Antidepressant mechanism of add-on repetitive transcranial magnetic stimulation in medication-resistant depression using cerebral glucose metabolism

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1. Introduction

Many patients with major depressive disorder do not improve appreciably despite several medication trials (Fekadu et al., 2009; Little, 2009). This phenomenon of medication-resistant depression (MRD) is associated with poor clinical and psychosocial outcomes (Kennedy and Foy, 2005; Little, 2009). Repetitive transcranial magnetic stimulation (rTMS) is an effective method in treating major depression (Brunelin et al., 2007; Fregni et al., 2006) and brings new hope to the treatment of these patients. Despite the fact that not all MRD patients seem to respond to rTMS, recent research has found that add-on high-frequency rTMS applied to the left dorsolateral prefrontal cortex...
(DLPCF) has promising antidepressant efficacy in patients with MRD (Avery et al., 2006; Bretlau et al., 2008; Fitzgerald et al., 2009; Rumi et al., 2005; Su et al., 2005).

Regarding the mechanism of depression, the limbic-cortical circuit is proposed as a critical neural network in depression; impaired reciprocal functional relationships between the cortical (e.g., dorsolateral, medial and ventral prefrontal cortex) and limbic (e.g., amygdala and hippocampus) structures are thought to correlate with emotional dysregulation and depression (Drevets et al., 2008; Mayberg, 2003). However, this proposed reciprocal dysregulation is not a universal finding in depression across studies. Furthermore, previous functional imaging studies showed inconsistent results in the exact location and direction of the regional cerebral metabolism in depression. Despite these findings being somewhat inconsistent, they have suggested the possibility of using pretreatment regional metabolic activities in various parts of the prefrontal and temporal cortex, anterior cingulum, hippocampus and midbrain to predict treatment response to antidepressants (Buchsbaum et al., 1997; Kennedy et al., 2001; Little et al., 1996; Little et al., 2005; Mayberg et al., 1997; Mayberg et al., 2000; Milak et al., 2009; Saxena et al., 2003), psychotherapy (Brody et al., 2001; Kennedy et al., 2007; Konarski et al., 2009) or sleep deprivation (Ebert et al., 1994; Wu et al., 1999). It is possible that the metabolism in those brain regions, such as the prefrontal cortex or anterior cingulum, could also predict the efficacy of add-on rTMS in patients with MRD. However, this clinically important issue on the early prediction of rTMS effects needs to be further studied.

The actual mechanism of rTMS as an antidepressant therapy is still unclear, but might involve a normalization of the limbic-cortical circuit. rTMS stimulated neurons in brain regions under the stimulation site (Pascual-Leone et al., 1994) and could also influence neuronal activity in remote or deeper brain regions (Kimbrell et al., 2002a). In functional imaging studies examining resting cerebral blood flow and glucose metabolism in depression, including those using positron emission tomography (PET) and single photon emission computed tomography (SPECT), depression is most often associated with hypoperfusion and hypometabolism in the left DLPCF and anterior cingulum (Paus and Barrett, 2004; Videbech, 2000). Therefore, the rTMS mechanism through the left DLPCF might be coming from increasing activities at the left DLPCF and anterior cingulum, and then normalize the limbic-cortical dysregulation underlying depression. The notion is partly supported by the results from an early rTMS study showing that a 2-week high-frequency rTMS at the left DLPCF affects both prefrontal and paralimbic activities in bipolar or unipolar depressed patients (Teneback et al., 1999). However, results so far have not been entirely consistent. For example, limited studies of combined TMS/PET or TMS/SPECT investigating the high-frequency rTMS antidepressant mechanism through the left DLPCF (Baeken et al., 2009; Kito et al., 2008; Speer et al., 2000; Teneback et al., 1999; Zheng, 2000) found that 2-week high-frequency rTMS significantly changes perfusion or metabolism in the remote paralimbic structures, such as the amygdala, basal ganglia, hippocampus/parahippocampus and uncus (Speer et al., 2000; Teneback et al., 1999), but some did not replicate such findings (Baeken et al., 2009; Zheng, 2000). Reasons for the discrepant findings are unclear, but may include clinical heterogeneity (for instance, whether unipolar and bipolar depression were studied together) and the fact that some studies have ignored examining the differences between responders and non-responders to rTMS.

