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ORIGINAL ARTICLE

Aberrant functional connectivity of dorsolateral prefrontal and cingulate networks in patients with major depression during working memory processing

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Background. In patients with major depressive disorder (MDD), functional neuroimaging studies have reported an increased activation of the dorsolateral prefrontal cortex (DLPFC) during executive performance and working memory (WM) processing, and also an increased activation of the anterior cingulate cortex (ACC) during baseline conditions. However, the functional coupling of these cortical networks during WM processing is less clear.

Method. In this study, we used a verbal WM paradigm, event-related functional magnetic resonance imaging (fMRI) and multivariate statistical techniques to explore patterns of functional coupling of temporally dissociable dorsolateral prefrontal and cingulate networks. By means of independent component analyses (ICAs), two components of interest were identified that showed either a positive or a negative temporal correlation with the delay period of the cognitive activation task in both healthy controls and MDD patients.

Results. In a prefronto-parietal network, a decreased functional connectivity pattern was identified in depressed patients comprising inferior parietal, superior prefrontal and frontopolar regions. Within this cortical network, MDD patients additionally revealed a pattern of increased functional connectivity in the left DLPFC and the cerebellum compared to healthy controls. In a second, temporally anti-correlated network, healthy controls exhibited higher connectivity in the ACC, the ventrolateral and the superior prefrontal cortex compared to MDD patients.

Conclusions. These results complement and expand previous functional neuroimaging findings by demonstrating a dysconnectivity of dissociable prefrontal and cingulate regions in MDD patients. A disturbance of these dynamic networks is characterized by a simultaneously increased connectivity of the DLPFC during task-induced activation and increased connectivity of the ACC during task-induced deactivation.

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Key words: Cingulate gyrus, fMRI, independent component analysis, major depression, prefrontal cortex, working memory.

Introduction

Major depressive disorder (MDD) is a mental disorder characterized by affective, cognitive and somatic symptoms. With regard to cognition, evidence from neuropsychological studies suggests that executive demands during high levels of working memory (WM) processing might be particularly affected in MDD patients (Zakzanis *et al*. 1998; Austin *et al*. 1999). The neuropsychological concept of WM implies that a limited capacity system that temporarily maintains and stores information on-line supports human thought processes by providing an interface between perception, long-term memory, executive function and actions requiring cognitive control (Baddeley, 2003). Functional neuroimaging studies have shown that the lateral prefrontal cortex is crucial for several component processes of WM, such as executive control and active maintenance (Bunge *et al*. 2000; D'Esposito *et al*. 2000; Kane & Engle, 2002; Zhang *et al*. 2003), although the brain networks associated with

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WM processing extend beyond prefrontal areas, involving parietal, temporal, cerebellar and subcortical regions (Wolf & Walter, 2005).

Using functional magnetic resonance imaging (fMRI), an increasing number of studies have explored the neural correlates of altered WM processing in patients with MDD (Harvey *et al*. 2005; Matsuo *et al*. 2007; Vasic *et al*. 2007), yielding divergent findings with regard to both behavioral and neurofunctional domains. Some studies confirmed previous neuropsychological results by showing task-related WM performance deficits (Okada *et al*. 2003; Hugdahl *et al*. 2004; Walter *et al*. 2007*b*), whereas other did not (Harvey *et al*. 2005; Rose *et al*. 2006*a*; Matsuo *et al*. 2007). On the functional level, both decreased and increased activation in WM-related prefrontal regions has been found, particularly in the dorsolateral prefrontal cortex (DLPFC; Vasic *et al*. 2007). The consideration of task performance for the analysis of the functional data seems to be of specific relevance as the fMRI analysis of incorrect trials might reveal different prefrontal activation patterns between groups (Walter *et al*. 2007*b*). Increased activation of the DLPFC in MDD patients has been discussed as a compensatory phenomenon (Harvey *et al*. 2005; Matsuo *et al*. 2007; Walter *et al*. 2007*b*), suggesting that an increased WM-related activation of lateral prefrontal areas is necessary to achieve or maintain an optimal or 'near-normal' level of cognitive performance.

