Mood related insights : functional and structural MRI studies in depression and anxiety disorders

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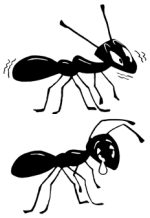

CHAPTER 2

REGIONAL BRAIN VOLUME IN DEPRESSION AND ANXIETY DISORDERS

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SUMMARY

Background: Major depressive disorder (MDD), panic disorder, and social anxiety disorder are among the most prevalent and frequently co-occuring psychiatric disorders in adults and may have, at least in part, a common etiology. In the present study we aimed to identify the unique and shared neuroanatomical profile of depression and anxiety, controlling for illness severity, medication use, sex, age of onset, and recurrence.

Methods: Sixty-eight patients with MDD, 88 patients with MDD and comorbid anxiety disorders, 68 patients with panic disorder and/or social anxiety disorder and/or generalized anxiety disorders, and 65 healthy controls were recruited from the Netherlands Study of Depression and Anxiety. Volumetric Magnetic Resonance Imaging was conducted for Voxel Based Morphometry analyses. We tested voxel wise for the effects of diagnosis, age of onset, and recurrence on gray matter density. Post hoc we studied the effects of use of medication, illness severity, and gender.

Results: We demonstrated lower gray matter volumes of the rostral anterior cingulate gyrus (ACC), extending into the dorsal ACC in MDD, comorbid depression-anxiety, and anxiety disorders without comorbid MDD, independent of illness severity, sex, and medication use. Furthermore, we demonstrated reduced right lateral inferior frontal volumes in MDD and reduced left middle/superior temporal volume in patients with anxiety disorders. Also, patients with onset of depression before 18 years of age showed lower volumes of the subgenual prefrontal cortex.

Discussion: Our findings indicate that reduced volume of the ventral anterior cingulate gyrus is a generic effect in depression and anxiety disorders, which is present independent of illness severity, medication use, and sex. This generic effect supports the notion of a shared etiology and may reflect a common symptom dimension related to altered emotional processing. Specific involvement of the inferior frontal cortex in MDD and lateral temporal cortex in anxiety disorders without MDD, on the other hand, may reflect disorder-specific symptom clusters. Early onset of depression is associated with a distinct neuroanatomical profile that may represent a vulnerability marker of depressive disorder.

Major depressive disorder (MDD), panic disorder, generalized anxiety disorder, and social anxiety disorder are among the most prevalent and most frequently co-occurring psychiatric disorders in adults. Estimations of the comorbidity of depression and anxiety range from 10% to more than 50% (Gorman, 1996; Gorman & Coplan, 1996; Ressler & Mayberg, 2007; Roy-Byrne et al., 2000). Therefore, it has been suggested that depression and anxiety have a similar etiology, also because they respond to the same treatment strategies (Ressler & Mayberg, 2007). The comorbid condition of depression and anxiety, however, may differ from MDD in clinical course and characteristics because it has been associated with worse outcome (Bruce et al., 2005; Gorman, 1996; Gorman & Coplan, 1996; Roy-Byrne et al., 2000; Rush et al., 2005) and more severe psychopathology (Kessler et al., 2005; Roy-Byrne et al., 2000; Rush et al., 2005). Also, the onset of anxiety often precedes the onset of the first depressive episode (Parker et al., 1999). However, in studies focusing on the neurobiology of depression and anxiety, comorbidity is rarely explicitly studied.

Major Depressive Disorder has frequently been associated with stress system dysregulation, as reflected by abnormal hypothalamus-pituitary-adrenal–axis function (Belmaker & Agam, 2008), of which the glucocorticoid cortisol is an end product. An abnormal glucocorticoid response has been associated with volumetric changes in the hippocampus, amygdala, and prefrontal cortex in animal studies (McEwen, 2005). These brain structures are rich in glucocorticoid receptors and are therefore a target for the putative neurotoxic action of excess glucocorticoids/cortisol. However, altered volumes of the hippocampus, amygdala, and prefrontal regions are likely to result from other pathogenetic mechanisms as well because abnormal cortisol levels have not been consistently found in MDD (Chida & Steptoe, 2009).

A number of neuroimaging studies of MDD have shown altered volumes in structures related to hypothalamus-pituitary-adrenal–axis function, emotion perception, and regulation, i.e. the hippocampus (Frodl et al., 2006; Lange & Irle, 2004; Mervaala et al., 2000; Sheline, Gado, & Kraemer, 2003; Wagner et al., 2008; Weniger, Lange, & Irle, 2006), amygdala (Hamilton et al., 2008; Sheline, Gado, & Price, 1998; Wagner et al., 2008), striatum (Hickie et al., 2007), medial prefrontal cortex (Bremner et al., 2002; Frodl et al., 2006; Wagner et al., 2008), and anterior cingulate gyrus (ACC) (Botteron et al., 2002; Caetano et al., 2006; Drevets et al., 1997; Hastings, Parsey, Oquendo, Arango, & Mann, 2004; Tang et al., 2007; Vasic, Walter, Höse, & Wolf, 2008; Yucel et al., 2008). Most studies have reported decreased volumes in these structures (Koolschijn et al., 2009), although findings have not been wholly consistent (Bremner et al., 2002; Caetano et al., 2001; Frodl et al., 2008a; Frodl et al., 2002; Frodl et al., 2003; Hastings et al., 2004; Koolschijn et al., 2009; Lange & Irle, 2004; Rusch, Abercrombie, Oakes, Schaefer, & Davidson, 2001; Vakili et al., 2000; Vasic et al., 2008; Weniger et al., 2006). Importantly, only few studies have explicitly controlled for comorbidity of anxiety, although morphometric changes have

been identified in anxiety disorders as well (Damsa, Kosel, & Moussally, 2009; Radua et al., 2010). Whereas volumetric studies in social anxiety disorder and generalized anxiety disorder have been rare, studies in panic disorder have fairly consistently found altered volumes of the amygdala (Asami et al., 2009; Hayano et al., 2009; Massana et al., 2003), insular cortex (Asami et al., 2009; Uchida et al., 2008), dorsomedial prefrontal cortex (Asami et al., 2009), and ACC (Asami et al., 2008; Asami et al., 2009; Uchida et al., 2008). In addition, altered brainstem (Asami et al., 2008; Protopopescu et al., 2006), orbitofrontal cortex (Asami et al., 2009; Mohlman et al., 2009), and superior temporal volumes (Asami et al., 2009; Yoo et al., 2005) have been implicated in the neuropathology of panic disorder. However, results of these studies may have been similarly confounded by the presence of comorbid depression (Asami et al., 2008; Uchida et al., 2008). In summary, volumetric studies appear to indicate specific involvement of the hippocampus in depression,the insular cortex and superior temporal areas in anxiety disorders, and prefrontal and amygdalar areas in both depression and anxiety disorders. Moreover, to our knowledge, no study to date studied the unique and common neuroanatomical profile of depression and anxiety.

