ORIGINAL ARTICLE

Evidence for Increased Glutamatergic Cortical Facilitation in Children and Adolescents With Major Depressive Disorder

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Context: Converging lines of evidence implicate the glutamate and γ-aminobutyric acid neurotransmitter systems in the pathophysiology of major depressive disorder. Transcranial magnetic stimulation cortical excitability and inhibition paradigms have been used to assess cortical glutamatergic and γ-aminobutyric acid–mediated tone in adults with major depressive disorder, but not in children and adolescents.

Objective: To compare measures of cortical excitability and inhibition with 4 different paradigms in a group of children and adolescents with major depressive disorder vs healthy controls.

Design: Cross-sectional study examining medication-free children and adolescents (aged 9-17 years) with major depressive disorder compared with healthy controls. Cortical excitability was assessed with motor threshold and intracortical facilitation measures. Cortical inhibition was measured with cortical silent period and intracortical inhibition paradigms.

Setting: University-based child and adolescent psychiatry clinic and neurostimulation laboratory.

Patients: Twenty-four participants with major depressive disorder and 22 healthy controls matched for age and sex. Patients with major depressive disorder were medication naive and had moderate to severe symptoms based on an evaluation with a child and adolescent psychiatrist and scores on the Children's Depression Rating Scale–Revised.

Main Outcome Measures: Motor threshold, intracortical facilitation, cortical silent period, and intracortical inhibition.

Results: Compared with healthy controls, depressed patients had significantly increased intracortical facilitation at interstimulus intervals of 10 and 15 milliseconds bilaterally. There were no significant group differences in cortical inhibition measures.

Conclusions: These findings suggest that major depressive disorder in children and adolescents is associated with increased intracortical facilitation and excessive glutamatergic activity.

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MAJOR DEPRESSIVE DISORDER (MDD) is a significant and common illness in childhood and adolescence, with a lifetime prevalence rate of 16% by adulthood.[1,2] Worldwide, it is one of the foremost causes of disease burden.[3,4] Current treatment is challenging because of the poor understanding of relevant pathophysiology[5] and suboptimal remission rates with standard treatments.[6] Recent controversy about the safety of selective serotonin reuptake inhibitors in young patients also underscores the necessity for a greater awareness of the underlying biological mechanisms of depression in children and adolescents.[7,8]

Multiple lines of evidence implicate the glutamate and γ-aminobutyric acid (GABA) neurotransmitter systems in the pathophysiology of MDD.[9-12] Glutamate is the primary excitatory neurotransmitter in the brain, playing key roles in cognition, promotion of synaptic plasticity, and facilitation of the production of neurotrophic factors.[13-15] Substantial evidence has demonstrated both the integral role of glutamate in the pathophysiology of depression in adults and its potential utility as a biomarker for MDD.[16-18] However, little is known about the role of this ubiquitous neurotransmitter in child and adolescent depression.[19,20] Conversely, GABA is the brain's principal inhibitory neurotransmitter.[21] Research demonstrates that deficient GABA

(GABA ionotropic receptor family A [$GABA_A$] and GABA metabotropic receptor family B [$GABA_B$]) neurotransmission plays a critical role in the pathophysiology of MDD in adults,[22-24] but there is little similar information about child and adolescent depression.[25,26]

Transcranial magnetic stimulation (TMS) is a promising method for examining glutamate and GABA functioning in children and adolescents.[27] Single- and paired-pulse TMS techniques involve the application of brief magnetic stimulations to the motor cortex while monitoring an electromyographic reading of motor evoked potential (MEP) in a hand muscle such as the abductor pollicis brevis (APB). These measures have good reliability and prior validation.[28-30]