Findings from positron emission tomography (PET), however, have shown activation abnormalities of the anterior cingulate cortex (ACC) in MDD patients at rest and during cognitive processes, suggesting that this brain region might play a pivotal role in the etiology of MDD (Drevets, 2000; Liotti *et al*. 2002; Mayberg 2003). In line with these findings, aberrant activation of the ACC in MDD patients has also been demonstrated by fMRI during task conditions with low cognitive demand or during a 'neutral' baseline condition (Rose *et al*. 2006*a*; Walter *et al*. 2007*b*). It is unclear, however, whether activation abnormalities of the ACC are independent of the requirements of a given cognitive task or whether they reflect an aberrant task-induced deactivation (TID) pattern. TID is a decrease in brain activity that occurs during the performance of an experimental task relative to a baseline condition (Raichle *et al*. 2001) or low cognitive demand (Greicius & Menon, 2004). The regions exhibiting a TID are thought to reflect a functional network that is active during rest and suppressed during a cognitively demanding task (Mazoyer *et al*. 2001; Raichle *et al*. 2001). The brain regions that were consistently found to show a TID, irrespective of the cognitive task, typically include midline brain areas such as the medial prefrontal cortex (mPFC), the anterior and posterior cingulate cortex (a/pCC), the precuneus, and the bilateral inferior parietal cortex (IPC). The strong anti-correlation of task-positive and task-negative networks (Fransson, 2005, 2006) suggests antagonistic psychological functions on these systems (Fox *et al*. 2005). In MDD patients, however, the functional connectivity of the lateral prefrontal cortex and the ACC, as well as their relationship to specific requirements of cognitive processing, has not been investigated so far.

In this study, we used a parametric WM activation task and event-related fMRI to investigate WM-related changes in the functional coupling of distinct networks within the frontal cortex in MDD patients. To assess the functional interaction between dorsolateral prefrontal and cingulate regions associated with WM processing, we used a multivariate statistical approach, i.e. independent component analysis (ICA). ICA is a statistical technique that maximizes the independence between the output components (Calhoun *et al*. 2001, 2004) of fMRI data, thus identifying a set of spatially non-overlapping and temporally synchronous brain networks. With regard to functional connectivity, spatial ICA reveals 'chronoarchitectonically' associated areas (Bartels & Zeki, 2004). In its application to fMRI data, ICA has been proven to be useful for revealing functionally related brain regions in healthy controls and in patients with neuropsychiatric disorders (Celone *et al*. 2006; Garrity *et al*. 2007). Compared to 'conventional' general linear model (GLM) approaches to fMRI data, which are more sensitive to detect primarily functional specificity, ICA is better suited to reveal characteristics of functional network connectivity (Jafri *et al*. 2008).

Based on previous functional neuroimaging findings (Vasic *et al*. 2007; Walter *et al*. 2007*b*), we were particularly interested in connectivity differences of lateral prefrontal and cingulate networks in MDD patients compared to healthy controls. We predicted that MDD patients would show an aberrant functional connectivity pattern in dorsolateral prefrontal regions associated with increased TIA. We also explored the connectivity pattern of TID networks in MDD patients compared to healthy subjects. In MDD patients, we predicted to find TID-related connectivity changes in a functional network comprising the ACC, as implied by previous PET and fMRI studies.

Materials and methods

Subjects

Fourteen right-handed subjects with MDD (six females) were recruited from among the in-patients being treated at the Department of Psychiatry and Psychotherapy at the University of Ulm. All patients

Table 1. *Demographic and clinical characteristics of patients with major depression and control subjects*

Characteristic	Patients with MDD ($n=14$)	Healthy controls ($n=14$)	p value
Age (years)	37.0 (8.6)	32.6 (9.0)	0.19
Laterality score[a]	91.8 (17.8)	85.5 (26.0)	0.46
Education (years)	12.0 (3.2)	11.6 (1.5)	0.65
Duration of illness (months)	39.6 (32.3)	N.A.	N.A.
Number of depressive episodes	2.8 (2.4)	N.A.	N.A.
HAMD score	16.5 (5.5)	N.A.	N.A.
MADRS score	23.0 (4.0)	N.A.	N.A.
BDI	20.5 (8.8)	0.7 (1.6)	0.0001
CGI	5.0 (1.8)	N.A.	N.A.

MDD, Major depressive disorder; HAMD, Hamilton Depression Rating Scale; MADRS, Montgomery–Asberg Depression Rating Scale; BDI, Beck Depression Inventory; CGI, Clinical Global Impression Scale; N.A., not applicable.

Values given as mean (standard deviation).