In this cross-sectional study, we investigated the shared and unique neuroanatomical profile of depression and anxiety, controlling for the effects of illness severity, use of selective serotonin reuptake inhibitors (SSRIs), and sex as potential confounders (Campbell & MacQueen, 2006). We also investigated the effects of recurrence of depression and age at onset, reflecting changes associated with prolonged illness duration (McKinnon, Yucel, Nazarov, & MacQueen, 2009) or increased vulnerability to depression and anxiety (MacMaster et al., 2006; MacMillan et al., 2003). Based on previous studies, we hypothesized that patients with MDD with or without comorbid anxiety disorders (panic disorder, social anxiety disorder, generalized anxiety disorder) would show decreased volumes in the hippocampus, amygdala, ACC, and medial prefrontal cortex. In addition, we predicted decreased volumes in the ACC, amygdala, insula, and superior temporal gyrus in patients with an anxiety disorder with or without comorbid MDD.

METHODS

PARTICIPANTS

Participants were recruited from the Netherlands Study of Depression and Anxiety (NESDA), a large-scale, multi-site, longitudinal, observational cohort study. The design has been described in detail elsewhere (Penninx et al., 2008). In short, NESDA was designed to be representative of those with depressive and anxiety disorders in different health care settings and stages of developmental history. Therefore, the sample is stratified for setting (community, primary care and specialized mental health) and set up to include a range of psychopathology.

Of the 2981 NESDA respondents (main sample), participants aged between 18 and 57 years were asked to participate in the NESDA neuroimaging study if

they met the DSM-IV criteria for a half year diagnosis of MDD and/or anxiety disorder (panic disorder, social anxiety disorder and/or generalized anxiety disorder) or no lifetime DSM-IV diagnosis (i.e., healthy controls). Personality disorders were not screened for and so were not used in the inclusion/exclusion criteria, although persons with known personality disorders (through information from clinics or through self-report) were not included in NESDA. Exclusion criteria for patients were the presence of axis-I disorders other than MDD, panic disorder, social anxiety disorder, generalized anxiety disorder and any use of psychotropic medication other than a stable use of SSRIs or infrequent benzodiazepine use (i.e., equivalent to 2 doses of 10 mg of oxazepam 3 times per week or use within 48 hrs prior to scanning). Exclusion criteria for participants were the presence or history of major internal or neurological disorder (e.g., contusio cerebri with loss of consciousness>15 min, diabetes mellitus type I), dependency or recent abuse (past year) of alcohol and/or drugs, hypertension, and general MRI contraindications. Diagnoses were established using the structured Composite International Diagnostic Interview (CIDI), according to DSM-IV algorithms were established using the structured Composite International Diagnostic Interview (CIDI) – lifetime version 2.1 (Robins et al., 1988), given by a trained interviewer. Controls were currently free of, and had never met criteria for, depressive or anxiety disorders or any other axis-I disorder and were not taking any psychotropic drugs.

Overall, 301 native Dutch speaking participants (MRI-sample; 235 patients and 66 controls) were included and underwent magnetic resonance imaging in one of the three participating centers: Leiden University Medical Center, Academic Medical Center Amsterdam, and University Medical Center Groningen. The Ethical Review Boards of each participating center approved this study. All participants provided written informed consent after receiving written information.

ADDITIONAL PSYCHIATRIC MEASUREMENTS

Severity of depression and anxiety at the day of scanning was assessed using Dutch versions of the Beck Anxiety Inventory (Beck, Epstein, Brown, & Steer, 1988), the Montgomery Åsberg Depression Rating Scale (Montgomery & Åsberg, 1979), the Inventory of Depressive Symptomology (Rush et al., 1986) and the Fear Questionaire (Marks & Mathews, 1979). Fear Questionnaire ratings were obtained from 243 subjects only.

IMAGE ACQUISITION

Imaging data were acquired using Philips 3-Tesla magnetic resonance imaging systems (Best, The Netherlands) located at the departments of Radiology of Leiden University Medical Center, Academic Medical Center Amsterdam, and University Medical Center Groningen, equipped with a SENSE-8 (Leiden University Medical Center and University Medical Center Groningen) or a SENSE-6 (Academic Medical Center Amsterdam) channel head coil. For each

subject, anatomical images were obtained using a sagittal 3-dimensional gradient-echo T1-weighted sequence (time to repetition=9 milliseconds, echo time =3.5 milliseconds; matrix 256x256; voxel size, 1x1x1mm; 170 slices, duration: 4.5 minutes).

STATISTICAL ANALYSIS

Demographic and clinical data were analyzed using SPSS 16.0 (SPSS Inc., Il, USA). Significance was set at $p<.05$, and post hoc paired tests were Bonferroni corrected for multiple comparisons.

Imaging data were analyzed using an optimized Voxel Based Morphometry (VBM) approach, following the Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL (Ashburner, 2007)) using Statistical Parametric Mapping software (SPM5) implemented in Matlab 7.1.0. (The MathWorks Inc., MA, USA). Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL) is a fully deformable method that is effectively unconstrained by number of degrees of freedom. It has proven good registration accuracy and has been recommended in favor of standard SPM normalization or the SPM unified segmentation approaches for whole brain and regional analysis without segmenting regions of interest (Yassa & Stark, 2009).

Preprocessing of VBM-DARTEL included (1) manually reorientation of the images to the anterior commissure; (2) segmentation of the anatomical images into gray matter, white matter and cerebrospinal fluid using the standard segmentation option implemented in SPM5; (3) applying the DARTEL approach for registration, normalization, and modulation, leaving the images in DARTEL space (in this approach, a DARTEL template is created based on the deformation fields that are produced during the segmentation procedure; next, all individual deformation fields were registered to this template); (4) smoothing of the gray matter and white matter images using an 8-mm full width at half maximum Gaussian kernel to increase signal to noise ratio and to maximize comparability with published VBM studies of depression and anxiety (Frodl et al., 2008b; Tang et al., 2007; Vasic et al., 2008; Wagner et al., 2008; Yoo et al., 2005). In the resulting images, each voxel represents an absolute amount of brain volume, equivalent to the brain volume per unit prior to normalization.

Based on previous articles on volumetric differences in MDD and anxiety disorders, we set the following a priori regions of interest: hippocampus, amygdala, medial PFC, orbitofrontal cortex, ACC, superior temporal gyrus, and insula. For the regions of interest, we set a threshold of $p<.001$, uncorrected, with an extent cluster threshold of 50 contiguous voxels. To further protect against type 1 error, small volume correction (SVC) was applied for the main comparison (effect of diagnosis) by centering a sphere of 16-mm around the peak voxel. The resulting volumes of interest had to meet $p<.05$, corrected for Family Wise Error (FWE) rate at the voxel level, to be considered significant. For non-regions of interest, a voxel level threshold of $p<.05$ whole brain FWE

corrected was set a priori.

