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Altered Transcranial Magnetic Stimulation–Electroencephalographic Markers of Inhibition and Excitation in the Dorsolateral Prefrontal Cortex in Major Depressive Disorder

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ABSTRACT

BACKGROUND: The neurophysiology of major depressive disorder (MDD) has become a particular focus of transcranial magnetic stimulation (TMS) investigational studies. TMS combined with electroencephalography (TMS-EEG) affords a window to directly measure evoked activity from the dorsolateral prefrontal cortex (DLPFC), which is of considerable interest in MDD. Our study examined TMS-EEG responses from the DLPFC in persons with MDD compared with those in healthy participants. Specifically, we examined TMS-EEG markers linked to inhibitory and excitatory neurophysiological processes and their balance.

METHODS: In all, 30 participants with MDD and 30 age- and sex-matched healthy participants underwent single-pulse TMS-EEG to assess inhibition and excitation from DLPFC. TMS-EEG waveforms were analyzed through global mean field amplitude.

RESULTS: MDD participants demonstrated abnormalities in TMS-EEG markers in the DLPFC. Inhibitory measures—N45 and N100—were larger in the MDD group than in healthy participants (N45 [$t = -4.894$, $p < .001$] and N100 [$t = -3.496$, $p = .001$]). In a receiver operating characteristic analysis, N45 amplitude predicted depression illness state with 80% sensitivity, 73.3% specificity, and 76.6% accuracy (area under the curve = 0.829, $p < .001$). The global mean field amplitude area under the curve, a neurophysiological measure of cortical reactivity, was significantly larger in persons with MDD ($t = -3.114$, $p = .003$), as was P60 ($t = -3.260$, $p = .002$). In healthy participants, there was a positive correlation between inhibitory N45 and excitatory global mean field amplitude area under the curve ($r = .711$, $p < .001$) that was not present in persons with MDD ($r = .149$, $p = .43$), demonstrating a potential imbalance between inhibition and excitation in MDD.

CONCLUSIONS: As the TMS-EEG waveform and its components index inhibitory and excitatory activity from the cortex, our results suggest abnormalities in these neurophysiological processes of DLPFC in persons with MDD.

Keywords: EEG, Excitation, GABA, Inhibition, Major depressive disorder, TMS

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Major depressive disorder (MDD) is a psychiatric illness affecting more than 300 million people (1). The World Health Organization lists MDD as the leading cause of disability worldwide (1). The burden is increasing (2), despite numerous treatment and pathophysiological advances over the past several decades. Specifically, the left dorsolateral prefrontal cortex (DLPFC) has been repeatedly linked to MDD pathophysiology (3) in several research modalities, including imaging and lesion studies (4,5), among others. Hypofunction of the left DLPFC in patients with MDD appears evident in functional imaging studies (6,7). Subsequently, the left DLPFC emerged as a viable target for repetitive transcranial magnetic stimulation (TMS) treatment, which is used at high frequencies to activate the DLPFC in MDD (8,9). Various neurochemical and pathophysiological abnormalities in MDD have been reported to date, which only highlights the urgent need for a clearer understanding of the neuropathology of MDD.

Glutamate, the most abundant and wide-reaching excitatory neurotransmitter, and altered glutamatergic neurotransmission have been repeatedly implicated in the pathophysiology of MDD. Glutamate plays key roles in cognition, synaptic plasticity, and facilitation of neurotrophic factor production (10–12). Decreases in glutamate concentration have been reported in patients with severe MDD (13) and unmedicated patients with moderate MDD (14). Moreover, the administration of the *N*-methyl-D-aspartate antagonist ketamine (15,16) rapidly improves symptoms. These data suggest that glutamate plays a considerable role in MDD pathophysiology and that

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neurophysiological measures approximating glutamatergic activity in MDD may prove useful as biomarkers (17–19).

Conversely, the inhibitory gamma-aminobutyric acid (GABA) system has also become a particular focus of investigation in MDD (20–22). GABA levels are significantly lower in MDD patients than in healthy subjects (20). In postmortem samples, Rajkowska *et al.* (23) examined patients with MDD and found decreased density and size of GABAergic interneurons in the DLPFC compared with those in healthy subjects. Patients with treatment-resistant depression appeared to have more profound GABA deficits, evidenced by magnetic resonance spectroscopy studies of the cortex (14,24).

TMS can be used to measure cortical inhibition and excitation. Short-interval intracortical inhibition (SICI) is a paired-stimulus motor condition linked to $GABA_A$ receptors (25). The cortical silent period (CSP), an inhibitory single-pulse motor TMS paradigm, has been closely associated with $GABA_B$ receptor–mediated neurotransmission (26–28). The resting motor threshold (RMT) is decreased by ketamine administration (29), reflecting an increase in cortical excitation. Intracortical facilitation (ICF) is a paired TMS stimulus condition linked to *N*-methyl-D-aspartate receptor–mediated neurotransmission (30–32). Both SICI and ICF paradigms can be applied in motor and nonmotor regions. Out of the above-mentioned TMS measures, CSP and SICI have been used to index inhibition in MDD, while RMT and ICF have been used to index excitation. Levinson *et al.* (33) demonstrated CSP deficits in MDD, and more extensive inhibitory deficits (CSP and SICI) in treatment-resistant depression, reinforcing that MDD is associated with aberrant GABAergic function—particularly in those with more severe illness. By contrast, there were no differences in RMT or ICF. However, Croarkin *et al.* (34) reported that adolescent MDD was associated with increased ICF but not SICI or CSP. Taken together, these findings suggest both inhibitory and excitatory abnormalities in MDD.

