Supplementary Material

Medial reward and lateral non-reward orbitofrontal cortex circuits change in opposite directions in depression

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Supplementary Methods

Participants

There were 421 patients with a diagnosis of major depressive disorder (MDD), and 488 controls. The patients were from Taiwan, Dongei and Xinan. 185 of the patients were not receiving medication at the time of the neuroimaging. Table S2 provides a summary of the demographic information and the psychiatric diagnosis of the participants. The subjects' consent was obtained according to the Declaration of Helsinki and the investigations were approved by the ethical committee of the institution in which the work was performed. Further details follow.

Xinan: Patients with MDD were recruited from the outpatient department of the First Affiliated Hospital of Chongqing Medical School in Chongqing, China. All were diagnosed according to the Structured Clinical Interview for DSM-IV, by independent assessments of two psychiatrists. They were also assessed for disease severity using the Hamilton Depression Rating Scale (HAMD) (Hamilton, 1960) and Beck Depression Inventory (BDI), illness duration and the medication status of the patients. Before the investigation, we excluded individuals who were not suitable for MRI scanning by interview and by the self-reported checklist. The MRI related exclusion criteria include claustrophobia, metallic implants, Meniere's Syndrome and a history of fainting within the previous half year. Exclusion criteria for both groups were as follows: current psychiatric disorders (except for MDD) and neurological disorders; substance abuse; and stroke or serious encephalopathy. Of note, all of the subjects in the control group did not meet DSM-IV criteria for any psychiatric disorders and did not use any drugs that could affect brain function. This study was approved by the Research Ethics Committee of the Brain Imaging Center of Southwest University and First Affiliated Hospital of Chongqing Medical School. Informed written consent was obtained from each subject. This study was conducted in accordance with the Helsinki Declaration as revised in 1989.

Taiwan: Patients were recruited from the Veteran General Hospital in Taipei, Taiwan. All participants were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorder-IV criteria for depression, and each participant's history of medical disease, psychiatric illness, and medication use was evaluated by interview and medical charts carefully. Experiments were conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Taipei Veterans General Hospital. Written informed consent was obtained from all participants after ensuring adequate understanding of the study. Any participants with the following conditions were excluded: (1) a comorbid substance-related disorder, (2) presence of neurobiological disorders, such as dementia, head injury, stroke, or Parkinson's disease; (3) presence of hypertension, diabetes, hyperlipidemia or coronary heart disease; (4) severe medical illness, such as malignancy, heart failure, or renal failure; (4) presence of ferromagnetic foreign bodies or implants that were anywhere in the body. Depression severity was evaluated by the psychiatrist-assessed Hamilton Depression Rating Scale (HAMD, 17 items) (Hamilton, 1960).

Dongbei: Participants were recruited from the outpatient clinics of the Department of Psychiatry, First Affiliated Hospital of China Medical University and the Mental Health Center of Shenyang, China. The absence or presence of Axis I disorders were independently assessed by 2 trained psychiatrists using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID). Patients were required to fulfill DSM-IV criteria for MDD, have no comorbid Axis I diagnosis. All the patients were assessed on the 17-item Hamilton Rating Scale for Depression (HAMD). The healthy control group were recruited from the community, Shengyang, China by advertisement. HC participants did not have a current or lifetime Axis I disorder or history of psychotic, mood, or other Axis I disorders in first-degree relatives. For all participant groups, individuals were excluded with a history of substance or alcohol abuse or dependence, head injury, neurologic or concomitant major medical disorder, and any contraindications for MRI. This study was approved by the Institutional Review Board of China Medical University, and all participants provided written informed consent after detailed description of the study.

Image Acquisition

Data for resting state functional connectivity analysis were collected in 3T MRI scanners in an 8 min period in which the participants were awake in the scanner not performing a task using standard protocols described in more detail next.

