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Depression and metabolic connectivity: insights into the locus coeruleus, HF-rTMS, and anxiety

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The use of repetitive Transcranial Magnetic Stimulation (rTMS) in treating major depressive disorder (MDD) is increasingly being explored in precision medicine. However, there's a notable lack of understanding of the underlying neurobiological effects, which limits our ability to correlate specific imaging features with treatment efficacy. As one possible neurobiological mechanism, clinical research has already shown that in MDD, lower norepinephrine release in the locus coeruleus (LC) triggers depressive symptoms, and pharmacological approaches that block norepinephrine reuptake boost its levels, easing depression. Surprisingly, the LC has not received a more pronounced focus in contemporary rTMS research. This study investigates the role of the LC in MDD and its response to high-frequency (HF)-rTMS using ^{18}FDG-PET imaging. We compared LC metabolic connectivity between MDD patients (n = 43) and healthy controls (n = 32). Additionally, we evaluated the predictive value of LC connectivity for HF-rTMS treatment outcomes and examined post-treatment changes in LC metabolic connectivity. Our findings revealed significant differences in LC metabolic connectivity between MDD patients and controls. Baseline LC metabolic connectivity did not predict HF-rTMS treatment outcomes. However, post-treatment analyses showed a significant correlation between improved clinical outcomes and attenuation of LC metabolic connectivity in regions associated with cognitive control and the default mode network. Notably, a reduction in state anxiety moderated this relationship, highlighting the role of anxiety in HF-rTMS efficacy for MDD treatment. Our findings suggest that LC metabolic connectivity, influenced by state anxiety levels, may be crucial in HF-rTMS efficacy, offering further insights for personalized MDD treatment strategies.

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INTRODUCTION

Repetitive Transcranial Magnetic Stimulation (rTMS), an evidence-based non-invasive brain stimulation (NIBS) therapy for Major Depressive Disorder (MDD), has exhibited consistent clinical efficacy over the past three decades [1]. However, despite its well-established clinical benefits, and certainly for high-frequency (HF) rTMS applied to the left dorsolateral prefrontal cortex (DLPFC), our understanding of the neurobiological mechanisms underlying its mood-improving effects has not undergone substantial expansion [2]. Moreover, the refinement and personalization of rTMS treatment protocols for individual MDD patients can significantly benefit from a better understanding of these mechanisms [3]. The beneficial effects of rTMS treatment on clinical outcomes can be attributed to complex neurobiological alterations, involving modifications in various neurotransmitter systems such as serotonin and dopamine, as well as changes in neurometabolites like glutamate and GABA [4, 5]. However, thus far, none of these components have been definitively established as the primary mechanism of action [6].

Besides the known involvement of other neurotransmitter systems, research in pharmacotherapy indicates that diminished norepinephrine release from the locus coeruleus (LC) in patients with MDD can precipitate hallmark depressive symptoms. These include compromised energy levels, attenuated motivation, and impaired concentration [7]. Chronic exposure to stressors, coupled with elevated activity in the LC-norepinephrine pathway, is known to amplify anxiety and accentuate depressive manifestations [8, 9]. Using a chemo-connectomics approach in mice it was shown that LC activation leads to rapid and specific reconfiguration of large-scale brain networks, relevance for psychiatric and stress-related disorders [10]. Disruptions in norepinephrine transmission from the LC have indeed been implicated in various stress-induced disorders, encompassing both anxiety and MDD [11]. Pharmaceutical interventions, specifically drugs that inhibit norepinephrine reuptake, are tailored to enhance synaptic norepinephrine concentrations, thereby ameliorating depressive symptomatology [12]. Certain classes of antidepressants, such as norepinephrine reuptake inhibitors (NRIs) and selected tricyclic antidepressants (TCAs), exert their therapeutic effects by acting on the LC. This action augments norepinephrine availability in neural circuits, mitigating depressive manifestations [13].

Despite the pivotal role of the LC in stress-related disorders such as MDD, and its intricate connections with numerous cortical and subcortical brain regions, including the prefrontal cortex and subcortical structures, it is noteworthy that the role of the noradrenergic system, particularly the LC, has not received a more

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pronounced focus in contemporary rTMS research. The existing literature has explored the role of the LC in modulating arousal, attention, and stress response [8, 9], but research specifically addressing the metabolic connectivity of the LC remains sparse. Recent studies have begun to uncover the metabolic underpinnings of LC function [14, 15], but a comprehensive understanding is still lacking. Nevertheless, focusing on the metabolic connectivity of the LC is important for several reasons in depression research, as the LC's extensive network of projections means that its metabolic health can significantly influence multiple brain regions [16]. Furthermore, the LC is involved in critical functions such as stress response, anxiety, and cognitive control, which are metabolically demanding [17].

Recent predictive brain imaging models suggest that "anxiety-related symptoms" in depressed patients may influence the outcome of (HF)rTMS treatment [18]. These MRI-based functional connectivity studies suggest that targeting a more dorsal region of the left DLPFC with HF rTMS may be more effective in patients with anxiosomatic symptoms. Anxiosomatic symptoms here include symptoms such as irritability/worry, sexual disinterest, and insomnia. Given this and the fact that our chosen left DLPFC target is also dorsally located, there is preliminary evidence linking stimulation of this area to inhibitory mechanisms, both in behavior [19] and neurochemicals such as γ-aminobutyric acid [20].