The aim of the present study was to investigate an add-on rTMS antidepressant mechanism and to characterize cerebral glucose metabolism abnormalities in MRD using 18F-FDG (fluorodeoxyglucose) and PET. To overcome some of the deficiencies in previous studies, we focused on unipolar depressed patients and stratified according to responder status. To investigate the rTMS neural mechanism, the baseline glucose metabolism of MRD patients who responded and who did not respond to a 2-week active rTMS treatment trial was compared. In addition, since most of the previous PET/SPECT studies conducted a second scan right after the rTMS treatment (Baeken et al., 2009; Kito et al., 2008; Speer et al., 2000; Teneback et al., 1999; Zheng, 2000), and in order to study the most important brain changes contributing to antidepressant effects from add-on rTMS, we followed and rescanned patients at 3 months after rTMS treatment. To characterize cerebral glucose metabolism abnormalities in MRD, we compared the MRD patients in symptomatic resolution with a group of healthy subjects. Post-rTMS scans from non-responders were not used in this comparison because a depressed status is a known significant factor influencing glucose metabolism (Drevets et al., 2008; Hoshikawa et al., 2009; Kimbrell et al., 2002b).

2. Material and methods

2.1. Study subjects

Twenty-three medication-resistant depressed patients (MRD; 6 male, 17 female) aged from 25 to 65 years were recruited at Taipei Veterans General Hospital from July 2008 to June 2009. The diagnoses were established by structural history-taking and administration of the MINI International Neuropsychiatric Interview (MINI), based on Diagnostic and Statistical Manual for Mental Disorders (DSM-IV) criteria (American Psychiatric Association, 1994). MRD was defined as patients meeting the DSM-IV criteria of MDD, having a history of failing to respond to at least 2 different kinds of adequate antidepressant trials (Avery et al., 2006) and with moderate severity scores ≥18 on the 17-item Hamilton Depression Rating Scale (HDRS-17) before rTMS treatment. Patients were recruited for the study only if they had no alcohol or substance abuse history, no major physical or neurological illness, and no comorbidity with schizophrenia, bipolar disorders, other major psychoses, obsessive–compulsive disorders, post-traumatic stress disorder, or Cluster B personality disorders.

2.2. PET and MRI procedures

All recruited MRD patients received two PET scans, including a baseline one before rTMS treatment and another follow-up one after 3 months. A group of gender- and handedness-matched healthy controls also received PET scans after interview and MINI screening; those with a strong family history of an axis I disorder were excluded.
PET scans of glucose utilization were acquired on a PET/CT scanner (Discovery VCT; GE Healthcare, USA) with a 3D brain mode. Patients fasted for at least 4 h before the PET examination. PET images were acquired 45 min after an intravenous injection of about 370 MBq of $^{18}$F-FDG. The brain acquisition time was 15 min. The system produces 47 consecutive slices over an axial length of 15.7 cm, with a slice thickness of 3.75 mm and a transaxial FOV of 70 cm. PET images were reconstructed in a 128 × 128 matrix, and corrected for attenuation using CT information with the ordered-subset expectation maximization iterative reconstruction algorithm (6 iterations and 14 subsets). Then the axial slices were realigned to yield sagittal and coronal images.

To accurately localize the rTMS stimulation site of the left DLPFC, magnetic resonance images (MRIs) were obtained before the first rTMS session for all patients. All MRI scans were performed on a 1.5 T MRI system (Excite II; GE Medical Systems, Milwaukee, WS, USA). T1-weighted images (T1-images) were acquired parallel to the anterior commissure-posterior commissure line by using a three-dimensional fluid-attenuated inversion-recovery fast spoiled gradient recalled echo (FLAIR-FSPGR) sequence. The imaging parameters were: TR = 8.548 ms, TE = 1.836 ms, TI = 400 ms, flip angle = 15°, field of view (FOV) = 26 × 26 cm, matrix size = 256 × 256, and 124 contiguous slices with the slice thickness = 1.5 mm.

