Relation between Structural and Functional Connectivity in Major Depressive Disorder

Supplemental Information

Supplemental Methods

Subjects

To rate depression and anxiety severity, we used the 17-item (scores ranging from 0 to 54) Hamilton Depression Rating Scale (HAM-D) (1) and the 14-item (scores ranging from 0 to 56) Hamilton Anxiety Rating Scale (HAM-A) (2). The Dutch Adult Reading Test (DART) was used to measure the intelligence level (3), which is the Dutch version of the National Adult Reading Test (4). Additional inclusion criteria for depressed subjects were age between 18 and 65 years and HAM-D ≥15. Exclusion criteria for depressed subjects were: 1) presence of severe alcohol or substance abuse (including nicotine), 2) primary anxiety disorder, eating disorder or psychotic disorder, 3) current or past psychotic or manic depressive episode, 4) use of psychotropic medication other than selective serotonin reuptake inhibitors, selective noradrenalin reuptake inhibitors or a tricyclic antidepressant, 5) major neurological disorders. Inclusion criteria for healthy controls were: 1) age between 18 and 65 years, 2) no current or past psychiatric disorders, 3) no current neurological disorder.

Facial Affect Paradigm

Neutral, fearful, angry, sad, happy and blurred faces with a left/right arrow in the middle were presented. For the fearful, angry, sad and happy faces two different intensities of emotion were included. In total, 6 blocks of 16 seconds (four faces) for every single emotion were presented, interspersed with a four second fixation cross between the blocks but not between the pictures. The blocks were presented in a pseudo-randomized order. Subjects had
to indicate the gender of the faces by clicking a left or right button. During the counterbalanced blurred faces trials, subjects had to indicate the direction of the arrow.

**MRI Data Acquisition**

For the structural scan a T1-weighted structural image was acquired for anatomical co-registration purposes with the following parameters: duration 383 s, echo time 4.6 ms, repetition time 9576 ms, flip angle 8°, matrix 256 x 256, number of slices 281, slice gap 0 mm, slice thickness 1.2 mm, ascending slice order, field of view 218 x 226 x 226 mm³, voxel size 2 x 0.883 x 0.883 mm³. Diffusion tensor imaging (DTI) data were acquired in alignment with the fMRI data. The diffusion weighting was performed along 32 directions, with a \( b \)-value of 600 s/mm². Four non-diffusion weighted images were acquired and averaged. Other DTI sequence parameters were: echo time 60 ms, repetition time 6721 ms, slice thickness 2 mm, field of view 224 x 224 x 140 mm³, voxel size 2 x 2 x 2 mm³. Total DTI scan time was 5 minutes.

**Structural Connectivity Analysis**

*Preprocessing*

The preprocessing of the DTI data was performed using in-house developed software, written in MATLAB (The MathWorks, Natick, MA). The preprocessing was executed on the Dutch Grid [www.biggrid.nl] using a web interface to the e-Bioinfra gateway (5,6). Head motion and deformations induced by eddy currents were corrected for by an affine registration of the diffusion weighted images (DWIs) to the non-diffusion weighted image. The gradient directions were corrected by the rotation component of the transformation. The DWIs were resampled isotropically. Rician noise in the DWIs was reduced by an adaptive noise filtering
method (7). Diffusion tensors were estimated in a non-linear least squares sense. From the tensors, fractional anisotropy (FA) and mean diffusivity maps were computed.

*Fiber tracking*

Fiber tracking is known to be an observer-dependent process. Therefore, the fiber tracking reproducibility was assessed by two blind observers (BdK and LH) reconstructing the uncinate fasciculus bilaterally for 10 subjects who were chosen randomly. The overlapping tract volume was computed relative to the total volume tracked by both observers. This resulted in an inter-observer reliability index of 90% ± 9%. Subsequently, one observer (BdK) performed the fiber tracking in all subjects. Figure S2 shows an example of the reconstructed uncinate fasciculus tract for one subject after fiber tracking.

*Statistical analysis*

To analyze specific FA differences within different parts of the tract, the generated 60 FA points along left and right uncinate fasciculus were divided into four parts, resulting in four averaged FA values per side (Figure S3). The first part contains fibers running from the medial temporal lobe to the first part of the curve that the uncinate fasciculus makes behind the external capsule inward to the insular cortex (8). The second part contains fibers running through the second part of the curve. The third part contains fibers connecting the anterior cingulate cortex and the orbitofrontal cortex. The fourth part only contains fibers connecting the orbitofrontal cortex.

*Functional Connectivity Analysis*

*fMRI data analysis*

For data analysis, Statistical Parametric Mapping (SPM8, Wellcome Trust Center for Neuroimaging, London, United Kingdom, http://www.fil.ion.ucl.ac.uk/spm/software/SPM8) was used for the following preprocessing steps: realignment to correct for subject motion;
slice timing; coregistration of functional and structural data; spatial normalization into standard stereotactic space using a template from the Montreal Neurological Institute; smoothing of data with an 8 mm Gaussian kernel. Head movement of all participants was <3 mm corresponding to one slice thickness.

**Figure S1.** For diffusion tensor imaging fiber tracking of the uncinate fasciculus, two regions of interest (ROIs) were placed in the most posterior coronal slice where the temporal and frontal lobes are separated. All fibers in the temporal lobe were included in the first ROI (1) and the entire projections of these fibers to the frontal lobe were distinguished with a second ‘AND’ ROI (2).

**Figure S2.** A sagittal view of a reconstructed uncinate fasciculus tract overlaid on a fractional anisotropy image color-coded for orientation. Fiber tracking was performed using DTIstudio (9).
Figure S3. This figure shows the population averaged and segmented left and right uncinate fasciculi in transparent red. The circles correspond to the population average fiber: starting with the blue circles in the medial temporal lobe and ending with the red circles in the orbitofrontal cortex (OFC). These points along left and right uncinate fasciculus were divided into four parts per side (a-d (left), e-h (right)). The first part (a and e) contains fibers running from medial temporal lobe to first part of the curve. The second part (b and f) contains fibers running through the second part of the curve and the third part (c and g) contains fibers connecting the anterior cingulate cortex with the OFC. The fourth part contains only fibers connecting to the OFC (d and h).
Supplemental References