Taking these factors into account, this study employed (F-18) fluorodeoxyglucose positron emission tomography (18FDG-PET) to assess locus coeruleus (LC) seed-based metabolic connectivity. As a proxy for glucose metabolism, ^{18}FDG-PET has been highly effective in predicting and detecting metabolic changes across various antidepressant treatments [21], making it well-suited for examining LC metabolic connectivity and its alterations in MDD patients. Metabolic connectivity was computed using the glucose metabolism (CMRglc) maps, a methodology previously utilized in our former CMRglc studies [22–24], alongside arterial spin labeling perfusion data [19].

Our first focus was to characterize the metabolic nature of LC metabolic connectivity by comparing a well-defined depressed sample with a never-depressed healthy control sample. Given the established role of the LC in MDD, we hypothesized that depressed individuals would exhibit heightened LC metabolic connectivity, particularly in stress-related regions, compared to healthy controls. Second, we expected baseline LC metabolic connectivity to predict clinical response to HF-rTMS treatment. Given that previous brain imaging models have suggested anxiety may influence clinical outcomes [18], and considering that our left DLPFC stimulation target is located more dorsally and has shown positive effects on behavioral inhibition [19], we hypothesized that higher anxiety levels might modulate the predictive value of baseline LC connectivity. Third, we anticipated that post-treatment reductions in LC metabolic connectivity would be associated with clinical improvement; Additionally, given the fact that the stimulation target was located more dorsally within the left DLPFC, we hypothesized that anxiety attenuation would moderate the relationship between post-treatment changes in LC metabolic connectivity and clinical improvement.

MATERIAL AND METHODS

Participants

Forty-three patients with MDD (Female:Male = 28:15; age = 47.88 y, sd = 10.78) with a diagnosis confirmed by the Dutch version of the Mini-International Neuropsychiatric Interview (MINI [25]) were included. All patients were considered to be at least stage III treatment-resistant (this means that all patients undergone at least two unsuccessful treatment attempts with serotonin reuptake inhibitors/noradrenaline and serotonin reuptake inhibitors (SSRI/NSRI) and one unsuccessful trial with a tricyclic antidepressant [26]). Patients with a neurological disorder, a history of bipolarity, psychosis, substance dependence, and a suicide attempt within six months prior to HF-rTMS treatment were excluded. All patients were off antidepressants, antipsychotics, and mood stabilizers for at least two weeks before the ^{18}FDG-PET scan. Depression severity was assessed before and after HF-rTMS treatment using the 17-item Hamilton Depression Rating Scale (HDRS [27]) by a licensed psychiatrist unaffiliated with the study. Thirty-two medication-free healthy volunteers (Female:Male = 18:14; age = 38.81 y, sd = 13.18) without any history of any psychiatric disorder (screened with the MINI) and with a score of less than thirteen on the 21-item Beck Depression Inventory (BDI-II [28]) were included. To examine the influence of state and trait anxiety on LC metabolic connectivity patterns, all participants were assessed using the Dutch version of the State-Trait Anxiety Inventory for adults (STAI) [29]. Detailed information regarding means and standard deviations can be found in Table 1.

All participants gave written informed consent, and the study was approved by the ethics committee of our university hospital (UZBrussel). This study was part of a larger project investigating neurobiological markers of HF-rTMS in MDD. Of note, this sample consists of two different cohorts of MDD patients with the same inclusion and exclusion criteria, but treated with a different HF-rTMS protocol, both applied to the left DLPFC under MRI guidance, stimulating the same anatomical site. Baseline ^{18}FDG-PET scans were also used to predict temperament dimension based on brainstem baseline metabolic activity [15]. Resting-state fMRI data was employed to investigate functional connectivity patterns in accelerated HF-rTMS [30, 31].

HF-rTMS treatment protocols. We used a Magstim Super Rapid magnetic stimulator (Magstim Company Limited, Minnesota, USA) connected to an eight-shaped coil of 70 mm diameter. The individual resting motor threshold (rMT) of the right abductor pollicis brevis muscle was determined by electromyography and set to a stimulation intensity of 110% rMT. The precise stimulation site was determined using three-dimensional magnetic resonance imaging (3D-MRI), with the central part of the mid-prefrontal gyrus marked as the left DLPFC target [32], with the corresponding MNI coordinates (−45, 30, 31) [22]. The coil was held tangentially to the skull with the coil handle pointing 45° antero-medially.

Table 1. Demographics.