2.3. rTMS procedures

Repetitive transcranial magnetic stimulation was administered by a trained psychiatrist (C.-T. L.) using a Magstim super rapid magnetic stimulator (Magstim Company, Ltd., Wales United Kingdom) with 4 booster modules equipped with a 700-mm air-cooled figure-eight-shaped coil. The detailed procedures were similar but modified from our previous study (Su et al., 2005). To increase the accuracy and consistency of coil placement to the left DLPFC, we used brain-navigation computer software and an infrared system (Brainsight, Rogue Research, Inc., Montreal, QC) to guide the coil to target the left DLPFC, which was defined as a spot between the junction of the Brodmann area (BA) 9 and 46 on each patient’s brain MRI (Fitzgerald et al., 2009). Ten daily rTMS treatment sessions were administered at the left DLPFC at 100% motor threshold (MT) for 2 consecutive weeks (5 sessions/week). Active 10-Hz rTMS stimulation was applied (4 s on and 26 s off) 40 times for a total of 1600 stimulations per 20-min session. The detailed procedures such as rTMS settings and the determination of MT were given in our previous study evaluating the efficacy of add-on rTMS in depressed patients (Su et al., 2005). Regarding antidepressant drugs, all patients continued their current antidepressant medications and no medication changes were allowed for at least 4 weeks preceding rTMS, and throughout the period of rTMS treatment and 3-month follow-up. However, if the depressed patients failed to respond to 2-week add-on rTMS (rTMS non-responders) or the rTMS responders had severely exacerbated depressed symptoms, changes in medications were permitted based on the clinician’s judgment. The medication changes in the non-responders or responders did not influence our main purpose of investigating the add-on rTMS antidepressant mechanism, because the second PET imaging data from these patients were not used for further comparisons with of post-rTMS responders (e.g., post-rTMS responders vs. healthy subjects, pre- vs. post-rTMS changes in responders). During the rTMS treatment course, patients were using the following medications: a selective serotonin reuptake inhibitor (fluoxetine [N = 2], escitalopram [N = 3]) or a serotonin-norepinephrine reuptake inhibitor (venlafaxine [N = 2], duloxetine [N = 3]), a norepinephrine dopamine reuptake inhibitor (bupropion [N = 7]) or a noradrenergic/specific serotonergic agent (mirtazapine [N = 3]), and these were used in combination treatments with antipsychotics (N = 8: 1 with fluoxetine and aripiprazole; 2 with duloxetine and quetiapine; 1 with bupropion and aripiprazole; 4 with bupropion and quetiapine).

The study was performed in accordance with the Declaration of Helsinki and was approved by the local Ethics Review Committee. All participants provided written informed consent. However, two patients withdrew their informed consent before rTMS treatment and another patient was also excluded after a positive MRI finding of pituitary adenoma.

2.4. Patient assessments

Severity of depression using HDRS-17 at baseline and at the end of week 2 and month 3 was assessed by another psychiatrist (T.-P. S.). The Beck Depression Inventory (BDI) was used to assess the patient’s subjective feelings. In case of rTMS-induced mania (Dolberg et al., 2001; Huang et al., 2004), the severity of manic symptoms were assessed by the same psychiatrist, using the Young Mania Rating Scales (YMRS). After a 2-week open-labeled add-on rTMS treatment, the patients were further subcategorized into responder and non-responder groups, according to their clinical responses to the rTMS treatment. Responders were defined by an objective improvement in HDRS-17 scores ≥ 50%.

2.5. Statistics

PET data were analyzed using Statistical Parametric Mapping version 2 (SPM2; Wellcome Department of Cognitive Neurology, Institute of Neurology, University College London, London, England) implemented in Matlab 7.0 (The Mathworks Inc., Sherborn, MA, USA). First, an $^{18}$F-FDG template was created from all subjects, including 20 MRD patients and 20 healthy controls, according to the procedures of spatial normalization (Gispert et al., 2003; Signorini et al., 1999). Then, each subject’s images were normalized to the $^{18}$F-FDG template and smoothed with a 3D Gaussian kernel (FWHM = 12 mm). The overall grand mean from the PET scans was centered and normalized to 100, and global variance across the scans was removed by analysis of covariance (ANCOVA) (Friston et al., 1990). To assess potential group differences of normalized brain glucose uptake, an ANCOVA with age, gender (Willis et al., 2002) and global gray matter values as covariates of no interest was used for between-group comparisons and the results of the main effects were reported [all baseline MRD vs. Healthy controls, Responders (post-rTMS) of MRD vs. Healthy controls]. The significant thresholds were first set at P < 0.05 uncorrected at the voxel level, followed by a cluster-level analysis with a corrected P< 0.05. A priori knowledge about the brain network of depression involves regions such as the
prefrontal cortex, anterior operculum-insula, cingulate cortex, basal ganglia, thalamus, precuneus, hippocampus/parahippocampal gyrus, amygdala and brainstem (Drevets et al., 2000; Speer et al., 2000), so for those only passing cluster-level uncorrected \( P < 0.05 \), the identified clusters were further examined by Small Volume Correction (SVC) with an anatomically defined regional mask in the relevant gray matter area (Friston, 1997), due to the concern about multiple comparisons. The clusters passing through SVC \( P \)-value \(< 0.05 \) were thought to be significant. However, within the clusters having shown significantly abnormal glucose uptake in all the baseline MRD versus controls, thresholds for identifying clusters where the responders had a significantly higher or lower glucose metabolism than the non-responder group were set to a cluster-level uncorrected \( P < 0.05 \). Since the regions of interest are predefined, the use of the "corrected" \( P \)-values is unnecessary and inappropriately conservative (Friston et al., 1996).

