Research paper

Two-week rTMS-induced neuroimaging changes measured with fMRI in depression

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Abstract

Objective: To study the neuroimaging mechanisms of repetitive transcranial magnetic stimulation (rTMS) in treating major depressive disorder (MDD).

Methods: Twenty-seven treatment-naive patients with major depressive disorder (MDD) and 27 controls were enrolled. All of them were scanned with resting-state functional magnetic resonance imaging (fMRI) at baseline, and 15 patients were rescanned after two-week rTMS. The amplitude of low frequency fluctuation (ALFF) and functional connection degree (FCD), based on voxels and 3 brain networks (default mode network [DMN], central executive network [CEN], salience network [SN]), were used as imaging indicators to analyze. The correlations of brain imaging changes after rTMS with clinical efficacy were calculated.

Results: At baseline, patients groups showed increased ALFF in the right orbital frontal cortex (OFC) and decreased ALFF in the left striatal cortex and medial prefrontal cortex (PFC), while increased FCD in the right dorsal anterior cingulate cortex and OFC and decreased FCD in the right inferior parietal lobe and in the CEN. After rTMS, patients showed increased ALFF in the left dorsolateral prefrontal cortex (DLPFC) and superior frontal gyrus, FCD in the right dorsal anterior cingulate cortex, superior temporal gyrus and CEN, as well as decreased FCD in the bilateral lingual gyrus than pre-rTMS. These rTMS-induced neuroimaging changes did not significantly correlate with clinical efficacy.

Conclusions: This study indicated that rTMS results in changes of ALFF and FCD in some brain regions and CEN. But we could not conclude this is the neuroimaging mechanism of rTMS according to the correlation analysis.

1. Introduction

Depression is one of the most common mental disorders and the second leading cause of mental disability in China according to a recent cross-sectional epidemiological study (Huang et al., 2019). Although pharmacotherapy and psychotherapy have been shown to be effective for depression, only 33% of patients achieve full remission with medication during treatment in the acute phase, with less than 50% of patients failing to achieve remission after multiple medication trials (Rush et al., 2006; Nelson et al., 2006). Repetitive transcranial magnetic stimulation (rTMS) has been approved by the US Food and Drug Administration for treating refractory major depressive disorder (MDD) (O’Reardon et al., 2007). Although rTMS can be used as an alternative treatment for depression, meta-analysis shows that its effective rate for depression is only 0.55 and varies greatly across patients (Schutter et al., 2009; Slotema et al., 2010).

With the development of neuroimaging technology and analysis methods, resting-state functional magnetic resonance imaging (rsfMRI) has become an effective and common method to study the human brain, with the advantages that it is non-invasive, conveniently operated and repeatedly measured (van et al., 2010; Biswal et al., 2010). Amplitude of low frequency fluctuation (ALFF) and functional connectivity density (FCD) are two fundamental fMRI parameters describing local and network properties of resting-state brain function, respectively (Liu et al., 2018). ALFF might reflect voxel-level local neural activity during resting state and has been used as an effective indicator of regional spontaneous neuronal oscillations (Liu et al., 2014; Liao et al., 2019). Functional connectivity density (FCD) is a graph-based measurement, which is defined as functional connectivity between a voxel and the rest of voxels across the whole brain, and is applied to identify abnormal

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connectivity for each voxel in the whole brain separately (Zhang et al., 2016). Although some previous studies revealed brain region functional changes using ALFF or FCD as method of measurement in MDD patients (Liu et al., 2014; Zhang et al., 2016; Ye et al., 2012). However, as far as we knew, these two measures were never used together in MDD patients, just in mild cognitive impairment with Depressive Symptoms (Liu et al., 2018). Therefore rarely studies of ALFF or FCD were used in rTMS depression treatment. While a electroconvulsive therapy (ECT) study found that the changes of FCD of the left pre-/post central gyrus (Pre-/post CG), left superior temporal gyrus (STG), and right STG were significantly correlated with the depression scores in MDD patients before and after ECT (Wang et al., 2018). And our previous study found the ALFF of left DLPFC and nucleus accumbens couldn’t predict the effects of rTMS (Du et al., 2018), however, the ALFF analysis based on whole brain, especially the network, has not been used to explore the prediction mechanisms of rTMS in MDD.