Group	Control group	All patients	*p*-value	Group 1	Group 2	*p*-value
Gender (F:M ratio)	18:14	28:15	0.44	14:9	14:6	0.61
Age (years)	38.81 (13.18)	47.88 (10.78)	<0.01	47.35 (9.24)	48.50 (12.54)	0.73
BDI baseline	3.53 (3.49)	33.95 (10.98)	<0.01	33.48 (11.75)	34.50 (10.31)	0.77
HDRS baseline	-	25.19 (5.13)	<0.01	25.17 (3.73)	25.20 (6.49)	0.86
Percent Change HDRS (%)	-	31.75 (33.77)	-	34.65 (30.17)	28.41 (38.02)	0.55
Duration current depressive episode (years)	-	6.23 (6.40)	-	4.98 (5.52)	7.68 (7.15)	0.17
Benzodiazepine dose (mg/day)	-	13.55 (19.09)	-	12.54 (22.99)	14.70 (13.83)	0.72
STAI state	32.65 (8.71)	60.42 (9.88)	<0.01	65.95 (9.52)	54.28 (5.93)	<0.01
STAI trait	33.74 (7.54)	62.92 (8.20)	<0.01	64.30 (8.19)	61.39 (8.17)	0.28
Percent Change STAI state (%)	-	8.38 (19.95)	-	14.24 (16.48)	−2.27 (22.01)	0.02
Percent Change STAI trait (%)	-	7.61 (18.94)	-	8.66 (17.69)	5.52 (22.09)	0.68

Patients were unaware of the type of stimulation; they wore earplugs and were blindfolded.

Twenty-four patients were included in group 1 and received open-label HF-rTMS treatment using a high-frequency (10 Hz) stimulation protocol [33]. Ten HF-rTMS sessions, one session per day, were administered over two weeks. Patients received forty trains of HF-rTMS of 3.9 s duration separated by an interval of 26.1 s, or 1560 pulses per session. Pre and post-HF-rTMS treatment 18FDG-PET scans were collected.

In group 2, nineteen patients were treated with an accelerated HF-rTMS (20 Hz) crossover protocol, with a total of 20 HF-rTMS sessions distributed over 4 days (5 sessions per day) [34]. Patients received forty trains of 1.9 s each, separated by a 12 s interval (1560 pulses per session). Here, patients were randomized for active or sham stimulation by flipping a coin. As such, patients initially received active HF-rTMS during the first week, followed by a sham treatment in the subsequent week, or vice versa. However, for this group, we only took the baseline and the final ^{18}FDG-PET scan into account.

Brain imaging

LC region of interest. Located in the brainstem, the LC is a small but crucial structure consisting of a cluster of densely packed noradrenergic neurons and is positioned in the dorsal part of the rostral pons near the floor of the fourth ventricle [35, 36]. The LC was identified using the Harvard Ascending Arousal Network Atlas (available at https://www.nmr.mgh.harvard.edu/resources/aan-atlas; see also Fig. 2C).

^{18}FDG-PET scans. All participants underwent a static ^{18}FDG-PET baseline scan using an Ecat Acell (Siemens) scanner 30 min after tracer administration, following an intravenous injection of 222 MBq ^{18}FDG. As previously described [37], emission data were acquired in three-dimensional mode over a ten-min period. Each scan was then spatially normalized to the Montreal Neurological Institute (MNI) space using the unified segmentation algorithm in SPM12 (Statistical Parametric Mapping, Welcome Department of Imaging Neuroscience, University College London, UK), and subsequently projected to fsaverage space [38].

LC seed-based covariance connectivity analysis. The individual LC seed-based covariance connectivity was calculated using the CMRglc map, a technique previously used in CMRglc studies [22–24], and arterial spin labeling perfusion data [19]. This method quantifies the similarity between the seed region and a 10 mm FWHM (full width at half maximum) neighborhood surrounding each vertex in the single CMRglc map of an individual subject. The covariance connectivity (CC) between regions *i*-th and *j*-th is defined by

$$CC(i,j) = e^{\frac{-\left(m_i - m_j\right)^2}{2\left(\sigma_i^2 + \sigma_j^2\right)}} \quad (1)$$

where m and σ denote the mean and standard deviation of the regional glucose metabolism, respectively. Of note, the weighted mean and standard deviation were calculated for the vertex neighborhood cluster, with the weight being proportional to the standard normal probability density function, as initially employed in a previous study [39]. Matlab code for seed-based covariance connectivity analysis will be made available within the rsHRF toolbox [40].

Statistical analysis

Demographic and clinical data were analyzed with SPSS 27 (IBM, Statistical Package for the Social Sciences, Chicago, IL, USA), using *t*-tests, Pearson χ^2, and Pearson correlation analyses where appropriate. The significance level was set at $p \leq 0.05$, two-tailed for all analyses.

Independent *t* -test was implemented in SPM12 to compare the baseline group differences in LC metabolic covariance connectivity strength between MDD patients and healthy controls, with age and sex as covariates. Multiple regressions were further performed to explore the relationship between baseline LC metabolic covariance connectivity and clinical improvement (percent changes: $(Baseline - PostrTMS)/Baseline$), as well as the relationship between changes in LC metabolic connectivity (percent changes: $(Baseline - PostrTMS)/Baseline$) and clinical improvement, with age, sex, and HF-rTMS treatment protocols as covariates. Additionally, we examined the moderating effect of trait/state anxiety on the correlations between LC metabolic connectivity (changes) and clinical improvement. We considered LC connectivity (changes) as the independent variable and clinical improvement as the dependent variable, with age, sex, and HF-rTMS treatment protocols as covariates. We applied a cluster-forming threshold defined by uncorrected vertex-wise *p*-values < 0.001, and we determined a cluster-extent threshold (k) using random field theory to maintain a controlled significance level of $q < 0.05$, accounting for the False Discovery Rate (FDR).

