T=4.91, punc<0.001).

Figure 1. Task activation differences

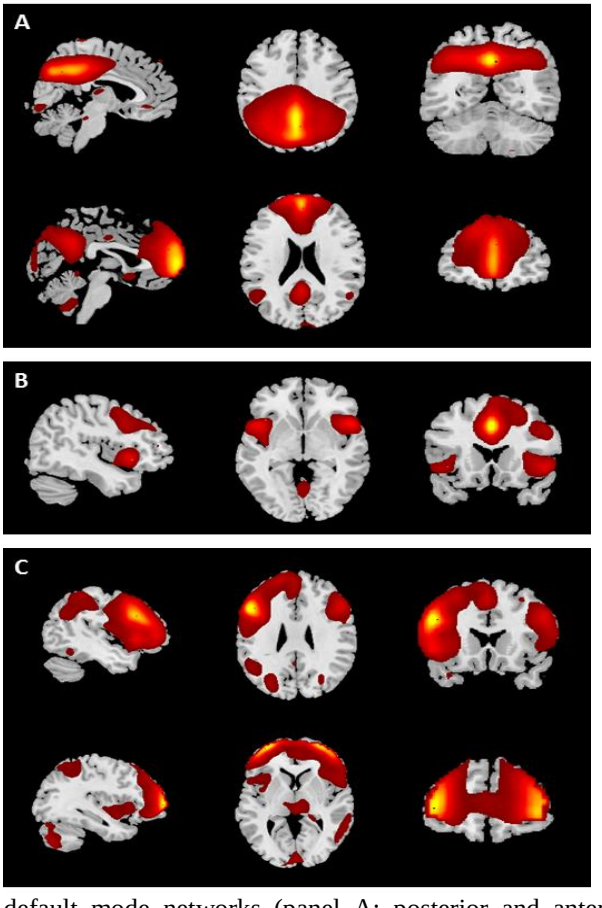


Activation differences for the self >semantic (panel A) and other>semantic (panel B) comparisons showed a default-mode network pattern in the whole group of participants. Depressed participants showed more activation than controls in the dorsomedial prefrontal cortex (medial BA8). Parameter estimates and 90% confidence intervals of post-hoc comparisons of task conditions against a resting baseline are shown in arbitrary units (panel C: left = depressed, self>rest, other>rest, semantic>rest, and right = healthy control, self>rest, other>rest, semantic>rest).

3.3.5. Component selection after group independent component analysis

The minimum description length indicated that 33 independent components could be estimated from the data. The ICASSO runs demonstrated a good stability for this number of components. After performing the group independent component analysis, the resulting 33 components were classified. Two default-mode, one salience and two frontoparietal control components were identified. The default-mode and frontoparietal control networks were separated into an anterior and posterior component. Of note, the posterior default mode component comprised the precuneus and posterior cingulate cortex. Importantly, there were no group differences in connectivity within the spatial maps of the networks of interest. The five networks are depicted in Figure 2.

Figure 2. Intrinsic Connectivity Networks



Visualization of the default mode networks (panel A: posterior and anterior DMN), salience network (panel B), and the frontoparietal control networks (panel C: posterior and anterior FPCN) that were identified after group independent component analysis.

3.3.6. Task effects in networks derived from independent component analysis

Several of the selected networks showed modulation of activation levels by the task conditions. The anterior DMN showed lower activation levels for semantic stimuli compared to rest. However, there were no differences in activation for the self and other stimuli compared to rest. The posterior DMN on the other hand was less active for each task condition, whereas the posterior frontoparietal control network was more active for each task condition compared to rest. Activation in the salience and anterior frontoparietal control networks was not strongly modulated by the task paradigm (Table 3). Importantly, there were no group differences in task-relatedness of the networks. Results were highly similar for the original and motion-corrected timecourses (Supplementary Table 4).