Recent studies have shown that depression is characterized by abnormal functional integration of brain networks (Kaiser et al., 2015; Liao et al., 2018), mainly the following 3 neural networks: the DMN, CEN and SN (Kaiser et al., 2015; Liao et al., 2018; Hamilton et al., 2013; Menon, 2011; Seeley et al., 2007; Fan et al., 2019). The DMN has been implicated in self-referential processing and episodic memory retrieval. The CEN, which plays a key role in executive function and emotion regulation, includes the DLPFC and lateral posterior parietal regions (Miller et al., 2001). The SN is involved in detecting, integrating and processing internal and external salient information, and it includes the dorsal anterior cingulate cortex (dACC), anterior insula, amygdala, and ventral striatum (Menon et al., 2010). The majority of studies exploring the mechanisms of rTMS have focused on assessing changes in brain network function by measuring functional connectivity between brain regions. Liston et al. pointed out that rTMS acted by reducing Subgenual anterior cingulate cortex (sgACC) to DMN connectivity and inducing negative connectivity between the DLPFC and DMN (Liston et al., 2014). The sgACC- DLPFC functional connectivity was indeed implicated in two rTMS studies (Fox et al., 2012, 2013). Kang et al. reported that reduction of connectivity strength between the DLPFC and the left caudate is positive correlation with symptom improvement in rTMS depression therapy (Kang et al., 2016). Philip et al. demonstrated that potential mechanisms of response to TMS in patients with comorbid Posttraumatic stress disorder (PTSD) and MDD was associated with reduced connectivity between the hippocampus and the SN (Philp et al., 2018). While utilizing independent components analysis (ICA) techniques in resting-state fMRI, two studies showed that SN may be key circuit that was associated with responsiveness to rTMS therapy in treatment-resistant depression (Ge et al., 2017; Iwabuchi et al., 2019). Few studies have analyzed fMRI data from the perspective of the overall function of a network. Fan et al. observed that network segregation of SN predicted symptom improvement following rTMS administered to the left DLPFC in MDD, but did not explore the changes of overall function in SN (Fan et al., 2019). Meanwhile, the subjects in those studies were patients with treatment-resistant depression who were not the best to explore treatment mechanisms because brain imaging might be affected by drugs.

Therefore, this study enrolled untreated patients with MDD who were treated with 10 Hz rTMS, and observed brain functional changes after treatment using resting-state fMRI. We calculated ALFF and FCD as indicators based on the whole brain and 3 brain networks. These findings could help us further understand the pathological mechanisms of depression and the effects of rTMS.

2. Materials and methods

2.1. Subjects

Twenty-seven medication-free depression outpatients (medication-free in the previous month and had been previously medicated for less than a week), between 20 and 55 years old, right-handed, with a single episode of or recurrent depressive episodes were recruited for this study. The diagnosis of MDD was confirmed by the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders IV (SCID-I/P, Chinese version). Patients were excluded if they had a history of alcohol or drug abuse, current or past psychotic disorders, any current clinically significant neurological disorder or other serious physical diseases, morphological abnormalities in the brain, and any electronic or metal implants. The choice of rTMS treatment for the MDD patients was decided by their clinicians, and then they were screened by the researchers to assess if they met the above inclusion/exclusion criteria. All patients were treated with rTMS, and 15 of them agreed to be rescanned with fMRI. A total of 27 HCs were recruited from community advertisements. The HCs were required to be between 20 and 55 years of age and right-handed. The information of the subjects in this study are partially overlapped with one of our previous article (Du et al., 2018). None of them had a lifetime history of Axis I psychiatric disorders as assessed with Structured Clinical Interview for the DSM (SCID). They were excluded for the following conditions: chronic or severe physical illness, pregnancy, drug abuse and the presence of electronic or metal implants. Written informed consent was obtained from all subjects. The study protocol was reviewed and approved by the Local Medical Ethics Committee of the First Affiliated Hospital of Chongqing Medical University.

