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Ultra high-resolution imaging of amygdala subnuclei structural connectivity in Major Depressive Disorder

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Abstract

Background: Major Depressive Disorder (MDD) is an increasingly common and disabling illness. Since the amygdala has been reported to have pathological involvement in mood disorders, we aimed to investigate potential changes to structural connectivity of individual amygdala subnuclei in MDD for the first time, using ultra high-field 7T diffusion MRI.

Methods: Twenty-four MDD patients (11 female) and twenty-four age-matched healthy control participants (7 female) underwent diffusion-weighted imaging with a 1.05mm isotropic resolution at 7 Tesla. Amygdala nuclei regions of interest were obtained through automated segmentation of 0.69mm resolution T1-weighted images and 0.35mm resolution T2-weighted images. Probabilistic tractography was performed on all subjects, with random seeding at each amygdala nucleus.

Results: The right lateral, basal, central and centrocortical amygdala nuclei exhibited significantly increased connection density to the rest of the brain, whereas the left medial nucleus demonstrated significantly lower connection density ($p_{FDR} < 0.05$). Increased connection density

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⁵Conflict of interest

Dr. Balchandani (the Principal Investigator in this study) is a named inventor on patents relating to magnetic resonance imaging (MRI) and RF pulse design. The patents have been licensed to GE Healthcare, Siemens AG, and Philips international. Dr. Balchandani receives royalty payments relating to these patents.

In the past 5 years, Dr. Murrough has provided consultation services to Boehringer Ingelheim, Sage Therapeutics, FSV7, Novartis, Allergan, Fortress Biotech, Janssen Research and Development, Medavante-Prophase and Global Medical Education (GME) and has received research support from Avanir Pharmaceuticals, Inc.

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in the right lateral and basal nuclei were driven by the stria terminalis and the significant difference in the right central nucleus was driven by the uncinate fasciculus. Decreased connection density at the left medial nucleus did not appear to be driven by any individual white matter tract.

Conclusions: By exploiting ultra-high resolution MRI, structural hyperconnectivity was demonstrated involving the amygdaloid nuclei in the right hemisphere in MDD. To a lesser extent, impairment of subnuclei connectivity was shown in the left hemisphere.

Keywords

Major Depressive Disorder; Diffusion MRI; Tractography; Amygdala; Ultra high-field; 7T; Neuroimaging

1. Introduction

Major depressive disorder (MDD) is a debilitating condition with significant worldwide prevalence and substantial mortality (1-3). There is an emerging body of literature implicating the amygdala as a particular brain region of interest in the pathology of MDD (4, 5). The amygdala plays an important functional role in emotional processing, fear conditioning and extinction, motivation, social salience and affective state, aberrant regulation of which form the depressive phenotype (6).

Concordant to its functional role, the amygdala has extensive intrinsic and extrinsic structural connections within the brain. Classically, the three main efferent white matter tracts from the amygdala are the anterior commissure, stria terminalis and the amygdalofugal pathway (7). The anterior commissure crosses the midline of the brain, connecting the anterior temporal lobes bilaterally (7). The stria terminalis originates from the central nucleus of the amygdala, projecting to a terminus dorsal to the anterior commissure via the lateral side of the fornix. The amygdalofugal pathway is thought to originate in the anteromedial temporal lobe, passing through the basolateral and central amygdala nuclei towards the midline (8, 9). Ascending branches of the amygdalofugal pathway cross through the nucleus accumbens and conclude in the area of the septal nuclei. Descending amygdalofugal white matter fibers project towards nuclei of the hypothalamus, and medial fibers reach the basal forebrain and olfactory areas. Similarly, the stria terminalis also connects the amygdala to the hypothalamus (7). Functionally, each efferent projection is considered to have a distinct purpose. The anterior commissure plays an important role in coupling the amygdalae and temporal lobe hemispheres and is involved in emotion, olfaction, instinctual behavior and memory consolidation (10, 11). The amygdalofugal tract is thought to exert downstream control over the hypothalamus and septal nuclei, influencing threat reactivity (12). Moreover, reports of amnesia in patients with damage to the amygdalofugal tract suggest an additional role in memory (13). The stria terminalis is theorized to regulate social and motivational conduct (14). Amino acid afferent neurotransmission from the amygdala in non-human primates has been described to reach the frontal, insular, cingulate and temporal cortices. Additionally, hippocampal cornu ammonis (CA) 1 and the entorhinal cortex give rise to significant amygdala input (15). Intrinsically, the lateral nucleus of the amygdala appears to project strongly to the basal nucleus (15). Additionally, there is evidence for a direct connection from the visual pulvinar