However, to further elucidate differences between responders and non-responders, activity passing a voxel-level uncorrected \( P < 0.05 \) was reported, too. On the other hand, voxel-based partial correlations were performed for investigating the association between the improvement in depressive symptoms (% HDRS change) and \(^{18}\)F-FDG uptake after covarying for age, gender and total gray matter counts; in those with cluster-level uncorrected \( P < 0.05 \), the clusters passing through SVC \( P \)-value \(< 0.05 \) were thought to be significant. A paired-\( t \) test was performed in patients before and 3 months following rTMS treatment. The significance thresholds were set at a cluster-level corrected \( P < 0.005 \) (Friston et al., 1991; Friston et al., 1996).

Statistical analysis of demographic and clinical data was performed by using SPSS 11.5 (SPSS Inc, Chicago, IL). One-way ANOVA (or Student’s \( t \) test) and Fisher’s chi-square test (or Yates’ correction) were used to compare the continuous and categorical variables among groups, respectively. \( P < 0.05 \) was deemed statistically significant.

3. Results

3.1. Demographic and clinical variables

Twenty MRD patients (5 male, 15 female) received 2-week rTMS treatments and PET scans at baseline and the 3-month follow-up, whereas 20 healthy controls (6 male, 14 female) received single PET scans (Table 1). Thirteen (65%) MRD patients met the criteria for responders at the 2-week end of the rTMS treatment and all of them had subjective improvement in BDI scores \( \geq 40 \% \) from the baseline to the end

### Table 1

Demographic and clinical characteristics among depressed patients and healthy control subjects.

<table>
<thead>
<tr>
<th>MRD (N=20)</th>
<th>( \text{tMS Responders} ) (N=13)</th>
<th>( \text{tMS Non-responders} ) (N=7)</th>
<th>( \text{Healthy Control} ) (N=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>5/8</td>
<td>0/7</td>
<td>6/14</td>
</tr>
<tr>
<td>Age (years), Mean (SD)</td>
<td>51.5 (11.3)</td>
<td>50.9 (6.9)</td>
<td>39.1 (11.0)</td>
</tr>
<tr>
<td>Married/unmarried (N)</td>
<td>9/4</td>
<td>4/3</td>
<td>12/8</td>
</tr>
<tr>
<td>Right-handedness, N (%)</td>
<td>13 (100)</td>
<td>7 (100)</td>
<td>20 (100)</td>
</tr>
<tr>
<td>Illness characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of onset (years), Mean (SD)</td>
<td>39.4 (14.3)</td>
<td>42.4 (8.6)</td>
<td>—</td>
</tr>
<tr>
<td>Illness duration (years), Mean (SD)</td>
<td>12.8 (8.2)</td>
<td>8.6 (6.7)</td>
<td>—</td>
</tr>
<tr>
<td>Past depressive episode (times), Mean (SD)</td>
<td>7.2 (4.1)</td>
<td>5.7 (3.0)</td>
<td>—</td>
</tr>
<tr>
<td>Past suicidal attempts (times), Mean (SD)</td>
<td>1.2 (2.7)</td>
<td>2.6 (2.6)</td>
<td>—</td>
</tr>
<tr>
<td>Psychomotor retardation features, N (%)</td>
<td>8 (61.5)</td>
<td>5 (71.4)</td>
<td>—</td>
</tr>
<tr>
<td>Psychosis features, N (%)</td>
<td>0 (0%)</td>
<td>1 (14.3%)</td>
<td>—</td>
</tr>
<tr>
<td>Pre-tMDS HDRS-17, Mean (SD)</td>
<td>23.5 (3.7)</td>
<td>24.6 (3.2)</td>
<td>—</td>
</tr>
<tr>
<td>2-week Post-tMDS HDRS-17, Mean (SD)</td>
<td>7.2 (2.8)</td>
<td>19.1 (4.1)</td>
<td>—</td>
</tr>
<tr>
<td>3-month Post-tMDS HDRS-17, Mean (SD)</td>
<td>7.0 (4.8)</td>
<td>19.9 (9.1)</td>
<td>—</td>
</tr>
<tr>
<td>Pre-tMDS BDI, Mean (SD)</td>
<td>29.0 (8.6)</td>
<td>31.1 (7.6)</td>
<td>—</td>
</tr>
<tr>
<td>2-week Post-tMDS BDI, Mean (SD)</td>
<td>12.1 (6.0)</td>
<td>27.9 (12.2)</td>
<td>—</td>
</tr>
<tr>
<td>3-month Post-tMDS BDI, Mean (SD)</td>
<td>14.5 (8.8)</td>
<td>29.9 (13.0)</td>
<td>—</td>
</tr>
<tr>
<td>Pre-tMDS YMRS, Mean (SD)</td>
<td>0.7 (1.1)</td>
<td>0.4 (0.8)</td>
<td>—</td>
</tr>
<tr>
<td>2-week Post-tMDS YMRS, Mean (SD)</td>
<td>0.8 (2.2)</td>
<td>0.4 (1.1)</td>
<td>—</td>
</tr>
<tr>
<td>3-month Post-tMDS YMRS, Mean (SD)</td>
<td>1.5 (3.8)</td>
<td>0.1 (0.4)</td>
<td>—</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antidepressants, N (SSRI/SNRI/others)</td>
<td>4/2</td>
<td>7</td>
<td>1/3</td>
</tr>
<tr>
<td>Combined antipsychotics, N (%)</td>
<td>5 (38.5%)</td>
<td>3 (42.8%)</td>
<td>—</td>
</tr>
<tr>
<td>Benzodiazepines, N (%)</td>
<td>11 (84.6%)</td>
<td>5 (71.4%)</td>
<td>—</td>
</tr>
</tbody>
</table>