Next, data were analyzed in the context of the general linear model (Friston et al., 1995). For our main comparison, we performed a 1x4 factorial analysis with group as random factor over all subjects. To test for effects of depression severity, anxiety severity, SSRI use, and sex, the mean signals of significant clusters were calculated and exported to SPSS. To test for the effects of depression severity, we divided the MDD and comorbid depression-anxiety patients into three subgroups (remitted, mild, moderate to severe), based on their current Montgomery Åsberg Depression Rating Scale score (Muller, Szegedi, Wetzel, & Benkert, 2000). In addition, a whole-brain voxel-wise analysis was performed in SPM to test for the effect of early (<18 year) vs. late (≥18 year) onset of depressive symptoms in MDD and comorbid depression-anxiety and for onset of anxiety symptoms within comorbid depression-anxiety and anxiety disorders without MDD, as compared with controls. Also, a whole-brain voxel-wise analysis was performed to test for effects of recurrent vs. a single major depressive episode in MDD and comorbid depression-anxiety.

Age, gray matter total volumes, and center (by means of two dummy variables) were entered as covariates in each comparison. White matter images were only used to verify whether white matter volume changes occurred in the same regions as gray matter volume changes. For each SPM comparison, groups were matched for age, sex, scan site, and handedness. A description of the total sample is given in the results section. Description of matching procedure and resulting samples for the additional analyses (exclusion of SSRI users, age of onset, recurrence) can be found in the supplemental material.

To achieve maximal sensitivity, optimize voxel residual smoothness estimation, and exclude false positives in non-gray matter tissue, voxel wise comparisons were masked using a comparison-specific explicit optimal threshold gray matter mask created using the Masking toolbox (Ridgway et al., 2009). To preserve optimal normalization accuracy, we left the normalized, modulated, and smoothed images in DARTEL space. Therefore, coordinates are not equivalent to Montreal Neurological Institute (MNI) coordinates. All regions are identified using the detailed brain atlas of Talairach and Tournoux (1988).

RESULTS

SAMPLE DESCRIPTIVE

Data from ten participants were excluded because of poor image quality. In addition, data from two controls were excluded because they had Montgomery Åsberg Depression Rating Scale scores >8 (Muller et al., 2000). We formed four groups based on Composite International Diagnostic Interview half-year diagnoses. Our final sample consisted of 289 subjects: 68 patients with MDD (MDD group), 88 with MDD and one or more comorbid anxiety disorder (CDA group: MDD and panic disorder and/or social anxiety disorder and/or generalized anxiety disorder), 68 with one or more anxiety disorder (panic

disorder, social anxiety disorder, generalized anxiety disorder) but no MDD (ANX group), and 65 controls.

Table 1 lists the sample characteristics. Groups were matched for sex, handedness, distribution of participants scanned over sites, and age, but not on education. The MDD and comorbid depression-anxiety group both had fewer years of education than controls (MDD: U=1370.5, p<.008; CDA: U=1594, p<.008). Furthermore, the comorbid depression-anxiety group included more SSRI users than the MDD and ANX groups. A main effect of group was found on Montgomery Åsberg Depression Rating Scale, Inventory of Depressive Symptomatology, Beck Anxiety Inventory, and Fear Questionnaire score. All diagnostic groups showed higher Montgomery Åsberg Depression Rating Scale, Inventory of Depressive Symptomatology, Beck Anxiety Inventory, and Fear Questionnaire total scores than controls (all z>-4.82; all p<.008). In addition, the comorbid depression-anxiety group reported higher Montgomery Åsberg Depression Rating Scale and Inventory of Depressive Symptomatology scores than the MDD and ANX group, and higher Beck Anxiety Inventory scores than the MDD group (all z>-2.9, p<.008).

Between the NESDA baseline interview (T1) and the magnetic resonance imaging session (T2), depressive scores decreased in all diagnostic groups (Inventory of Depressive Symptomatology; t>3.45; p<.001). The MDD group showed an additional decrease in Beck Anxiety Inventory scores (t_{65}=3.15, p=.002). Post hoc tests showed that currently remitted and mildly depressed subgroups but not the moderately to severely depressed subgroups, showed lowered depressive scores at the time of scanning than at time of baseline interview: At T1, all groups showed moderate/severe depressive scores. The MDD and comorbid depression-anxiety groups did not differ in onset at the first MDD episode, and the comorbid depression-anxiety and ANX groups did not differ in age at onset of the first anxiety disorder. Within the comorbid depression-anxiety group, onset of anxiety generally preceded onset of the first depressive episode (Z=-5.22, p<.001).

VBM RESULTS

Groups did not differ in total gray matter and white matter volumes (gray matter $F_{3,284}$=.25; p=.34 and white matter $F_{3,284}$=.25; p=.86). Lower regional gray matter density of the left rostral ACC (Brodmann area [BA] 24b/c and 32; [1]) was observed in patients compared with controls, extending into the dorsal ACC (BA 32') (Figure 1a). Voxel based comparison of the MDD, comorbid depression-anxiety, and ANX group with controls showed that the rostral and dorsal ACC reduction was most robust in the comorbid depression-anxiety group and was borderline significant in the MDD and ANX groups (MDD: MNI coordinates: [x=0 y=32 z=-11], Z=2.99; p=.001; ANX: MNI coordinates: [x=0 y=41 z=1], Z=3.08; p=.001). Gray matter results are listed in Table 2. The region surrounding the rostral and dorsal ACC gray matter reductions showed white matter volumetric reductions as well (Table 2).

1 For identifying subregions of the ACC, we used the definition described by Bush et al. (2000).

TABLE 1: CLINICAL CHARACTERISTICS OF THE TOTAL SAMPLE (N=289)

MDD= Major Depressive Disorder; CDA= Comorbid MDD and anxiety; ANX= anxiety without MDD; HC= healthy controls; a=17 MDD+GAD, 17 MDD+PD, 9 MDD+PD+GAD, 9 MDD+SAD, 15 MDD+SAD+GAD, 12 MDD+PD+SAD, 9 MDD+PD+SAD+GAD; b=20 PD, 2 PD+GAD, 25 SAD, 3 SAD+GAD, 14 PD+SAD, 4 PD+SAD+GAD (of which 18 PD w/o AGO & 22 PD w/ AGO) & 22 PD w/ AGO). H= Kruskal Wallis non parametric multiple sample test; U= Mann-Whitney non-parametric 2 sample test; X^2 = Chi-square test for categorical variables; df=degrees of freedom; SSRI= Selective Serotonin Reuptake Inhibitor; T1: baseline measurement; T2: MRI-measurement; MADRS: Montgomery Åsberg Depression Rating Scale; BAI: Beck Anxiety Inventory; IDS: Inventory of depressive symptomatology; FQ: Fear Questionnaire; rem=remitted depressive scores (MADRS: 0-8), mild=mild depressive scores: (MADRS 9-8), mod_sev=moderate to severe depressive scores (MADRS>19); interval= interval between T1 and T2; GM=grey matter total volume; WM=white mattter total volume.