Xinan: All images were acquired on a 3.0-T Siemens Trio MRI scanner using a 16-channel whole-brain coil (Siemens Medical, Erlangen, Germany). High- resolution T1-weighted 3D images were acquired using a magnetization-prepared rapid gradient echo (MPRAGE) sequence (echo time (TE) = 2.52 ms; repetition time (TR) = 1900 ms; inversion time (TI) = 900 ms; flip angle = 9 degrees; slices = 176; thickness = 1.0 mm; resolution matrix = 256×256; voxel size = 1×1×1 mm3). For each participant, 242 functional images were acquired with a gradient echo type Echo Planar Imaging (EPI) sequence (echo time (TE) = 30 ms; repetition time (TR) = 2000 ms; flip angle = 90 degrees; slices = 32; slice thickness = 3.0 mm; slice gap = 1 mm; resolution matrix = 64×64; voxel size 3.4×3.4×3〔mm〕^3). During image acquisition, participants were instructed to keep their eyes closed while keeping their head as still as possible without falling asleep. All participants stayed awake during the MRI imaging as confirmed by the participants after the session.

Taiwan: fMRI scanning was performed at National Yang-Ming University in Taiwan using a 3.0-T Siemens MRI Scanner (Siemens Magnetom Tim Trio, Erlangen, Germany) with a 12-channel head coil. During the experiments, the participants were instructed to relax with their eyes closed, without falling asleep. After the resting state experiment, participants were asked whether they fell asleep during the resting state scan session, and participants were rescanned if they had fallen asleep during the resting state scan. T2*-weighted images with BOLD contrast were measured using a gradient echo- planar imaging (EPI) sequence (repetition time, TR: 2,500 ms, echo time, TE: 27 ms, field of view, FoV: 220 mm, flip angle: 77 degree, matrix size: 64 x 64, and voxel size: 3.44×3.44×3.40 mm). For each run, 200 EPI volume images were acquired in the anterior and posterior commissure (AC – PC) plane. High-resolution structural T1 images were acquired with three-dimensional (3D) magnetization-prepared rapid gradient-echo sequence (3D-MPRAGE; TR: 2,530 ms, TE: 3.5 ms, TI: 1,100 ms, FoV: 256 mm, and

flip angle: 7 degree, 192 sagittal slices, voxel size = 1.0 mm x 1.0 mm 1.0 mm, no gap). For each participant, the whole fMRI scanning lasted about 16 min (T1: 8min, Resting: 8min).

Dongbei: Magnetic resonance imaging was performed on a GE Signa HDX 3.0T MRI scanner with a standard head coil at the First Affiliated Hospital of China Medical University, Shenyang, China. Ear plugs were provided for each participants to reduce noise interference during the scan. Head motion was minimized with restraining foam pads. Functional images were collected using a gradient-echo planar imaging sequence sensitive to BOLD contrast: TR = 2000ms, TE = 30ms, flip angle = 90°, FOV = 24 × 24cm, matrix = 64 × 64. Thirty-five axial slices were collected with 3 mm thickness without a gap. Participants were instructed to rest with their eyes closed but remain awake during scanning. No participant reported falling asleep during the scan when routinely asked immediately after scanning. Structural images were performed using a T1-weighted, 3D fast spoiled gradient-echo (FSPGR) sequence with the following parameters: TR/TE = 7.2/3.2 ms, Flip = angle 13°, field of view (FOV) =240×240 mm, 176 slices, voxel size = 1 mm^3.

Global signal removal

Global signal regression was not used in the results described in the main part of the paper, for the following reasons.

Global Signal Regression (GSReg) involves regressing out signal averaged over the entire brain (Fox *et al.* , 2009). This can change resting state correlations in ways that dramatically alter correlation patterns and hence conclusions about brain functional connectedness (Saad *et al.* , 2012). An intuitive example helps to introduce some of the effects. Consider that only visual areas drift up for a short period. Then during this period, the signal in other brain areas including those that have nothing to do with the visual cortical areas will be forced to show negative 'signal' values, because the mean global signal is being set to zero. This in turn will introduce negative correlations between the visual and the other brain areas, even though there may not be functional connections between them. Thus negative correlations may be artefactual, and are very difficult to interpret. Worse than this, GSReg can fundamentally alter inter-regional correlations within a group of individuals, or their differences between groups (Saad *et al.* , 2012). This can potentially spread underlying group differences to brain regions that may never have had any functional connectivity differences (Saad *et al.* , 2012). Further, in a study of functional connectivity in autism spectrum disorder, it was shown that GSReg, rather than simply altering the mean or range of correlation values amongst pairs of brain regions, systematically alters the rank ordering of values in addition to introducing negative values; leads to a reversal in the direction of group correlation differences relative to other preprocessing approaches, with a higher incidence of both long-range and local correlation differences that favored the patient group; and results in locations showing group differences that no longer agree with those showing correlations with behavioral symptoms (Gotts *et al.* , 2013). Further, in a study on schizophrenia, increased cortical power and variance was identified in schizophrenia, an effect predictive of symptoms yet obscured by GSreg (Yang *et al.* , 2014).The finding was absent in bipolar patients, confirming diagnostic specificity (Yang *et al.* , 2014). Thus GSreg may obscure other information of potential interest in resting state functional connectivity datasets. Further, in a study in macaques, it was shown that the global