Note. MRD, Medication-Resistant Depression; tMDS, repetitive transcranial magnetic stimulation; HDRS-17, 17-item Hamilton Depression Rating Scales; BDI, Beck Depression Inventory; YMRS, Young Mania Rating Scale; S.D, standard deviation; SSRI, specific serotonin reuptake inhibitor; SNRI, serotonin norepinephrine reuptake inhibitor; others including NDBI (norepinephrine dopamine reuptake inhibitor) and NaSSA (Noradrenergic/Specific Serotonergic Agent).

a Fisher’s exact or Yates’ correction chi-square test.

b ANOVA, \( F \) (df = 2.37).

** \( P < 0.01 \).

*** \( P < 0.001 \).
of treatment. Five patients had co-morbid dysthymic disorder (non-responders:responders (N = 2:3): 4 had panic disorder (N = 1:3) and 15 had generalized anxiety disorder (N = 6:9). The responders and non-responders did not differ in demographic characteristics, despite the fact that all the non-responders were female (responders vs. non-responders, Yates’ chi-square \(P = 0.0176\)). The MRD patients were significantly older than the healthy controls. Thus, gender and age were included in the subsequent SPM models as variables of no interest. There were no differences between responders and non-responders in illness characteristics such as age at onset, duration of illness, previous depressive episodes, past suicidal attempts and features of psychosis or psychomotor retardation. At the baseline assessment, there was no difference in the pretreatment severity of MRD between groups on the HDRS-17 (\(P = 0.242\)), BDI (\(P = 0.587\)) and YMRS (\(P = 0.586\)). Baseline medication was similar for responders and non-responders (all \(P > 0.76\), Table 1). All patients tolerated the 2-week add-on rTMS treatment well, with no patients switching due to experiencing mania, although 4 patients (20%) reported temporary headache during the first days of rTMS treatment. As expected, at the end of the 2-week rTMS course, the responders had significantly lower depressive scores than the non-responders on the HDRS-17 (\(P < 0.001\)) and BDI (\(P < 0.001\)), but not on the YMRS (\(P = 0.713\)). It was surprising to note that 84.6% (11/13) of initial responders maintained their status of symptomatic relief at the end of the 3-month follow-up. In summary, our data suggest that add-on rTMS is an effective and safe method to treat MRD and that the antidepressant effect can last at least 3 months.

3.2. Regional brain glucose metabolism between patients with MRD and normal subjects

Compared with healthy controls, all MRD patients at the baseline (\(N = 20\)) and the post-rTMS responders that maintained symptomatic relief at the end of the 3-month follow-up (\(N = 11\)) presented a similarly lower normalized metabolism in bilateral prefrontal areas (dorsolateral, orbital and medial PFC), the anterior and middle cingulum, as well as the inferior frontal gyrus extending to the anterior insula and temporal poles bilaterally (all cluster-level \(P_{\text{corrected}} < 0.001\)). In contrast, they showed an abnormally higher normalized metabolism in several limbic and subcortical regions, including the bilateral striatum, amygdala, hippocampus/parahippocampus, fusiform gyri, cerebellum and part of the brainstem (cluster-level \(P_{\text{corrected}} < 0.001\) for baseline comparison and SVC-\(P_{\text{corrected}} < 0.05\) for post-rTMS comparison with healthy subjects). After the depressed mood had been controlled by rTMS, the abnormal pattern still existed in the post-rTMS MRD responders (Fig. 1). These findings suggest the pathophysiology underlying MRD is similar to the proposed model of cortico-limbic dysregulation in depression (Mayberg, 1997), and might be a stable trait marker of MRD.