[a] As rated by the Edinburgh Handedness Inventory (Oldfield, 1971).

were diagnosed according to DSM-IV criteria, excluding subjects with concurrent Axis I disorders. In addition to a detailed interview conducted by an experienced clinical psychiatrist, case-notes were reviewed to corroborate a definitive diagnosis. Psychopathology was rated by means of the Brief Psychiatric Rating Scale (BPRS), the 21-item Hamilton Depression Scale (HAMD-21), Montgomery–Asberg Depression Rating Scale (MADRS) and the Clinical Global Impression Scale (CGI) (see Table 1). All of the patients were treated with antidepressants: seven patients were treated with citalopram alone (20–40 mg/day), two were treated with venlafaxine alone (150 and 300 mg/day), one was medicated with citalopram (40 mg/day) and mirtazapine (30 mg/day), and four were medicated with a monotherapy of mirtazapine (30 mg/day), reboxetine (4 mg/day), fluoxetine (30 mg/day) and tranylcypromine (40 mg/day). None of the patients were receiving a stable regime of benzodiazepines at the time of the fMRI measurements. All patients were assessed within their first day/s of admission, that is during a symptomatic phase before or shortly after changing a previous antidepressant drug regime. Given previous evidence suggesting a relationship between WM performance and the number of affective episodes (Harvey *et al*. 2004), these clinical data were collected retrospectively by evaluating the patient's history and complementary case-notes (Table 1).

The healthy control group consisted of 14 right-handed healthy subjects (seven females) matched for age, handedness and education. Subjects with a history of neurological or psychiatric disorder or a family history of mood disorders, substance abuse or dependence were excluded. The study was approved by the local Institutional Ethics Committee. After complete description of the study to the subjects, written informed consent was obtained. Complementary results derived from a GLM-based analysis using this control sample and 12 out of 14 patients investigated in this study have been reported and discussed elsewhere (Walter *et al*. 2007*b*).

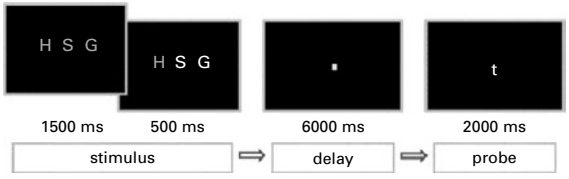

Fig. 1. Activation paradigm, shown for a trial of load level 2. In this example, the letters S and G were highlighted and subjects had to subsequently memorize the letters T and H (manipulated set). The probe-letter t is a part of the previously manipulated set, that is a positive probe (see section on fMRI activation paradigm for further details).

fMRI activation paradigm

We used a modified version of the Sternberg Item Recognition Paradigm (Sternberg, 1966), which has been shown previously to elicit robust prefrontal and parietal activation in both depressed patients and healthy controls (Fig. 1); for a detailed description of the task see Wolf & Walter (2005). Each trial started with the presentation of three gray capital letters on a black screen for a period of 1500 ms. One, two or three

of these letters were highlighted at the end of the stimulus phase for a period of 500 ms. All participants were instructed that during the subsequent delay period they were to focus only on those letters that had become highlighted and to memorize the letter/s that followed them in the alphabet. In the probe period of 200 ms, a lower-case letter was presented, and the subjects had to indicate using their right index or middle finger whether this letter was or was not part of the previously manipulated letters. The control condition displayed three gray upper-case Xs and required a stereotype button press in response to the presentation of a lower-case x during the probe phase.

Functional data acquisition

Data were acquired using a 1.5-T Magnetom Vision (Siemens, Erlangen, Germany) whole-body MRI system equipped with a standard head volume coil. T_2*-weighted images were obtained using echo-planar imaging in an axial orientation [repetition time (TR) = 2400 ms, echo time (TE) = 40 ms, field of view (FoV) = 192 mm, 64×64 matrix, 24 slices, slice thickness = 4 mm, gap = 2 mm]. Stimuli were presented through LCD video goggles (Resonance Technologies, Northridge, CA, USA) and both reaction times and accuracy indices were recorded. Head movement was minimized using padded ear-phones. The fMRI protocol was a rapid event-related design with a pseudo-randomized time-jitter of 1.5 ± 0.5 TR inter-trial interval, with trial duration of 10 s + 2.4–4.8 s. There were three sessions in total, each including 28 trials, comprising 164 volumes (492 volumes in total). The first eight volumes of each session were discarded to allow for equilibration effects.