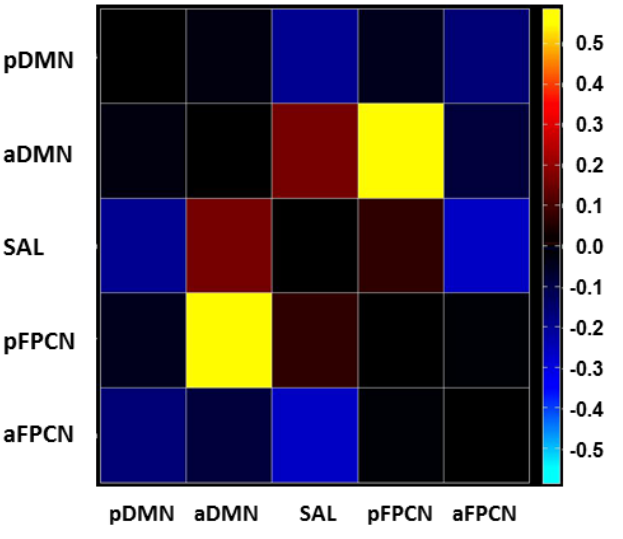
Table 3. Component activation differences for self>rest, other>rest, and semantic>rest (results based on original timecourses of posterior/anterior DMN, salience, and posterior/anterior FPCN).

Component	Self		Other		Semantic	
	T	p_{unc}	T	p_{unc}	T	p_{unc}
pDMN	**-4.655**	**<0.01**	**-2.574**	**0.01**	**-4.684**	**<0.01**
aDMN	-0.169	0.86	0.945	0.35	**-4.407**	**<0.01**
SAL	-0.168	0.87	-2.031	0.05	-1.626	0.11
pFPCN	**3.453**	**<0.01**	**3.539**	**<0.01**	**4.325**	**<0.01**
aFPCN	-0.734	0.47	-1.740	0.09	-2.252	0.03

3.3.7. *Functional connectivity between networks derived from independent component analysis*

The salience network showed connectivity with each of the other networks (Figure 3). Associations with the anterior DMN and posterior FPCN were positive, although the association with the posterior FPCN was largely diminished after motion correction (Supplementary Figure 1). Of note, the anterior DMN and posterior FPCN also showed very strong positive connectivity with each other ($r>0.5$). On the other hand, the salience network showed negative connectivity with the posterior DMN and anterior FPCN. The anterior FPCN showed negative connectivity with both of the DMN components. Importantly, there were no group differences in functional connectivity between the networks in the original and motion-corrected timecourses.

Figure 3. Correlation matrix of functional network connectivity between default mode (top rows), salience (middle row) and frontoparietal control networks (bottom rows). Yellow-red denotes positive connectivity, blue denotes negative connectivity. Abbreviations: a = anterior, p = posterior.



3.3.8. Post-hoc exploration of group differences in connectivity between the dorsomedial prefrontal cortex and networks derived from the independent component analysis

The post-hoc exploration showed positive connectivity between the dorsomedial prefrontal cortex and the anterior default mode (r=0.21), salience (r=0.24), anterior (r=0.20), and posterior frontoparietal control (r=0.13) networks in the total sample ($p<0.001$). However, no significant group-by-condition interactions, nor main effects of group ($p>0.05$) were found. Thus, there were no group differences in connectivity between the dorsomedial prefrontal cortex and the networks of interest.

3.4. Discussion

The aim of this study was to examine neural correlates of self-reflection in depressive patients by investigating focal regional activation and task-related modulation of activation in the default mode network (DMN), salience network (SAL) and frontoparietal control network (FPCN). More specifically, we investigated activation and connectivity within and between these networks during self-reflection processing. The behavioral results indicated lower self-esteem and more negative self-evaluations in depressed patients compared to controls. In terms of activation, we observed a focal increase in the dorsomedial prefrontal cortex during self-reflection in depressed patients, replicating previous studies. We also confirmed task-related modulation in the DMN and FPCN. The anterior DMN deactivated during semantic processing, but not during self- and other-reflection, whereas the posterior FPCN was activated during all task conditions compared to rest. Notably, we did not find alterations in activation or connectivity in depressed patients at the network level.