		MDD	**CDA a**	**ANX b**	**HC**	*H*	*F*	*U*	X^2	*df*	*p*	
N		68	88	68	65							
gender	male/female; N	24/44	29/59	18/50	24/41				*1.93*	*3*	*.59*	
scan site	amc/lumc/umcg; N	18/26/24	28/35/25	21/20/27	27/26/12				*.23*	*3*	*.97*	
handedness	left/right; N	6/62	6/82	5/63	5/60				*9.7*	*6*	*.14*	
SSRI use	yes/no; N	18/50	40/48	21/47	0/65				*6.88*	*2*	*.03*	*
age	in years; mean ± sd	37.16 ± 10.24	37.27 ± 10.64	35.96 ± 9.45	40.54 ± 9.71	*7.24*				*3*	*.07*	
education	in years; mean ± sd	12.67 ± 2.91	11.62 ± 3.13	13.11 ± 3.21	14.28 ± 2.86	*26.13*				*3*	*<.001*	*
MADRS	total score; mean ± sd	13.01 ± 9.18	19.94 ± 9.16	10.93 ± 8.66	1.05 ± 1.86	*147.73*				*3*	*<.001*	*
	range	0 – 39	0 – 49	0 – 35	0 – 7							
	rem/mild/mod_sev; N	24/25/19	9/35/43	-	-				*15.68*	*2*	*<.001*	*
IDS T1	total score; mean ± sd	27.68 ± 9.96	33.02 ± 11.51	22.79 ± 11.91	5.14 ± 3.51	*150*				*3*	*<.001*	*
IDS T2	total score; mean ± sd	19.85 ± 11.86	29.49 ± 11.16	19.26 ± 10.81	3.79 ± 3.58	*144.01*				*3*	*<.001*	*
	range	1 – 57	5 – 57	4 – 49	0 - 17							
BAI T1	total score; mean ± sd	11.68 ± 8.86	18.41 ± 9.10	15.22 ± 9.9	1.89 ± 3.11	*137.33*				*3*	*<.001*	*
BAI T2	total score; mean ± sd	8.95 ± 8.2	18.23 ± 8.97	14.12 ± 9.60	2.19 ± 2.57	*125.30*				*3*	*<.001*	*
	range	0 – 50	1 – 46	0 – 42	0 – 10							
FQ	total score; mean ± sd	21.1 ± 15.39	36.35 ± 19.09	37.17 ± 20.48	9.05 ± 7.71	*44.41*				*3*	*<.001*	*
	range	0 – 79	6 – 88	3 – 84	0 – 29							
interval	in days; mean ± sd	71.4 ± 59.1	57.9 ± 49.5	69.9 ± 33.2	63.7 ± 28.8		*1.47*			*3*	*.22*	
onset MDD	age in years; mean ± sd	25.62 ± 10.36	23.40 ± 11.38	-	-			*2505.5*			*.13*	
onset ANX	age in years; mean ± sd	-	17.67 ± 10.27	15.47 ± 11.27	-			*2156*			*.20*	
recurrence MDD	single/recurrent episode; N	29/39	39/49	-	-							
ANX	diagnosis in lifetime; N	21	87	68	0							
	diagnosis in past year; N	9	87	68	0							
MDD	diagnsois in lifetime; N	68	87	37	0							
	diagnosis in past year; N	68	87	4	0							
GM	ml; mean ± sd	728.7 ± 67.64	729.98 ± 75.11	739.98 ± 76.95	725.48 ± 76.58		*.48*			*3,29*	*.70*	
WM	ml; mean ± sd	486.12 ± 63	500.81 ± 64.76	493.99 ± 64.38	489.19 ± 63.66		*.78*			*3,29*	*.78*	

Furthermore, in the MDD group, gray matter volume reductions in the right inferior frontal cortex were observed (Figure 1b). The ANX group showed less left middle and superior temporal gyrus volume compared to controls (Figure 1c). In both regions, white matter reductions were observed as well. The reverse contrasts (MDD, CDA, and ANX groups > controls) did not reveal significant clusters.

EFFECTS OF ILLNESS SEVERITY, SEX, AND SSRI-USE

Analysis with SPSS showed that the rostral/dorsal ACC gray matter volume reduction occurred in all diagnostic groups relative to controls. (all p <.05, Bonferroni corrected), whereas the right inferior frontal gyrus and middle/superior temporal gyrus volume reductions were specific to the MDD and ANX groups, respectively (p<.05, Bonferroni corrected).

No effect of depressive state was observed within group on ACC (MDD: $F_{2,66}$=.98; p=.38; CDA: $F_{2,80}$=.09; p=.91) or inferior frontal gyrus volume within MDD ($F_{2,67}$=2.14, p=.13). Adding Beck Anxiety Inventory and Fear Questionnaire scores to these models did not change the results (rostral/dorsal ACC: MDD: $F_{2,64}$=.51, p=.61; CDA: $F_{2,78}$=.1, p=.91; inferior frontal gyrus: $F_{2,64}$=2.06, p=.14).

Beck Anxiety Inventory and Fear Questionnaire scores were not predictive of rostral/dorsal ACC volume in patients (BAI: β=05; p=.22; FQ: β=-.06; p=.1), of inferior frontal gyrus volumes in MDD (BAI: β=-.02; p=.85; FQ: β=.14; p=.24), and of middle/superior temporal gyrus volumes in patients with anxiety disorders (BAI: β=-.06; p=.52; FQ: β=.02; p=.86). No interaction of sex and diagnosis was observed in any region (rostral/dorsal ACC: $F_{3,287}$=1.46; p=.23; inferior frontal gyrus: $F_{3,187}$=.56; p=.64; middle/superior temporal gyrus: $F_{2,287}$=1.6; p=.19) and omission of the SSRI users from the analysis did not affect the results. Table S-1 lists the sample characteristics of the SSRI-free diagnostic groups.

AGE AT ONSET

A voxel-wise, whole-brain analysis showed that patients with early onset of depression (MDD and comorbid depression-anxiety) had lower gray matter volumes of the right subgenual ACC (BA 25) extending into the medial orbitofrontal gyrus compared with controls (Table 2c, Figure 1d), and no effect of sex was observed. No volumetric differences were observed in the subgenual ACC in persons with late-onset depression vs. controls, and no white matter reductions occurred in this region. Also, no effect of early vs. late onset of the first anxiety disorder was observed in the ANX and comorbid depression-anxiety group. Table S-2 lists the sample characteristics of the early vs. late onset subgroups.

A voxel-wise, whole-brain analysis showed no effect of single vs. recurrent depressive episodes in the MDD and comorbid depression-anxiety group. Table S-3 lists the sample characteristics of the single vs. recurrent episode MDD groups.

FIGURE 1: VBM EFFECTS

A) main effect of patients < healthy controls showing gray matter reductions in the rostral and dorsal anterior cingulate gyrus; B) lower right inferior frontal gyrus volume in MDD as compared to HC; C) lower right middle/superior temporal gyrus volume in ANX as compared to HC; D) MDD patients (MDD and CDA) with onset of the first depressive episode before the age of 18 are characterized by lower subgenual OFC volumes than HC. All effects are displayed at p<.005, uncorrected.