Data analysis

Behavioral data analysis

Task accuracy during the WM task was recorded as percentage of correct responses during target and non-target trials as well as reaction times (RTs) of correctly performed trials. Changes in task accuracy and RT with increasing load were assessed separately using a repeated-measures analysis of variance (ANOVA; $p < 0.05$) with the factors group and load for accuracy and RT, followed by a *post-hoc* analysis using Fisher's least-significant-difference (LSD) test.

Analysis of fMRI data

Data preprocessing. Preprocessing of the functional data was performed using SPM5 (Wellcome Department of Cognitive Neurology, London, UK) and Matlab 7.2 (MathWorks, Natick, MA, USA). The functional images were corrected for slice timing differences and corrected for motion artifacts, then spatially normalized to the MNI template with a final voxel resolution of $3 \times 3 \times 3$ mm. All images were spatially smoothed with a 9-mm full-width at half-maximum isotropic Gaussian kernel.

Independent component analysis (ICA). A spatial independent component analysis was performed using a Group ICA for fMRI Toolbox (GIFT; http://icatb.sourceforge.net) (Correa *et al*. 2005). The dimensionality of the functional data for each subject was reduced using three consecutive steps of principal component analysis (PCA) alternated with data concatenation across the subjects, resulting in one aggregate mixing matrix for all the subjects. An ICA decomposition using the Infomax algorithm was used to extract 17 ICs, consisting of group spatial maps and related time-courses. The number of ICs was estimated using the minimum description length criteria (Li *et al*. 2007). These ICs were used for a back reconstruction into individual ICs using the aggregate mixing matrix created during the dimensionality data reduction steps.

The individual ICs consisting of individual spatial independent maps and time-courses were eventually temporally sorted using the events of the task. A parametric design matrix was computed for each subject using SPM5. For each session, stimulus and target periods were modeled as regressors independent of the WM load level. The delay period was parametrically modeled using a first-order polynomial expansion. Individual head movement parameters were used as regressors of no interest. Two components of interest (COIs) that showed the greatest positive and negative temporal correlation with both the delay period and the parametrically modeled regressor were chosen for the second-level within- and between-group analyses. For each subject's spatial COI, the voxel weights across all sessions were used as random effects variables and analyzed using SPM5. For within-group analyses, voxel-wise one-sample t tests against the null hypothesis of zero magnitude were used to calculate within-group maps for each COI. The statistical threshold for these analyses was set at $p < 0.05$, family-wise error (FWE) corrected for multiple comparisons. To compare spatial maps between healthy controls and MDD patients, an ANOVA was calculated using accuracy indices at load level 3 as nuisance variable. The between-group comparisons were masked by a combination of the main effects maps of both groups for each COI ($p < 0.001$). The statistical threshold for these analyses was set at $p < 0.05$ corrected for multiple comparisons using the false discovery rate (qFDR) (Genovese *et al*. 2002;

Table 2. *fMRI task performance of patients with major depression and control subjects*

	Patients with MDD ($n=14$)		Controls ($n=14$)	
Working memory condition	Reaction time (ms)	Accuracy (%)	Reaction time (ms)	Accuracy (%)
Control condition	692 (108)	98.6 (3.9)	608 (189)	98.3 (4.5)
Load level 1	866 (127)	93.1 (10.0)	760 (122)	92.4 (13.8)
Load level 2	1020 (167)	83.6 (12.9)	870 (95)	90.0 (12.9)
Load level 3	1186 (180)	71.6 (17.1)	976 (91)	89.1 (12.6)

fMRI, Functional magnetic resonance imaging; MDD, major depressive disorder.
All values given as mean (standard deviation).