We also aimed to determine if the metabolic connectivity differences in the LC between individuals with MDD and those without depression align with the distribution of noradrenergic neurotransmitter systems. To do this, we utilized the neuromaps toolbox [41] to assess if the differences in LC metabolic connectivity we observed were due to general noradrenergic activity [42]. In line with earlier findings [22], we also examined whether there was an overlap between the changes in LC metabolic connectivity and the distribution of the electric field (E-field) as it may relate to clinical outcome. Due to the unavailability of MRI data for all participants (i.e., Group 1), we employed SimNIBS software (version 3.2.6) to simulate the E-field distribution on MNI152 mesh for all participants (see Fig. 2C). As before, the center of the coil was precisely positioned over the targeted left dorsolateral prefrontal stimulation site (MNI coordinates = −45, 30, 31). The stimulation parameters for simulation were set to dI/dt = 1 A/ms, with a coil orientation of 45° to the midline and a coil-to-scalp distance of 4 mm, using a Magstim 70-mm figure-of-eight coil.

Finally, we used the BrainStat toolbox [43] to conduct meta-analytic decoding with precomputed Neurosynth maps in order to investigate the relationship between meta-analytic cognitive terms and LC metabolic connectivity group difference t-statistic maps (considering only significant clusters), the correlation maps between (changes in) LC metabolic connectivity and clinical improvement, as well as interaction (moderation analysis), effects maps. The statistical significance was assessed using a generative null model [44] and adjusted for multiple comparisons with the Benjamini–Hochberg FDR method (q<0.05).

RESULTS

Behavioral results

When comparing the healthy control group to the MDD group (consisting of MDD groups 1 and 2) there were no differences in sex distribution, although the control group was significantly younger than the MDD group. As expected, MDD patients scored significantly higher on the HDRS and BDI-II at baseline. MDD patients also scored significantly higher than the control group on the State-Trait Anxiety Inventory (STAI) state and trait questionnaires. No significant demographic differences were observed between the two MDD groups. Although baseline STAI state scores were significantly lower for group 2, no differences in baseline HDRS and BDI-II scores or clinical outcome results (percent changes: $(Baseline - PostrTMS)/Baseline$) were observed between both MDD groups. The average scores on the HDRS showed a substantial reduction from their initial baseline. By the end of the study, the average score was 16.95, with a standard deviation of 8.56. This change was statistically significant, as shown by a *t*-value of 6.29 and a *p*-value of less than 0.01. The effect size, measured by Cohen's dav, was large at 1.21, indicating a reduction in HDRS scores by approximately 31.75% from the starting point. See also Table 1.

LC seed-based covariance connectivity results

MDD vs healthy controls. According to the visual data in Fig. 1A and the statistical details in Table 2, we found that LC metabolic connectivity was significantly greater in MDD patients, especially noted in the prefrontal cortical regions and the right superior parietal gyrus. Furthermore, LC metabolic connectivity was significantly lower in the right postcentral gyrus when comparing MDD patients to the control group. When examining the influence of anxiety, as measured by the STAI, no significant clusters indicating an effect on LC metabolic connectivity patterns were found. This suggests that, within our study parameters, anxiety did not significantly alter LC connectivity patterns.

Our analysis did not reveal a significant spatial correlation between the map of group differences and the noradrenergic distribution map (r= 0.04, Spin permutation test [45] $p_{spin} = 0.35$),