The dorsomedial prefrontal cortex (dmPFC; medial BA8) was more strongly activated during self- and other-reflection (compared to a semantic baseline condition) in depressed participants than in non-depressed participants. This is a replication of previous studies using a similar self-reflection task (Lemogne et al., 2009; Yoshimura et al., 2010). One study reported increased dmPFC activation for self in comparison to a distant other (Sarsam et al., 2013). Of note, post-hoc visualization showed that activation levels for reflection on a close other were intermediate between self-reflection and semantic processing. Increased dmPFC activation during self-reflection could be interpreted as the neural substrate of excessive self-focus in depression, possibly signaling the discrepancy between actual and ideal self. This interpretation fits with theoretical models of self-reflection in the brain, which relate the dmPFC to evaluation and decision making processes (e.g. van der Meer et al., 2010). It is also in line with our behavioral data, demonstrating lower self-esteem, negative self-evaluations and slowed decision making during self-evaluation in depressed patients.

It is somewhat counterintuitive that the dmPFC was not differently activated between self- and other-reflection in depressed patients. However, reflection on close others is likely to involve self-conceptualizations and self-comparisons (and thus is intricately linked to self-reflection). Indeed, recent studies suggest that close other

reflection may result in highly comparable activations as self-reflection (Murray et al., 2012). Some studies therefore choose a more distant other for this task condition, which may also have drawbacks (i.e., less familiarity may then become a confounder). An alternative account for the findings is the emotional content of the sentences, which may elicit activation increases in depressed patients. However, sentences were balanced on emotional valence and emotional tasks do not elicit consistently increased activation in the dmPFC in depression (Groenewold et al., 2013). The subthreshold activation increase in the left inferior frontal gyrus was reported previously in a study with a similar task-design. We found no group differences in the ventromedial prefrontal areas, however this has solely been reported in event-related task designs contrasting self with a passive reference condition (Lemogne et al., 2009).

Task-related modulation of activation was observed in several intrinsic connectivity networks. Consistent with our hypotheses, there was an absence of task-induced deactivation during self and other reflection conditions in the anterior DMN. Task-induced deactivation was observed during the semantic condition, providing evidence that the selected network was indeed part of the DMN. These findings support the notion that the DMN should not be conceptualized as a task-negative network per se (Spreng, 2012), as it is involved in internally focused and social evaluative processing (Amft et al., 2014). A meta-analysis indeed showed overlapping activation for self-related processing and resting state activation in anterior DMN areas (Qin and Northoff, 2011). Our study adds to these findings, by demonstrating for the first time that one intrinsic functional connectivity network shows comparable activation levels across self-reflection, other-reflection and fixation cross resting periods.

The posterior DMN component comprising precuneus and posterior cingulate cortex was deactivated during all task conditions. Of note, this posterior part of the DMN has been found to be the most active area of the brain during rest (Gusnard and Raichle, 2001) and is considered a central part of the DMN (Fransson and Marrelec, 2005). The posterior FPCN was activated during all task conditions, whereas activation of the anterior FPCN component was not strongly dependent on task condition. Thus, the independent component analysis appeared to split the DMN and FPCN into separate components that differed on degree of task-modulation. The posterior networks followed the pattern of task-negative (DMN) and task-positive results (FPCN) that was previously

reported in the literature (Marchetti et al., 2012). Other factors might influence activation in the anterior FPCN component such as sustained attention (Dosenbach et al., 2008).

In addition, we investigated whether there was connectivity between the networks during the task. The salience network was confirmed to be the central network that is connected to both DMN and FPCN components (Sridharan et al., 2008). The connectivity with task-involved networks was positive, suggesting that the salience network plays a role in selecting and recruiting networks during self-reflection task performance. Of note, connectivity between the salience network and posterior FPCN was diminished in the motion-corrected interpolated timecourse. Surprisingly, the strongest positive connectivity was observed between the anterior DMN and posterior FPCN. This is contrary to expectations based on resting state studies and the conceptualizations as task-negative and task-positive networks (Marchetti et al., 2012). However, positive connectivity between DMN and FPCN has also been reported during a future planning task (Gerlach et al., 2014). The positive connectivity may reflect the integration of cognitive control and reflective capacity required to meet task demands, by directing attention inwards.

