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Widespread reductions in gray matter volume in depression

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ABSTRACT

Abnormalities in functional limbic–anterior cingulate–prefrontal circuits associated with emotional reactivity, evaluation and regulation have been implicated in the pathophysiology of major depressive disorder (MDD). However, existing knowledge about structural alterations in depression is equivocal and based on cohorts of limited sample size. This study used voxel-based morphometry (VBM) and surface-based cortical thickness to investigate the structure of these circuits in a large and well-characterized patient cohort with MDD.

Non-geriatric MDD outpatients (n = 102) and age- and gender-matched healthy control participants (n = 34) provided T1-weighted magnetic resonance imaging data during their baseline visit as part of the International Study to Predict Optimized Treatment for Depression. Whole-brain VBM volumetric and surface-based cortical thickness assessments were performed voxel-wise and compared (at $p < 0.05$ corrected for multiple comparisons) between the MDD and control groups.

MDD participants had reduced gray matter volume in the anterior cingulate cortex, regions of the prefrontal circuits, including dorsolateral and dorsomedial prefrontal cortices, and lateral and medial orbitofrontal cortices, but not in limbic regions. Additional reductions were observed cortically in the posterior temporal and parieto-occipital cortices and, subcortically in the basal ganglia and cerebellum. Focal cortical *thinning* in the medial orbitofrontal cortex was also observed for the MDD group. These alterations in volume and cortical thickness were not associated with severity of depressive symptoms.

The findings demonstrate that widespread gray matter structural abnormalities are present in a well-powered study of patients with depression. The patterns of gray matter loss correspond to the same brain functional network regions that were previously established to be abnormal in MDD, which may support an underlying structural abnormality for these circuits.

1. Introduction

Major depressive disorder (MDD) is a leading cause of disability and has an economic impact in the United States of $42 billion per year (Mathers and Loncar, 2006). Advances in imaging technology are helping to progress the understanding of the pathophysiology of MDD and the targeting of new treatments. A substantial body of imaging data demonstrates spatially distributed functional abnormalities occurring throughout the brain in MDD (Drevets et al., 2008). The key regions that have gained consensus for an involvement in MDD include the limbic cortex (amygdala, hippocampus), the cingulate cortex, dorsal striatum and the frontal lobe (dorsolateral prefrontal cortex [DLPFC] and medial prefrontal cortex [MPFC]). These data have informed prevailing theories of the mechanisms of MDD, which highlight an imbalance between an overactive salience network (involving in the putamen, amygdala, and anterior cingulate) and decreased responsiveness in regions

Abbreviations: AAL, Automated Anatomical Labeling; ACC, Anterior Cingulate Cortex; BAs, Brodmann Areas; CVNA, Change in Volume expected in that region through Normal Aging; DLPFC, Dorsolateral Prefrontal Cortex; DTI, Diffusion Tensor Imaging; FDR, False Discovery Rate; fMRI, functional Magnetic Resonance Imaging; GM, Gray Matter; $HRSD_{17}$, 17-Item Hamilton Rating Scale for Depression; iSPOT-D, International Study to Predict Optimized Treatment in Depression; MDD, Major Depressive Disorder; MPFC, Medial Prefrontal Cortex; MRI, Magnetic Resonance Imaging; OFC, Orbitofrontal Cortex; PFC, Prefrontal Cortex; VBM, Voxel-Based Morphometry.

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involved in contextual processing (dorsal striatum and dorsolateral prefrontal cortex — DLPFC) (Hamilton et al., 2012). Although much of the imaging data is derived from PET and fMRI studies, convergent evidence from structural studies suggests similar widespread changes can be seen using structural methods. A recent analysis of gray matter structural networks highlighted the presence of global organizational abnormalities that are associated with a diagnosis of depression (Singh et al., in press). In this paper we evaluate the pattern and extent of gray matter (GM) changes in MDD, and examine whether these patterns of GM loss correspond to the same regions known to be functionally abnormal in MDD.

While it is clear that volumetric GM regional loss occurs in MDD, the precise pattern of this loss has not been clearly defined. Existing studies in patients with MDD have shown loss of GM volume across a range of cortical brain regions, including the medial prefrontal regions of the anterior cingulate cortex and orbitofrontal cortex (OFC), the lateral prefrontal cortex, the temporal cortex (Ballmaier et al., 2004), and the pre- and post-central gyri (Taki et al., 2005). Moderate GM loss has also been observed in subcortical regions including the putamen and caudate nucleus, as well as the amygdala and thalamus (Egger et al., 2008; Tang et al., 2007). Considered overall, the data that supports these changes is limited due to the small number of participants in most studies, the large amount of variation in the regions identified and the enormous variation in methodology. The heterogeneity between studies is echoed in two recent meta-analyses which show convergent findings for cingulate and OFC regions but differ in their findings relating to striatal and limbic structures (Egger et al., 2008; Tang et al., 2007). Another region which has been consistently identified in functional imaging studies of MDD is the DLPFC; however, the data relating to GM abnormalities in this region are mixed, with at least 50% of studies failing to report GM changes in this area (Bora et al., 2012). A further factor that likely contributes to the variability in the nature of the structural literature relating to MDD is the heterogeneity of the condition itself.