Motor threshold (MT) and intracortical facilitation (ICF) are measures of cortical excitability.[31,32] The MT is influenced by voltage-gated sodium channels.[33] Prior work indicates that ICF indexes glutamatergic *N*-methyl-D-aspartate (NMDA) receptor functioning. This is supported by studies of NMDA antagonists that decrease ICF and studies in which the delay of excitatory postsynaptic potentials mediated by NMDA are consistent with the time interval of ICF.[34,35] This measure involves a subthreshold conditioning pulse followed by a suprathreshold pulse with a 10- to 20-millisecond interstimulus interval.[36] The MEP of the suprathreshold pulse is measured to determine the degree of increased output with the conditioning pulse. Neurophysiological measures of cortical inhibition include intracortical inhibition (ICI)[37,38] and the cortical silent period (CSP).[37,39] In ICI, a subthreshold conditioning pulse precedes a suprathreshold pulse with an interstimulus interval of 1 to 5 milliseconds.[40] The MEP of the suprathreshold pulse is measured to determine the degree of reduced output with the conditioning pulse. This ICI measure (often referred to as short-interval ICI) is thought to index $GABA_A$ receptor–mediated neurotransmission on the basis of prior research demonstrating that $GABA_A$ agonists potentiate this measure.[41] The CSP measure is collected with simultaneous TMS of the motor cortex while the subject contracts the muscle of interest, thus providing background electromyographic activity. This stimulation produces a quiescent period on the electromyographic monitoring after the TMS pulse.[37,39] The CSP duration corresponds to the amount of cortical inhibition. In pharmacologic studies, $GABA_B$ agonists potentiate the CSP; hence, it is argued that this measure is an index of $GABA_B$.[41]

To date, these TMS measures have not been examined in children and adolescents with mood disorders to our knowledge. Prior work on adults with MDD demonstrated that depressed subjects have deficits in CSP and ICI measures.[42-44] The purpose of our study was to examine measures of cortical excitability (MT and ICF) and inhibition (CSP and ICI) in medication-naive children and adolescents with moderate to severe MDD compared with healthy controls. We hypothesized that depressed children and adolescents would demonstrate excessive cortical excitability (measured by MT and ICF) compared with healthy controls. In addition, we postulated that depressed children and adolescents would have deficits in cortical inhibition (measured by CSP and ICI) compared with healthy controls.

METHODS

In this cross-sectional study, a clinical assessment preceded TMS cortical excitability and inhibition measures during a single session. These TMS measures were collected bilaterally and included MT, ICF, CSP, and ICI.

STUDY PARTICIPANTS

The sample consisted of 24 medication-naive child or adolescent patients with MDD and 22 healthy controls matched for age and sex. Depressed patients and healthy controls were not taking antidepressants or any other psychotropic medications prior to enrollment or during this study. Subjects were not receiving any type of psychotherapy during the study. Depressed patients were recruited through the Child and Adolescent Psychiatry Clinic of the Children's Medical Center, Dallas, Texas. Healthy controls were recruited through advertisements in the Dallas area. This study was approved by the institutional review board of the University of Texas Southwestern Medical Center, Dallas. Prior to clinical evaluation or any study activities, the purpose of the investigation and the study procedures was explained to participants and their parents. Participants provided written assent and their parents provided written informed consent. All participants were assessed by a board-certified child and adolescent psychiatrist (P.E.C.). Assessment included a physical examination, a neurologic examination, and a urine pregnancy test for female participants who had reached menarche. Clinical assessments included the Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children Present and Lifetime (K-SADS-PL) semistructured psychiatric interview,[45] the Children's Depression Rating Scale–Revised (CDRS-R),[46] and the Quick Inventory of Depressive Symptomatology, Adolescent Version, Self-report.[47]

INCLUSION AND EXCLUSION CRITERIA

Eligible participants were aged 7 to 18 years, male or female, with no risk factors for seizures (history of unprovoked seizures, seizure disorder, history of febrile seizures, family history of epilepsy, prior neurosurgery, or brain tumor), no unstable medical conditions, and no implanted metal. These criteria were confirmed with the TMS Adult Safety Screen[48] and a clinical interview to ensure the safety of participants during the study. Handedness in all participants was confirmed with Oldfield's Edinburgh Handedness Inventory.[49]