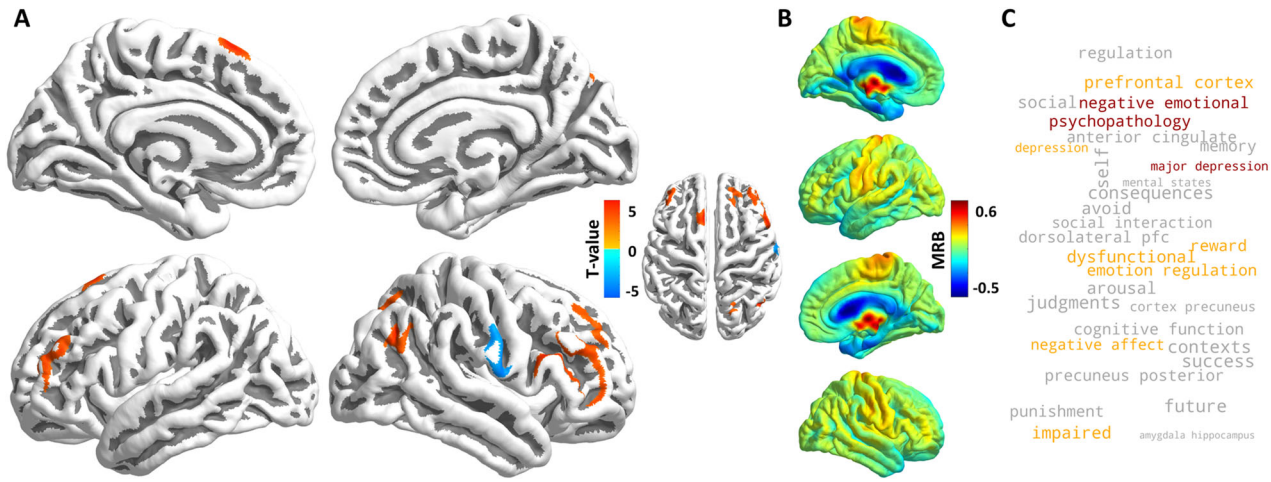

Fig. 1 Locus coeruleus metabolic connectivity in major depressive disorder (MDD) compared to healthy controls. A 3D brain renderings illustrate the regions of increased locus coeruleus (LC) metabolic connectivity in patients with Major Depressive Disorder (MDD) relative to healthy controls. The renderings show multiple perspectives: lateral (top left and bottom right), medial (bottom left), and superior (top right) views of the brain. Areas with significant positive T-value are marked in warmer colors (orange to red spectrum), with the most intense areas indicating the highest level of connectivity difference. The prefrontal cortical areas along with the right superior parietal gyrus exhibit pronounced increases in LC connectivity in MDD patients. Areas with significant negative T-value (MDD < HC) are marked in cold colors (blue), here with the right postcentral gyrus. For detailed statistical analysis of cluster size and location, we refer to Table 2. **B** Visualization of the noradrenergic density distribution map (MRB, Ding et al. [42]; obtained from the neuromaps toolbox). **C** Word cloud of cognitive functions associated with brain regions that exhibited altered LC metabolic connectivity in MDD (**A**). The font size of a given cognitive term corresponds to the correlation coefficient (*r*) of the thresholded group difference T-value map and meta-analytic map of that term generated by Neurosynth. All correlations are significant (FDR $q < 0.05$).

Table 2. Locus coeruleus metabolic connectivity in major depressive disorder (MDD) compared to healthy controls (HC).

	Cluster size (#vertices)	Anatomical region	Hemisphere	T-value	Peak MNI coordinates (x,y,z) (mm)
MDD > HC	500	Middle frontal gyrus	Left	4.34	−40, 50, 17
	293	Superior frontal gyrus	Left	5.36	−3, 23, 63
	743	Middle frontal gyrus	Right	4.34	41, 44, 27
	346	Superior frontal gyrus	Right	3.81	21, 51, 36
	406	Superior parietal gyrus	Right	3.83	20, −67, 51
HC > MDD	413	Postcentral gyrus	Right	3.21	63, −11, 37

indicating that the differences in LC metabolic connectivity we found could be more specific to the condition and not simply a reflection of widespread noradrenergic activity in the brain (see Fig. 1B).

We found that regions exhibiting modified LC metabolic connectivity in MDD were correlated with several meta-analytic cognitive terms mainly involved in emotional (de)regulation processes (Fig. 1C).

Predictive value LC metabolic connectivity to HF-rTMS treatment outcome in MDD. Due to the unavailability of follow-up ^{18}FDG-PET scans and the loss of some STAI questionnaires required for the moderation analysis, for this part of the analysis, the final sample size was reduced to 39 MDD patients.

We initially theorized that a higher baseline level of metabolic activity in the LC would be associated with a more favorable response to HF-rTMS targeting the left DLPFC. Contrary to our expectations, our research did not uncover any significant patterns of connectivity that supported this theory. Furthermore, including baseline anxiety levels (assessed through both state and trait anxiety scales of the STAI) as a potential influencing factor did not reveal any significant impact on LC metabolic connectivity.

HF-rTMS antidepressant treatment effects on LC metabolic connectivity. Our research found a significant positive correlation between changes in LC metabolic connectivity $((Baseline - PostrTMS)/Baseline$ and clinical improvement $((Baseline - PostrTMS)/Baseline)$, as measured by the HDRS, following HF-rTMS treatment. This means that those MDD patients with a stronger decrease in LC metabolic connectivity following HF-rTMS treatment showed better clinical improvement. These changes were primarily observed in the left-sided regions of the prefrontal, temporal, and parietal cortices. For detailed data and visual representation of these findings, we refer to Table 3 and Fig. 2A.

Our results revealed a significant spatial correlation between the E-field intensity and the LC metabolic connectivity change that corresponded to improved clinical outcomes ($r = 0.24$, $p_{spin} < 0.01$). Patients with MDD whose E-field and LC metabolic connectivity maps overlapped demonstrated better clinical improvements than those without such an overlap. This observation suggests that mapping the E-field distribution at the stimulation site may potentially enhance predictions of patient responses to HF-rTMS treatment.

Moreover, our findings revealed a significant interaction effect between state anxiety and these correlations (see Table 3 and Fig. 2B). Unlike trait anxiety scores from the STAI, which showed no such effect, reductions in state anxiety $((Baseline - PostrTMS)/Baseline$ significantly influenced the positive correlation between LC metabolic connectivity changes and clinical improvement. The moderated hierarchical regression analysis further indicates that the attenuation of LC metabolic

Table 3. Locus coeruleus (LC) metabolic connectivity changes related to clinical improvement following HF-rTMS treatment in major depressive disorder (MDD), and interaction with state anxiety changes.