In this paper, we present a highly powered, single-site study of structural GM changes in MDD designed to address the gap in the literature outlined above. We use whole-brain analysis methods, permitting a systematic evaluation of GM abnormalities occurring throughout the entire brain. The International Study to Predict Optimized Treatment for Depression (iSPOT-D) is a large trial of treatment prediction in depression (Grieve et al., 2013; Williams et al., 2011). Our cohort is well characterized and non-geriatric, and the methods are highly standardized, which is in contrast to much of the available literature. We have recently presented data from this study supporting the concept that functional and white matter Diffusion Tensor Imaging (DTI) structural deficits underlie the fronto-limbic dysfunction observed in MDD (Grieve et al., 2013; Korgaonkar et al., 2011, 2012). A large part of the motivation for this study is to understand the GM changes occurring in MDD in order to put into these in context of the connectivity abnormalities that are being seen in both in our work, and by others. The global patterns of the abnormalities that are being identified using DTI and functional MRI suggest that associated GM changes may also be present which might reflect these abnormal networks. We therefore hypothesize that the GM volume changes will involve multiple regions but will be most marked in regions characterized by hypofunction in functional data or by decreased connectivity in DTI tractography studies. Specifically we hypothesize that the significant structural changes will occur in the DLPFC — a region characterized by decreased activity in MDD. In addition, based on the literature and on the strong involvement of the cingulate bundle in our previous DTI analysis, we hypothesize (i) that the cingulate cortex and (ii) the OFC—which has a large number of projection fibers extending from the cingulate region—will both show GM volume reductions that reflect underlying circuit abnormalities present in MDD.

2. Material and methods

2.1. Participants

Magnetic resonance imaging (MRI) data were drawn from the first 102 MDD and 34 age- and gender-matched healthy participants who provided MRI data at Westmead Hospital (Sydney Medical School, University of Sydney) as part of the baseline data collection for the iSPOT-D study. MDD participants in the study were aged 18–65, fluent in English, had a total $HRSD_{17} \geq 16$ and met the DSM-IV criteria for single or recurrent nonpsychotic MDD established by MINI Plus and did not have suicidal tendencies, history of bipolar, schizophrenia, schizoaffective disorder or psychosis, current primary diagnosis of anorexia, obsessive–compulsive disorder or primary post traumatic disorder. They did not have substance dependence, history of brain injury, had suffered loss of consciousness for greater than five minutes or any contraindications for MRI. A complete description of the iSPOT-D study protocol, clinical assessments, inclusion/exclusion criteria and diagnosis procedures of the overall trial is provided in Williams et al. (2011) and a description of the imaging sub-study is provided in Grieve et al. (2013). This study was conducted according to the principles of the Declaration of Helsinki 2008. After the study procedures were fully explained in accordance with the ethical guidelines of the institutional review board, participants provided written consent.

Demographic features (e.g., age, gender, years of education) were gathered at baseline. The 17-item Hamilton Rating Scale for Depression ($HRSD_{17}$) was gathered at baseline to measure depressive symptom severity.

2.2. Image acquisition

Magnetic resonance images were acquired using a 3.0 T GE Signa HDx scanner (GE Healthcare, Milwaukee, Wisconsin). Acquisition was performed using an 8-channel head coil. Three-dimensional T1-weighted magnetic resonance images were acquired in the sagittal plane using a 3D SPGR sequence (TR = 8.3 ms; TE = 3.2 ms; Flip Angle = 11°; TI = 500 ms; NEX = 1; ASSET = 1.5; Frequency direction: S/I). A total of 180 contiguous 1 mm slices were acquired with a 256×256 matrix, with an in-plane resolution of 1 mm × 1 mm resulting in isotropic voxels.

2.3. Voxel-based morphometry analysis

T1 image data was pre-processed and analyzed using the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm.html) and the SPM8 software package (http://www.fil.ion.ucl.ac.uk/spm). Images were corrected for bias-field inhomogeneity; tissue-classified into GM, white matter and cerebrospinal fluid; and registered to standard space using high-dimensional DARTEL normalization (Ashburner, 2007). The segmentation approach is based on an adaptive maximum, a posterior technique which does not need a priori information about tissue probabilities (Rajapakse et al., 1997). The segmentation procedure is further refined by accounting for partial volume effects and by applying a hidden Markov random field model which incorporates spatial prior information of the adjacent voxels into the segmentation estimation (Tohka et al., 2004). The warped tissue type images were modulated to preserve the volume of a particular tissue within a voxel by multiplying voxel values in the segmented images by the Jacobian determinants derived from the spatial normalization step. The analysis of these modulated images allows testing for regional differences in absolute volume of tissue class. Finally, images were smoothed with a full-width half-maximum kernel of 8 mm. Statistical analyses were performed on these images for whole-brain comparison of volume between the MDD and control groups. Regions were labeled with reference to the Automated Anatomical Labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002). Brodmann areas (BAs) are also provided to enable

cross-referencing to this commonly used nomenclature. For this purpose, the publically available atlas distributed with MRIcro was used (www.mccauslandcenter.sc.edu/mricro/mricro/lesion.html#brod).