2.2. rTMS protocol

rTMS was delivered in sessions by a YRD CC1-I magnetic simulator (YIRUIDE Inc., Wuhan, China). The patients received a total of ten sessions of rTMS (five sessions per week for 2 weeks). The stimulation parameters were guided by recently published international safety guidelines: 100% magnetic field strength relative to the patient’s observed resting motor threshold (MT) (Du et al., 2018), at ten pulses per second for 3 s, with an interval of 21 s. Treatment sessions lasted for 20 min (50 trains) and consisted of 1500 pulses. The stimulated position in the left DLPFC was located using the ‘5 cm’ method that was at a site 5 cm anterior to the optimal site for motor response (Du et al., 2018).

2.3. Clinical assessment

All the 27 patients were assessed using both the 17-item Hamilton Depression Scale (HAMD) and the 14-item Hamilton Anxiety Scale (HAMA) at baseline, and 15 patients were reassessed at the end of the 2 weeks of rTMS treatment (Du et al., 2018).

2.4. Functional MRI acquisition and preprocessing

Data were acquired on a 3.0 Tesla MRI system (GE Medical Systems, Waukesha, WI, USA) at the First Affiliated Hospital of Chongqing Medical University. The subjects were instructed to relax with their eyes closed and not move their heads while remaining awake during the MRI scan. Functional images were acquired using an echo-planar imaging sequence (repetition time, 2000 ms; echo time, 30 ms; flip angle, 90°; field of view, 240 × 240 mm2 matrix, 64 × 64; slice thickness, 5 mm; and 33 axial slices). A total of 240 volumes were collected for a total scan time of 480 s. Subsequently, 3D T1-weighted anatomical images were acquired (repetition time, 8.35 ms; echo time, 3.27 ms; flip angle, 12°; field of view, 240 × 240 mm2; matrix, 256 × 256; slice thickness, 1 mm; and 156 sagittal slices). The subjects were later asked whether they had fallen asleep.

Both functional and anatomical images were preprocessed using DPARSF v2.3 software. The first ten functional images were excluded, and subsequent images were corrected for temporal differences by slice-time and for head motion by alignment. The head motion parameters (three translations and three rotations) for each volume were obtained.
using rigid body transforms. The subjects' data were excluded from further analysis if either their translation or rotation parameters for any single volume exceeded 3 mm or 3°. We also calculated the individual mean framewise displacement (FD) by summing the absolute values of the differences of the six realignment parameters to express the relative transformation parameters. Individual 3D T1-weighted anatomical images were coregistered to the functional images. The coregistered 3D T1-weighted images were segmented into gray matter, white matter, and cerebrospinal fluid using SPM8 (www.fil.ion.ucl.ac.uk/spm). The segmented gray matter was then normalized to Montreal Neurological Institute (MNI) space. These spatial transformation parameters were then applied to the functional images. The normalized fMRI data were resliced at a resolution of 3 × 3 × 3 mm3 and spatially smoothed with a 6-mm full-width Gaussian kernel at half-maximum.

Several sources of spurious variance (24 head motion parameters, averaged signals from the CSF and white matter, and the global brain signal) were eliminated by multiple linear regression (Ji et al., 2019). Subsequently, linear trends were removed from the time courses. Temporal band pass filtering (0.01–0.08 Hz) was performed. Finally, because FC is sensitive to the confounding factor of head motion, scrubbing regression was performed for motion correction to reduce the negative influence.

2.5. ALFF and FCD analysis based on whole brain and 3 networks

We used ALFF to describe the intrinsic local activity of each voxel. After obtaining the ALFF value of each voxel, the average ALFF value of all voxels in the CEN, DMN and SN networks was calculated, which represented the ALFF value of the whole network. Then, the processed data were analyzed to calculate the global function connection degree. Before the calculation, the number of functional connections ki was first defined. Ki was defined as the number of Pearson correlations between voxel i and other voxels that exceeded a given threshold T (here, r = 0.25, p = 0.05) (Tomasi et al., 2010). According to the definition of Tomasi and Volkow, FCD for voxel I was defined as the number of significant functional connections between i and all other voxels that exceeded the threshold T (Tomasi et al., 2010). FCD values for all voxels in the whole brain were calculated to obtain the global functional connectivity density map for each subject. After obtaining the global FCD value of each voxel, the average global FCD value of all voxels in the CEN, DMN and SN networks for each subject were calculated, which represented the FCD value of every network. The CEN, DMN and SN network templates were obtained by meta-analysis (http://www.neurosynth.org/).