signal may reflect some underlying neurophysiological effects that may be of interest, such as effects related to gamma frequencies in the local field potential recorded in even distant cortical areas (Scholvinck *et al.* , 2010). There are thus good reasons for not regressing out the global mean signal.

However, for completeness we also measured the functional connectivity differences between depressed patients and the controls in the same dataset after global signal removal. Some of the voxels with altered functional connectivity were identified in corresponding areas, including OFC13, OFC47/12, the medial temporal lobe from the pole to the parahippocampal gyrus, the anterior cingulate cortex, the thalamus, the precuneus, and the angular gyrus. The functional connectivity differences between the depressed patients and the controls between this set of brain regions was broadly similar to those identified without GSreg and reported in the main text of this paper.

However, a number of other areas became prominent with GSReg, including many occipital / calcarine visual areas far distant from the areas identified without GSReg. There was also more pronounced voxel connectivity differences apparently present involving the thalamus with GSReg. These extra regions evident with GSReg often had negative r values for the functional connectivity in the controls. The indication is thus that these extra regions reflected the effect of the removal of the global signal, and we had little confidence in them. For these reasons, we report only the effects without GSReg in the main text of the paper.

Effects of medication

The effects of medication were assessed by comparing the functional connectivity in 185 patients not receiving medication, and 182 patients receiving medication, from the Dongbei and Xinan datasets. It was suggested to us that this analysis be performed, but we note that these are supplementary data, and the study was not designed as an investigation of the effects of medication with matched groups with and without medication (Wells *et al.* , 2014). However, we do present the patient demographic and clinical characteristics of the groups, as follows, where M is the medicated and UM is the unmedicated group: Age M 38.5 ± 13.8 (mean ± sd) years; UM 34.5 ± 13.1. Sex M male 117 female 65; UM male 127 female 58. Education M 11.7 ± 3.4; UM 12.0 ± 3.6. Illness duration M 4.9 ± 5.9; UM 2.4 ± 4.0 $p=4.4\times10^{-5}$. Hamilton depression Rating scale (HAMD) M 18.9 ± 7.4; UM 22.4 ± 5.7 $p=8.1\times10^{-7}$. The analysis approach was the same as in the full investigation, with a whole brain voxel-level analysis performed now separately for patients not receiving medication, and for those receiving medication. Where there were more than 10 voxels in any AAL2 region, the average functional connectivity with the 10 or more voxels in the other AAL2 areas was measured.

The functional connectivity results are shown in Fig. S1A and B. The overall pattern of functional connectivity differences between patients and controls is similar for the unmedicated (Fig. S1A) and the medicated (Fig. S1B) subgroups of patients, providing evidence that the main differences between patients and controls shown in Figs. 1-3 were found in depressed patients whether or not they were receiving medication.