2.4. Cortical thickness and sub-cortical volume analysis

Cortical surface reconstruction and volumetric segmentation was performed in an automated manner using the FreeSurfer image analysis suite (version 4.3, http://surfer.nmr.mgh.harvard.edu/). The technical details of these procedures have been previously described elsewhere (Grieve et al., 2011). Briefly, for each participant, the boundary between the GM and white matter and the outer surface of the cortex (the pial surface) was segmented. Cortical thickness measurements at each point across the cortical mantle were calculated as the closest distance from the GM/white matter boundary to the GM/cerebrospinal fluid boundary (Fischl and Dale, 2000). This method uses both intensity and continuity information from the entire three-dimensional magnetic resonance volume in segmentation and deformation procedures to produce representations of cortical thickness. The surface representations are created using spatial intensity gradients across tissue classes and are therefore not simply reliant on absolute signal intensity. The surfaces produced are not restricted to the voxel resolution of the original data and thus are capable of detecting sub-millimeter differences between groups. Cortical segmentation and labels were manually inspected for accuracy. Each participant dataset was then normalized to an average reference surface template in standard space—provided with FreeSurfer—to allow for cortical thickness evaluations at every surface point across participants. Registration was performed in a spherical surface-based coordinate system that is adapted to the folding pattern of each individual dataset, allowing a much higher localization accuracy of structural features of the brain across participants (Fischl et al., 1999). These images were then entered for statistical evaluation of MDD vs. control groups as described below.

2.5. Statistical analyses

Volume and cortical thickness structural differences between the MDD and healthy participant groups were evaluated using independent sample t-tests. Analyses were performed at every voxel for the VBM data and at every surface point (vertex) for the cortical thickness data. For the MDD group only, depressive severity was assessed for correlations with both VBM and cortical thickness voxel/vertex-wise. Since our cohort was age and gender matched we did not co-vary for these parameters. A statistical threshold of $p < 0.05$ (false discovery rate [FDR] corrected for multiple comparisons) was used. Volume and cortical thickness values for the significant clusters were extracted using a 5 mm sphere ROI centered on the cluster peak and used to calculate percent differences between the two groups. For the purposes of giving a regionally specific comparator, we also express these local differences in terms of the volume loss expected in these regions via the normal aging process in years (i.e., Change in Volume expected in that region through Normal Aging [CVNA]) using the same ROI. These comparisons are based on our previously presented data on normal aging in 476 individuals without a psychiatric or other medical diagnosis (Grieve et al., 2005, 2011). We use this convention throughout the remainder of the text.

3. Results

3.1. Participant characteristics

Demographic information for the MDD and healthy participant groups is summarized in Table 1. As expected, the MDD group had greater depressive severity ($HRSD_{17}$) compared to healthy participants.

Table 1
Selected demographic features, global and lobar gray matter volumes.

Measures	Controls (n = 34)	MDD (n = 102)	p value
Demographic/clinical measures			
Age (years)	31.5 ± 12.4	33.8 ± 13.1	0.390
Gender	18 M:16 F	48 M:54 F	0.871
Education (years)	14.7 ± 3.5	14.2 ± 2.9	0.420
Spotcor (IQ measure)	46.8 ± 5.7	46.2 ± 6.6	0.594
$HRSD_{17}$ baseline[a]	1.0 ± 1.2	21.0 ± 3.9	<0.0001
Age of onset (years)	–	22.1 ± 12.2	–
Disease duration (years)	–	11.3 ± 11.8	–
Melancholic subtype	–	31%	–
Volumetric GM measures			
Whole brain (ml)[a]	885.5 ± 56.9	846.7 ± 79.9	0.010
Frontal lobe (ml)[a]	237.4 ± 22.0	224.5 ± 23.9	0.007
Temporal lobe (ml)[a]	144.1 ± 10.6	137.1 ± 12.9	0.005
Parietal lobe (ml)[a]	126.7 ± 10.1	121.6 ± 13.1	0.038
Occipital lobe (ml)[a]	97.7 ± 7.2	93.3 ± 9.2	0.014
Cerebellum (ml)	125.4 ± 7.8	120.4 ± 15.3	0.072

Abbreviations: $HRSD_{17}$, 17-item Hamilton Rating Scale for Depression; MDD, Major Depressive Disorder.
[a] Indicates significant difference ($p < 0.05$).

3.2. Global and lobar GM volume measurements

The global and lobar GM volumes for MDD vs. controls are summarized in Table 1. Compared to healthy controls, MDD participants had 4.4% less global GM volume than did healthy controls ($p < 0.05$). These volumetric differences were greatest in the frontal and temporal lobes (5.4% and 4.8%, respectively), with significant differences also present in the parietal and occipital lobes. The global GM difference between depressed participants and controls equated to a CVNA of 14 years. The lobar GM volume differences equated to CVNA values of 12 years (frontal), 10 years (parietal), 15 years (temporal) and 14 years (occipital). Cerebellar volume differences between the two groups were not significant.