	Cluster size (#vertices)	Anatomical region	Hemisphere	T-value	Peak MNI coordinates (x,y,z) (mm)
Correlation					
Positive	170	Middle temporal gyrus	Left	3.93	−59, −31, −17
	188	Pars Opercularis	Left	3.83	−53, 17, 10
	291	Supramarginal gyrus	Left	4.15	−58, −45, 24
Negative	No significant clusters emerged				
Interaction					
Positive	138	postcentral gyrus	Left	4.79	−35, −33, 42
	370	supramarginal gyrus	Left	4.80	−37, −39, 42
	258	isthmus cingulate	Right	4.86	2, −46, 31
	224	posterior cingulate	Right	4.80	3, −27, 37
	215	precuneus	Right	4.80	3, −55, 19
Negative	No significant clusters emerged				

connectivity in relation to the higher clinical improvement in the MDD patients with a higher level of decrease in state anxiety (percent changes >9.1%), but not in individuals with a lower level of decrease in state anxiety. This moderation effect was particularly notable in the posterior regions of the cerebrum, including the posterior cingulate cortices and the precuneus. As before, we wanted to investigate if changes in LC metabolic connectivity and clinical outcome, influenced by variations in state anxiety, were associated with the distribution of noradrenergic neurotransmitter systems [42]. Our findings revealed a significant spatial correlation between the map showing how state anxiety changes moderate LC metabolic connectivity in relationship to clinical improvement and the map of noradrenergic system distribution ($r = 0.10$, $p_{spin} = 0.01$). This suggests that the way in which state anxiety influences the changes in LC metabolic connectivity—and in turn, clinical outcomes—could be aligned with the overall distribution of noradrenergic activity in the brain. It also indicates that while changes in anxiety after HF-rTMS treatment may affect the noradrenergic system broadly, the specific areas of influence regarding the moderation by state anxiety are localized to regions in the posterior brain. This points to a targeted rather than a generalized noradrenergic effect on the relationship between LC metabolic connectivity and depressive symptoms moderated by state anxiety improvement as they respond to HF-rTMS. The association analysis with precomputed Neurosynth maps may further indicate a significant correlation with the meta-analytic cognitive terms involved in mental states (Fig. 2D), anxiety, and TMS (Fig. 2E), respectively.

DISCUSSION

In this study, using ^{18}FDG-PET, we conducted a comparison of metabolic connectivity in the LC between patients with MDD and healthy individuals who have never experienced depression. Our comparative analysis between individuals with MDD and healthy controls showed a significant increase in metabolic connectivity between the LC and the bilateral dorsolateral prefrontal cortices, which are key components of the cognitive control network (CCN). The CCN is crucial for managing various cognitive tasks, such as decision-making, working memory, and cognitive flexibility, functions that are essential for adaptive behavior and thought processes [46]. Furthermore, we noted increased LC metabolic connectivity with the superior parietal gyrus, part of the dorsal attention network (DAN). The DAN is involved in directing attention to important stimuli and sustaining focus through "top-down" attention processes [47]. In MDD, difficulties in diverting attention from negative information can lead to persistent negative thinking, a symptom potentially linked to disruptions in the DAN [48]. This hypermetabolic connectivity may reflect a compensatory mechanism aimed at preserving cognitive function, a hypothesis supported by other neuroimaging studies of MDD (e.g., [49]). Conversely, we found a decrease in metabolic connectivity between the LC and the postcentral gyrus in MDD patients. The postcentral gyrus is part of the somatosensory network (SSN), processing bodily sensations like touch, temperature, pain, and stress [50]. Abnormalities in the SSN are associated with impaired pain discrimination and bodily awareness, common complaints in MDD [51]. Importantly, our (spatial) association analysis revealed that the differences in LC metabolic connectivity observed between individuals with MDD and those who have never been depressed are specific to the condition. This finding suggests that these differences are not merely due to general noradrenergic activity throughout the brain, but rather are unique to the disorder itself.

Our investigation into the predictive value of baseline LC metabolic connectivity for clinical outcomes following HF-rTMS treatment showed no significant patterns. This lack of correlation held true even when accounting for individual variations in anxiety levels. Despite the acknowledged role of the LC in mood regulation and stress response, other brain regions, neurotransmitter systems, and pathways may be involved, potentially more suitable for outcome predictions [52]. Based on our findings, we can only conclude that LC metabolic connectivity does not serve as a reliable biomarker for predicting the efficacy of HF-rTMS treatment targeting the left DLPFC. It is important to note that only a few potential - but not robust - brain biomarkers of rTMS outcome have been identified to date [53].