For participants with MDD, the diagnosis was confirmed with a K-SADS-PL semistructured psychiatric interview conducted by a board-certified child and adolescent psychiatrist (P.E.C.) and a PsyD-level child and adolescent psychologist (B.D.K.). Further inclusion criteria included a score of 40 or higher on the CDRS-R. The K-SADS-PL and CDRS-R were used in the interview with each subject and with the subject's caregiver. Exclusion criteria included comorbid posttraumatic stress disorder, obsessive-compulsive disorder, pervasive developmental disorders, mental retardation, bipolar disorder, schizophrenia, tic disorder, conduct disorder, eating disorders, and substance use disorders.

Healthy controls had to be in excellent health and not meet the criteria for any current or lifetime *DSM-IV-TR* diagnoses based on the K-SADS-PL semistructured psychiatric interview. Other exclusion criteria for healthy controls were any psychiatric treatment or psychotropic medications ever and family psychiatric history in a first- or second-degree relative.

TMS TESTING

Testing with TMS was conducted as previously described in reports of adult studies.[40,44] Subjects were seated in a comfortable chair and wore a swim cap during the procedure. All subjects and research team members wore earplugs during testing sessions. Electromyographic readings were recorded from the APB. Muscle relaxation during the procedure was monitored with audio feedback. The TMS was applied to the hand area of the contralateral cortex with a figure-of-8 magnetic coil (diameter, 70 mm per loop) using the Magstim 200 magnetic stimulator device (Magstim Co Ltd). For a determination of resting MT, the TMS coil was held tangentially on the head with the handle backward at 45° laterally from midline. The optimal coil position for stimulation was identified as the location producing the largest MEP with moderately suprathreshold intensities in a resting APB. The optimal coil position was located by moving the coil in 1-cm increments over the presumed motor cortex area. The optimal stimulation site was marked with a black marker to ensure continuity throughout the experiment. The resting MT was defined as the stimulation intensity eliciting an MEP greater than 50 μV in 5 of 10 trials with a relaxed APB. For ICI and ICF (**Figure 1**) measurements, a subthreshold conditioning stimulus set to 80% of resting MT preceded a suprathreshold test stimulus, which was calibrated to produce an average MEP of 0.5- to 1.5-mV peak-to-peak amplitude in the contralateral APB. Conditioning stimuli were delivered to the motor cortex prior to the test stimulus in 1 of 5 random interstimulus intervals: 2 milliseconds (ICI-2) and 4 milliseconds (ICI-4) for ICI measures; 10 milliseconds (ICF-10), 15 milliseconds (ICF-15), and 20 milliseconds (ICF-20) for ICF measures. The sequence of administration was counterbalanced to prevent order effects. For ICF and ICI, the change in test stimulus MEP amplitude of each interstimulus interval was expressed as a percentage of the mean unconditioned MEP amplitude. The CSP was measured with a tonically active APB (a 20% maximum contraction), with simultaneous stimulation at 140% of resting MT delivered to the contralateral motor cortex. Ten trials were performed and averaged. The entire process was executed bilaterally to collect cortical excitability and inhibition measures from each hemisphere.