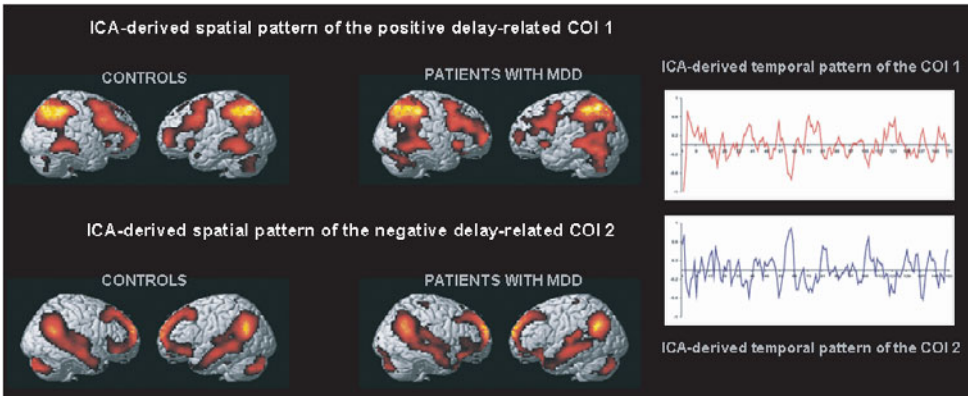

Fig. 2. *Left*, upper panel: independent component analysis (ICA)-derived spatial pattern of the positive delay-related component of interest (COI) 1 in healthy controls and major depressive disorder (MDD) patients. Results of the second-level within-group analyses, $p<0.05$ family-wise error (FWE) corrected. *Left*, lower panel: ICA-derived spatial pattern of the negative delay-related COI 2 in healthy controls and MDD patients. *Right*: ICA-derived time courses for the COI 1 and COI 2 showing an anti-correlated time-course pattern across all three functional magnetic resonance imaging (fMRI) sessions (456 scans in total).

Storey & Tibshirani, 2003) and a spatial contiguity criterion of 10 adjacent voxels (Forman *et al*. 1995). All anatomical regions and denominations are reported according to the atlases of Talairach & Tournoux (1988) and Duvernoy (1999). Coordinates are maxima in a given cluster according to the standard MNI template.

Results

Performance during the working memory task

In both groups we found increasing RTs with increasing WM manipulation load [$F(3,78)=98.938$, $p=0.00001$]. MDD patients were slower than healthy controls [$F(1,26)=10.777$, $p=0.003$]. A significant group by load interaction was not found [$F(3,78)=2.2786$, $p=0.09$]. We observed a significant linear decline in accuracy with increasing load in both groups [$F(3,78)=13.489$, $p=0.00001$]. MDD patients did not perform significantly worse than healthy controls overall [$F(1,26)=2.6320$, $p=0.12$]. A significant group by load interaction was found [$F(3,78)=4.2366$, $p=0.008$], due to the different accuracy only at load level 3, as revealed by *post-hoc* analyses ($p=0.02$); see Table 2 for details on task accuracy and RTs.

Functional imaging results

Within-group analyses

In both depressed subjects and healthy controls, two COIs were identified that were correlated with the parametrically modulated regressor of the WM task. These COIs were temporally associated with the delay period, and included voxels that were either positively (delay-related COI 1: $r=0.356$) or negatively correlated (COI 2: $r=-0.453$) with the component-related time-courses (Fig. 2). These spatial maps represent voxels that showed a maximal MR signal increase or decrease during the increasing manipulation and maintenance of verbal stimuli.

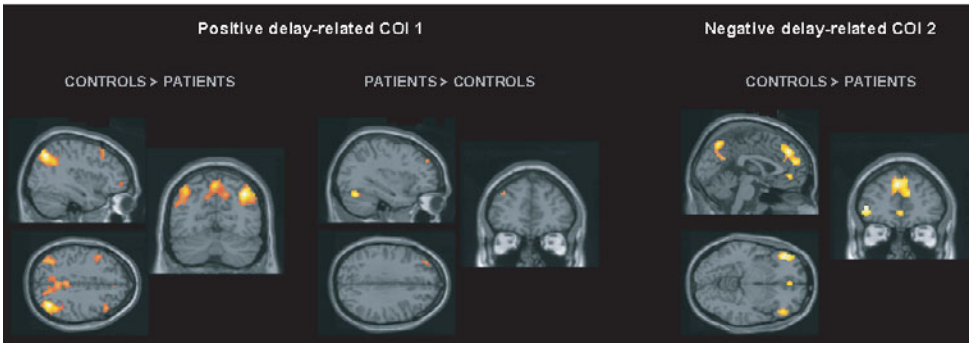

Fig. 3. *Left*: Between-group activation maps of the positive delay-related component of interest (COI) 1 showing decreased connectivity in major depressive disorder (MDD) patients in the bilateral parietal cortex [Brodmann area (BA) 40], the bilateral middle frontal gyrus (BA 8/9 and BA 10), and also increased connectivity in the patient group in the left dorsolateral prefrontal cortex (DLPFC) (BA 9 and 46) and the cerebellum. *Right*: Between-group activation maps of the negative delay-related COI 2 showing increased connectivity in the bilateral inferior frontal gyrus (BA 47), the left superior frontal cortex (BA 8/9) and the cingulate gyrus (BA 24/32) in the control group compared to MDD patients. Results of the second-level between-group analyses, $p < 0.05$ false discovery rate (FDR) corrected. (See Table 3 for detailed stereotaxic coordinates and Z values.)