Post-treatment analyses revealed that patients who responded displayed a positive correlation between clinical outcome and metabolic connectivity of the LC with components of both the CCN and the Default Mode Network (DMN). The DMN is typically characterized by hyperactivity and increased connectivity in MDD, while dysregulated activity and connectivity in the CCN are also implicated in the condition [54]. The LC, as the primary source of norepinephrine (NE) in the brain, with extensive axonal connections, may therefore exert significant influence over cognitive functions. This includes attention, working memory, rumination, and cognitive flexibility [55]. Our observations suggest that HF-rTMS when successful may recalibrate the noradrenergic system by aligning LC activity and its projections to relevant cerebral regions, potentially resulting in increased cognitive efficiency, reduced ruminative tendencies, and more skillful emotional regulation. Using an intersectional chemogenetic approach designed to selectively and reliably stimulate tonic LC-NE activity

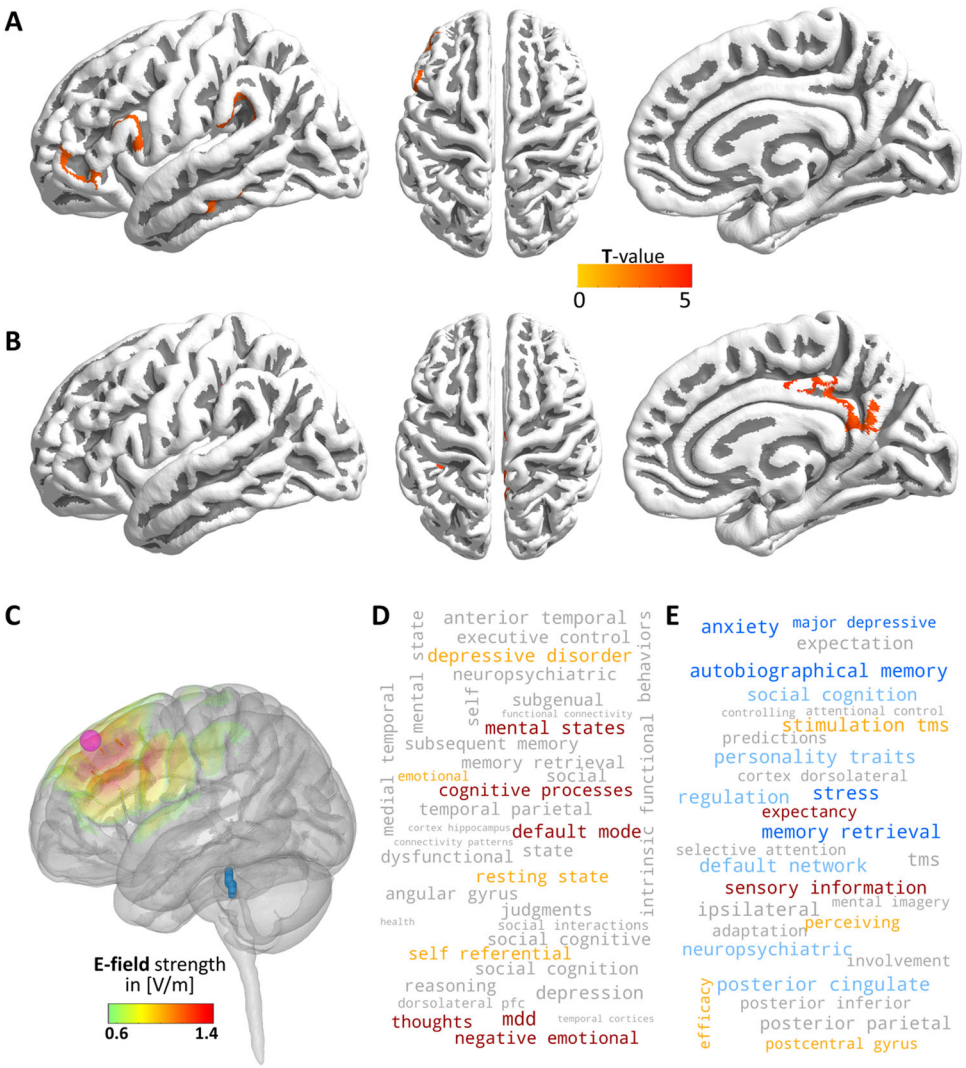

Fig. 2 HF-rTMS antidepressant treatment effects on locus coeruleus metabolic connectivity. A 3D brain renderings illustrate the regions resulting from the changes in LC metabolic connectivity with clinical improvement. Areas with significant positive correlation are marked in red. No significant negative correlation clusters were detected. For detailed statistical analysis of cluster size and location, we refer to Table 3. **B** 3D brain renderings illustrate the regions resulting from the interaction between changes in state anxiety and LC metabolic connectivity that affect clinical improvement. Areas with significant positive interaction are marked in red. No significant negative interaction clusters were detected. For detailed statistical analysis of cluster size and location, we refer to Table 3. **C** Distribution of the electric field norm (displaying only the top 5th percentile value). The coil center (purple dot) was positioned directly above the left prefrontal stimulation site (DLPFC: MNI = 45, 30, 31). Visualization of the Locus Coeruleus in blue. **D** Word cloud of cognitive functions associated with the significant statistical correlation map between changes in LC metabolic connectivity and clinical improvement ($p<0.05$, cluster FDR corrected). **E** Word cloud of cognitive functions associated with statistical map depicted in (**B**). All correlations are significant (FDR $q < 0.05$). Warm/grey colors represent positive correlations, while cold colors indicate negative correlations.

in mice, the study by Oyarzabal et al. revealed substantial changes in DMN connectivity, synchronous low-frequency activity, and glucose uptake [56]. Of interest, patients with MDD whose E-field and LC metabolic connectivity maps overlapped demonstrated better clinical improvements than those without such an overlap. This observation could indicate that mapping the E-field distribution at the stimulation site can potentially enhance predictions of patient responses to HF-rTMS treatment. This finding aligns with recent advancements in rTMS treatment strategies [3].