The motor cortex is not intuitively associated with MDD pathophysiology. The combination of TMS with electroencephalography (TMS-EEG) has afforded investigations of neurophysiological indices in brain regions more closely associated with MDD (e.g., DLPFC). TMS-EEG evokes a variety of cortical responses that were initially characterized in the motor cortex, including N45, P60, and N100. Previous pharmacological studies following motor cortex stimulation demonstrated that administration of alprazolam and zolpidem (modulators specific to the $GABA_A$ receptor) increase the N45 deflection, potentially linking N45 with $GABA_A$ inhibitory activity (35). While the origins of the P60 component have not been well elucidated, it is enhanced during ICF and negatively correlated with inhibitory SICI (36), suggesting a potential relationship with glutamatergic activity. The N100 component, a large negative deflection recorded 80 to 120 ms after TMS pulse, increases with coadministration of baclofen, a specific $GABA_B$ receptor agonist, while baclofen appeared to have no effect on earlier negative components of the TMS-evoked potential (35). Lastly, the area under the TMS-induced waveform can be assessed as a whole using the measure global mean field amplitude (GMFA) (37). Partial or local mean field power has previously been utilized by Pellicciari *et al.* (38) to assess motor cortical excitability in healthy participants. GMFA area under the TMS-EEG curve (GMFA-AUC) represents overall brain activity induced by TMS and therefore may be interpreted as an index of cortical reactivity or excitation (39,40). Individual TMS-EEG components (positive and negative) and the TMS-EEG waveform as a whole have only recently been studied in this manner and therefore should be cautiously interpreted as such. There is more clear evidence that N45 and N100 components are associated with inhibitory neuronal processes (35). Regarding positive components and the waveform as a whole, while the evidence to date reinforces the above interpretations (39,40), further investigation is required to more definitively establish that the P60 and GMFA-AUC represent excitation of the cortex.

To our knowledge, there have been no previous investigations evaluating TMS-EEG measures of inhibition and excitation from DLPFC in patients with MDD in comparison with those in healthy subjects. Understanding these differences is a topical area of investigation in relation to both identifying neurophysiological mechanisms of illness and treatment response (41). Therefore, in this study, we aimed to examine differences in TMS-EEG measures of inhibition and excitation from the DLPFC in individuals with MDD and healthy subjects. We hypothesized that in individuals with MDD, TMS-EEG neurophysiological measures of inhibition and excitation in the DLPFC would be significantly altered. Specifically, given previous TMS-electromyography investigations into cortical inhibition in MDD (33), and the hypofrontality observed in MDD (3), we hypothesized that TMS-EEG measures of cortical activity would demonstrate deficits in both inhibition (N45 and N100) and excitation (P60 and GMFA-AUC).

METHODS AND MATERIALS

Recruitment

Sixty participants (30 with MDD and currently experiencing a depressive episode, and 30 sex-matched healthy individuals age-matched to within 2.1 years) were recruited at the Centre for Addiction and Mental Health in Toronto, Ontario, Canada. The group of individuals with MDD was composed of a subset of participants in a repetitive-TMS treatment trial (clinicaltrials.gov identifier NCT01515215, https://clinicaltrials.gov/ct2/show/NCT01515215?id=NCT01515215&rank=1) who received TMS-EEG as part of baseline assessments prior to entering treatment arms. All participants were right-handed. All participants gave written informed consent, and the protocol was approved by the Centre for Addiction and Mental Health in accordance with the Declaration of Helsinki. The Structured Clinical Interview for the DSM-IV (42) confirmed the diagnosis of MDD, and a score of >20 on the 17-item Hamilton Depression Rating Scale (HDRS-17) confirmed an active major depressive episode (43). In healthy participants, the Structured Clinical Interview for the DSM-IV (42) ruled out psychopathology. Participants were excluded if they had a history of DSM-IV substance dependence in the previous 6 months (excluding nicotine) or DSM-IV substance abuse in the previous month; met DSM-IV criteria for borderline or antisocial personality disorder with the Structured Clinical Interview for DSM-IV Axis II Disorders (44); had a significant unstable medical illness or history of seizures; or were taking >2 mg lorazepam daily (or equivalent) during the previous 4 weeks. The Antidepressant

Treatment History Form (ATHF) (45) was performed in the MDD group to assess levels of treatment resistance in the current episode.

Transcranial Magnetic Stimulation

Monophasic TMS pulses were administered over the left motor cortex and DLPFC using a 7-cm figure-of-eight coil and two Magstim 200 stimulators connected via a BiStim module (Magstim Company Ltd., Whitland, United Kingdom). To localize the motor cortex, the coil was placed between electrodes FC3 and C3, in the optimal position for eliciting motor-evoked potentials (MEPs) from the right abductor pollicis brevis. To identify the optimal location of DLPFC, neuronavigation was performed with the miniBIRD system (Ascension Technologies, St. Louis, MO) (46). Stimulation was directed at the junction of the middle and anterior one third of the middle frontal gyrus [Talairach coordinates (x, y, z) = −50, 30, 36] corresponding with posterior regions of Brodmann area 9, overlapping with the superior section of Brodmann area 46. In both regions, the optimal position was marked on the EEG cap with a felt pen, to ensure consistent coil placement, with the handle of the coil pointed backward, perpendicularly to the presumed direction of the central sulcus, 45° to the midsagittal line.