Specifically, a positive delay-related component (COI 1) revealed a consistent and robust fronto-parieto-cerebellar pattern of task-induced activation across both groups. The COI 1 included the bilateral ventrolateral prefrontal cortex [VLPFC, Brodmann areas (BAs) 44 and 47], the DLPFC (BA 9 and 46), the frontopolar cortex (BA 10), the ACC (BA 32), the superior frontal cortex (BA 6 and 8), the insula, the putamen, the thalamus, the cerebellum and the left superior and inferior parietal lobule (BA 7 and 40). By contrast, a negative delay-related component (COI 2) that was temporally anti-correlated to the COI 1 revealed a pattern of TID including the bilateral VLPFC (BA 47), the frontopolar cortex (BA 10), the superior frontal cortex (BA 8/9), the precuneus (BA 7), the cuneus (BA 31), the temporal cortex (BA 20 and BA 37/38), the cingulate cortex (BA 24/32) and the cerebellum bilaterally; see also Fig. 2, detailed stereotaxic coordinates and Z values available on request.

Between-group analyses

Delay-related COI 1. Compared to healthy controls, depressed patients showed a decreased parietal, superior prefrontal and frontopolar connectivity pattern. Decreased connectivity in the patient group was found in the parietal cortex bilaterally (BA 40), the middle frontal gyrus bilaterally (BA 8/9), the left superior frontal gyrus (BA 6) as well as the middle frontal gyrus (BA 10) bilaterally. The inverse comparisons (MDD patients > healthy controls) revealed a pattern of increased connectivity in the DLPFC (BA 9 and 46), the VLPFC (BA 44) and the bilateral cerebellum (Fig. 3 and Table 3).

Delay-related COI 2. Compared to healthy controls, depressed patients showed a decreased connectivity pattern in the bilateral inferior frontal gyrus (BA 47), the left superior frontal cortex (BA 8/9) and the cingulate cortex (BA 24/32) (Fig. 3); see Table 3 for detailed stereotaxic coordinates and Z scores. The inverse comparisons (MDD patients > healthy controls) showed increased connectivity in depressed patients in the bilateral superior temporal cortex (BA 38), the left cuneus (BA 31) and the right lingual gyrus (BA 18).

Discussion

In this study, we used fMRI and a WM activation paradigm to investigate the functional coupling of task-related neural networks underlying WM processing in patients with major depression. Functional connectivity analyses were performed using ICAs, thus comparing chronoarchitectonically characterized spatial maps in healthy controls and MDD patients. Our data revealed two main findings: first, connectivity abnormalities were detected within a dorsolateral prefrontal/parietal network (COI 1), which was positively correlated with the delay period of the WM task. Specifically, a decreased functional connectivity pattern comprising inferior parietal, superior prefrontal and frontopolar regions was found in depressed patients when compared to control subjects. Within the same network, however, MDD patients additionally showed a pattern of increased functional connectivity in the left DLPFC and the cerebellum. Second, connectivity abnormalities were also detected within a ventrolateral prefrontal/cingulate network, which was anti-correlated to the ICA-derived COI 1,

Table 3. *Functional connectivity differences between healthy controls and depressed patients, shown for both delay-related components of interest (COI 1 and 2); results of the second-level between-group analysis, $p < 0.05$ false discovery rate (FDR) corrected. x, y and z are Talairach coordinates of the most significant center of activation within an activated cluster*