Depressed participants showed no differences in connectivity within or between networks compared to healthy participants, in contrast to results from independent component analyses in resting state studies (e.g. Zhu et al., 2012; Tahmasian et al., 2013; Manoliu et al., 2014). One recent study in depressed patients did not find connectivity differences during internal-focus, but only during external-focus (Belleau et al., 2014). Thus, irregular connectivity between default-mode and control networks might be more important for impairments in the suppression of self-reflection (requiring negative connectivity) rather than the process of self-reflection itself (requiring positive connectivity). In line with this reasoning, connectivity alterations during rest have also been proposed to indicate an increased tendency to engage in self-reflective or ruminative processing in depressed patients (Berman et al., 2011). According to this viewpoint, impairments in suppression of self-reflection could be of such a severity that depressed patients are unable to truly rest when they receive resting instructions.

The focal result in the dorsomedial prefrontal cortex suggests that node-level abnormalities are more important than network-level abnormalities during self-reflection in depression. The focus of activation was spatially consistent with the dorsal nexus and it

showed connectivity with the default-mode, salience and frontoparietal control networks, indicating that the activation focus serves as a hub node between these networks. However, no group differences in connectivity emerged. Notwithstanding, increased connectivity may be more readily observed during rest due to fundamental differences in the way depressed participants experience a resting period. Moreover, connectivity differences may be more localized and more readily observed at the node-level than the network-level.

This study is characterized by several methodological strengths. The primary value lies in the combination of voxel-wise analyses using a general linear model, and network-level analyses using independent component analysis on the same dataset. The voxel-wise activation analyses built on hypotheses from previous studies, and used a slightly more stringent threshold. Motion correction was applied to the time-courses, which is particularly important for connectivity analyses. Limitations are the moderate depression severity, and relatively large proportion of depressed patients (42%) taking antidepressant medication. Antidepressants have been shown to normalize activation and connectivity abnormalities to a certain extent (Harmer et al., 2009; Schaefer et al., 2014). Thus, group differences may have been underestimated. In addition, no resting state scan was performed, so we were unable to compare connectivity patterns in a true resting period. Increasing the number of components may increase sensitivity to detect more fine-grained group differences. However, the estimated number of components was based upon the MDL, which is a data-driven statistic.

3.5. **Conclusion**

To conclude, this study replicated heightened focal activation in the dorsomedial prefrontal cortex of depressed patients during self-reflection. This focus of activation demonstrated connectivity with default mode, salience and frontoparietal networks, and thus might be seen as a hub node that corresponds to the previously identified “dorsal nexus”. Contrary to expectations, depressed patients recruited networks of interest in a similar way as non-depressed participants during self-reflective processing. These findings suggest that the previously reported increases in connectivity within the DMN and reduced connectivity between the DMN and FPCN in depressed patients do not generalize to self-reflective processing. Future research should address the relevant

context in which abnormal connectivity between networks arises, and study the role of node-level abnormalities such as those observed in the dorsal nexus.