Resting Motor Threshold

At the beginning of each experiment, RMT was determined by applying single pulses of TMS to this optimal position in the motor cortex. RMT was defined as the minimum stimulus intensity required to elicit an MEP of $>50\mu V$ in ≥ 5 of 10 trials (47) and was determined once prior to positioning the EEG cap and once after.

EEG Recording and Analysis

EEG was performed using a 64-channel SynAmps 2 EEG system (Compumedics Neuroscan, Melbourne, Victoria, Australia). All electrode (silver/silver chloride ring electrodes) impedance was lowered to ≤ 5 kΩ. Electrodes were referenced to an electrode positioned posterior to the Cz electrode. In addition, four electrodes were placed on the outer corner of each eye, as well as above and below the left eye, to assess any eye movement artifact. For each subject, we determined stimulus intensity that elicited a mean peak-to-peak MEP amplitude of 1 mV in 20 trials. EEG signals were recorded using direct current and a low-pass filter, an anti-aliasing filter of 200 Hz, at 20 kHz sampling rate, which had been shown previously to avoid saturation of amplifiers and minimize TMS-related artifact (48). Analyses of EEG data were performed using EEGLAB (49), FieldTrip (50), and custom scripts in MATLAB (R2015a; The MathWorks, Inc., Natick, MA). Data were epoched around the TMS pulse (−1000 to 1000 ms) and were baseline corrected (−500 to −200 ms), and data around the TMS pulse (−2 to 20 ms) were removed and linearly interpolated. Data were downsampled and visually inspected for extreme noise (e.g., muscle movements, bad electrodes) (51). A first round of independent component analysis (fastICA with "tanh" function) was applied to detect and remove TMS tails and large-amplitude muscle artifacts (52). Data were filtered (zero-shift, second order, Butterworth) with bandpass (1–100 Hz) and bandstop (58 Hz, 62 Hz) filters. A second round of independent component analysis was applied to remove other artifacts (i.e., eye blinks, eye movements, and muscle artifacts) from data. Missing electrodes were then replaced by spherical Spline interpolation and data re-referenced to the average (over all electrodes) for further analysis. EEG analyses and artifact removal were performed as per Rogasch *et al.* (53).

GMFA Analysis

GMFA was calculated for individual participants in each group using the GMFA equation as adapted from Lehmann and Skrandies (37).

$$\mathrm{GMFA}(t) = \sqrt{\sum_{i}^{K} \left(V_i(t) - V_{mean}(t)\right)^2 \Big/ K}$$

GMFA calculates the maximum amplitude of the evoked field (37) and is used to assess the global brain response to TMS-EEG (40,54). For individual participants, amplitude and latency of component peaks within the GMFA (i.e., local maxima) were calculated. All components were visually inspected prior to final analysis. N45 amplitude was calculated as the average amplitude of GMFA between 35 and 55 ms. P60 amplitude was calculated as the average amplitude of GMFA between 50 and 70 ms. N100 amplitude was calculated as the average amplitude of GMFA between 80 and 120 ms. GMFA-AUC was calculated by summation of GMFA amplitude from 55 to 275 ms after TMS pulse.

Statistical Analysis

All statistical analyses were conducted using statistical software (SPSS for Mac 22.0; IBM Corp., Armonk, NY). Mean scores for HDRS-17 and ATHF were calculated for the MDD group. Question 3 of the HDRS-17, pertaining to suicide (HDRS-17 suicide subscore), was extracted and analyzed separately for each participant in the MDD group. Distribution of data was assessed using the Kolmogorov-Smirnov test of normality; as neither group's data were normally distributed, the data were logarithmically transformed. Independent *t* tests were performed to compare N45, P60, and N100 amplitudes and GMFA-AUC values between groups. Given the potential for multiple comparisons, we then set the alpha at .0125 to reflect four tests (N45, P60, N100, GMFA-AUC). Spearman's correlation analyses were performed in the MDD group between GMFA components and ordinal variables (HDRS-17 scores, HDRS-17 suicide subscores, ATHF scores, and sex) and between GMFA components and sex in all participants. Pearson's correlation analyses were performed in both groups between GMFA components and age (in years) (both continuous variables). Receiver operating characteristic (ROC) analyses were performed with several variables to ascertain predictive associations with TMS-EEG variables.

RESULTS

Demographics

There were 60 individuals in the sample, consisting of 30 participants with MDD and 30 age- and sex-matched healthy participants, 15 men and 15 women per group. The mean age

and SD of the MDD sample were 39.13 years (10.89 years), and the mean and SD of the HDRS-17 score were 24.8 (3.47) (Table 1). The mean and SD of the ATHF score were 3.37 (1.33). Medication information for the group of MDD patients is found in Table 1. In healthy subjects, the mean age was 37.03 years (SD 11.03 years) (Table 1). There was no significant difference between the two groups in RMT (t = 0.261, p = .795).

GMFA Component Analysis

N45 Amplitude. The mean N45 amplitude was significantly larger in the MDD group than in the healthy group ($t_{41.248}$ = −4.894, $p < .001$; Bonferroni corrected $p < .001$; d = 1.227) (Figure 1A and Table 2). As the distribution of N45 amplitude was not normal, a logarithmic transformation was performed, with the same results. The mean logarithmically transformed N45 amplitude was significantly larger in the MDD group than in the group of healthy participants (t_{58} = −5.029, $p < .001$; Bonferroni corrected $p < .001$). There was no correlation between N45 amplitude and age, sex, ATHF score, number of lifetime episodes of depression, HDRS-17 score, or HDRS-17 suicide subscore in participants with MDD (Supplemental Table S1). An ROC analysis was then performed between N45 and illness status (as a binary variable: 0 = healthy, 1 = MDD). The N45 amplitude positively predicted depressed illness status with 80% sensitivity, 73.3% specificity, and 76.6% accuracy (AUC = 0.829, $p < .001$) (Figure 2).