of the thalamus to the amygdala, possibly enabling emotional salience attribution to simplistic visual information relayed from the visual cortex to facilitate swift and unconscious selective attention switching (16). In fact, this subcortical pathway has been shown to originate in the superior colliculus in the macaque (17) and has been reported to continue efferently from the amygdala to visual cortices 2 and 4 (18).

Given the amygdala's broad and extensive structural connectivity profile and functional relevance to emotional pathology, it is not surprising that it is a structure frequently associated with MDD pathology. Seed-based resting state fMRI analyses have revealed significantly reduced amygdala connectivity with the cerebellum, occipital cortex, caudate, superior and middle temporal lobes and insula in MDD patients. Additionally, increased amygdala-temporal pole functional connectivity was negatively correlated with depression symptom severity and anxiety ratings (19). In adolescent depression, the amygdala has been reported to exhibit lower positive functional coupling between the amygdala and hippocampus, parahippocampus and brainstem and increased connectivity between the amygdala and precuneus. Where functional connectivity was significantly increased compared to control subjects, blood-oxygenation-level dependent (BOLD) response was inversely correlated with general depression, lassitude and dysphoria scores (20). Longitudinal assessment of high-risk MDD resilient individuals revealed significantly increased functional connectivity between the amygdala and the orbitofrontal cortex, which correlated with positive life events (21). Connectivity between the prefrontal cortices and the limbic system has been cited as frequently altered in MDD (22), and uncinate fasciculus volume, as a proxy for fronto-temporal structural connectivity known to involve the amygdala, has been shown to correlate with both amygdala volume and trait anxiety (23).

Due to past constraints of MR spatial resolution, imaging time and the availability of technology, studies considering the amygdala in depression have often treated the region as a single, unified structure in analyses. However, both animal and human studies have shown that the amygdala is comprised of at least thirteen anatomically separate and discrete subnuclei (24, 25). Several studies support the notion that these amygdala subregions are functionally distinct during emotional processing (26-28). We therefore set out to utilize a recently developed automated segmentation technique and ultra-high resolution diffusion MRI to investigate the role of amygdala subnuclei connectivity in major depression at a high level of granularity for the first time.

2. Methods

2.1. Participants

Twenty-four participants (mean age = 38.7 years, SD = 9.8 years, 11 females) with a primary diagnosis of MDD were recruited through the Mood and Anxiety Disorders Program at the Icahn School of Medicine at Mount Sinai. Twenty-four age-matched controls were also recruited (mean age = 39.8 years, SD = 12.3 years, 7 females). All participants were English-speaking and between 18 and 65 years of age. Age was not significantly different between groups ($p = 1.0$). Eligible MDD patients had no psychotic features, and were assessed by the Structured Clinical Interview for DSM-IV disorders (SCID-IV) or the Structured Clinical Interview for DSM-5 Research Version (SCID-5-RV) (29, 30). They

were antidepressant free for at least 4 weeks prior to study participation and were currently experiencing a major depressive episode. No depressed participants had previously undergone electroconvulsive therapy. Healthy controls had no current or lifetime psychiatric disorder as determined by the SCID-IV or SCID-5-RV (29, 30). Participants with a current diagnosis of obsessive compulsive disorder (OCD), alcohol or substance abuse in the previous year, or lifetime history of a psychotic illness, bipolar disorder, or neurological disease were excluded. In the MDD group, 33% had co-morbid social phobia, 16% had generalized anxiety, 4% had binge eating disorder, 4% had panic disorder and 4% had post-traumatic stress disorder. Participants with MRI contraindications, unstable medical conditions, or positive urine toxicology on day of scan were also excluded. The mean age of illness onset in the depression sample was 17.5 years (SD = 10.4 years) and the mean duration of depressive episode was 75.0 months (SD = 84.0 months). All participants gave fully-informed written consent prior to investigation. Participant demographics and summary statistics are given in Table 1. This protocol was approved by the local Institutional Review Board.