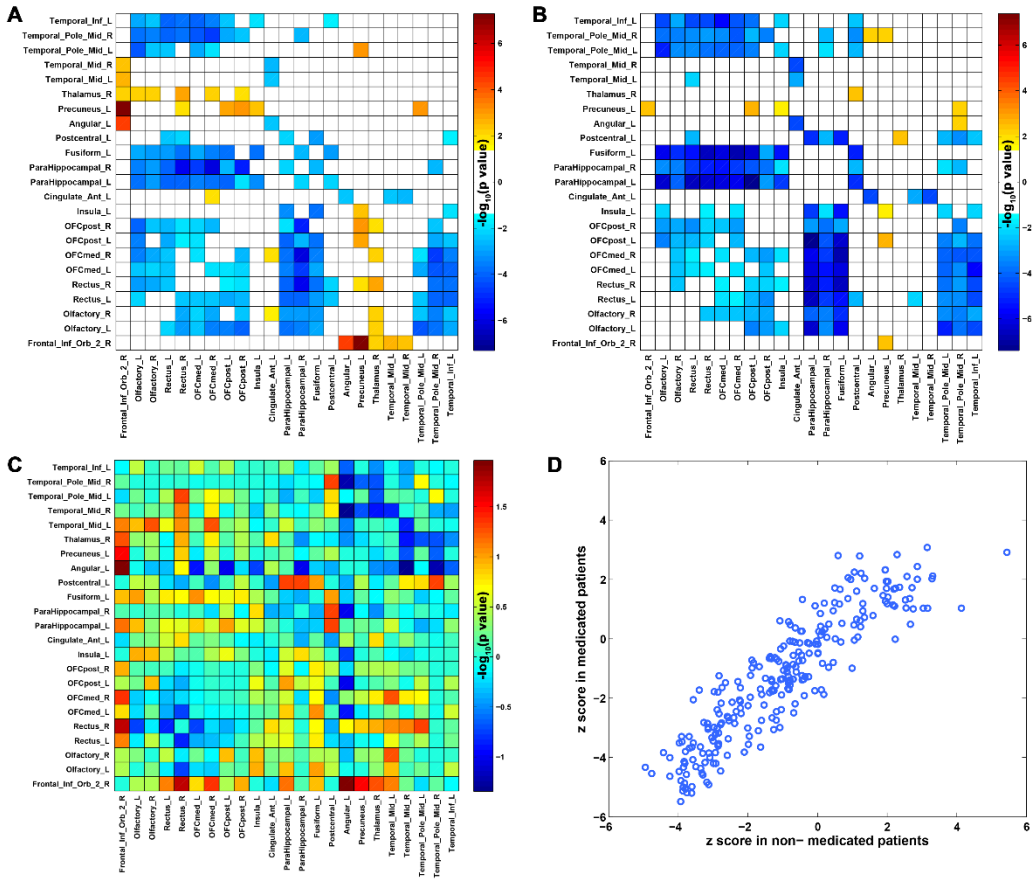

Fig. S1. Comparison of functional connectivity in 182 depressed patients receiving medication with functional connectivity in 185 depressed patients not receiving medication. A. The voxel-based ROI connectivity difference between non-medicated patients and controls. The p-values represent the difference of the functional connectivity of the significant voxels within different automated anatomical atlas (AAL2 (Rolls *et al.* , 2015)) areas. B. The voxel-based ROI connectivity difference between medicated patients and controls. C. For the corresponding AAL2 areas, the p value of the functional connectivity difference for the different AAL2 areas between medicated and unmedicated patients. A positive p-value indicates a higher functional connectivity in unmedicated than in medicated patients. (A t value of 1.66 is significant two-tailed with these degrees of freedom.) D. The z-score representing the difference from controls of the functional connectivities for each of the AAL2 areas in (A-C) for medicated vs unmedicated depressed patients. The correlation between the z-scores for medicated and unmedicated patients was high ($r=0.91$, $p<0.0001$).

Although the overall pattern of functional connectivity is similar in the subgroup without medication (Fig. S1A) and with medication (Fig. S1B), to test for significant differences, a t-test was performed between these two functional connectivity matrices, with the results shown in Fig. S1C. As one of the main findings for differences of functional connectivity between patients and controls was increased functional connectivity of the lateral orbitofrontal cortex BA 47/12 with the pre-cuneus, angular gyrus and Mid-temporal gyrus, and to relieve the burden of multiple comparisons, we tested whether these three functional connectivity links were weaker in medicated than in un-medicated patients. For the Frontal_Inf_Orb_2R with

precuneus link there is a significantly smaller functional connectivity in the medicated than the unmedicated group ($t=2.17$, $p<0.015$, one-tailed test of the specific prediction in all 3 cases). For the Frontal_Inf_Orb_2R with angular gyrus link there is a significantly smaller functional connectivity in the medicated than the unmedicated group ($t=2.55$, $p=0.005$). For the Frontal_Inf_Orb_2R with temporal_Mid_R link there is a significantly smaller functional connectivity in the medicated than the unmedicated group ($t=1.76$, $p=0.039$). The results overall are thus consistent with the hypothesis that the increased functional connectivity of the lateral orbitofrontal cortex BA 47/12 with the precuneus, angular gyrus, and mid-temporal gyrus is related to depression, and that treatment with medication reduces the functional connectivity of these three links.