Table 1. Demographics of Healthy and MDD Groups, Clinical Scores of MDD Group, and Current Medications of MDD Group

	Healthy Participants	Participants With MDD	t (p)
Demographics			
Age, years, mean (SD)	37.03 (11.02)	39.13 (10.89)	−0.472 (.461)
Sex, male, *n*	15	15	
Clinical			
HDRS score, mean (SD)	–	24.80 (3.48)	
HDRS suicide subscore, mean (SD)	–	1.33 (0.844)	
ATHF score, mean (SD)	–	3.37 (1.33)	
Medications			
Antidepressant, *n*			
SSRI	–	14	
SNRI	–	9	
Mirtazapine	–	2	
Bupropion	–	6	
TCA	–	0	
MAOI	–	1	
Benzodiazepine, *n*	–	11	
Zopiclone, *n*	–	4	
Antipsychotic, *n*	–	7	
Mood stabilizer, *n*	–	2	
Stimulant, *n*	–	2	

ATHF, Antidepressant Treatment History Form; HDRS, Hamilton Depression Rating Scale; MAOI, monoamine oxidase inhibitor; MDD, major depressive disorder; SNRI, serotonin and norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant.

N100 Amplitude. The mean N100 amplitude was also significantly larger in the MDD group than in the group of healthy participants ($t_{40.652}$ = −3.496, p = .001; Bonferroni corrected p = .003; d = 1.395) (Figure 1B and Table 2). As the distribution of N100 amplitude was not normal for either the healthy or the MDD group, a logarithmic transformation was performed, with the same results. The mean logarithmically transformed N100 amplitude was significantly larger in the MDD group than in the healthy group (t_{58} = −3.375, p = .001; Bonferroni corrected p = .001). There was no correlation between N100 amplitude and age, sex, ATHF score, number of lifetime episodes of depression, or HDRS-17 suicide subscore in the participants with MDD (Supplemental Table S1). There was a positive correlation between N100 amplitude and overall HDRS-17 score in the participants with MDD (Spearman's ρ = .420, p = .021). However, an ROC analysis for the N100 component amplitude and change in HDRS-17 score variables was not significant, nor was the ROC analysis between N100 amplitude and illness status (Supplemental Table S1).

Effects of Benzodiazepines on N45 and N100 in MDD. Because of the effects of benzodiazepines on cortical inhibition (25), we compared 11 participants in the MDD group who were on benzodiazepines at the time of study recruitment to the remaining 19. There were no significant differences in N45 or N100 between benzodiazepine and nonbenzodiazepine-treated MDD participants. (N45: t_{28} = −0.970, p = .341, N100: t_{28} = −1.572, p = .127).

P60 Amplitude. The mean amplitude of the P60 component was significantly larger in the MDD group than in the healthy group ($t_{51.674}$ = −3.260, p = .002; Bonferroni corrected p = .003; d = 0.839) (Figure 1C and Table 2). As the distribution of P60 amplitude was not normal for either the healthy or the MDD group, a logarithmic transformation was performed, with the same results. The mean logarithmically transformed P60 amplitude was significantly larger in the MDD group than in healthy participants (t_{58} = −3.436, p = .001; Bonferroni corrected p = .001). There was no correlation between P60 amplitude and age, sex, ATHF score, number of lifetime episodes of depression, HDRS-17 score, or HDRS-17 suicide subscore in the MDD participants (Supplemental Table S1). As there was no association found between any clinical variables and the P60 component, an ROC analysis was not conducted. An ROC analysis between P60 amplitude and illness status was not significant (Supplemental Table S1).

Area Under the Curve (GMFA-AUC). The GMFA-AUC was significantly larger in the MDD group than in healthy participants (t_{58} = −3.114, p = .003; Bonferroni corrected p = .006; d = 0.740) (Figure 1D and Table 2). As the distribution of the GMFA-AUC was not normal for either the healthy or the MDD group, a log transformation was performed, with the same results. The mean log transformed GMFA-AUC was significantly larger in the MDD group than in healthy participants (t_{58} = −3.051, p = .003; Bonferroni corrected p = .003). There was no correlation between GMFA-AUC amplitude and age, sex, ATHF score, number of lifetime episodes of