2.2. MRI acquisition

Magnetic resonance imaging data was acquired for all participants on a 7 Tesla whole body scanner (Magnetom, Siemens Healthcare, Erlangen, Germany). A SC72CD gradient coil was used with a single coil transmit and a 32-channel receive head coil (Nova Medical, Wilmington, MA, USA). A T1-weighted MP2RAGE sequence (31) was performed on each participant, with a $0.7\text{mm} \times 0.7\text{mm} \times 0.7\text{mm}$ voxel resolution. Field of view (FOV) was $225 \times 183\text{mm}$, acquisition matrix was $320 \times 260\text{mm}$, orientation of scan was coronal, repetition time (TR) was 6000ms and echo time (TE) was 3.62ms. Number of slices for a single slab was 240. A coronal-oblique T2-weighted turbo spin echo (T2-TSE) sequence was also obtained for all participants, with a $0.43\text{mm} \times 0.43\text{mm} \times 2.0\text{mm}$ voxel resolution. Number of acquired slices was 66. Acquisition matrix was $512 \times 408\text{mm}$, FOV was 222×177 , TR was 9000ms and TE was 69ms.

A diffusion-weighted imaging sequence was performed in the same scanning session for all participants. TE was 67.6ms and TR was 7200ms. FOV was $210 \times 210\text{mm}$, acquisition matrix was $200 \times 200\text{mm}$, number of slices was 132, flip angle was 90° , spatial resolution of the diffusion data was 1.05mm isotropic and number of gradient directions was 64, with five $b=0 \text{ s/mm}^2$. The five $b=0$ acquisitions were interleaved during the acquisition to correct for artefacts, at time points 0.0s, 115.2s, 223.2s, 338.4s and 453.6s. The b-value for the sequence was 1500s/mm^2 . Two dMRI reverse-direction scans were acquired to correct gradient distortions.

2.3. Structural data processing

The T1-weighted images were preprocessed using the FreeSurfer version 6.0 “recon-all” pipeline, non-parametric non-uniform intensity correction, intensity normalization, skull stripping and neck removal, automatic segmentation and parcellation steps (32). Multispectral amygdala segmentation was carried out in FreeSurfer development version 6.0, utilizing the T1-weighted and T2-weighted high-resolution images, producing masks for the basal, lateral, accessory basal, central, medial and cortical nuclei (33). The FreeSurfer

automated amygdala segmentation was developed using an *ex vivo* dataset comprised of 10 autopsied human brain hemispheres and 7T field strength MRI scanning with a 0.1mm isotropic resolution. Amygdala nuclei were verified by a neuroanatomist and the automated atlas was built using an algorithm based on Bayesian inference. Validation using publicly available datasets showed that the amygdala automatic atlas significantly outperformed estimations of amygdala volume as a whole by FreeSurfer version 5.1 and performed with 84% accuracy in Alzheimer's disease discrimination from age-matched controls (33). The nuclei were also grouped into a basolateral complex (accessory basal, basal and lateral nuclei) and a centrocortical complex (central, cortical and medial nuclei) by summing individual nucleus metrics. All outputs were manually inspected to ensure quality of preprocessing and accuracy of segmentation.

2.4. Diffusion data processing

Diffusion data was denoised using MRTrix two-shell phase-reversed processing (34, 35). FreeSurfer segmented and parcellated images were used for whole brain masking in the image processing (32). B₁ field inhomogeneity correction was performed for the diffusion images (36). Fiber orientation distributions (FOD) were estimated from the diffusion data using spherical deconvolution (36), and diffusion tensor estimation was carried out using iteratively reweighted linear least squares estimator (37).