A full-color image can be found on the supplementary sheet.

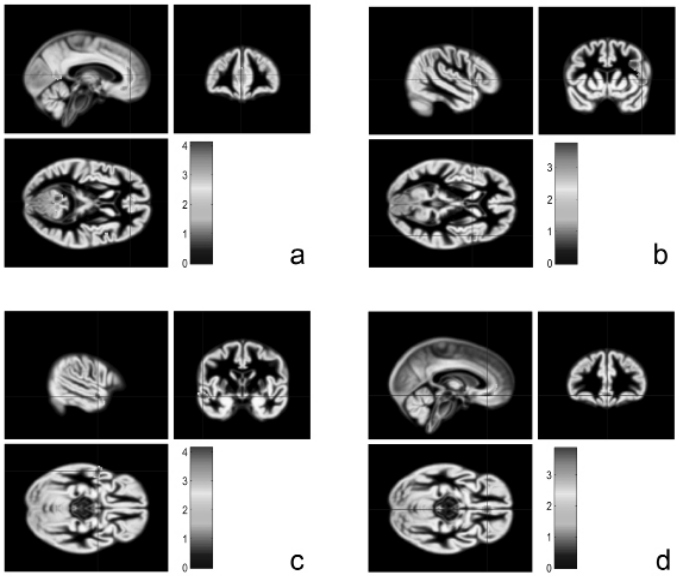

TABLE 2: VBM RESULTS

MDD= Major Depressive Disorder; CDA= Comorbid MDD and anxiety; ANX= anxiety without MDD; HC= healthy controls; SSRI= Selective Serotonin Reuptake Inhibitor; DARTEL-coordinate= coordinates of the voxel showing peak significance in mean DARTEL space, R/L= Right/Left hemisphere; BA= Brodmann area; k= cluster size; SVC= Small Volume Correction.

A: Gray matter group comparisons, including SSRI-users

					DARTEL-coordinate			
comparison	R/L	BA	region	k	x	y	z	Z-value
pt < HC	L	24b/c/32	rostral anterior cingulate gyrus	152	0	42	4	3.54
	L	32'	dorsal anterior cingulate gyrus		-2	39	16	3.52
MDD<HC	R	44	inferior frontal gyrus	178	36	11	27	3.69
	R	45	inferior frontal gyrus	115	47	11	4	3.56
CDA<HC	R	24b/c/32	rostal anterior cingulate gyrus	83	2	42	6	3.50
ANX<HC	L	21	middle/superior temporal gyrus	181	-53	-3	-12	4.07

Statistics, coordinates and cluster sizes of the comparisons between pt (patients; MDD, CDA and ANX), and HC. SVC corrected at *p* <.05. SPM smoothness 11.2, 11.8, 11.3 mm; 698.7 resels.

B: White matter group comparisons, including SSRI-users

			k	DARTEL-coordinate			Z-value	
comparison	R/L	region		x	y	z		
pt < HC	R	rostral anterior cingulate gyrus	407	9	42	10	4.32	*
	L	rostral anterior cingulate gyrus	11	-9	41	10	3.17	

Statistics, coordinates and cluster sizes of the comparisons between pt (patients; MDD, CDA and ANX), and HC. Uncorrected at *p* <.001; * SVC FWE corrected at *p* <.05; SPM smoothness 10.6, 10.9, 10.6 mm; 633.3 resels.

C: Gray matter comparisons of early onset vs late onset of MDD in MDD and CDA

					DARTEL-coordinate			
comparison	R/L	BA	region	k	x	y	z	Z-value
EO<HC	R	25/11	subgenual ACC/ orbitofrontal gyru	127	2	32	-11	3.77

Statistics, coordinates and cluster sizes of the comparisons between early onset (EO) MDD and CDA vs. HC. SVC corrected at *p* <.05. SPM smoothness 11.2, 11.8, 11.3 mm; 690.5 resels.

DISCUSSION

In this study we investigated the neuroanatomical characteristics of depression and anxiety in a large sample of outpatients with MDD, panic disorder, social anxiety disorder, and/or generalized anxiety disorder. We used a whole brain, DARTEL-VBM approach, tested explicitly for the effects of comorbidity of depression and anxiety, and controlled for the effects of illness severity, SSRI use, and sex. In addition, we tested voxel-wise for the effects of age at onset and recurrence of depression.

We demonstrated lower gray matter volumes of the rostral ACC, extending into the dorsal ACC in patients with mood and/or anxiety disorders. This rostral ACC decrease occurred in patients with MDD, comorbid depression-anxiety, and anxiety disorders without comorbid MDD, independent of depressive state or anxiety severity, and no effect of SSRI use or sex was observed. Also, white matter reductions occurred in the region bordering the gray matter ACC reduction. Furthermore, we demonstrated reduced right inferior frontal gyrus volumes in MDD and reduced left middle/superior temporal gyrus volume in anxiety disorders compared with healthy controls. In addition, depressed subjects (MDD and comorbid depression-anxiety) with onset of the first depressive episode before 18 years of age showed lower volumes in the subgenual ACC, extending into the medial orbitofrontal gyrus. Finally, patients with MDD and comorbid depression-anxiety with recurrent episode depression showed no volumetric differences compared with a single episode of depression.

To our knowledge, this is the first study to examine the neuroanatomical (i.e. neuroradiological) correlates of both depression and anxiety while explicitly testing for the effects of their co-occurrence. Our findings indicate that reduced volume of the ACC, and more specifically the regions of the ACC that are part of the rostral-ventral affective subdivision (Bush et al., 2000) is a generic effect in depression and anxiety disorders, present independently of depressive state or anxiety severity. This generic ACC reduction supports the notion of a shared etiology in depression and anxiety and may reflect a common pathophysiological mechanism related to altered emotion processing. The rostral ACC region has been found to be primarily involved in salience assessments of emotional and motivational information, while the dorsal ACC has been implicated in effortful processing (Bush et al., 2000), motivational processes, and regulating negative mood (Mak, Wong, Han, & Lee, 2009). The ventral part of the ACC, (including the rostral ACC) has been associated with executive inhibition (Matthews et al., 2009), induced sadness (Liotti et al., 2002; Wang et al., 2008), and negative emotion processing (Shin et al., 2000) in both patients with MDD and controls. The volume reduction observed in this study most likely reflects loss of glial cell density and neuronal size (Cotter, Mackay, Landau, Kerwin, & Everall, 2001), and may be the result of hypothalamus-pituitary-adrenal axis dysregulation (Belmaker & Agam, 2008). The rostral-ventral affective subdivision of the ACC has extensive connections with the orbitofrontal cortex, amygdala, and anterior

insula (Devinsky, Morrell, & Vogt, 1995) and therefore is an important hub in emotion perception and regulation. Also, abnormal ACC morphometry has been associated with worse outcome and/or worse treatment response in MDD (Chen et al., 2007; Frodl et al., 2008c; Yucel et al., 2008).