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Supplements

Supplementary Table 1. Task activation for the self-reflection condition

Location		Cluster		Peak				MNI		
Label	BA	p_{FWE}	# voxels	P_{FWE}	T	Z	p_{unc}	x (mm)	y (mm)	z (mm)
OFC/dmPFC	10	<0.001	3913	<0.001	13.48	Inf	<0.001	0	57	9
	10			<0.001	10.48	Inf	<0.001	-3	60	27
	9			<0.001	9.14	7.69	<0.001	-21	51	36
Prec / Par	7	<0.001	2269	<0.001	12.96	Inf	<0.001	-6	-60	36
	40			<0.001	8.56	7.32	<0.001	39	-36	45
	40			<0.001	8.33	7.17	<0.001	45	-30	42
Cerebellum	-	<0.001	595	<0.001	10.92	Inf	<0.001	27	-78	-36
	-			<0.001	10.29	Inf	<0.001	48	-63	-39
	-			0.017	4.94	4.64	<0.001	9	-87	-18
Cerebellum	-	<0.001	796	<0.001	9.47	Inf	<0.001	-45	-63	-39
	-			<0.001	8.35	7.19	<0.001	-30	-81	-39
	-			<0.001	7.6	6.68	<0.001	-33	-51	-36
TPJ	39	<0.001	262	<0.001	8.32	7.17	<0.001	-51	-63	39
	39			<0.001	8.1	7.02	<0.001	-48	-57	30
ITG	20	0.001	52	<0.001	8.21	7.09	<0.001	-48	3	-33
DLPFC	46	<0.001	111	<0.001	7.2	6.39	<0.001	-39	21	42
Striatum	-	<0.001	215	<0.001	7.11	6.33	<0.001	-12	6	3
	-			<0.001	6.25	5.69	<0.001	0	12	-12
SFG	6	<0.001	84	<0.001	6.64	5.99	<0.001	-27	-9	54
ITG	20	0.002	39	<0.001	6.48	5.87	<0.001	48	6	-33
Insula/IFG	47	<0.001	77	<0.001	6.2	5.65	<0.001	-33	18	-12
	45			0.005	5.27	4.91	<0.001	-51	18	0
Midbrain	-	0.004	26	0.001	5.61	5.19	<0.001	0	-21	-18
Parietal	40	0.001	40	0.010	5.1	4.78	<0.001	-45	-30	45

Supplementary Table 2. Task activation for the other-reflection condition

Other > Semantic										
Location		Cluster		Peak				MNI		
Label	BA	p_{FWE}	# voxels	P_{FWE}	T	Z	p_{unc}	x (mm)	y (mm)	z (mm)
Precuneus	7	<0.001	1737	<0.001	18.22	Inf	<0.001	-6	-60	36
OFC	10	<0.001	2740	<0.001	12.96	Inf	<0.001	3	60	9
	10			<0.001	12.8	Inf	<0.001	-3	60	27
	10			<0.001	12.49	Inf	<0.001	3	63	21
ITG / MTG	20	<0.001	354	<0.001	11.94	Inf	<0.001	-48	6	-33
	21			<0.001	10.84	Inf	<0.001	-60	-6	-18
	21			<0.001	9.28	7.77	<0.001	-57	-12	-12
Cerebellum	-	<0.001	294	<0.001	11.56	Inf	<0.001	27	-78	-36
	-			<0.001	7.84	6.84	<0.001	48	-63	-42
ACC	25-	<0.001	193	<0.001	11.33	Inf	<0.001	0	12	-12
TPJ	-	<0.001	396	<0.001	11.22	Inf	<0.001	-48	-60	30
ITG / MTG	21	<0.001	240	<0.001	11.16	Inf	<0.001	60	-6	-21
	20			<0.001	9.52	Inf	<0.001	48	6	-33
TPJ	39	<0.001	343	<0.001	9.78	Inf	<0.001	48	-60	30
Cerebellum	-	<0.001	219	<0.001	8.64	7.37	<0.001	-27	-81	-36
Cerebellum	-	<0.001	131	<0.001	6.96	6.22	<0.001	-9	-57	-48
	-			<0.001	6.44	5.83	<0.001	3	-60	-48
Insula	47	0.006	19	0.005	5.3	4.93	<0.001	-33	18	-15

Supplementary Table 3. Activation differences between the self- and other-reflection conditions