Amygdala nuclei regions of interest were co-registered to diffusion space using nearest neighbor interpolation in statistical parametric mapping software (SPM12), with the B0 image as the reference image, the T2-weighted image as the source image and the amygdala nuclei regions of interest as additional images. MRTrix software was used to carry out probabilistic tractography (38), performed using each amygdala nucleus mask as an individual seed region for 250,000 random seeds. Streamlines were thresholded using an FOD amplitude cutoff of 0.15 and at a maximum angle between successive streamline generation steps of 60°. The spherical deconvolution (SIFT2) algorithm was applied to all tracks, to eliminate streamlines that were unlikely to be biologically accurate and allow ground-truth accuracy of streamline count (39, 40). Whole brain fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD) and axial diffusivity (AD) maps were created, and using MRTrix's 'tcksample', microstructural measurements of FA, MD, RD and AD were extracted from every voxel along every streamline. Mean measurements per streamline were produced, and averaged to output one mean FA, MD, RD and AD value per amygdala nucleus ROI per participant. Microstructural order is used to refer to the degree of diffusivity and anisotropy with regards to the tissue structure, as factors such as axon diameter, packing density and membrane permeability affect diffusion metrics without being necessarily a correlate of structural integrity (41). Streamline count was used as extracted, using the MRTrix 'tckstat' command, applying SIFT2 weightings to improve accuracy to ground truth white matter and to eliminate spurious streamlines. The resultant metric is referred to as connection density.

A secondary analysis was carried out with the aim of elucidating which particular amygdalae-associated white matter tracts contributed to significant differences in connection density in MDD at a subnuclei level. Regions of interest (ROI's) were manually drawn

around the previously produced tracks of the stria terminalis, uncinate fasciculus, amygdalofugal tract, inferior fronto-occipital fasciculus and anterior commissure for each participant. An additional tractography was then performed using the amygdala nuclei that exhibited significant between group differences in streamline density as seeding regions and the individual tract ROI's as inclusion criteria for streamlines. Again, 250,000 random seeds were used per amygdala nucleus, streamlines were thresholded using an FOD amplitude cutoff of 0.15 and the maximum angle between successive streamline generation steps was 60°. All outputs were visually quality checked to ensure streamlines accurately tracked the correct white matter bundles. Streamline counts of the amygdala nuclei individual tracts were extracted with applied SIFT2 weightings.

2.5. Statistical analysis

Microstructural and streamline count metrics underwent between group analyses using non-parametric Mann-Whitney U testing of the null hypothesis. Testing was carried out both with and without age adjustment. Non-parametric regressions using the R package 'rfit' were carried out on the data for the MDD group only, to minimize bias caused by between group differences (42). Non-parametric statistical approaches were used to account for non-normal data distributions. Tractography metrics were regressed against age of illness onset and duration of depressive episode. Statistical results were corrected for multiple comparisons using false-discovery rate (FDR). All statistical analyses were carried out in R, version 3.3.3 (43) and a statistical significance threshold of 0.05 was used.

3. Results

3.1. Qualitative description of anatomical connections of the amygdala nuclei

The present ultra-high resolution probabilistic tractography analysis revealed five distinct white matter tracts connecting with the amygdala (Fig. 1). In keeping with prior reports of amygdala structural connectivity, we identified the three main efferent pathways of the amygdala in all participants: the stria terminalis, the anterior commissure and the amygdalofugal tract. A subcortical-visual pathway was also seen in the tractographies, appearing to pass along the inferior fronto-occipital fasciculus. While directionality cannot be assessed using probabilistic tractography, previous literature is suggestive these tracts may represent reciprocal connectivity with the visual areas (17, 18). The uncinate fasciculus was also tracked.

Our analyses did not consider discrete end point regions of interest, and anatomical connections were not investigated in a quantitative manner. Qualitatively however, virtual rendering of the amygdala subnuclei structural connectivity shows that a) nuclei exhibit differing profiles of streamlines and b) the basal and lateral nuclei exhibit shorter range connections than the medial, central and cortical nuclei.

3.2. Tissue microstructure

Several differences in the depression group compared to controls indicated an increase in microstructural order. In the left hemisphere, there was evidence of decreased microstructural order in the MDD group. However, when non-parametric between group

testing was performed with an additional adjustment for age, no significant differences in tissue microstructure in the MDD group compared to controls survived. These findings are presented as supplemental information.