3.3. Regional brain glucose metabolism in responders and non-responders at baseline

When we compared responders and non-responders, the responders had higher metabolic activities in the bilateral medial prefrontal cortex and the rostral anterior cingulum (2756 voxels, cluster-level uncorrected \(P = 0.009\)), but not on the YMRS (\(P = 0.713\)). It was surprising to note that 84.6% (11/13) of initial responders maintained their status of symptomatic relief at the end of the 3-month follow-up. In summary, our data suggest that add-on rTMS is an effective and safe method to treat MRD and that the antidepressant effect can last at least 3 months.
the responders (Fig. 3, upper right). This finding provides evidence that patients who responded well to rTMS had a less dysregulated limbic-cortical metabolic pattern (Fig. 3, lower).

3.4. Relationship between regional glucose metabolism and depressive symptom improvement

By conducting a partial correlation test on all the patients (\(P_{\text{p}} < 0.05\), small volume corrected), we found a significant correlation between the percent improvement of the HDRS-17 scores after a 2-week rTMS treatment and the baseline regional glucose metabolism in the left parahippocampal and fusiform gyri (3751 voxels, \(P = 0.001\)) (Fig. 4, lower). This area also encompassed parts of the middle and inferior temporal gyri. Furthermore, improvement in depression was significantly correlated with regional glucose metabolism in the rostral anterior cingulum (1070 voxels, \(P = 0.029\)) (Fig. 4, upper).

3.5. Before-versus-after changes of glucose metabolism associated with rTMS treatment

In the rTMS responders maintaining symptomatic relief at the end of the 3-month follow-up (\(N = 11\), 2 were excluded due to symptom exacerbation and medication changes after rTMS treatment), the glucose metabolism in the left fusiform gyrus and left middle temporal cortex decreased significantly (4210 voxels, \(P = 0.003\)) after rTMS treatment, while the glucose metabolism in the middle cingulum, bilateral somatosensory areas and precuneus increased significantly (8201 voxels, \(P < 0.001\)) (Fig. 5). This pattern was not found in the non-responders. Instead, the non-responders showed an increased metabolism in the bilateral temporal cortex and fusiform gyri, which was correlated with worsening depressive symptoms. Our findings suggest that rTMS antidepressant efficacy was associated with reversing the hypermetabolism of the left fusiform gyri and left middle temporal cortex. However, the effects occurred only in the responders, suggesting that higher baseline activities in the bilateral medial prefrontal cortex and rostral anterior cingulum, as shown in Fig. 2, play a critical role in the add-on rTMS antidepressant mechanism.

4. Discussion

By using a combined PET/rTMS design and following patients for 3 months, we were able to elucidate the antidepressant mechanism of rTMS and the central abnormalities underlying MRD. First, our findings suggest that the add-on rTMS antidepressant mechanism is associated with suppression of hyperactivity in the left limbic and paralimbic structures, perhaps through enhancing the function of the anterior cingulum. Second, our results showed a possible value in predicting rTMS antidepressant effects with baseline neuronal activity in the left parahippocampal and fusiform gyri, medial prefrontal cortex and rostral anterior cingulum. Third, our results indicate that the range of severities that depressed patients present can influence not only the presentations of central glucose metabolism (Hosokawa et al., 2009; Kimbrell et al., 2002b), but also the responsiveness of patients to treatment. Furthermore, we found persistent central metabolic abnormalities in the MRD patients including hypometabolism in the prefrontal cortex and hypermetabolism in several limbic and subcortical areas. These suggest the persistent limbic-cortical dysregulation could be a trait marker underlying the central mechanism of MRD.