3.3. Whole-brain voxel-level GM volume analysis

The VBM analysis showed large areas of decreased GM volume in MDD distributed across the brain, with no regions of increased GM volume. The dominant findings were of three large bilateral clusters, with each cluster spanning several contiguous anatomical regions. For convenience, since the three clusters were bilaterally symmetric, the right and left components are referred to below as the same cluster number. These clusters are broadly characterized as follows: (1) the DLPFC (BA 45, 46) with extension inferiorly to the lateral OFC (BA 47) and superiorly to involve the pre/post central gyri (Fig. 1, top row), (2) a large midline region spanning the medial orbitofrontal cortex/posterior gyrus rectus, entire cingulate cortex and the calcarine/lingual gyri (Fig. 1, middle row), and (3) a broad region in the posterior temporal lobe spanning the inferior/middle/superior temporal gyri (Fig. 1, bottom row). These three regions are presented in Table 2, broken down by anatomically distinct regions classified using both AAL and Brodmann descriptors. In addition, several discrete, focal regions of significant GM volume reduction were present, which are presented by lobe in Table 2. These dominant findings are summarized below:

3.3.1. Dorsolateral prefrontal cortex (BA 8, 9, 45, 46), (part of the large cluster 1)

The DLPFC component encompasses most of BA 45 and the lateral components of BA 46. The GM difference centered at the maxima in BA 46 corresponded to a 24% volume difference on the left (CVNA: 32 years) and a 27% volume difference on the right (CVNA: 31 years).