In addition to these preplanned tests, it is noted that for the Frontal_Inf_Orb_2R with both the Rectus-R, and OFCmed-R links there are significantly smaller functional connectivities in the medicated than the unmedicated group ($t=2.37$ $p=0.009$, and $t=2.02$ $p=0.043$ respectively).

It is noted that this supplementary data analysis was not a main aim of this investigation and has some limitations, in that this is a cross-sectional not longitudinal comparison, and that the mean illness duration was 4.9 years in the medicated group and 2.4 years in the medicated group.

Table S1. The anatomical regions defined in each hemisphere and their label in the automated anatomical labelling atlas AAL2 (Rolls *et al.* , 2015). Column 4 provides a set of possible abbreviations for the anatomical descriptions.

NO.	ANATOMICAL DESCRIPTION	LABEL aal2.nii.gz	POSSIBLE ABBREVIATION
1,2	Precentral gyrus	Precentral	PreCG
3, 4	Superior frontal gyrus, dorsolateral	Frontal_Sup	SFG
5, 6	Middle frontal gyrus	Frontal_Mid	MFG
7, 8	Inferior frontal gyrus, opercular part	Frontal_Inf_Oper	IFGoperc
9, 10	Inferior frontal gyrus, triangular part	Frontal_Inf_Tri	IFGtriang
11, 12	IFG pars orbitalis,	Frontal_Inf_Orb	IFGorb
13, 14	Rolandic operculum	Rolandic_Oper	ROL
15, 16	Supplementary motor area	Supp_Motor_Area	SMA
17, 18	Olfactory cortex	Olfactory	OLF
19, 20	Superior frontal gyrus, medial	Frontal_Sup_Med	SFGmedial
21, 22	Superior frontal gyrus, medial orbital	Frontal_Med_Orb	PFCventmed
23, 24	Gyrus rectus	Rectus	REC
25, 26	Medial orbital gyrus	OFCmed	OFCmed
27, 28	Anterior orbital gyrus	OFCant	OFCant
29, 30	Posterior orbital gyrus	OFCpost	OFCpost
31, 32	Lateral orbital gyrus	OFClat	OFClat
33, 34	Insula	Insula	INS
35, 36	Anterior cingulate & paracingulate gyri	Cingulate_Ant	ACC
37, 38	Middle cingulate & paracingulate gyri	Cingulate_Mid	MCC
39, 40	Posterior cingulate gyrus	Cingulate_Post	PCC
41, 42	Hippocampus	Hippocampus	HIP
43, 44	Parahippocampal gyrus	ParaHippocampal	PHG
45, 46	Amygdala	Amygdala	AMYG
47, 48	Calcarine fissure and surrounding cortex	Calcarine	CAL
49, 50	Cuneus	Cuneus	CUN
51, 52	Lingual gyrus	Lingual	LING
53, 54	Superior occipital gyrus	Occipital_Sup	SOG
55, 56	Middle occipital gyrus	Occipital_Mid	MOG
57, 58	Inferior occipital gyrus	Occipital_Inf	IOG
59, 60	Fusiform gyrus	Fusiform	FFG
61, 62	Postcentral gyrus	Postcentral	PoCG
63, 64	Superior parietal gyrus	Parietal_Sup	SPG
65, 66	Inferior parietal gyrus, excluding supramarginal and angular gyri	Parietal_Inf	IPG
67, 68	SupraMarginal gyrus	SupraMarginal	SMG
69, 70	Angular gyrus	Angular	ANG
71, 72	Precuneus	Precuneus	PCUN
73, 74	Paracentral lobule	Paracentral_Lobule	PCL
75, 76	Caudate nucleus	Caudate	CAU
77, 78	Lenticular nucleus, Putamen	Putamen	PUT
79, 80	Lenticular nucleus, Pallidum	Pallidum	PAL
81, 82	Thalamus	Thalamus	THA
83, 84	Heschl's gyrus	Heschl	HES
85, 86	Superior temporal gyrus	Temporal_Sup	STG
87, 88	Temporal pole: superior temporal gyrus	Temporal_Pole_Sup	TPOsup
89, 90	Middle temporal gyrus	Temporal_Mid	MTG
91, 92	Temporal pole: middle temporal gyrus	Temporal_Pole_Mid	TPOmid
93, 94	Inferior temporal gyrus	Temporal_Inf	ITG

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