In the present study, the antidepressant response rate to add-on rTMS was as high as 65%. As for rTMS monotherapy in treating depression, the reported response rates were usually 30–45% (Brunelin et al., 2007; Schutter, 2009). However, in non-psychotic drug-refractory depression, the response rate to high-frequency rTMS at the left DLPFC was reported to be higher, around 55% (7/11 responders) (Grunhaus et al., 2000). Our results support the notion that depressive patients without psychotic symptoms respond to rTMS better than those with vivid psychotic symptoms, since almost none of
Fig. 3. Comparisons of normalized baseline glucose metabolism between rTMS responders and non-responders under a voxel-level uncorrected $P<0.05$ (Upper left: Responders > Non-responders; Upper right: Responders < Non-responders; Lower: Responders > Non-Responders [in red], Responders < Non-Responders [in blue], with transverse slices in Axis-Z of −40 to 40), suggesting patients with less impaired limbic-cortical dysregulation responded better to rTMS.
our patients had psychotic symptoms. Other possible causes of the higher efficacy in the present study could be the combination of rTMS and antidepressants, so-called add-on rTMS, and a high accuracy in targeting the stimulation site to the left DLPFC. Add-on rTMS has the ability to augment the efficacy of antidepressants and is effective in treating refractory depression (Bretlau et al., 2008; Rumi et al., 2005). We previously showed that active add-on rTMS had a high response rate of 60% vs. 10% in a sham-controlled group in a randomized and double-blind study (Su et al., 2005). In the present study, to increase the accuracy of targeting to the left DLPFC, we used a brain-navigation method to help guide the coil, based on each patient’s MRI. Compared to the standard 5-cm method for localization, the neuro-navigated method to target the left DLPFC has been shown to enhance response to rTMS treatment in depression (Fitzgerald et al., 2009). In contrast to some previous studies, a strength of our study was its clinical homogeneity, since we focused only on MRD. Among our MRD patients, the anterior and middle cingulum, anterior operculum-insula and large parts of the frontal areas showed an abnormally lower metabolism; in contrast, the depressives had a higher glucose metabolism in the bilateral subcortical and limbic structures. Most of the abnormalities remained, even after depression was controlled. This pattern of results implied that the pathophysiology underlying MRD involves a dysregulation of the limbic-cortical system, echoing a depression model proposed by Mayberg (Mayberg, 1997). The functionally reciprocal relationships between limbic structures and the frontal cortex have been elucidated in primates and in humans (Carmichael...
and Price, 1994; Mayberg et al., 1999; Porrino et al., 1981), and are associated with emotional processing in both healthy and depressed subjects (Mayberg et al., 1999). The persistence of limbic-cortical dysregulation during symptomatic resolution could be associated with a high risk for the development of depressive relapse. This is in line with a previous study which reported that some depressed patients during remission still had a persistently elevated amygdala metabolism (Drevets, 1999); also, our findings support the findings that treatment-resistant depressives had significantly higher hippocampus/amygdala blood perfusion than non-treatment-resistant depressives (Hornig et al., 1997). By comparing a homogeneous depression group to healthy subjects, we found that the bidirectional phenomenon of limbic-cortical reciprocity could be a trait phenomenon of MRD.

Although rTMS was directly applied to the left DLPFC, metabolism in the anterior cingulum was better correlated with rTMS treatment efficacy than that in the left DLPFC. The anterior cingulum is structurally and functionally connected to both the prefrontal cortex and several limbic structures, such as the amygdala and hippocampus at the temporal cortex (Carter and van Veen, 2007; Devinsky et al., 1995; Morgane et al., 2005). The anterior cingulum (BA 24, 32) monitors conflict and engages top-down control over the DLPFC to solve problems (Carter and van Veen, 2007; Devinsky et al., 1995). The finding of pretreatment activity in the anterior cingulum and medial prefrontal cortex as possible predictors of an antidepressant response is consistent with previous research with different treatment modalities, such as antidepressants (Kennedy et al., 2001; Mayberg et al., 1997; Saxena et al., 2003), psychotherapy (Kennedy et al., 2007) and sleep deprivation (Ebert et al., 1994; Wu et al., 1999). This implies that the underlying mechanism of the antidepressant efficacy of add-on rTMS may be partly in common with that of other antidepressant treatment modalities.