The results are in concordance with previous imaging studies that have demonstrated affective ACC abnormalities in depression and anxiety disorders, however without consistently controlling for comorbidity (Asami et al., 2009; Botteron et al., 2002; Drevets et al., 1997; Tang et al., 2007; Yucel et al., 2009). Although our comorbid depression-anxiety group displayed more severe depressive and anxiety related pathology, the two depressive groups were characterized by a similar rostral ACC gray matter volume reduction. Also, within groups, no associations between illness severity and ACC volume were observed. The latter finding appears to be at odds with studies that did report a negative correlation between illness severity and ACC volumes (Frodl et al., 2008c; Yucel et al., 2009). For example, Frodl et al. (2008c) in their sample of inpatients who were receiving medication, demonstrated a moderate correlation of depression severity with total right ACC volume but not left total ACC, or subregions within the ACC. However, our findings are in agreement with studies that also failed to demonstrate a relation between illness severity and ACC volume (Caetano et al., 2006; Vasic et al., 2008; Yucel et al., 2008), as was confirmed in a recent meta-analysis (Koolschijn et al., 2009). In this study, we further demonstrated rostral ACC volume reductions even in (recently) remitted patients with depression, consistent with findings of abnormal rostral ACC activation following mood induction in remitted patients (Liotti et al., 2002). Finally, our negative findings regarding SSRI use on ACC volume are in agreement with work of Asami et al (2008). Recent animal studies also indicated that the neurogenesis promoting effects of SSRIs may only be achieved in youth, not in adulthood or old age (Couillard-Despres et al., 2009; Navailles, Hof, & Schmauss, 2008).

In addition to our rostral ACC findings, we also demonstrated reduced right inferior frontal gyrus gray matter volumes in MDD. This finding is in agreement with earlier VBM results of both decreased inferior frontal gyrus concentration (Vasic et al., 2008) and density (Frodl et al., 2008a)[2] in inpatients with MDD in which comorbid anxiety disorders were excluded as well. Data from the present study indicate that this finding is specific for patients with MDD without comorbid anxiety disorders, independent of depression severity and SSRI use. The right inferior frontal gyrus has been implicated in inhibitory processes relevant to executive performance (Matthews et al., 2009; Wang et al., 2008), selective response suppression (Forstmann, van den Wildenberg, & Ridderinkhof, 2008) and cognitive processes related to negative affect (Lieberman et al., 2004), functions that are likely to be impaired in MDD. Therefore, reduced right inferior frontal gyrus volume may represent a neuroanatomical basis for these abnormalities. The left middle/superior temporal reduction in anxiety disorders is also in agreement with previous research (Uchida et al., 2003; Vythilingam et

2 Concentration refers to the proportion of gray matter in each voxel, in which the changes in voxel size have not been accounted for, i.e., the voxel value has not been modulated with the Jacobian determinant derived from the spatial normalization. This does not allow for comparison of the absolute voxel value (Good et al., 2001).

al., 2000) and has repeatedly been linked to the pathophysiology of panic disorder, presumably reflecting impaired evaluation of interoceptive information (Uchida et al., 2008) and altered threat processing (Phillips et al., 1998), as has been shown in functional neuroimaging studies. Depressed subjects (MDD and comorbid depression-anxiety) with onset of the first depressive episode before 18 years of age showed lower gray matter volumes of the subgenual ACC relative to controls, extending into the medial orbitofrontal, an effect that was not observed in patients with onset of the first depressive episode after 18 years of age. This finding is in line with results of Botteron and colleagues (2002), who reported subgenual ACC volume decreases in adolescent-onset MDD, although no direct comparison with late-onset MDD was made. The subgenual ACC volume reduction may represent an early neurobiological lesion resulting in increased vulnerablility to depressed mood, as abnormal subgenual ACC activity during sad mood induction through autobiographical episodes, even after full remission of depression, has been proposed as a trait marker for depression (Liotti et al., 2002). Also, the subgenual ACC has been implicated in fear extinction and emotion regulation and appears to serve as a regulatory hub between the dorsolateral PFC and amygdala, modulating responsiveness in the latter (Delgado, Nearing, Ledoux, & Phelps, 2008). Disruption of this area may therefore lead to altered emotion processing. Finally, subgenual ACC volume reduction may reflect a genetically determined predisposition to MDD because early onset depression is associated with increased familial risk of MDD (Jaffee et al., 2002). This hypothesis has received empirical support from findings of Nolan and coworkers (2002). Moreover, Drevets et al. (1997) reported subgenual ACC reductions in a predominantly familial MDD group in adulthood. Although it may be argued that subgenual ACC reduction merely reflects longer disease duration, this suggestion conflicts with the observed subgenual ACC reduction in the adolescent female cohort of Botteron and colleagues (2002).

In this study, we failed to demonstrate hippocampal and amygdalar volume reductions in MDD, panic disorder, social anxiety disorder and generalized anxiety disorder. Post hoc analyses revealed hippocampal volume reduction only at z=2.17 (p=.02, uncorrected), far below our a priori threshold, and no amygdala volume changes were observed. One may argue that VBM techniques are less sensitive to detect volumetric alternations in small structures or that non-linear registration methods are less sensitive to pick up shape-related alterations compared with region of interest segmentation approaches. It should be noted that post mortem studies did not found support of apoptosis, massive cell loss, or loss of plasticity in the human hippocampus (Campbell & MacQueen, 2004; Swaab, Bao, & Lucassen, 2005), although reports have been conflicting (Stockmeier et al., 2004). Moreover, post mortem studies have mainly included the brains of patients who committed suicide, a subgroup that may not be representative of patients with MDD. Also, most of our depressed patients, although representative of our local outpatient population, were not severely depressed, and therefore did not show hippocampal atrophy.

However, hippocampal reductions were also absent in our severely depressed subgroups (data not shown). In their meta-analysis, MacKinnon et al. (2009) showed that hippocampal atrophy is more likely to occur in pediatric or elderly samples with recurrent MDD. Therefore, decreased hippocampal volume is considered the result of the disease process, as supported by the longitudinal findings of Frodl et al (2008b). Regarding the amygdala, Hamilton et al. (2008) concluded in their meta-analysis that volume of this region s dependent on medication use in MDD. However, this conclusion is not supported by our data; we did not observe amygdalar atrophy in our analysis nor depressed and anxious patients who did not take an SSRI.

In this study, we were unable to identify gray matter reductions outside our a priori regions of interest that survive whole-brain corrections for multiple comparisons. We did, however, observe reductions in the bilateral posterior cingulate cortex (BA 30/23; Z=4.04; p<.001 uncorrected) in addition to the rostral/dorsal ACC reductions described previously in our patient groups. Because most imaging studies to date have focused on frontal and subcortical regions, this region was not included in our a priori regions of interest. However, inclusion of the posterior cingulate cortex as region of interest in future imaging studies will likely be useful in further unraveling the complex neurodynamics of depression and anxiety. Notably, this region has been included in the default mode network as one of the highest energy consuming regions and has been associated with larger deactivations during emotional facial processing (Gentili et al., 2009) and threat processing in anxious patients (Zhao et al., 2007). The posterior cingulate cortex has shown negative connectivity with the amygdala (i.e. increased posterior cingulate cortex activation is correlated with decreased amygdalar activation) and positive connectivity with the dorsal ACC (BA 32) (Stein et al., 2007), indicating that the posterior cingulate cortex is also involved in regulatory interactions with the amygdala, directly and via the ACC.