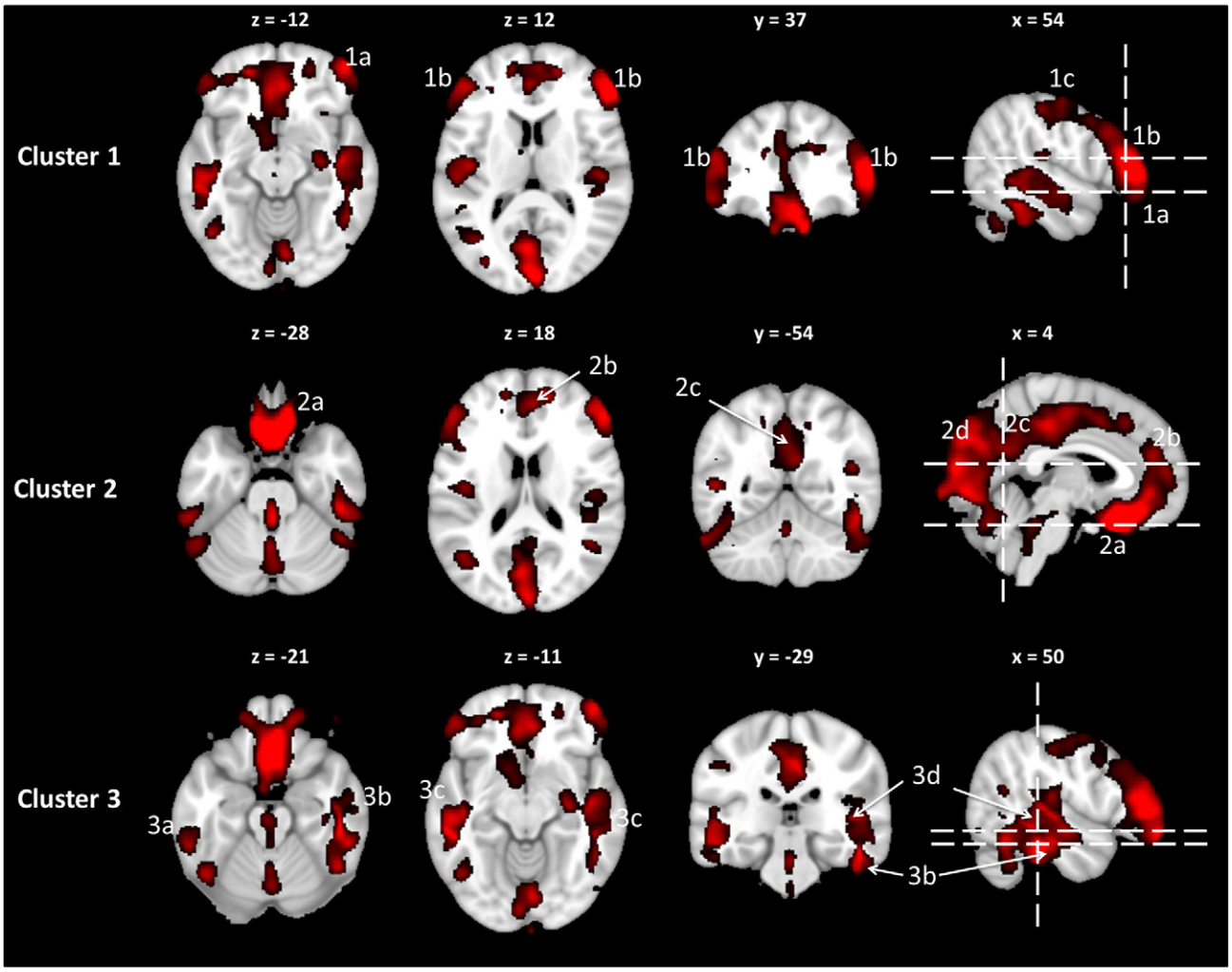

Fig. 1. Voxel based morphometry volume differences between the MDD and control groups (MDD < controls at $p < 0.05$ FDR-corrected). The dominant three bilateral clusters are shown in two axial slices (columns 1 & 2), one coronal slice (column 3) and one sagittal slice (column 4) (the location of the axial and coronal slices is marked on the sagittal slice for reference): (top row) cluster 1 — 1a — dorsolateral prefrontal cortex, 1b — lateral orbitofrontal cortex, 1c — precentral gyrus; (middle row) cluster 2 — 2a — gyrus rectus, 1b — anterior cingulate cortex, 1c — posterior cingulate cortex, 1d — precuneus; and (bottom row) cluster 3 — 3a — fusiform gyrus, 3b — inferior temporal gyrus, 3c — middle temporal gyrus and 3d — superior temporal gyrus. Abbreviations: FDR, False Discovery Rate; MDD, Major Depressive Disorder.

3.3.2. Medial and lateral orbitofrontal cortices (BA 10, 11, 47), (part of clusters 1 & 2)

The cluster maxima corresponding to the medial OFC were present in the middle frontal gyrus (BA 10/11) and the gyrus rectus. The decreased GM volume in this cluster corresponded to a difference of approximately 8–10% (CVNA: 23–25 years). The changes were anatomically symmetric. The gyrus rectus GM differences (26%) were profound but restricted to the posterior portion (CVNA: >50 years). For the lateral OFC, the peak cluster was located in the middle frontal gyrus (GM difference of 19%, CVNA: 24 years).

3.3.3. Dorsomedial prefrontal cortex (BA 10), (part of cluster 2)

Cluster maxima were found in the bilateral medial frontal gyrus within the dorsomedial prefrontal cortex (PFC) region. A GM difference of 11% on the left (CVNA: 37 years) and 11% on the right (CVNA: >50 years) was found for these clusters.

3.3.4. Anterior cingulate cortex (BA 24, 25, 32), (part of cluster 2)

The anterior cingulate cortex (ACC) component of cluster 2 was maximal in the caudal ACC (11%) but involved the whole of the ACC quite homogenously (global ACC ranged from 8 to 11%). The maximal ACC volume differences were symmetric and corresponded to approximately CVNA: 26 years.

3.3.5. Mid- and posterior cingulate cortices (BA 23, 26), (part of cluster 2)

The GM volume decrease involved the entire mid- and posterior cingulate cortices. The volume differences were quite homogenous and similar in magnitude to that seen in the ACC, ranging from 8 to 11% (CVNA: 32–42 years). The changes were anatomically symmetric.

3.3.6. Posterior temporal lobe (BA 18, 19), (part of cluster 3)

Large bilateral regions of decreased GM volume in the MDD group involve the posterior portions of the temporal lobe. The volume decreases in the medial temporal lobe correspond to an average regional volumetric difference of 10–13% (CVNA: 21–48 years).

3.3.7. Occipital cortex (BA 18, 19)

The GM changes in the occipital region were predominantly midline (calcarine and lingual sulci) and were contiguous with the medial PFC and cingulate clusters. Additional occipital lobe clusters were also present in the middle occipital gyrus (bilateral) and the right inferior occipital gyrus. The significant regions of volume differences in the occipital lobe were 8–14% (CVNA: 12–38 years).

3.3.8. Other regions

Small focal decreases in GM in the MDD cohort were present in the left pre- and post-central gyri (continuous with cluster 1). Scattered

Table 2
Summary of significant clusters with VBM and cortical thickness differences between controls and MDD participants at p = 0.05 (FDR corrected).