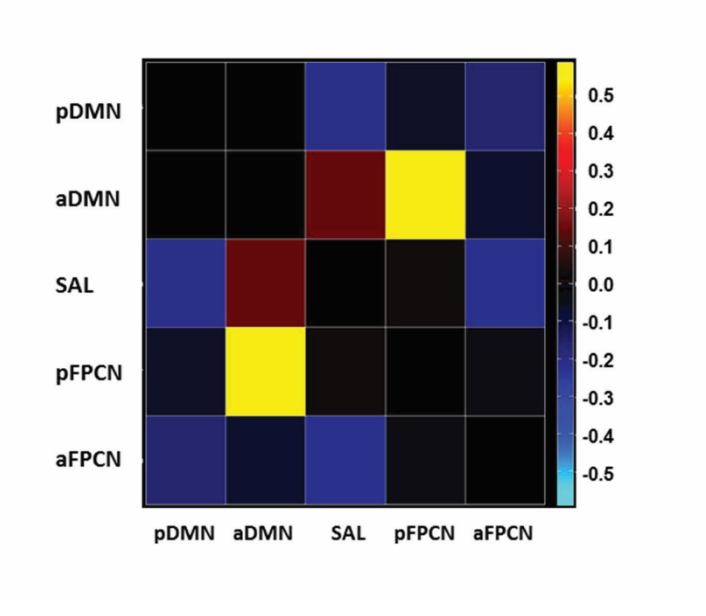
Self > Other										
Location		Cluster		Peak				MNI		
Label	BA	p_{FWE}	# voxels	P_{FWE}	T	Z	p_{unc}	x (mm)	y (mm)	z (mm)
dmPFC	6	<0.001	151	<0.001	6.45	5.84	<0.001	12	9	54
	6			<0.001	5.94	5.45	<0.001	21	12	57
Parietal	40	0.001	49	0.001	5.72	5.28	<0.001	54	-30	42
DLPFC	46	<0.001	74	0.002	5.57	5.16	<0.001	33	45	24
	45			0.015	4.98	4.68	<0.001	42	45	9
ACC	32	0.001	50	0.003	5.44	5.06	<0.001	6	21	36
Insula	48	0.007	16	0.004	5.34	4.97	<0.001	-33	21	9
Cerebellum	-	0.009	13	0.013	5.03	4.71	<0.001	-33	-54	-33
Cerebellum	-	0.011	11	0.016	4.95	4.65	<0.001	36	-54	-33

Other > Self										
Location		Cluster		Peak				MNI		
Label	BA	p_{FWE}	# voxels	P_{FWE}	T	Z	p_{unc}	x (mm)	y (mm)	z (mm)
MTG	21	<0.001	139	<0.001	8.83	7.50	<0.001	-60	-3	-21
				<0.001	8.07	7.00	<0.001	-57	-9	-15
MTG	21	<0.001	131	<0.001	8.78	7.46	<0.001	60	-6	-21
Precuneus	7	<0.001	365	<0.001	7.61	6.69	<0.001	0	-60	33
ACC	25	0.003	27	<0.001	6.72	6.05	<0.001	0	15	-12
TPJ	39	<0.001	99	<0.001	6.27	5.71	<0.001	45	-60	27
ITG	20	<0.001	60	<0.001	6.20	5.65	<0.001	-45	9	-33
TPJ	39	0.001	46	0.002	5.52	5.12	<0.001	-45	-63	27

Supplementary Table 4. Component activation differences for self>rest, other>rest, and semantic>rest (results motion-corrected timecourses of posterior/anterior DMN, salience, posterior/anterior FPCN).

Component	**Self**		**Other**		**Semantic**	
	T	p_{unc}	T	p_{unc}	T	p_{unc}
pDMN	**-4.656**	**<0.01**	**-2.641**	**0.01**	**-4.685**	**<0.01**
aDMN	-0.242	0.81	0.855	0.40	**-4.559**	**<0.01**
SAL	-0.234	0.82	-2.106	0.04	-1.721	0.09
pFPCN	**3.504**	**<0.01**	**3.588**	**<0.01**	**4.306**	**<0.01**
aFPCN	-0.637	0.53	-1.618	0.11	-2.154	0.04

Supplementary Figure 1. Correlation matrix of functional connectivity between default mode (top rows), salience (middle row) and frontoparietal control networks (bottom rows). Yellow-red denotes positive connectivity, blue denotes negative connectivity. Abbreviations: a = anterior, p = posterior.



Part 2 – the neural correlates of cognitive and vascular depression risk factors