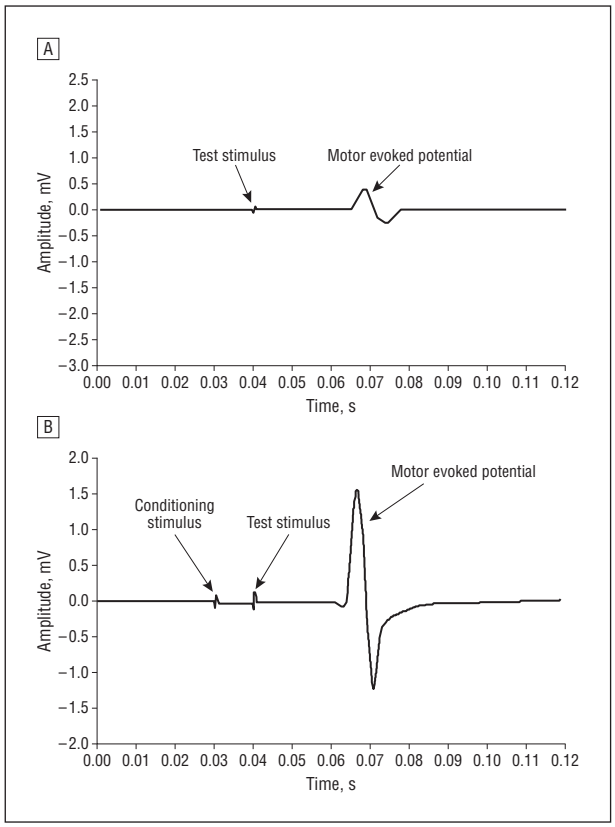

Figure 1. Intracortical facilitation. These tracings are averaged electromyographic waveforms from cortical excitability testing in an adolescent patient. A, A suprathreshold test stimulus produces motor evoked potentials with a near 1-mV peak-to-peak amplitude. B, In the intracortical facilitation paradigm, a subthreshold conditioning stimulus precedes the test stimulus with an interstimulus interval of 10 to 20 milliseconds. This paired stimulation culminates in a motor evoked potential with an increased amplitude compared with that of the test stimulus alone (A). Differences in motor evoked potential amplitudes are expressed as a percentage of the unconditioned motor evoked potential. Prior work suggests that this measure indexes cortical glutamatergic *N*-methyl-D-aspartate receptor functioning.

DEPENDENT AND INDEPENDENT VARIABLES AND COVARIATES

The primary outcome measures were MT, ICF, CSP, and ICI. The primary independent variable was patients with MDD vs healthy controls (a binary, categorical, independent variable, with healthy controls as the reference group). The total score on the CDRS-R, sex, and age in years were included as covariates in the models to bolster precision in the evaluation of the relationship between MDD and healthy controls on each outcome measure.

STATISTICAL ANALYSIS

Demographic and clinical characteristics of the 2 groups were reported using mean (standard deviation) for continuous variables and frequency (percentage) for categorical variables. To identify any differences between the characteristics of the 2 groups, we used the 2–independent sample *t* test with the Satterthwaite method for unequal variances for continuous variables and the χ^2 test or Fisher exact test for categorical variables.

The primary data analysis was a 2-group (MDD and healthy control) by 2-region (left hemisphere and right hemisphere) linear mixed model analysis of repeated measures. A separate mixed model analysis was conducted for each primary outcome. Restricted maximum likelihood estimation and type 3 tests of fixed effects were used, with the Kenward-Roger correction applied to the variance components covariance structure. The model contained fixed-effects terms for group, region, and group × region interaction. Intercept was included as a random effect. Simple group effects in each region were assessed, as were simple region effects within each group. The total score on the CDRS-R, age, and sex were included as covariates in the model. We performed all statistical analyses using SAS version 9.2 statistical software (SAS Institute, Inc). The mixed model procedures of PROC MIXED in SAS were used for the mixed model analysis. The level of significance for all tests was set at $\alpha = .05$ (2-tailed). For multiple testing on the tests of main effects, interaction effects, and post hoc tests of simple effects, *P* values were adjusted using the false discovery rate.[50]

RESULTS

SUBJECTS

The sample consisted of 24 medication-naive children and adolescents with MDD (aged 9-17 years; mean [SD]

Table 1. Characteristics of 24 Children and Adolescents With Major Depressive Disorder Compared With 22 Healthy Controls[a]