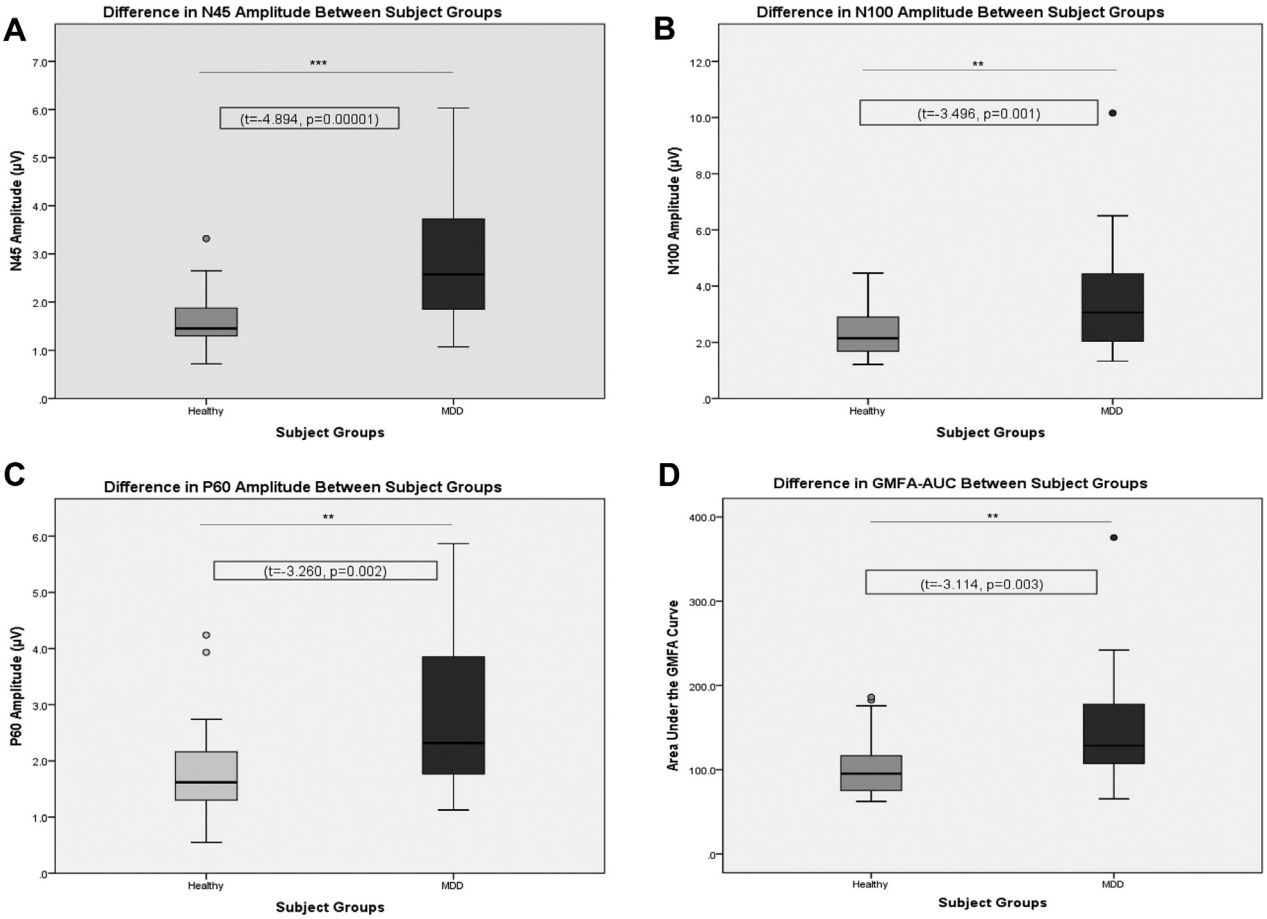

Figure 1. Differences in markers for transcranial magnetic stimulation combined with electroencephalography between healthy participants and patients with major depressive disorder (MDD). Specifically, inhibitory components including the **(A)** N45 and **(B)** N100 and excitatory components including the **(C)** P60 and the **(D)** area under the curve for global mean field amplitude (GMFA-AUC).

depression, or HDRS-17 score in the MDD participants (Supplemental Table S1). There was a negative correlation between GMFA-AUC and HDRS-17 suicide subscore in the MDD participants (Spearman's $\rho = -.435$, $p = .016$). An ROC analysis was not significant when conducted between GMFA-AUC and presence or absence of suicidal ideation in the MDD participants or between GMFA-AUC and illness status (Supplemental Table S1). In healthy participants, a positive correlation was shown between inhibitory N45 amplitude and excitatory GMFA-AUC (Pearson's $r = .711$, $p \leq .0001$), not present in the MDD group (Pearson's $r = .149$, $p = .43$). A Fisher z test was conducted ($z = 2.648$, $p = .008$), and the results demonstrated that the two correlations are significantly different, representing a potential imbalance between inhibition and/or excitation associated with illness status (Figure 2).

Table 2. Comparison of TMS-EEG Markers of Cortical Inhibition and Excitation Between Healthy and MDD Groups

TMS-EEG Markers (μV)	Healthy Participants	Participants With MDD	t (p)
GMFA Component Amplitude, Mean (SD)			
N45	1.56 (0.57)	2.80 (1.31)	−4.894 (.000016)
N100	2.35 (0.86)	3.55 (1.90)	−3.496 (.001)
P60	1.80 (0.89)	2.71 (1.25)	−3.260 (.002)
AUC, Mean (SD)	105.34 (36.63)	142.87 (61.69)	−3.114 (.003)

AUC, area under the curve; GMFA, global mean field amplitude; MDD, major depressive disorder; TMS-EEG, transcranial magnetic stimulation combined with electroencephalography.

As participants in the MDD group were taking several different classes of medication, we compared the 14 patients taking selective serotonin reuptake inhibitors with the 16 who were not, the 9 patients taking serotonin and norepinephrine reuptake inhibitor with the 21 who were not, and the 9 patients taking antipsychotics or mood stabilizers with the 21 who were not. There were no significant differences in N45 amplitude, P60 amplitude, N100 amplitude, or GMFA-AUC between the patients taking the medications and those who were not in any of these subanalyses (Supplemental Table S1).

There was no correlation between N45 amplitude, N100 amplitude, P60 amplitude, or GMFA-AUC and age or sex in healthy participants.