Region	MNI space (mm)			Cluster size	Z score	FDR corrected p-value	% change
	x	y	z				
Cortical thickness differences							
Controls < MDD							
L — Superior frontal gyrus (BA 11)	−21	56	−13	334[a]	5.13	<0.001	16.4%
L — Lateral orbitofrontal	−22	47	−12	334[a]	4.19	0.002	12.8%
Controls > MDD							
L — Medial frontal gyrus (BA 25)/medial orbitofrontal	−6	17	−16	8	4.08	0.003	16.1%
Gray matter volume differences							
Controls > MDD							
Frontal							
Dorsolateral prefrontal cortex and lateral OFC							
L — Middle frontal gyrus (BA 45/46)	−53	32	21	51416[b]	4.25	0.002	24.1%
R — Inferior frontal gyrus (BA 45/46)	54	38	12	8820[a]	5.68	<0.001	27.3%
L — Inferior frontal gyrus (BA 44)	−47	15	18	51416[b]	3.37	0.015	9.2%
R — Middle frontal gyrus (BA 47)	48	52	−14	8820[a]	4.99	<0.001	19.0%
Medial orbitofrontal cortex/posterior gyrus rectus							
R — rectal gyrus (BA 11)	4	20	−29	51416[b]	6.66	<0.001	26.2%
L — Superior/middle frontal gyrus (BA 11)	−20	50	−12	51416[b]	4.45	0.001	8.1%
R — Superior/middle frontal gyrus (BA 10/11)	15	47	−21	51416[b]	4.13	0.002	9.9%
Dorsomedial prefrontal cortex							
L — Medial frontal gyrus (BA 10)	−14	56	5	51416[b]	4.75	<0.001	10.7%
R — Medial frontal gyrus (BA 10)	17	52	13	51416[b]	4.62	0.001	11.1%
Cingulate cortex							
L — Anterior cingulate (BA 32)	−21	14	42	51416[b]	4.89	0.001	10.9%
R — Anterior cingulate (BA 32)	6	45	20	51416[b]	4.07	0.003	11.4%
R — Mid cingulate (BA 23)	5	−27	45	51416[b]	4.64	0.001	10.3%
L — Posterior cingulate (BA 23/26)	−3	−38	24	51416[b]	3.57	0.009	7.9%
L — Precuneus/posterior cingulate (BA 7/23)	−5	−69	38	51416[b]	4.58	0.001	11.1%
R — Precuneus/posterior cingulate (BA 7/23)	6	−57	24	51416[b]	4.04	0.003	10.0%
Other frontal regions							
L — Superior frontal gyrus (BA 6)/supplementary motor area	−9	11	54	51416[b]	2.89	0.041	7.0%
R — Precentral gyrus (BA 4)	56	−8	50	8820[a]	3.69	0.007	20.5%
L — Precentral gyrus (BA 4/6)	−60	−7	42	85	3.20	0.021	17.2%
Temporal							
L — Fusiform gyrus (BA 37)	−45	−60	−21	1681[c]	4.28	0.002	10.8%
R — Fusiform/inferior temporal gyrus (BA 37)	50	−54	−17	9745[d]	4.47	0.001	10.3%
R — Inferior temporal gyrus (BA 20)	50	−30	−21	9745[d]	5.09	<0.001	12.9%
L — Middle temporal gyrus (BA 20)	−54	−34	−11	4330[e]	4.93	<0.001	13.0%
L — Middle temporal gyrus (BA 21/39)	−51	−54	6	1375	4.16	0.002	11.6%
R — Superior temporal gyrus (BA 22)	46	−22	0	9745[d]	4.72	0.001	9.5%
Parietal							
L — Postcentral gyrus (BA 2/3)	−45	−25	40	261	3.49	0.011	10.3%
R — Postcentral gyrus (BA 2/3)	54	−23	48	8820[a]	3.79	0.006	13.7%
L — Inferior parietal (BA 40)	−39	−48	36	868	3.63	0.008	12.1%
R — Inferior parietal (BA 7)	33	−61	42	41	3.15	0.024	10.9%
Occipital							
L — Cuneus/lingual/calcarine gyrus (BA 17/18)	−5	−78	26	51416[b]	5.16	<0.001	14.0%
R — Cuneus/lingual/calcarine gyrus (BA 17/18)	2	−92	−12	51416[b]	5.28	<0.001	8.1%
L — Middle occipital gyrus (BA 18/19)	−32	−82	10	193	3.75	0.006	12.3%
R — Middle occipital gyrus (BA 19)	26	−70	25	606	3.71	0.007	11.6%
R — Inferior occipital gyrus (BA 19)	32	−81	−5	35	3.29	0.017	10.2%
Subcortical							
L — Thalamus	−8	−4	13	28	3.00	0.032	9.3%
L — Globus pallidus	−27	−13	−3	107	3.39	0.014	7.2%
L — Caudate	−9	9	8	51416[b]	2.88	0.042	8.4%
Cerebellum							
L — Crus I	−57	−57	−32	1681[c]	4.02	0.003	23.4%
R — Crus I	52	−54	−30	9745[d]	3.71	0.007	23.4%
R — Lobule VIIb	28	−63	−48	25	2.98	0.034	8.8%
L — Lobule VIIb	−28	−63	−45	43	2.97	0.035	8.2%
L — Medial crus I	−27	−69	−36	186[b]	2.94	0.037	7.4%

[a], [b], [c], [d] and [e] indicate part of the same cluster.
Abbreviations: BA, Brodmann Area; FDR, False Discovery Rate; L, left; MDD, Major Depressive Disorder; MNI, Montreal Neurological Institute; OFC, Orbitofrontal Cortex; R, right; VBM, Voxel-Based Morphometry.

GM volume differences were present in the cerebellum; the largest of these were located bilaterally in the posterior crus I region (23%; CVNA: >50 years). Focal regions of GM volume difference were also present in the left anterior thalamus, left putamen and left caudate (GM volume decrease: 9%, 7% and 8%, respectively; CVNA 13–15 years).