3.3. Connection density

Streamline count was significantly increased in the MDD group compared to controls in tractography seeded at the right lateral nucleus ($p_{FDR} = 0.03$), the right basal nucleus ($p_{FDR} = 0.03$), the right central nucleus ($p_{FDR} = 0.02$) and the right centrocortical complex ($p_{FDR} = 0.03$) (Fig. 2). In the left hemisphere, there was a significant decrease in streamline count originating from the left medial nucleus ($p_{FDR} = 0.03$) in the MDD group (Fig. 3). All results remained significant with statistical adjustment for age.

3.4. Connection density of individual white matter bundles

Our findings of significant differences in connection density in white matter tracks seeded at the right lateral, right basal, right central and left medial nuclei in MDD were robust to both statistical adjustment for age and correction for multiple comparisons. For this reason, a secondary analysis was carried out on these nuclei to determine which individual white matter tracts contributed to depression-related changes in structural connectivity. The stria terminalis, anterior commissure, inferior fronto-occipital fasciculus, uncinate fasciculus and amygdalofugal pathway were isolated for each nucleus in each participant (Fig. 4). False discovery rate corrected non-parametric between group testing with adjustment for age revealed that the stria terminalis seeded from both the right lateral and basal nuclei had significantly increased connection density in the MDD group ($p_{FDR} = 0.01$, $p_{FDR} = 0.02$ respectively) (Fig. 5). The anterior commissure, inferior fronto-occipital fasciculus, uncinate and amygdalofugal pathway showed no significant differences in the major depression group when seeded from the right lateral and right basal nuclei. Significantly increased general connection density in streamlines seeded at the right central nucleus in MDD was driven by the uncinate fasciculus ($p_{FDR} = 0.01$) (Fig. 5). No other white matter tract showed significant between group differences when seeded at the right central nucleus. Lower general connection strength in streamlines seeded at the left medial nucleus did not appear to be driven by any of the single white matter tracts isolated in this study, with no significant differences identified in the MDD group in the stria, uncinate, anterior commissure, amygdalofugal pathway or inferior fronto-occipital fasciculus.

3.5. Association with clinical variables

Non-parametric regression in the depression group with covariation for age revealed that RD of streamlines was positively correlated with age of onset at the left basolateral nucleus ($p = 0.01$) and left accessory basal nucleus ($p = 0.01$). MD of streamlines seeded at the left basolateral complex were also significantly positively associated with age of illness onset ($p = 0.01$). However, none of these associations survived correction for multiple comparisons. No association was identified between diffusion imaging metrics and duration of depressive episode. Similarly, no significant relationship was identified using non-parametric age-controlled regression between connection density metrics of the individual white matter tracts and depression onset age or episode duration.

4. Discussion

In prior analyses of limbic structures in MDD, the amygdala has commonly been treated as a unified structure at conventional MRI field strengths (44-47). However, histologically and anatomically, reports suggest that over thirteen distinct subnuclei make up the amygdala (48). We present here a study that utilizes 7 Tesla field strength, ultra-high resolution imaging and a recently developed segmentation technique to carry out an analysis more closely resembling ground-truth biology. We show that three of the right amygdala nuclei display an increased connection density, suggesting structural hyperconnectivity of the right amygdala. Furthermore, we show that significantly increased connection density in MDD is driven by the stria terminalis in tracks seeded at the right lateral and basal nuclei, and the uncinate fasciculus in tracks seeded at the right central nucleus. Left medial amygdala nucleus tracks showed changes in count suggestive of hypoconnectivity. Importantly, we show differential changes in the amygdala lateral, basal, central and medial substructures that are specific to MDD, indicating that the amygdala nuclei do not react uniformly to MDD status. Furthermore, we provide here both a recommendation for the consideration of the amygdala nuclei as distinct entities and further characterization of structural brain changes in depression.