In the temporal cortex, we found a significant correlation between rTMS-related depressive improvement and baseline regional glucose metabolism in the left parahippocampal and fusiform gyri, as well as in part of the middle/ inferior temporal gyrus. Three months after rTMS treatment, the glucose metabolism in the left fusiform gyrus and left middle temporal cortex had decreased significantly only in the rTMS responders. Some previous voxel-based PET/SPECT/rTMS studies investigating how blood flow changes after rTMS found that central blood flow was changed in the remote medial temporal structures, such as amygdala, hippocampus/parahippocampus and uncus (Speer et al., 2000; Teneback et al., 1999), but the fusiform gyrus and middle temporal cortex were not consistently identified. The inconsistent results across studies may result from the heterogeneity of the study populations. For example, most studies recruited both unipolar and bipolar patients that were not treatment-resistant subjects (Speer et al., 2000; Teneback et al., 1999), while some focused on only male subjects with depression (Kito et al., 2008). Increased activities in the limbic structures are associated with depression and anxiety (Drevets et al., 2008; Etkin and Wager, 2007). The fusiform gyrus is tightly connected to the parahippocampus, a brain region in the limbic system, and both of them were found to be related to face and scene recognition and perception (Nakamura et al., 2000). Depressed patients tended to respond to sad, but not happy facial expressions and had preferentially increased neural responses in the fusiform gyrus (Surguladze et al., 2005). A greater tendency to focus attention on the self and ruminating over sad memories and scenes from the past are common symptoms in depression (Watkins and Teasdale, 2004). On the other hand, the function of the middle temporal lobe is also associated with facial recognition. Depressive patients are more sensitive to negative emotions such as disgust, which is evidenced by the finding of abnormally higher activation in the left middle/inferior temporal gyrus when depressed patients were exposed to facial expressions of strong disgust (Surguladze et al.). Higher levels of rumination and distraction are both linked to medication resistance in depression (Bagby et al., 1999). This may partly account for our findings on the increased metabolism in the left temporal cortex of medication-resistant depressive patients. Taken together, our results suggest that high-frequency add-on rTMS at the left DLPFC might increase the ability of the anterior cingulum in modulating and engaging the left DLPFC in the top-down control of dysfunctional limbic and associated structures, and normalize the limbic-cortical dysregulation underlying depression.

Enhanced serotonergic transmission could also be involved in the antidepressant effects of rTMS. A molecular imaging study has shown that rTMS in the left DLPFC could modulate serotonin metabolism in limbic areas, including the left temporal parahippocampal gyrus (Sibon et al., 2007). The limbic system is thought to play a critical role in emotional processing and depression, and is structurally and functionally connected to the prefrontal cortex, cingulate cortex and midbrain (Morgane et al., 2005). Add-on rTMS might boost the ability of the antidepressants to increase central serotonergic transmission or stimulate serotonergic transmission in other mood-related structures, such as the limbic system, that cannot be achieved through the use of antidepressant drugs alone. This also explains why add-on rTMS is more effective in treating MRD patients (Bretlau et al., 2008; Rumi et al., 2005). In sum, our results indicate that MRD showed dysregulation in the limbic-cortical system, and those who presented less impairment in this system responded better to high-frequency rTMS plus antidepressant medications.

The interpretation of our findings needs to be tempered by some existing limitations. First, our sample size was relatively small and a placebo effect due to active rTMS treatment cannot be excluded, since there was no placebo arm. Second, the second scan was not done right after rTMS treatment in the present study. Since all the patients had a medication-resistant history, we assumed that medications alone might have little beneficial impact on clinical symptoms or brain metabolism, if the patients had used them for a period. However, the metabolic changes after rTMS might not be attributed only to specific rTMS antidepressant effects; instead, they might reflect a combination of specific rTMS-dependent effects and any other unspecific rTMS-independent treatment effects over time. Third, the patients were not drug-free, but were all under antidepressant treatment. However, continued medication treatment is ethically sound in treating MRD and also necessary to demonstrate the differences between responders and non-responders to add-on rTMS treatment, in terms of the generalizability of the results. Fourth, the psychiatric comorbidities in the depressives could affect the interpreting of our
results. However, there were no significant differences in the rate of occurrence of comorbidities between the responders and non-responders. Finally, we did not measure plasma input function or estimate the absolute glucose metabolic rate. Instead, we focused on the relative differences between the groups. However, some investigators were in favor of using semi-quantitative data because relative regional glucose uptake values have a much smaller variability than absolute values themselves (Signorini et al., 1999).

5. Conclusion

MRD and rTMS provided us a great opportunity to better understand the pathophysiology of depression. The present study shows the persistent limbic-cortical dysregulation underlying the central mechanism of MRD. Patients who responded well to rTMS were less impaired in the limbic-cortical system. Instead of the baseline activities in the DLPCF correlated with rTMS efficacy, higher pretreatment glucose metabolism in the anterior cingulum and lower metabolism in the left parahippocampus and fusiform gyrus could characterize add-on rTMS efficacy. After successful rTMS treatment, significantly decreased metabolism in the left middle temporal cortex and fusiform gyrus was associated with rTMS antidepressant efficacy. Our findings suggest that the add-on rTMS antidepressant mechanism is associated with suppression of hyperactivity of the left limbic and paralimbic structures, perhaps through enhancing the function of the anterior cingulum.

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Conflict of interest

None of the authors in this study has a conflict of interest to declare.

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References