This study has a number of strengths. First, we were able to include large samples of patients and controls who were extensively screened and phenotyped according to the NESDA protocol; therefore, we could define subgroups of patients based on clinical comorbidity. Second, only stable SSRI use was allowed in our study, and less than half of our patients were taking antidepressant medication at the time of scanning. Consequently, we were able to control for the effects of SSRI on regional brain volume. Third, we used a whole brain approach (VBM-DARTEL) that is rater-unbiased in its segmentation. Therefore, we did not restrict our analysis to only a limited number of brain structures but were able to detect volumetric changes across the brain and to verify if gray matter changes were accompanied by white matter changes in the same region. Voxel-based methods have been found to show satisfactory correlations with manual segmentation approaches (Asami et al., 2008; Uchida et al., 2008; Zhao et al., 2007).

Several potential limitations should also be noted. First, because the epidemiological NESDA cohort was recruited through general practitioners

and outpatient clinics, we may not have been fully able to capture the most severe end of the depressive spectrum. Second, patients with MDD and comorbid depression-anxiety differed slightly from controls in years of education. However, adding years of education as a covariate did not change the results of the main comparison; if anything, the rostral ACC reduction was more robust. Third, assessment of onset and recurrence of depression was based on self-report, which theoretically may have resulted in both underdiagnosis and overdiagnosis of past depression and anxiety. However, this would have biased our results to the null and therefore have led to underestimation of the true associations. Fourth, although persons with identified posttraumatic stress disorder where not included in the NESDA sample, subjects were not systematically screened for it. Therefore, we tested (post hoc) for the effect of trauma on ACC volume because data regarding the experience of emotional and physical trauma were available but no effect of self-reported trauma was observed on ACC volume and no interaction with diagnosis was observed (See the supplemental material for detailed information). Fifth, although similar Philips 3 tesla systems were used at each site in this multi-center study, variability in image acquisition may have occurred owing to minor differences in hardware (receiver coil) and timing of software upgrades. However, no diagnosis x scan site bias occurred. Moreover, reliability of multiscanner VBM has been proven good (Stonnington et al., 2008).

In conclusion, our results suggest a generic involvement of the ventral-rostral ACC in (comorbid) MDD, panic disorder, social anxiety disorder, and generalized anxiety disorder, extending into the dorsal ACC that is independent of symptom severity. Although our results do not directly address pathogenetic mechanisms involved in depression and anxiety, they support the notion of a shared pathogenetic mechanism in these disorders that may reflect impaired emotion processing and regulation, presumably through intricate connections of the ventral ACC with other limbic structures (i.e., amygdala, orbitofrontal cortex, and anterior insula) that have been implicated in mood regulation models as well. Psychometric and functional imaging studies focusing on the common and distinct symptom profiles of depression and anxiety, may further aid in unravelling the common and distinct phenomenological and neurobiological correlates of these disorders. In addition to this generic ACC effect, we showed disorder specific involvement of the right inferior frontal gyrus in MDD, and superior temporal gyrus in panic disorder and/or social anxiety disorder and/or generalized anxiety disorder. Longitudinal studies and prospective studies should clarify whether these volumetric abnormalities are the result of the disease process or represent a vulnerability factor for the development of depression and anxiety in adulthood.

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M.J. van Tol had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

EFFECTS OF SSRIs: DESCRIPTION OF GROUP

After omission of the SSRI using participants, groups were matched on age, gender, scan site, and handedness. Our renewed sample consisted of 207 subjects, i.e. 50 MDD, 48 CDA, 47 ANX, and 62 HC. Table S1 lists all characteristics for the medication free sample. Groups were comparable on all clinical characteristics to the groups that include the SSRI-using participants. Also, no effect of group exists with respect to total gray matter ($F_{3,203}$=.34, p=.80) and total white matter ($F_{3,203}$=.18, p=.91).

AGE OF ONSET

Groups were matched on age, edu, gender and handedness. Within diagnostic groups, early onset (EO) did not differ in MADRS and BAI scores. Clinical characteristics are listed in Table S2. Gray matter images were entered in a one-way ANOVA with group as independent variable and age, gender, gray matter totals, and center as covariates.

RECURRENCE

MDD and CDA were pooled and splitted into a single episode MDD group and a recurrent episode MDD group. Groups (single, recurrent, and healthy controls) were matched on age, gender and handedness. Groups differed in years of education, MADRS scores and BAI scores (edu: $F_{2,204}$=.14.19, p<.001; MADRS: $F_{2,204}$=74.8, p<.001; BAI: $F_{2;204}$=45.11, p<.001). Post hoc tests showed that healthy controls had more years of education and lower scores on MADRS and BAI than single and recurrent MDD. Clinical characteristics are listed in Table S3. Gray matter images were entered in a one-way ANOVA with group as independent variable and age, gender, gray matter totals, and center as covariates.

EFFECT OF TRAUMA ON ACC VOLUMES

Data regarding the experience of emotional and physical trauma were available (assessed with the Nemesis trauma interview; De Graaf et al, 2002). In our sample of 289 subjects, 55.9 % of MDD, 69.3% of CDA, 57.4% of ANX, and 38.5 % of healthy controls reported to have ever experienced emotional or physical trauma in their lives (ranging from 'once' to 'very often'). To test for the effect of trauma on ACC volumes, we set up ANCOVAs with gray matter ACC volume as dependent factor, and diagnosis and trauma (yes/no) as independent factor. Age, gray matter totals, and scan center were entered as covariates. No effect of self-reported trauma was observed on ACC volume ($F_{1,277}$=.37, p=.54], and no interaction with diagnosis was observed ($F_{3,277}$=.37, p=,26). The experience of a physical or emotional trauma more than once, occurred in 34 % of MDD, 46% of CDA, 40% of ANX, and 17% of the healthy controls before the age of 16. Again, (severe) childhood trauma was not associated with ACC volume when compared to subjects that never experienced trauma($F_{1,215}$=1.22, p=.27), and no interaction with diagnosis was observed ($F_{3,215}$=.84, p=.48).

TABLE S-1: CLINICAL CHARACTERISTICS OF THE SSRI FREE SAMPLE (N=207)

MDD= Major Depressive Disorder; CDA= Comorbid MDD and anxiety; ANX= anxiety without MDD; HC= healthy controls; c=9 MDD+GAD, 12 MDD+PD, 5 MDD+PD+GAD, 7 MDD+SAD, 7 MDD+SAD+GAD, 5 MDD+PD+SAD, 3 MDD+PD+SAD+GAD; d= 16 PD, 2 PD+GAD, 20 SAD, 7 PD+SAD, 2 PD+SAD+GAD; SSRI = Selective Serotonin Reuptake Inhibitor; T1: baseline mearurement; T2: MRI-measurement; MADRS: Montgomery Åsberg Depression Rating Scale; BAI: Beck Anxiety Inventory; IDS: Inventory of depressive symptomatology; FQ: Fear Questionnaire; rem= remitted depressive scores (MADRS: 0-8), mild= mild depressive scores: (MADRS 9-18), mod_sev= moderate to severe depressive scores (MADRS>19); GM= grey matter total volume; WM= white mattter total volume.