Our findings of increasing microstructural order without age adjustment in streamlines seeded at the right basal nucleus, right lateral nucleus, right central nucleus, right centrocortical complex and left lateral nucleus, combined with statistically robust increases in streamline count, suggest that these nuclei are hyperconnected with the rest of the brain in MDD. Previous studies of the structural connectivity of the amygdala in depression are concordant with these results (49, 50). Decreased MD and increased FA within the left amygdala was identified in remitted depressed individuals, suggesting a greater cell density and number of fibers. Additional tractography analyses in the same cohort revealed an increase in structural connectivity between the left amygdala and the hippocampus, cerebellum and brainstem (49). Increased correlation strength between grey matter volume of the amygdala and angular gyrus was also identified in MDD compared to controls (50). In adolescent depression, significantly lower FA and increased RD was reported in the bilateral uncinate fasciculus, a major white matter tract connecting the amygdala to frontal regions (51). Furthermore, healthy adolescents with high familial risk for MDD revealed significantly reduced FA in a tract-based spatial statistics analysis in the uncinate and inferior fronto-occipital fasciculi, both of which structurally involve the amygdalae (52). A meta-analysis of seven voxel-based diffusion imaging studies confirmed FA alterations compared to controls in the inferior fronto-occipital fasciculus in major depression (53).

In non-human primates, the amygdala nuclei are structurally connected to a larger number of regions in juveniles, and connectivity is pruned and refined over time (54). Developmental white matter changes are particularly protracted in the uncinate fasciculus (54) and perhaps for this reason, the uncinate is particularly vulnerable to psychiatric illness status. Functional MRI studies have shown replicable alterations in temporal lobe – prefrontal coupling in major depression (55), the principal structural correlate of which is the uncinate fasciculus (56). Uncinate microstructure has previously been associated with apathy in humans, and is considered to be important in episodic memory, social ability and emotional function (57).

The central nucleus is a major site for efferent projections from the amygdala, and it is highly involved in the mediation of behavioral responses to stress (58). Our results show for the first time evidence of central subnucleus-specific involvement of the uncinate fasciculus in MDD. The stria terminalis is another major amygdala efferent, projecting dorsally from multiple subnuclei and branching to the hypothalamic and septal nuclei (59). Given its terminus at the hypothalamus, it is unsurprising that the stria is involved in motivation and reactivity to stress, both of which are foci of dysregulation in MDD (7). The present methodology allows us to implicate connection density of the stria terminalis streamlines seeded specifically at the right basal and lateral amygdala nuclei in depression, which is a unprecedented level of detail in humans to date. Interestingly, the basal, lateral and central nuclei are the three amygdala subregions that have been implicated in significant developmental pruning of their connections (54). It is possible that the maturation and plasticity required at these nuclei during development make them liable to deviations which associate with depression status.

In agreement with a portion of previously published results in MDD (19, 46), we also report a finding of significantly decreased connection density in depressed patients, localized in streamlines seeded at the medial nucleus. This finding did not appear to be driven by any individual white matter tract, but was an effect observed in medial nucleus streamlines generally to the whole brain. Left lateral nucleus MD, left basolateral complex RD and left lateral nucleus RD along emanating streamlines were also significantly increased when analysis was carried out without age-adjustment, suggesting a possible loss in microstructural order. Taken together, the present results illustrate an important feature concerning amygdala connectivity in MDD: the amygdala's structural connectivity appears to be differentially enhanced or weakened based on the particular nuclei and hemisphere. Interestingly, we also reveal a significant positive association with three microstructural measures and age at illness onset, although the results did not survive correction for multiple comparisons. The range of onset ages in the sample may therefore be potential confounding factor in the dataset, showing that our finding of significantly decreased structural connectivity in MDD is sensitive to the age at which individuals develop MDD, and as such should be interpreted with caution.

Our study is a promising demonstration of how ultra high-field MRI may enable differential interrogation of connectivity among amygdala subnuclei in MDD, there are several limitations to the study which should be noted. Firstly, duration of illness, participant age and age at onset of the first episode of MDD were varied within the sample, which may be considered confounding factors. Secondly, although a sizable sample for a 7T investigation, the sample size used here was relatively small, due in part to more cautious MRI screening protocols at 7T than at conventional field strengths. A significant limitation of the methodology is also that probabilistic tractography cannot differentiate between fibers emanating from each nucleus and fibers passing through the nuclei. Given that the amygdala subnuclei are extensively interconnected in a manner well-defined by the non-human primate literature (56, 60), efferent, afferent or origination status of streamlines cannot be accurately identified in the present study. Additionally, longitudinal study of the participants was not carried out, and therefore the significant differences in structural connectivity in MDD could either pre-exist onset of depression and represent a possible vulnerability, or be

a consequence of the illness. Nevertheless, the presented findings are a useful pilot demonstration of the advantages of high-resolution imaging in MDD and segmentation of heterogenous brain regions.