	Controls > Patients with MDD						Controls > Patients with MDD					
	Anatomical region	x	y	z	*Z* value	BA	Anatomical region	x	y	z	*Z* value	BA
COI 1	Right inferior parietal lobule	39	−66	42	6.83	40	Left cerebellum	−39	−66	−21	5.88	–
	Left inferior parietal lobule	−39	−66	45	4.54	40	Right cerebellum	33	−45	−39	4.99	–
	Right superior frontal gyrus	6	27	57	4.15	8	Left inferior frontal gyrus	−57	18	27	4.46	44
	Right precuneus	3	−54	45	4.13	7	Left precentral gyrus	−54	−6	39	3.90	6
	Left middle frontal gyrus	−45	12	39	3.86	8/9	Left inferior frontal gyrus	−48	33	12	3.89	46
	Right middle frontal gyrus	42	24	42	3.74	8/9	Left superior frontal gyrus	−33	45	33	3.73	9
	Left superior frontal gyrus	−24	3	63	3.40	6						
	Right middle frontal gyrus	42	51	−6	2.90	10						
	Left middle frontal gyrus	−42	48	−6	2.76	10						
COI 2	Left medial frontal gyrus	−3	45	36	5.51	8/9	Left superior temporal gyrus	−39	24	−27	6.05	38
	Left inferior frontal gyrus	−45	36	−9	5.51	47	Right superior temporal gyrus	30	27	−21	5.79	38
	Left middle frontal gyrus	−27	66	9	3.93	10	Left cuneus	−15	−63	18	5.76	31
	Left precuneus	−9	−66	45	4.71	7	Left middle temporal gyrus	−60	−6	−9	4.70	21
	Right inferior temporal gyrus	60	−21	−18	4.63	20	Right lingual gyrus	6	−78	0	4.39	18
	Right cerebellum	39	−66	−36	4.61	–						
	Left cerebellum	−36	−63	−39	4.34	–						
	Left middle temporal gyrus	−57	−60	6	4.19	37						
	Cingulate gyrus	0	45	−6	3.93	24/32						
	Right inferior frontal gyrus	48	33	−6	3.86	47						

MDD, Major depressive disorder; BA, Brodmann area.

thus most likely reflecting TID. In healthy controls, TID connectivity was increased in the ACC, the ventrolateral and the superior prefrontal cortex compared to MDD patients. Conversely, MDD patients showed increased connectivity in the bilateral superior temporal cortex (BA 38), the left cuneus (BA 31) and the right lingual gyrus (BA 18).

The increased connectivity of the left DLPFC in MDD patients is suggestive of a compensatory recruitment of this region during WM processing within a lateral prefrontal/parietal/cerebellar network. This finding is consistent with previous functional neuroimaging studies demonstrating an increased activation of the left DLPFC in patients with depression using a GLM approach (Matsuo *et al*. 2007; Walter *et al*. 2007*b*). In a recently published longitudinal fMRI study using the n-back task, depressed patients showed a greater quadratic load-response in the inferior frontal cortex and a greater linear load-response activity in the inferior parietal and superior temporal cortex as compared with control subjects (Walsh *et al*. 2007). The authors hypothesized that, with increasing cognitive demand, patients with depression require greater recruitment of these regions to maintain task performance. The finding of increased activation in widely distributed cortical areas with increasing WM load indicates a complex, load-dependent pattern of functional abnormalities within a WM-related network, rather than a regional dysfunction of circumscribed lateral and medial prefrontal regions in MDD. This notion is in good accordance with our findings of disturbed WM-related functional connectivity in superior frontal areas and the parietal cortex in MDD patients, as shown within the COI 1.

However, although we were able to confirm increased connectivity of the left DLPFC in MDD patients, we also found increased connectivity in cerebellar regions compared to healthy controls. Apart from its role in motor coordination and control, recent evidence indicates that the cerebellum is involved in the organization of higher order function. Besides of the lateral prefrontal cortex, the role of the cerebellum during WM processing has been increasingly recognized (Owen *et al*. 2005). In addition, recent findings highlight the relevance of a functionally intact prefrontocerebellar network in neuropsychiatric patients. For instance, patients with cerebellar lesions exhibit deficits in planning, set-shifting, verbal fluency, abstract reasoning, WM (Schmahmann & Sherman, 1998; Tavano *et al*. 2007) and attentional processes (Gottwald *et al*. 2004). The neuropsychological pattern in patients with cerebellar lesions suggests a disruption of neural circuits that link prefrontal, posterior parietal, superior temporal and limbic cortices with the cerebellum (Schmahmann & Sherman, 1998; Desmond *et al*. 2003; Ziemus *et al*. 2007). In patients with MDD, the cerebellum has been previously identified as a part of a functional network subserving executive processes (Walter *et al*. 2007*a*). Based on our findings of a spatiotemporally coherent prefrontocerebellar network positively correlated with cognitive processing during the delay period, we may hypothesize that the functional coupling between the cerebellum and the left DLPFC could partly account for the optimization of cognitive performance in MDD patients. In MDD patients, the notion of a neural compensation mechanism comprising lateral prefrontal regions is supported by previous findings of increased prefrontal activation during cognitive performance (Matsuo *et al*. 2007; Walter *et al*. 2007*b*). Yet it is unclear whether neural compensation mechanisms in MDD patients are regionally confined to lateral prefrontal regions, or whether neural compensation occurs on the neural network level, for example by increasing the connectivity strength within a prefrontocerebellar network. In this study, however, a significant correlation between the connectivity strength in cerebellar and prefrontal regions and accuracy measures was not found. Importantly, previous studies on WM processing in MDD patients did not consistently report significant correlations between activation in prefrontal or cerebellar regions of interest and task-related accuracy indices, possibly because of the relatively small sample sizes. Thus, although our study suggests performance-related compensatory prefrontocerebellar processes in MDD patients, the precise contribution of the lateral prefrontal cortex and its functional coupling with the cerebellum in patients with MDD clearly necessitates further investigation at this stage of research.