3.4. Whole brain cortical thickness analysis

In comparison to the widespread differences in volume, the measured cortical thickness differences between the MDD and control groups were limited. The cortical thickness analysis showed a discrete region of thinned cortex in those with MDD which was centered in

the left medial orbitofrontal cortex (16% decrease; $p = 0.003$). The remainder of the regions identified in the VBM analysis showed no significant differences at the whole-brain correction level. Interestingly, when the contrast MDD > controls was tested, MDD participants were found to have a focal region of left lateral OFC with *increased* cortical thickness compared to controls; however, the cluster size for this finding was very small at 8 vertices (16% increase; FDR-corrected p-value < 0.001).

3.5. Volume and cortical thickness association with depressive severity

In the MDD group, no clusters were found to have significant correlations between depressive severity (using $HRSD_{17}$) and VBM volume or cortical thickness.

4. Discussion

Our data demonstrate conclusively that widespread GM volume abnormalities are present in patients with depression. These alterations are substantial, corresponding to the amount of GM volume loss that, when averaged over the whole brain, would be expected from nearly 14 years of normal aging. The GM loss is also highly regionally specific, with focal regions showing decreases in GM volumes of nearly twice the magnitude of the global measure. The distributed and regionally specific nature of these alterations provides compelling support for considering MDD as a condition that involves the impairment of networks across the brain. Previous studies have used MRI to detect brain structural abnormalities in patients with MDD, but they have produced heterogeneous and often conflicting results (Arnone et al., 2012; Bora et al., 2012; Koolschijn et al., 2009). Ours is the largest study to specifically examine GM volumetric changes in MDD and it provides a very robust level of evidence documenting the structural pattern of GM loss. Our cohort is well characterized and non-geriatric, with an average age of 34 years (standard deviation: 13 years). The whole-brain analysis approach used in the study also means that no a priori regional bias was added to our findings.

The magnitude of the GM volume differences found in this study is striking. This is especially apparent when compared to the volumes of GM that are lost during the aging process. We have shown that a diagnosis of MDD in a non-geriatric cohort is associated with focal decreases of GM volume that are in excess of that expected from 11 to 50 years of normal aging, depending on the region. Regional GM differences were present in the DLPFC (27%), ACC (11%) and the medial orbital frontal cortex (10%), all regions that have an established role in the pathophysiology of MDD. Additional regions of the brain were also found to have significant GM loss with MDD, including the medial temporal lobe (13%), the gyrus rectus (26%) and the occipital lobe (8–14%).

There is a scarcity of prior data that specifically examines GM volume changes in the DLPFC with MDD. Therefore, our demonstration of a large DLPFC volume reduction represents an important addition to the literature and is in keeping with the central role of this region in the dominant models of MDD. The regional changes correlate well with the abnormal fMRI responses (hypoactivation of the dorsolateral prefrontal cortex during working memory updating and during conscious negative emotion processing) seen in our previous analysis of fMRI data from this sample (Korgaonkar et al., 2013) and also seen in other studies (Fitzgerald et al., 2006). While it seems surprising that such a large effect has not previously been demonstrated, the heterogeneity of findings in this region identified in meta-analyses suggests that both methodological and cohort factors may impact on observations of structural change in this region (Arnone et al., 2012; Bora et al., 2011). The key methodological factors contributing to this are the variability in delineating this region across previous studies employing a region of interest approach and the large inter-individual variability for this region (Sanches et al., 2009). While a VBM analysis is an unbiased method to tackle these issues, the necessity for a severe correction for type 1 error at the whole brain level may limit statistical power especially with smaller cohorts. The relatively young age of our cohort is also important since the DLPFC undergoes accelerated GM loss during "normal" aging (Grieve et al., 2005), so in a geriatric MDD cohort these changes may be attenuated relative to the normal population due to age-related changes common to both groups. Given the increased prevalence of depression that occurs with age (Mirowsky and Ross, 1992), it is interesting to speculate that age-related GM volume loss in this region may actually predispose to depression.

The second highest regional GM volume loss occurred in the gyrus rectus (26% reduction in volume; greater than 50 years CVNA). Previous data have highlighted the role of this region in emotional regulation and in depression (Fitzgerald et al., 2008). Substantial GM volume loss was additionally present in the medial OFC, which is also in agreement with prior studies (Arnone et al., 2012; Bora et al., 2012; Koolschijn et al., 2009). GM loss in the medial OFC region was part of a large paramedian cluster of significant GM volume loss that included the posterior gyrus rectus, cingulate cortex and medial occipital lobe. This medial OFC region has been specifically implicated as a key component in the circuitry of depression (Drevets et al., 2008). We also observed that participants with MDD showed a reduction in cortical thickness in this region. fMRI data supports a distinct role of this region in emotional regulation (Golkar et al., 2012). A longitudinal study demonstrated a relationship between this cortical structure as an adult and emotional reactivity measured at 4 months of age, data that implies the presence of a "hard-wired" structural predisposition to emotional reactivity (Schwartz et al., 2010). Our findings of discrete GM volume and thickness loss in the gyrus rectus replicate previous data (Ballmaier et al., 2004). Importantly, the medial orbital prefrontal cortex/gyrus rectus is badly affected by magnetic susceptibility artifact in fMRI studies, and to a lesser extent in DTI studies, due to the sensitivity of the echo planar imaging sequence to magnetic field gradients that occur adjacent to the paranasal sinuses. These gradients reduce the contrast-to-noise of the Blood Oxygenation Level-Dependent response and also cause geometric distortion (Cusack et al., 2005; Grieve, 2000). Due to these effects, global fMRI studies may understate the importance of this region. Perhaps targeted fMRI studies using localized shims or spin-echo fMRI may be required to fully understand the importance of this region.