Characteristic	Patients With MDD (n = 24)	Healthy Controls (n = 22)	*P* Value (FDR-Adjusted *P* Value)
Age, y			.87 (.99)
Mean (SD)	13.87 (2.11)	13.77 (2.18)	
Range	9-17	9-17	
Sex			.57 (.89)
Male	10 (42)	11 (50)	
Female	14 (58)	11 (50)	
Left-handed	2 (8)	2 (9)	.99 (.99)
Family history of mood disorder	13 (54)	0	<.001 (.002)
Anxiety disorders	8 (33)	0	.009 (.02)
ADHD	4 (17)	0	.14 (.26)
Oppositional defiant disorder	1 (4)	0	.97 (.99)
Type 1 diabetes mellitus	1 (4)	0	.97 (.99)
Ethnicity			.02 (.04)
White	15 (62)	8 (36)	
African American	2 (8)	11 (50)	
Hispanic	3 (12)	1 (5)	
Other	4 (17)	2 (9)	
Episode duration, mo			NA
Mean (SD)	10.9 (9.7)	NA	
Range	1-48	NA	
CDRS-R score			<.001 (.003)
Mean (SD)	58.9 (8.5)	19.6 (1.6)	
Range	44-77	17-24	
QIDS-A_{17}-SR score			<.001 (.003)
Mean (SD)	12.5 (5.6)	3.4 (1.9)	
Range	4-25	0-7	

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; CDRS-R, Children's Depression Rating Scale–Revised; FDR, false discovery rate; MDD, major depressive disorder; NA, not applicable; QIDS-A_{17}-SR, Quick Inventory of Depressive Symptomatology, Adolescent Version, Self-report.
[a]Values are expressed as number (percentage) unless indicated otherwise.

age, 13.87 [2.11] years; 14 female) and 22 healthy controls (aged 9-17 years; mean [SD] age, 13.77 [2.18] years]; 11 female). Of the 46 adolescents in this study, 13 (28%) had a family history of mood disorder. Family history of mood disorder occurred in 13 of the 24 depressed adolescents (54%) and in none of the 22 healthy controls. The characteristics of the study participants are summarized in **Table 1**.

CORTICAL EXCITABILITY

Motor Threshold

The MT least squares mean (SE) values were similar for the MDD and healthy control groups (60.90 [5.89] and 54.96 [6.28], respectively). The mixed model repeated-measures analysis revealed no significant main effects of group ($F_{1,41}$ = 0.26; raw P = .61; adjusted P = .71), region ($F_{1,43.4}$ = 0.79; raw P = .38; adjusted P = .58), or group × region interaction effect ($F_{1,43.4}$ = 0.02; raw P = .87; adjusted P = .87). No significant simple group effects emerged (raw $P > .60$; adjusted $P > .72$). No significant simple region effects emerged (raw $P > .46$; adjusted $P > .74$).

Table 2. Intracortical Facilitation Results in 24 Children and Adolescents With Major Depressive Disorder Compared With 22 Healthy Controls

TMS Measure	Least Squares Mean (SE)		
	Patients With MDD (n = 24)	Healthy Controls (n = 22)	*P* Value (FDR-Adjusted *P* Value)
ICF-10	2.09 (0.24)	0.86 (0.26)	.01 (.03)[a]
Right hemisphere	1.99 (0.26)	0.84 (0.28)	.03 (.11)[b]
Left hemisphere	2.19 (0.25)	0.87 (0.27)	.01 (.04)[b]
ICF-15	2.49 (0.26)	0.61 (0.28)	.001 (.007)[a]
Right hemisphere	2.46 (0.28)	0.53 (0.30)	<.001 (.005)[b]
Left hemisphere	2.53 (0.27)	0.69 (0.29)	.001 (.007)[b]
ICF-20	1.59 (0.32)	1.12 (0.35)	.47 (.71)[a]
Right hemisphere	1.51 (0.34)	1.12 (0.37)	.55 (.72)[b]
Left hemisphere	1.68 (0.33)	1.13 (0.36)	.41 (.72)[b]

Abbreviations: FDR, false discovery rate; ICF-10, intracortical facilitation with 10-millisecond interstimulus interval; ICF-15, intracortical facilitation with 15-millisecond interstimulus interval; ICF-20, intracortical facilitation with 20-millisecond interstimulus interval; MDD, major depressive disorder; TMS, transcranial magnetic stimulation.
[a]The FDR-adjusted *P* value is for tests of group main effects.
[b]The FDR-adjusted *P* value is for tests of simple group effects within each region.