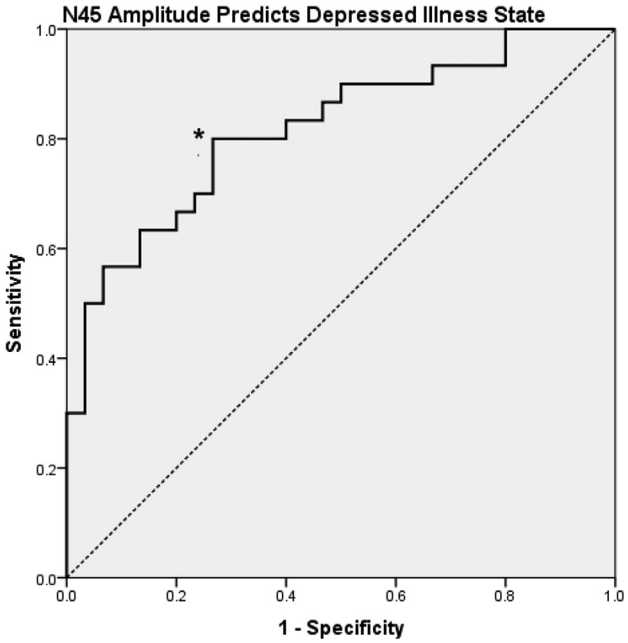

Figure 2. Results of receiver operating characteristic analysis: N45 amplitude predicts MDD illness state with 80% sensitivity, 73.3% specificity, and 76.6% accuracy.

DISCUSSION

Our results provide evidence of DLPFC abnormalities in TMS-EEG inhibition and excitation measures in MDD (Figures 3 and 4) compared with those of healthy participants. Specifically, measures associated with inhibition (i.e., N45 amplitude, N100 amplitude) were larger in the MDD group (Figure 1A, B). Further, an ROC analysis revealed that N45 amplitude predicted those in the MDD group with 80% sensitivity, 73.3% specificity, and 76.6% accuracy (Figure 2). Cortical excitation in the DLPFC—indexed through GMFA-AUC—was also significantly larger in the MDD group, as was P60 amplitude (Figure 1C, D). Lastly, we found an excitation/inhibition imbalance in the MDD group when compared with that of healthy participants, as the close coupling of inhibition and excitation in the healthy group was not observed in MDD participants (Figure 5).

We demonstrated both N45 and N100 amplitudes were larger in MDD compared with those in healthy participants (Figure 1A, B). These measures are related to inhibition involving GABAergic neurotransmission; Premoli *et al.* (35) reported that $GABA_A$ agonists (alprazolam and diazepam) specifically increased N45 amplitude. Zolpidem increased N45 amplitude only, while baclofen increased N100 amplitude (35). Our findings also suggest that N45 amplitude is a significant indicator of MDD status (Figure 2), based on an ROC analysis. Further, there was an excitation/inhibition imbalance in the MDD group relative to that of healthy participants (Figure 5), with no association between inhibitory and excitatory measures in MDD, whereas tight coupling was seen in healthy participants. Lastly, in addressing a potential benzodiazepine effect on inhibitory measures, there were no significant differences between MDD participants treated with benzodiazepines and those not—mitigating any potential pharmacological confound in overall group differences.

Our findings appear in contrast to those of Levinson *et al.* (33), who reported deficient inhibitory neurotransmission in the

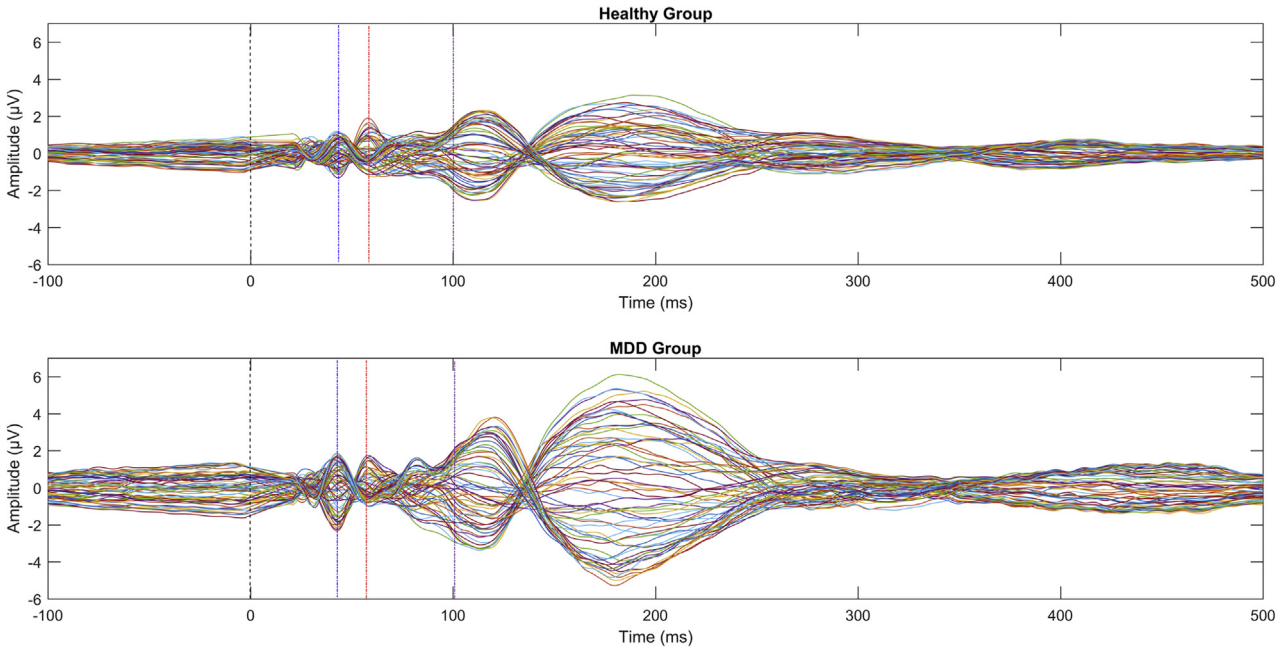

Figure 3. Butterfly plot of waveform from transcranial magnetic stimulation combined with electroencephalography for healthy participants (top panel) and participants in the major depressive disorder (MDD) group (bottom panel). The black dashed line represents time of transcranial magnetic stimulation pulse at time 0. The blue dotted line represents N45, the red dotted line represents P60, and the purple dotted line represents N100.