2.6. Statistical analysis

Two-sample t-tests were used to compare ALFF and FCD at baseline between patient with MDD and control groups, and paired t-tests were used between pre- and post-rTMS treatment. At baseline, Pearson correlation analyses were used to calculate the correlations between ALFF and FCD values in those brain regions with significant differences and symptom scores. After rTMS treatment, Pearson correlation analyses were used to study whether the changes in ALFF and FCD values were correlated with the changes in clinical symptoms.

3. Results

3.1. Clinical outcomes

Demographics of the participants are summarized in Table 1. There were no significant differences between the depression group and the normal control group in age, sex and education level (p > 0.05). After 2 weeks of rTMS treatment, the total scores on the HAMD and HAMA were significantly decreased (p < 0.05) (Table 2).

| Table 1 | Demographics and clinical characters of controls and depression patients. |
|---|---|---|---|---|---|---|
| Demographics | HC (n = 27) | MDD (n = 27) | T-value | P-value |
| Age (years) | 41.00 ± 8.04 | 41.22 ± 12.71 | 0.94 | 0.36 |
| Sex (Male:Female) | 6/21 | 8/19 | 0.53 | 0.38 |
| Education (years) | 12.26 ± 2.90 | 12.44 ± 3.40 | 0.83 | 0.22 |
| Age of onset (years) | 36.62 ± 13.81 |  |

Abbreviations: MDD, major depressive disorder; HC, healthy control.

The values are illustrated as mean ± SD.

3.2. Neuroimaging comparisons at baseline between the patients with MDD and HCs

The analysis based on whole-brain voxel levels showed that ALFF was increased in the right orbital frontal cortex (OFC) and decreased in the left striatal cortex (caudate nucleus and putamen) and medial prefrontal cortex in the patient group (Fig. 1a, Table S1 in Supplement), while FCD was increased in the right dorsal anterior cingulate cortex (dACC) and OFC and decreased in the right inferior parietal lobe (Fig. 1b, Table S2 in Supplement) in the patient group compared with the control group. We used cluster threshold at p < 0.05 (height p < 0.001, and size of voxels > 53).

The analysis based on networks showed no significant differences in ALFF in the DMN, CEN or SN between the two groups (Fig. 2a), while the FCD of the CEN was significantly lower in the patient group, and there were no significant differences in FCD in the DMN and SN between the two groups (Fig. 2b).

Correlation analysis at baseline showed that only FCD of the CEN was negatively correlated with Hamilton anxiety (HAMA) scale scores (r = −0.41, p = 0.03) (Fig. 2c).

3.3. Neuroimaging comparisons between pre- and post-rTMS scans

The analysis based on voxels across the whole brain showed that ALFF in the left dorsolateral prefrontal cortex and superior frontal gyrus was increased after rTMS treatment (Fig. 3a, Table S3 in Supplement), while FCD in the right dorsal anterior cingulate cortex and superior temporal gyrus was increased and that in the bilateral lingual gyrus decreased after rTMS treatment (Fig. 3b, Table S4 in Supplement).

The analysis at the brain network level showed that there was no significant difference in ALFF in all 3 brain networks after rTMS treatment (Fig. 3c); however, FCD in the CEN was significantly higher after rTMS (p = 0.031), while there was no significant difference in FCD in the DMN and SN (Fig. 3d).

There were no significant correlations between clinical efficacy of rTMS and brain imaging changes in those regions with significance during comparison of pre- and post-rTMS.

4. Discussion

The present study demonstrated that rTMS was an effective treatment for treatment-naive depression and examined neuroimaging changes with fMRI after rTMS. We focused on three core networks that
have been implicated in the pathophysiology of MDD and found that rTMS induced alterations not only in regional brain areas but also in whole neural networks. Specifically, we discovered that abnormal function in the CEN was significantly reversed after TMS. This study was the first to explore the neuroimaging mechanisms of rTMS in patients with MDD by using ALFF and FCD analyses at both the whole brain level and the 3 important brain networks level.