Intracortical Facilitation

ICF-10. For ICF-10 values, the mixed model repeated-measures analysis revealed no significant group × region interaction effect ($F_{1,40.9}$ = 0.54; raw P = .47; adjusted P = .87) and no significant region main effect ($F_{1,41.1}$ = 0.96; raw P = .33; adjusted P = .58), but it did reveal a significant group main effect ($F_{1,38.3}$ = 6.51; raw P = .01; adjusted P = .03). The pattern of the overall adjusted least squares mean (SE) revealed that ICF-10 values were significantly higher for the MDD group than for the healthy control group (2.09 [0.24] vs 0.86 [0.26], respectively; raw P = .01; adjusted P = .03) (**Table 2**). Furthermore, this pattern was found with simple group effects in the left hemisphere (raw P = .01; adjusted P = .04) but not in the right hemisphere (raw P = .03; adjusted P = .11). No significant simple region effects emerged for adjusted ICF-10 values (raw $P > .22$; adjusted $P > .74$). The adjusted least squares means for ICF-10 are reported in Table 2.

ICF-15. For ICF-15 values, the mixed model repeated-measures analysis revealed no significant group × region interaction effect ($F_{1,40.3}$ = 0.16; raw P = .69; adjusted P = .87) and no significant region main effect ($F_{1,40.5}$ = 1.17; raw P = .29; adjusted P = .58), but it did reveal a significant group main effect ($F_{1,38.3}$ = 12.77; raw P = .001; adjusted P = .007). The pattern of the overall adjusted least squares mean (SE) revealed that ICF-15 values were significantly higher for the MDD group than for the normal controls (2.49 [0.26]) vs 0.61 [0.28], respectively; raw P = .001; adjusted P = .007). Furthermore, simple group effects were significant within both the right hemisphere (raw $P < .001$; adjusted P = .005) and the left hemisphere (raw P = .001; adjusted P = .007). No significant simple region effects emerged for adjusted ICF-15 values (raw $P > .31$; adjusted $P > .74$). The

adjusted least squares means for ICF-15 are reported in Table 2.

ICF-20. For ICF-20 values, the mixed model repeated-measures analysis revealed no significant group × region interaction effect ($F_{1,37.1}$ = 0.29; raw P = .59; adjusted P = .87), no significant region main effect ($F_{1,37.3}$ = 0.38; raw P = .54; adjusted P = .63), and no significant group main effect ($F_{1,34.9}$ = 0.54; raw P = .47; adjusted P = .71). No significant simple group effects emerged for adjusted ICF-20 values (raw $P > .41$; adjusted P = .72). No significant simple region effects emerged for adjusted ICF-20 values (raw $P > .41$; adjusted $P > .74$). The adjusted least squares means for ICF-20 are reported in Table 2.

CORTICAL INHIBITION

Cortical Silent Period

The CSP least squares mean (SE) values were similar for the MDD and healthy control groups (169.2 [17.31] and 176.6 [16.98] milliseconds, respectively). In the MDD group, the CSP least squares mean (SE) of the right hemisphere was 178.6 (17.82) milliseconds, whereas it was 159.8 (17.73) milliseconds for the left hemisphere. The mixed model repeated-measures analysis revealed no significant group × region interaction effect ($F_{1,41.1}$ = 2.36; raw P = .13; adjusted P = .52) and no significant group main effect ($F_{1,38.4}$ = 0.05; raw P = .82; adjusted P = .82), but it did reveal a trend toward a significant region main effect ($F_{1,41.1}$ = 3.33; raw P = .07; adjusted P = .49). Simple region effects revealed a trend toward a significant region difference (left hemisphere vs right hemisphere) on the adjusted CSP values within the MDD group (raw P = .02; adjusted P = .14) but not within the control group (raw P = .84; adjusted P = .95). No significant simple group effects emerged for adjusted CSP values (raw $P > .63$; adjusted $P > .72$).