The functional role of the cingulate cortex—and specifically the ACC—in depression is well described (Drevets et al., 2008). Our data shows a large midline cluster of GM volume loss, which contiguously involves the cingulate cortex, extending from the medial OFC to the precuneus and medial occipital cortices. These regions are also the key components of the default mode network (Greicius et al., 2003). Abnormal resting state functional connectivity of these same regions has been described in MDD (Greicius et al., 2007). Our previous work using DTI data showed that connectivity in the cingulate portion of the cingulate bundle has structural changes with MDD at baseline and also has an impact on remission (Korgaonkar et al., under review). The OFC has extensive connections with the limbic system, including from the cingulate cortex (Ongür and Price, 2000). The presence of structural changes in both the ACC and the OFC supports the notion that MDD may be conceived as primarily a network abnormality.

One of the most striking findings in our study was that no significant hippocampal or amygdala GM volume loss was present with MDD. Hippocampal volume loss has been identified as a significant feature of MDD in one recent meta-analysis of structural brain changes in depression, but not in a second meta-analysis. Neither of these studies demonstrated amygdala volume loss (Arnone et al., 2012; Bora et al., 2011). While some of the reasons for this may be methodological such as MR image acquisition parameters, post acquisition processing and the method used for volumetric assessment (Geuze et al., 2005; Morey et al., 2009), it is clear that volumetric changes of the hippocampus that occur with age, with various neuropsychiatric disorders, and also in response to stress are quite heterogeneous. Our cohort is a relatively

young population and has an average duration of depression of 11 years. Therefore, our finding of a lack of changes to the hippocampus in this group may suggest that GM volume loss in the hippocampus does not play a key role in non-geriatric depression. Although, we observe strong abnormal fMRI measures affecting the amygdala in the current cohort, the associated GM volume changes in the amygdala were weak (Grieve et al., 2013) and may not be as robust to observe for assessments made at the whole brain level. Another important finding was no significant association of depressive severity (using the $HDRS_{17}$ scale) with both volume and cortical thickness for the MDD cohort. This has not been a consistent finding in previous studies. While the lack of correlation in our study replicates previous studies that found the same (McKinnon et al., 2009; Zou et al., 2010), a significant association between GM volume and depression severity has also been previously reported (Chen et al., 2007; Cheng et al., 2010).

Our study presents a highly powered, highly standardized cross-sectional analysis of a well-described cohort with depression, providing valuable and novel insights into the GM volume changes that characterize depression. Given the size of the health and economic impact of this disorder and the "hit and miss" nature of treatments, there is a clear need to understand the depressive phenotype in greater detail. Cross-sectional data is unable to clarify whether these volume changes precede the development of depression or whether they occur secondary to the depressive state. Prospective studies with both structural and functional imaging, correlated with genetics and clinical data in young cohorts, are needed to provide the data to answer this question.

Our cortical thickness data confirmed the GM changes present in the medial OFC, but did not detect cortical thinning elsewhere. In contrast to the GM findings, we found a very focal region of increased thickness in the left lateral OFC; however the significance of this finding is unclear. This finding may be secondary to the loss in sensitivity implicit in a whole-brain analysis, or may be a reflection that the GM volume changes are not adequately captured by cortical thickness measures. Heritability analyses using twins data have demonstrated that there are different genetic drivers for GM volume and cortical thickness measures (Winkler et al., 2010). Previous studies (with different MDD cohorts) that looked at both of these measures have shown mixed data, with some having shown no cortical thickness changes while others have shown changes. Further work is clearly needed to understand the morphological nature of the GM volume loss that we have demonstrated.

In conclusion, we have demonstrated that substantial GM volumetric abnormalities exist in individuals with MDD compared to individuals without MDD. These changes are widespread and involve key regions known to be involved in depression, including the DLPFC, OFC and ACC. In addition to these "usual suspects", we identified a generalized midline GM volume loss that correlates anatomically with the midline resting state default mode network regions which have been implicated in depression, as well as strong GM loss in the medial temporal lobe, cerebellum and pre/post central gyrus. Further work is required to understand the significance of these volumetric changes, including prospective studies and studies that integrate fMRI, DTI and volumetric data.

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