By incorporating such imaging data, it may become possible to stratify and individualize treatment plans, enhancing their effectiveness for each patient based on their unique neural characteristics [57]. Indeed, Siddiqi et al. proposed that targeting the left DLPFC more anteriorly and laterally could lead to reductions in sadness and symptoms characteristic of melancholia [18]. In contrast, stimulation of the DLPFC in a more dorsal location, which aligns with the target area in our study, seemed to influence 'anxiosomatic' symptoms—physical symptoms often associated with anxiety. Our perfusion data from another accelerated rTMS treatment paradigm also supports this idea [19]. These studies suggest that more dorsally located targets might enhance treatment outcomes in patients with higher anxiety levels, with these effects likely being regulated by the noradrenergic activity of the LC. Corroborating these findings, our current study observed a significant moderating effect of reduced state anxiety on LC metabolic connectivity, which was associated with antidepressant outcomes. Specifically, MDD patients who showed a decrease in state anxiety, as assessed by the STAI state measure, also exhibited attenuated LC metabolic connectivity within the posterior DMN, which is involved in self-referential thinking and rumination, including regions like the isthmus cingulate, posterior cingulate, and precuneus. These areas are

also connected to self-awareness, which can be heightened by anxiety, possibly elucidated by the precuneus' role in self-consciousness [48]. Considering the supramarginal gyrus' involvement in emotional processing and social anxiety, the observed interaction between decreased anxiety and LC metabolic connectivity changes might also shed light on HF-rTMS's beneficial effects. Lastly, while the postcentral gyrus is not traditionally associated with core MDD pathology, our findings suggest that anxiety may significantly modulate the somatosensory abnormalities observed in depression [58]. To date, rTMS has not been established as a primary treatment for anxiety disorders, as noted by Lefaucheur et al. [1]. However, research by Vicario et al. suggests that rTMS may have the potential to lessen the severity of anxiety symptoms [59]. In addition to this, our current observations also point to a targeted rather than a generalized noradrenergic effect on the relationship between LC metabolic connectivity and depressive symptoms moderated by state anxiety improvement as they respond to HF-rTMS. The overarching implication is that the modulation of the noradrenergic system by HF-rTMS appears to have a dual benefit: it not only helps alleviate depressive symptoms but also seems to exert a regulatory effect on the symptoms of anxiety within the context of MDD.

Several limitations warrant a cautious interpretation of the results: (1) The ^{18}FDG-PET technique measures metabolic activity within the LC, which is central to the brain's noradrenergic system. However, this measure does not directly reflect noradrenaline levels. Therefore, while the findings suggest changes in metabolic activity, they cannot conclusively indicate alterations in noradrenaline levels, and as mentioned before, other neurotransmitter systems could also be involved. (2) Ascribing the clinical effects solely to changes in noradrenergic activity would be an oversimplification, given that neurotransmitter systems are involved in MDD pathophysiology and treatment response. (3) The study's conclusions are specific to treatment-resistant MDD and may not generalize to patients with different treatment histories or responses. (4) The study did not differentiate between treatment responders and remitters due to the small sample size, which is typically a key consideration in larger clinical trials. (5) Although we do not have definitive reasons for why frequency differences in the high-frequency range (10 vs. 20 Hz) or differences between daily and accelerated stimulation applications might lead to varying treatment outcomes or metabolic connectivity patterns, we have accounted for these variables in our statistical analyses. Still, the possibility that they influenced our metabolic connectivity findings cannot be entirely dismissed. (6) Finally, as already mentioned in [60], the interpretations of the alteration maps and meta-analytic cognitive terms from the Neurosynth database should be considered as an indirect brain-cognition association in MDD.

In conclusion, our research found significant differences in metabolic connectivity between the locus coeruleus and specific brain regions in MDD patients compared to healthy controls, highlighting the involvement of the LC in depression-related neural dysfunction. While baseline ^{18}FDG-PET imaging of LC connectivity was not predictive of treatment outcome, patients with greater overlap between their E-field and LC connectivity maps showed improved clinical response to HF-rTMS. Furthermore, reductions in state anxiety moderated the association between changes in LC connectivity and clinical improvement. These findings suggest that the integration of ^{18}FDG-PET imaging with personalized E-field modeling and the consideration of anxiety levels may lead to more tailored (HF)rTMS treatment parameters, thereby improving therapeutic efficacy.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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AUTHOR CONTRIBUTIONS

GW: Conceptualization, Methodology, Validation, Formal analysis, Data curation, Writing – original draft, Visualization. CB: Conceptualization, Methodology, Validation, Investigation, Resources, Writing – original draft, Project administration, Funding acquisition.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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