Our results further suggest that within a TID-related network, the ACC is more deactivated in healthy controls than in MDD patients. We hypothesize that an increased baseline level of activation in the ACC in MDD patients contributes to a failure of deactivation in the presence of cognitive effort. Consistent with this notion, increased levels of activation in the ACC were reported in depressed patients by some fMRI studies during conditions with low cognitive demand compared to those with high cognitive demand (Walter *et al*. 2007*b*). In addition, regionally specific smaller magnitudes of decrease in activation with increased task difficulty in depressed patients have been described in the ACC (Rose *et al*. 2006*a*). Walsh *et al*. (2007) found that MDD patients with the lowest linear load-response activity in the dorsal portion of the ACC at baseline showed the greatest clinical improvement after therapy, suggesting that increased activation in the ACC during WM processing might represent a negative prognostic

factor with regard to clinical recovery. At present, only one study has examined functional connectivity in depressed subjects using ICA. Greicius *et al*. (2007) identified increased functional connectivity in patients with MDD in the subgenual cingulate, the thalamus, the orbitofrontal cortex and the precuneus during the brain resting ('default') state. The connectivity in the ACC correlated positively with the duration of the current depressed episode and was therefore characterized as a measure of depression refractoriness. These ICA-derived findings suggested that, in MDD patients, the connectivity of the ACC is already altered during the resting state, and thus might reflect a trait characteristic in contrast to the activation differences associated with cognitive processing. Nevertheless, connectivity abnormalities of the ACC can also be detected within a task-related network of TID, indicating the persistence of ACC dysfunction beyond the resting state.

One possible limitation of this study is that all patients received various types of antidepressants that could have potentially biased the functional findings. However, current functional neuroimaging evidence suggests that a subacute administration of selective serotonin reuptake inhibitors (SSRIs) in healthy controls does not affect WM performance or the hemodynamic function to a magnitude greater than one standard deviation unit (Rose *et al*. 2006*b*). Thus, as more than half of the patients were treated with SSRIs (seven with citalopram alone), the potential bias arising from antidepressant drug administration does not seem to sufficiently explain findings of disturbed cortical connectivity in this patient sample. Furthermore, fMRI studies on WM performance in untreated depressed patients have reported similar findings of increased task-related left lateral prefrontal and ACC activation (Matsuo *et al*. 2007; Walsh *et al*. 2007), indicating that medication alone does not sufficiently explain aberrant activation of these cortical regions. Eventually, the possibility of a beneficial 'neuroprotective' effect of antidepressant therapy on cerebral tissue cannot be ruled out in our patient sample, as antidepressant treatment has been demonstrated to increase brain-derived neurotrophic factor (BDNF) levels in both animal models and humans (Haynes *et al*. 2004; Kosten *et al*. 2008). Therefore, we sought to minimize additional confounds arising from aging processes, substance abuse, and a longer total duration of drug treatment by including a carefully selected, relatively young patient sample without Axis I comorbidity, psychotic symptoms or a history substance abuse or dependence.

Nevertheless, these ICA-derived results complement previous functional neuroimaging findings by revealing a disturbed coupling of prefrontal, temporal, parietal and cingulate regions in patients with depression during WM processing. Aberrant WM-associated brain function in MDD is not sufficiently characterized by regionally disturbed lateral and medial prefrontal areas, but rather within a framework of functional network connectivity. Our findings also suggest that ICA is a practicable and effective statistical method to investigate the neural correlates of WM processing in MDD patients in terms of functional network connectivity, by identifying brain networks underlying both task-induced activation and deactivation. Studies examining functional brain connectivity in MDD patients over time, in conjunction with resting state examinations including larger patient samples, could essentially contribute to and eventually identify the precise neural mechanisms of cognitive impairment in MDD.

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Declaration of Interest

None.

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