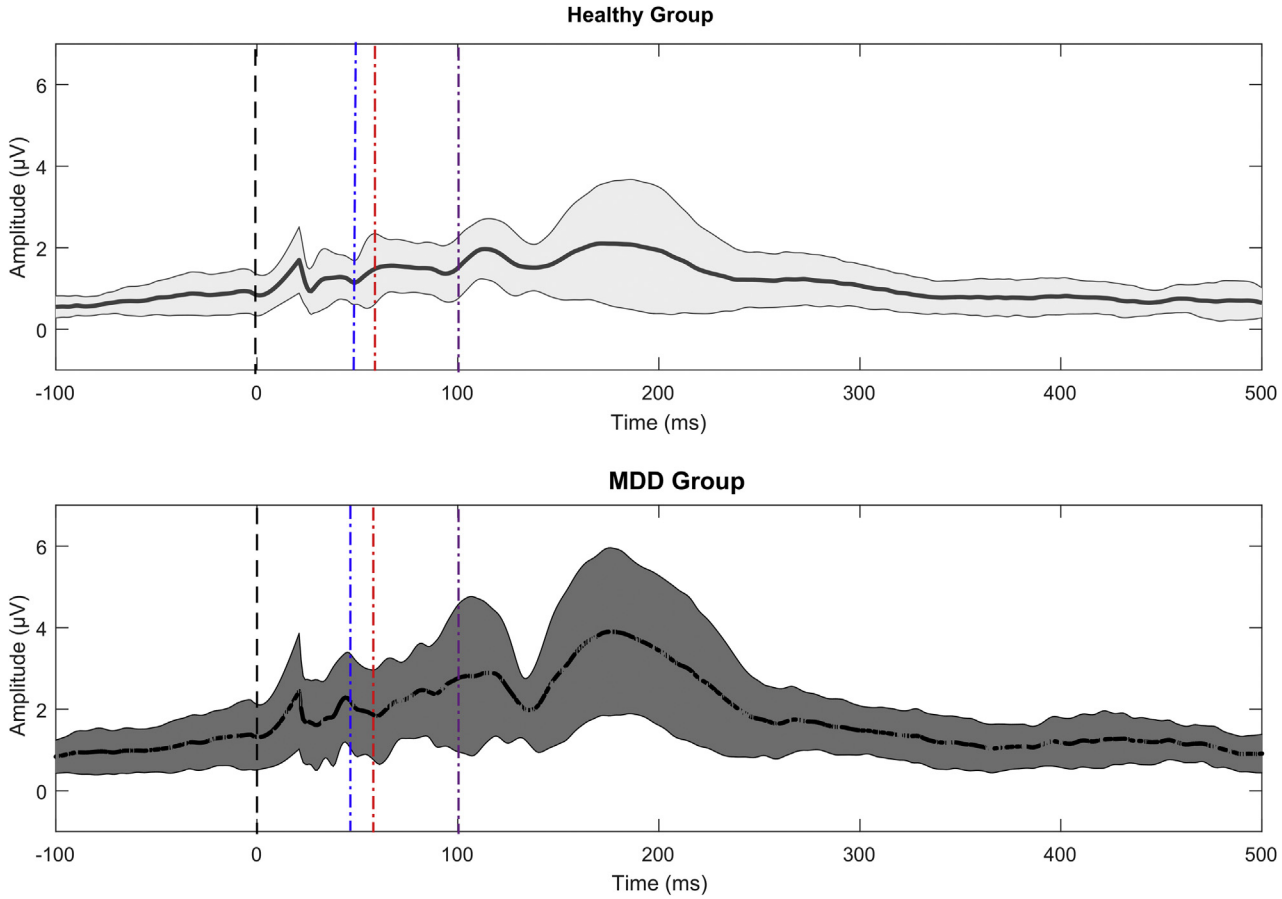

Figure 4. Global mean field amplitude for healthy individuals (top panel) and individuals in the major depressive disorder (MDD) group (bottom panel). The black dashed line represents time of transcranial magnetic stimulation pulse at time 0. The blue dotted line represents N45, the red dotted line represents P60, and the purple dotted line represents N100.

motor cortex in MDD. These differences may be reconciled in several key ways. Perhaps most crucial is the difference in cortical regions; our present findings compared measures in the DLPFC, whereas Levinson *et al.* (33) reported deficits in the motor cortex. Rajkowska *et al.* (23) reported reductions in GABAergic neuronal density in the prefrontal cortex in postmortem studies of MDD patients. Importantly, while these findings showed reduction in GABAergic neuronal density and size, it is unclear whether these reductions reflect physiological activity of inhibitory neurotransmission. That is, decreases in GABA concentration may be related to increased GABA turnover postsynaptically, potentially resulting in increased inhibitory neurotransmission. Regardless of directionality, our evidence suggests pervasive abnormalities in inhibitory neurotransmission in MDD relative to that of healthy participants and further highlights the importance of additional experiments to elucidate the role of GABA in MDD.