		MDD	CDA c	ANX d	HC
N		50	48	47	62
gender	male/female; N	20/30	12/36	13/34	23/39
scan site	amc/lumc/umcg; N	12/20/18	16/21/11	14/15/18	26/25/11
handedness	left/right; N	6/44	4/44	3/44	5/57
History of SSRI use	1/2/3/4 *; N	7/3/4/36	5/7/5/31	3/7/3/34	
age	in years; mean ± sd	36.22 ± 10.02	36.58 ± 10.9	35.11 ± 9.85	39.89 ± 9.46
education	in years; mean ± sd	12.56 ± 2.45	12.04 ± 3.4	13.23 ± 3.27	14.34 ± 2.85
MADRS	total score; mean ± sd	12.56 ± 9.78	20.17 ± 9.58	10.87 ± 8.76	1 ± 1.78
	range	0-39	2-43	0-35	0-7
	severity class: rem/mild/mod_sev; N	19/18/13	6/17/25	-	-
IDS T1	total score; mean ± sd	27.54 ± 10.17	32.65 ± 10.61	22.51 ± 11.91	5.03 ± 3.52
IDS T2	total score; mean ± sd	20.58 ± 15.43	28.56 ± 11.15	19 ± 10.83	3.59 ± 3.64
	range	1 - 57	5 - 55	4 - 49	0 - 17
BAI T1	total score; mean ± sd	11.62 ± 9.38	17.90 ± 9.09	15.26 ± 9.73	1.84 ± 3.14
BAI T2	total score; mean ± sd	8.78 ± 8.59	17.94 ± 9.22	15.30 ± 10.28	1.84 ± 3.14
	range	0 - 50	1 - 46	0 - 42	0 - 10
FQ	total score; mean ± sd	20.58 ± 15.43	24.65 ± 21.47	29.98 ± 23.82	7.62 ± 8.10
	range	0 - 79	0 - 78	0 - 84	0 - 29
onset MDD	age in years; mean ± sd	25.26 ± 9.49	22.36 ± 11.72	-	-
onset ANX	age in years; mean ± sd	-	17.5 ± 10.54	15.38 ± 11.49	-
recurrence MDD	single/recurrent episode; N	21/29	21/27	-	-
ANX	diagnosis in lifetime; N	14	-	-	0
	diagnosis in past year; N	6	-	-	-
MDD	diagnsois in lifetime; N	-	-	25	0
	diagnosis in past year; N	-	-	1	-
GM	ml; mean ± sd	735.07 ± 57.90	725.2 ± 73.7	738.44 ± 79.77	728. 69 ± 75.26
WM	ml; mean ± sd	495.04 ± 60.85	489.65 ± 60.18	489.70 ± 67.78	486.55 ± 59.83

TABLE S-2: CLINICAL CHARACTERISTICS OF MATCHED EARLY AND LATE DEPRESSION ONSET GROUPS VS. HC

MDD= Major Depressive Disorder; CDA= Comorbid MDD and anxiety; HC= healthy controls; EO= early onset MDD (onset of first episode before age of 18); LO= late onset MDD (onset of first episode after age of 18); SSRI= Selective Serotonin Reuptake Inhibitor; MADRS: Montgomery Åsberg Depression Rating Scale; BAI: Beck Anxiety Inventory; GM= gray matter total volume; WM= white mattter total volume.

		MDD		CDA		HC
		EO	LO	EO	LO	
N		23	38	32	40	48
gender	male/female; N	8/15	12/26	8/24	14/26	17/31
scan site	amc/lumc/umcg; N	4/8/11	12/16/10	10/11/11	16/14/10	19/22/7
handedness	left/right; N	1/22	5/33	2/30	3/37	5/43
SSRI use	current yes/no; N	7/16	7/31	14/18	21/19	0/48
age	in years: mean ± sd	34 ± 10.5	37 ± 8.1	32.4 ± 9.9	37 ± 9.6	37.7 ± 8.9
education	in years: mean ± sd	13 ± 2.9	12.3 ± 2.6	12.3 ± 2.6	12.6 ± 3.1	13.7 ± 2.2
MADRS	total score: mean ± sd	14.4 ± 9.4	13.2 ± 9.4	19.7 ± 8.8	19.1 ± 9.7	.8 ± 1.6
BAI	total score: mean ± sd	9.2 ± 7.2	8.9 ± 9.1	20.3 ± 8.7	16.3 ± 9.1	1.9 ± 2.6
onset MDD	age in years: mean ± sd	15.17 ± 2.55	30.13 ± 7.32	13.22 ± 3.52	28.18 ± 7.37	-
GM	in ml: mean ± sd	742.2±65.8	724.7 ± 68.8	746.5 ± 69.3	732.9 ± 78.9	738.4 ± 75.4
WM	in ml: mean ± sd	467.1 ± 62.2	491.1 ± 62.6	485.6 ± 59.6	509.5 ± 71.1	486.4 ± 58.8

TABLE S-3: CLINICAL CHARACTERISTIC OF MATCHED SINGLE EPISODE VS. RECURRENT EPISODE MDD

MDD= Major Depressive Disorder; CDA= Comorbid MDD and anxiety; HC= healthy controls; SSRI= Selective Serotonin Reuptake Inhibitor; MADRS: Montgomery Åsberg Depression Rating Scale; BAI: Beck Anxiety Inventory; GM= gray matter total volume; WM= white mattter total volume.

		MDD + CDA		HC
		single	recurrent	
N		68	85	58
gender	male/female; N	27/41	24/61	21/37
scan site	amc/lumc/umcg; N	19/35/14	26/26/33	17/26/22
handedness	left/right; N	5/63	7/78	5/53
diagn	MDD/CDA; N	29/39	36/49	-
SSRI	current use yes/no; N	26/42	30/55	-
age	in years; mean ± sd	37.37 ± 10.13	37.51 ± 9.60	38.9 ± 8.96
education	in years; mean ± sd	13.08 ± 3.12	12.45 ± 3.11	14.59 ± 2.6
MADRS	total score: mean ± sd	10.31 ± 11.11	16.14 ± 10.1	.98 ± 1.83
BAI	total score: mean ± sd	9.37 ± 10.4	13.2 ± 9.2	1.96 ± 2.46
GM	ml; mean ± sd	732.34 ± 77.97	727.94 ± 63.91	730.83 ± 75.06
WM	ml; mean ± sd	492.33 ± 65.12	491.09 ± 61.52	486.67 ± 60.76