Overall, we report significant changes in white matter connection density in MDD compared to controls that imply both increased and decreased structural connection strength. Increases in connection density in the right hemisphere were driven specifically by the uncinate fasciculus and stria terminalis. Importantly, we show results that differ between amygdala subnuclei, demonstrating the importance and clinical significance of the separate consideration of amygdala substructures in major depression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

An axial, coronal and sagittal view of probabilistic tractography seeded at all amygdala nuclei combined. White matter tracts reconstructed include: the stria terminalis, the uncinate fasciculus, a subcortical visual pathway passing along the inferior fronto-occipital fasciculus, the amygdalofugal tract and the anterior commissure. The color of streamlines is determined by their directionality.

Fig. 2.

Significantly increased streamline density of tracks seeded at the right lateral nucleus, right basal nucleus, right central nucleus and right centrocorcal complex in the MDD group compared to controls.

Fig. 3. Significant decrease in streamline density in the MDD group compared to controls in streamlines emanating from the left medial nucleus.

Fig. 4. Isolated white-matter tracts seeded from the amygdala nuclei: a) the stria terminalis b) anterior commissure c) amygdalofugal tract d) inferior fronto-occipital fasciculus and e) uncinate fasciculus. The color of streamlines is determined by their directionality.

Fig. 5.

Significantly increased connection density of the stria terminalis in streamlines seeded at the right lateral and right basal nuclei and significantly increased connection density of the uncinatus fasciculus seeded from the right central nucleus.

Table 1.

Participant demographics and summary statistics

	MDD	Control
Gender, % Female	45%	29%
Age (years), Median (Mean) [Minimum, Maximum]	42.5 (43.2) [24, 63]	37.3 (33.5) [22, 55]
Age (years), 1 st Qu. / 3 rd Qu.	31 / 47.5	29 / 51.25
Ever treated for a psychiatric illness, %	87%	4%
Talk therapy only, %	25%	0%
Ever hospitalized for mental health condition, %	4%	0%
Received ECT, %	0%	0%
Age of onset (years), Median (Mean) [Minimum, Maximum]	15 (16) [7, 47]	-
Age of onset (years), 1 st Qu. / 3 rd Qu.	10.75 / 21.5	
Recurrent MDD, %	79%	0%
Duration of current episode (months), Median (Mean) [Minimum, Maximum]	48 (69.6) [2, 252]	-
Duration of current episode (months), 1 st Qu./ 3 rd Qu.	5 / 156	-
Marijuana smoked in past month, %	4%	0%
MADRS rating of MDD symptom severity, Median (Mean) [Minimum, Maximum]	25.5 (14.25) [13,43]	-
MADRS rating of MDD symptom severity, 1 st Qu. / 3 rd Qu.	27.5 / 34.5	-

KEY RESOURCES TABLE

Resource Type	Specific Reagent or Resource	Source or Reference	Identifiers	Additional Information
Add additional rows as needed for each resource type	Include species and sex when applicable.	Include name of manufacturer, company, repository, individual, or research lab. Include PMID or DOI for references; use “this paper” if new.	Include catalog numbers, stock numbers, database IDs or accession numbers, and/or RRIDs. RRIDs are highly encouraged; search for RRIDs at https://scicrunch.org/resources .	Include any additional information or notes if necessary.
Antibody				
Bacterial or Viral Strain				
Biological Sample				
Cell Line				
Chemical Compound or Drug				
Commercial Assay Or Kit				
Deposited Data; Public Database				
Genetic Reagent				
Organism/Strain				
Peptide, Recombinant Protein				
Recombinant DNA				
Sequence-Based Reagent				
Software; Algorithm	MRTrix	http://www.brain.org.au/software/mrtrix/	RRID:SCR_006971	
Software; Algorithm	FreeSurfer	http://surfer.nmr.mgh.harvard.edu/	RRID:SCR_001847	
Transfected Construct				
Other				