In contrast to the extensive TMS-EEG literature regarding inhibitory neurotransmission, little has been written directly evaluating excitatory neurotransmission in the DLPFC. The P60 appears to be linked to excitatory mechanisms (36), specifically *N*-methyl-D-aspartate and/or glutamatergic activity (55). In this sense, our finding of increased P60 amplitude in the MDD group aligns with recent evidence of motor cortical hyperfacilitation in patients with vascular depression (56). Moreover, several excitability studies of other cortical regions demonstrate altered levels of glutamate and changes in ICF in MDD (34,57). The larger GMFA-AUC in our MDD participants aligns with findings from earlier EEG studies showing "hyperactivation" in MDD, which normalized with drug treatment (58). Taken together, these data reveal abnormalities in both inhibitory and excitatory neurotransmission in MDD relative to that in healthy participants.

In a similar manner to that of Noda *et al.* (36), we examined associations between inhibitory processes (N45 amplitude) and excitation (GMFA-AUC), with the calculation of GMFA-AUC adjusted to 55 to 275 ms after TMS pulse to exclude the inhibitory N45 component and thus a possible confound. In healthy participants, there was a significant correlation between inhibition and excitation (Pearson's $r = .702$, $p < .001$), absent in the MDD group (Pearson's $r = -.043$, $p = .822$) (Figure 5), suggesting uncoupling of inhibition and excitation in MDD in contrast to strong association in healthy participants. These results reinforce our assertion that MDD is

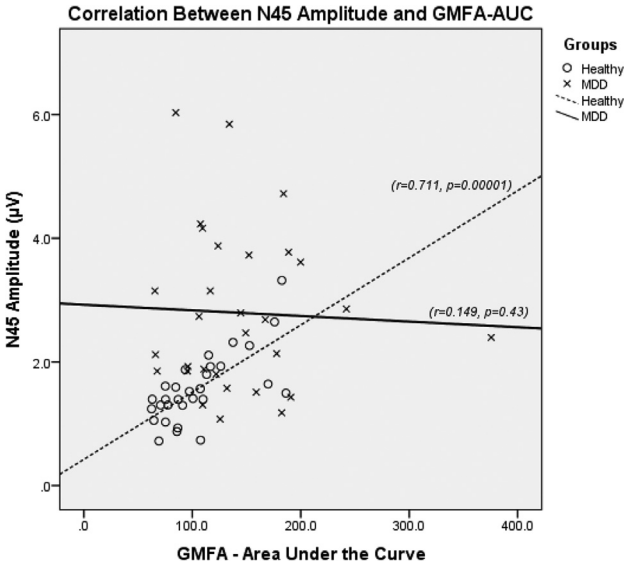

Figure 5. A correlation of cortical inhibition (N45 amplitude) with excitation (area under the curve for global mean field amplitude [GMFA-AUC]) is present in healthy participants but absent in patients with major depressive disorder (MDD).

associated with an imbalance between inhibition and excitatory neurotransmission.

As stated above, the results from our study align with extant evidence regarding the neuropathology of MDD. That is, our results highlight aberrant activity in the GABAergic system in DLPFC, in line with the GABAergic dysfunction hypothesis of MDD (59,60). Moreover, the aberrant TMS-EEG components, which represent neurophysiological indices of GABAergic activity, support previous findings of abnormal DLPFC GABAergic interneuron density in postmortem examinations of MDD patients (23). Overall, our findings corroborate the consensus of abnormal GABAergic function in the DLPFC (60,61) in MDD. What remains to be elucidated is the directionality of these abnormal TMS-EEG components when the DLPFC is stimulated directly, in association with GABAergic interneuronal deficits found postmortem in MDD.

There are some limitations to our study. First, TMS-EEG in this study was performed without auditory masking to minimize the auditory "click" in the N100-P200 complex of the TMS-EEG waveform. However, both healthy and MDD groups underwent TMS-EEG with the same paradigm, rendering the contribution of the "click" largely moot. Importantly, our main finding of N45 amplitude predicting illness state is external to the N100-P200 complex. While the N100-P200 complex was thought to be part of an auditory response to the TMS "click" (62), investigations using sound masking and deaf participants have shown that TMS-evoked cortical activation of N100 exists underneath the auditory evoked response, albeit to a smaller degree (63). A second limitation relates to our use of monophasic TMS and the minimal literature regarding TMS-EEG measurements of cortical excitability. Some emerging research has noted the potential for biphasic TMS to be more effective than monophasic TMS in eliciting early components of TMS-evoked potentials, which may represent cortical excitability (64). When stimulating the motor cortex, biphasic TMS appears more effective in producing MEPs (65). This area requires further exploration and may benefit from large-scale direct comparisons of monophasic to biphasic TMS. The final limitation regards potential important additional variables. Information on smoking status was not collected, and most MDD participants in this study were treated with antidepressants at the time of TMS-EEG testing, although the action of traditional antidepressants is not mediated by the GABA system. Nevertheless, we cannot rule out the possibility that these findings are at least partially driven by antidepressants. Future studies should examine unmedicated patients to directly address this potential confound.

In summary, our results demonstrate abnormalities in TMS-EEG measures that have been associated with both inhibition and excitation. Perhaps most importantly, we found that N45 amplitude, previously linked with $GABA_A$ neurotransmission, shows potential promise as a neurophysiological biomarker of the DLPFC to identify depressed state. While future studies are needed, this holds promise for uncovering a potential biological marker of MDD based in DLPFC physiology.

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