After rTMS treatment, we found some significant changes in ALFF and FCD based on whole brain analysis. Among those results, the following seem to be interesting. Firstly, ALFF were increased in the left DLPFC and superior frontal gyrus (SFG), which was the target region of rTMS and nearby. Numerous previous studies have reported that the activity of the left DLPFC in depression was weakened (Liston et al., 2014; Fox et al., 2012). Moreover, randomized clinical trials using rTMS of 10 Hz to stimulate the left DLPFC in refractory depression achieved positive clinical effects (Schutter et al., 2009), thus left DLPFC was regarded as one of the most common target site of rTMS in MDD. Previous studies pointed out that, by applying repeated pulses (rTMS) at low frequencies (e.g., 1 Hz), one can suppress underlying cortical activity and high-frequency stimulation (e.g., 20 Hz) can result in excitatory changes (Wagner et al., 2007; Kobayashi et al., 2003; Hallett et al., 2007). Our results further confirmed the thesis that high-frequency rTMS could increase the excitability of neurons. Meanwhile, we found that after rTMS treatment, FCD in the right dACC and superior temporal gyrus (STG) were increased, while FCD in the bilateral lingual gyrus was decreased. Previous studies have shown that the severity of depression was related to the connections of the dACC (Pannekoek et al., 2014), which is an important node in the SN. We found that FCD in the right dACC was increased in the patients with MDD at baseline, which was consistent with previous findings of enhanced dACC connections in the SN (Manoliu et al., 2013; Wu et al., 2016). After rTMS treatment, FCD in the dACC further increased, suggesting that increased connectivity might play a protective effect against depression and a positive role in the treatment of depression. However, whether increased functional connectivity of the dACC is an impairment or a compensation at baseline still needs to be verified by further research.

Secondly, the CEN, also known as the frontal control network, included the DLPFC and the inferior parietal lobe. The cognitive theory of depression has proposed that disconnection in this network led to impaired regulation of emotional processing and continuous depression (Kaiser et al., 2015). Extensive damage of the connections in the frontal parietal regions was detected in executive control and behavioral monitoring (Miller et al., 2001). Our study found that the connections of

![Fig. 1.](image1)

**Fig. 1.** a Brain regions showing significant differences in amplitude of low frequency fluctuation (ALFF) between the MDD group and the normal control group at baseline. The warm color denotes the region where ALFF in the MDD group is higher than that in the control group. This region is located in the orbital frontal cortex (OFC). The cool color denotes the regions where the ALFF in the MDD group is lower than that in the control group. The regions are located in the inferior parietal lobe (IPL) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

![Fig. 2.](image2)

**Fig. 2.** a The MDD group shows abnormal ALFF compared to the control group in the central executive network (CEN), default mode network (DMN) and salience network (SN), without significant differences. Error bars indicate standard deviations across subjects. The between-group differences were computed by using two-sample t-tests. b The MDD group shows abnormal functional connectivity density (FCD) compared to the control group in the central executive network (CEN), default mode network (DMN) and salience network (SN). Error bars indicate standard deviations across subjects. The between-group differences were computed by using two-sample t-tests. The MDD patients showed significantly lower FCD in CEN than the HC patients (p = 0.0002). c The correlation analysis between zFCD values in the CEN and HAMA scores at baseline using Pearson’s correlation is shown. The zFCD values in CEN were negatively correlated with HAMA scores at baseline (r = −0.41, p = 0.03).
the whole CEN measured by FCD values were weakened in the depressed patients compared to the controls. Previous studies on the CEN generally found that the connections between the DLPFC and other regions were also weakened (Ye et al., 2012; Liston et al., 2014; Alexopoulos et al., 2012). After rTMS treatment, the lower FCD value in the CEN at baseline was significantly improved. The left DLPFC, as a direct stimulus target of rTMS, as an important node in the CEN, in which the FCD was also significantly increased after rTMS treatment in this study. Therefore, we speculate that the influence of rTMS on the CEN might be induced by the activated function in the left DLPFC. Although there were no significant changes in ALFF or FCD of all the nodes in the DMN and SN, we suspect that the functional connections of the 3 networks might have changed through the obvious rTMS-induced increased CEN function. A recent rTMS study demonstrated that the CEN might play a role in the clinical improvement produced by rTMS for depression by mediating structural networks but not functional networks (Ge et al., 2019), which supports the importance of CEN, but the exact mechanism still needs further verification in the future. How rTMS exerts its modulatory effects by stimulating the left DLPFC is beyond the scope of this article. Moreover, we didn’t find any correlation between the neuroimaging changes and changes of clinical symptoms, it cannot be concluded that these changes in left DLPFC and CEN are the therapeutic mechanism of rTMS.

In the baseline comparison between the depression group and the control group, we found that ALFF was decreased in the left striatal cortex and medial prefrontal cortex while was increased in the right orbital frontal cortex. The medial prefrontal and striatal cortices are also important components of the DMN and SN, respectively, and are both closely related to depression. However, decreased ALFF in these two areas had not been reported, and even opposite result in some previous reports (Liu et al., 2014; Zhang et al., 2016b; Wang et al., 2012). The possible explanation is that the subjects selected in the previous studies were from different samples, either all male or all female or with first-onset depression. In addition, the sample size of these studies (including ours) was small, with only 30 patients in the largest, which might have affected the stability of the results.

Recently, some studies have explored changes of FCD in depression, and the findings have mainly focused on the prefrontal lobe and cingulate gyrus, but the directions of these changes were opposite. Most studies found decreased FCD in the frontal lobe, especially the medial prefrontal lobe (Murrough et al., 2016; Abdallah et al., 2017), while a few studies found increased FCD in the frontal lobe (Zhang et al., 2016). In addition, some studies found decreased FCD in the cingulate (Zhang et al., 2016; Murrough et al., 2016), while others found increased FCD in the cingulate (Abdallah et al., 2017). Our study further supports the abnormal function in the ACC and OFC, with increased FCD in the patients with MDD. However, the contradictory directions of these findings have made it somewhat confusing to interpret, and the
stability of FCD should be verified in future studies. Notably, our correlation analyses demonstrated that only FCD of the CEN correlated negatively with HAMA scale scores at baseline in MDD group. Our result was consistent with previous studies which revealed that individuals with high suicide or an anxiety disorder have decreased functioning of the CEN (Sylvester et al., 2012). Furthermore, other study showed that the CEN correlated negatively with the Beck Depression Inventory-II (BDI) scores in remitted MDD (Dong et al., 2019). This finding thus reflected that the functional impairment of CEN may be related to depressive symptom severity in MDD.

Some limitations of our study should be noted. First, the rescanned sample size was small. Possibly due to time constraints and unbearable MRI noise, only 15 patients were rescanned with fMRI after rTMS treatment. This small sample size might be why we did not find a significant correlation between brain imaging changes and clinical outcomes. Second, we failed to detect the effect of a sham rTMS control versus active rTMS, which would have made it more convincing that these neuroimaging changes as results of rTMS.

5. Conclusion

In conclusion, this study explored the neuroimaging mechanisms of rTMS in patients with MDD using ALFF and FCD based on the whole-brain and the three important brain networks. Our study found that rTMS induced increased regional function in the left DLPFC, the targeted area, and the average functional connectivity density of the CEN. This indicated that the function of the CEN in the left DLPFC, which participates in the CEN as an important node, were probably brain imaging mechanisms of rTMS treatment in patients with MDD. These findings have implications for future research efforts on rTMS as well as for our understanding of the neuroimaging pathophysiology of depression.

Author contributions

L.D. conceived and designed the experiments. H.L. and R.Q.Y prepared the samples and did fMRI for patients. W.Y.D. did rTMS in MDD patients. L.D., A.H.Z., Z.W.Z., Z.X. and Y.S.J.X. participated in interpreting and analyzing the data. A.H.Z. and L.D. wrote the paper.

Author statement

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). She is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from fmridul@126.com.

Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Supplementary materials

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References