Intracortical Inhibition

ICI-2. The ICI-2 least squares mean (SE) values were similar for the MDD and healthy control groups (0.51 [0.10] and 0.41 [0.11], respectively). The mixed model repeated-measures analysis revealed no significant group × region interaction effect ($F_{1,39.9}$ = 0.09; raw P = .77; adjusted P = .87), no significant region main effect ($F_{1,40.1}$ = 0.17; raw P = .68; adjusted P = .68), and no significant group main effect ($F_{1,37.5}$ = 0.26; raw P = .61; adjusted P = .71). Simple group effects showed that adjusted ICI-2 values were also statistically similar between the 2 groups within each hemisphere. No significant simple region effects emerged for adjusted ICI-2 values (raw $P > .62$; adjusted $P > .93$).

ICI-4. The ICI-4 least squares mean (SE) values were similar for the MDD and healthy control groups (0.68 [0.14] and 0.45 [0.15], respectively). The mixed model repeated-measures analysis revealed no significant group × region interaction effect ($F_{1,41}$ = 2.13; raw P = .15; adjusted P = .52), no significant region main effect ($F_{1,41.2}$ = 0.66; raw P = .42; adjusted P = .58), and no significant group main effect ($F_{1,39.1}$ = 0.70; raw P = .41; adjusted P = .71). No significant simple group effects emerged for adjusted ICI-4 values. No significant simple region effects emerged for adjusted ICI-4 values (raw $P > .12$; adjusted P = .74).

TESTING FOR THREAT TO VALIDITY BY FAMILY HISTORY OF MOOD DISORDER

To examine whether family history of mood disorder affected the basic interpretation of our findings on cortical excitability and inhibition, we conducted similar linear mixed model repeated-measures analyses with family history of mood disorder (along with age, sex, and CDRS-R total score included as covariates in each model). The basic results and conclusions did not differ from those reported herein (results not reported).

COMMENT

To our knowledge, this is the first study using TMS to evaluate cortical excitability and inhibition in children and adolescents with MDD. Our results suggest that depression in children and adolescents is associated with increased ICF, a direct neurophysiological corollary of excessive glutamatergic neurotransmission. However, contrary to the inhibition deficits previously reported in adults with depression, no deficits in inhibition, which are mediated through GABAergic mechanisms, were found in children and adolescents with MDD.

Excessive ICF, a neurophysiological index of increased cortical glutamatergic activity, is noteworthy because glutamate dysregulation plays a decisive role in depression and mood disorders. Glutamatergic neurons and synapses make up a major portion of relevant limbic and cortical neurocircuitry.[11] Prior animal research suggests that stress and glucocorticoids may collectively upregulate glutamate neurotransmission through increased presynaptic release and reduced clearance.[16]

Our findings suggest that children and adolescents with MDD have excessive cortical excitability mediated by NMDA receptors. Prior magnetic resonance spectroscopy (MRS) work with depressed adult subjects demonstrated reductions of glutamate metabolites (glutamate/glutamine, glutamate, and glutamine) in the anterior cingulate cortex,[51,52] left dorsolateral prefrontal cortex,[53] dorsomedial prefrontal cortex,[54] and ventromedial prefrontal cortex.[54] Initial MRS work with depressed children and adolescents demonstrated decreased glutamate/glutamine and glutamate concentrations in the anterior cingulate cortex.[19] Currently, drawing definitive conclusions about the pathophysiological implications of our findings in the context of prior MRS studies is problematic because of the complexity and nature of glutamatergic neurotransmission and the vast differences in methodologic approaches.

Although our findings may superficially appear to be at odds with previous findings of deficient cortical glutamate in depressed subjects, collectively this difference may simply suggest that there is less free glutamate with

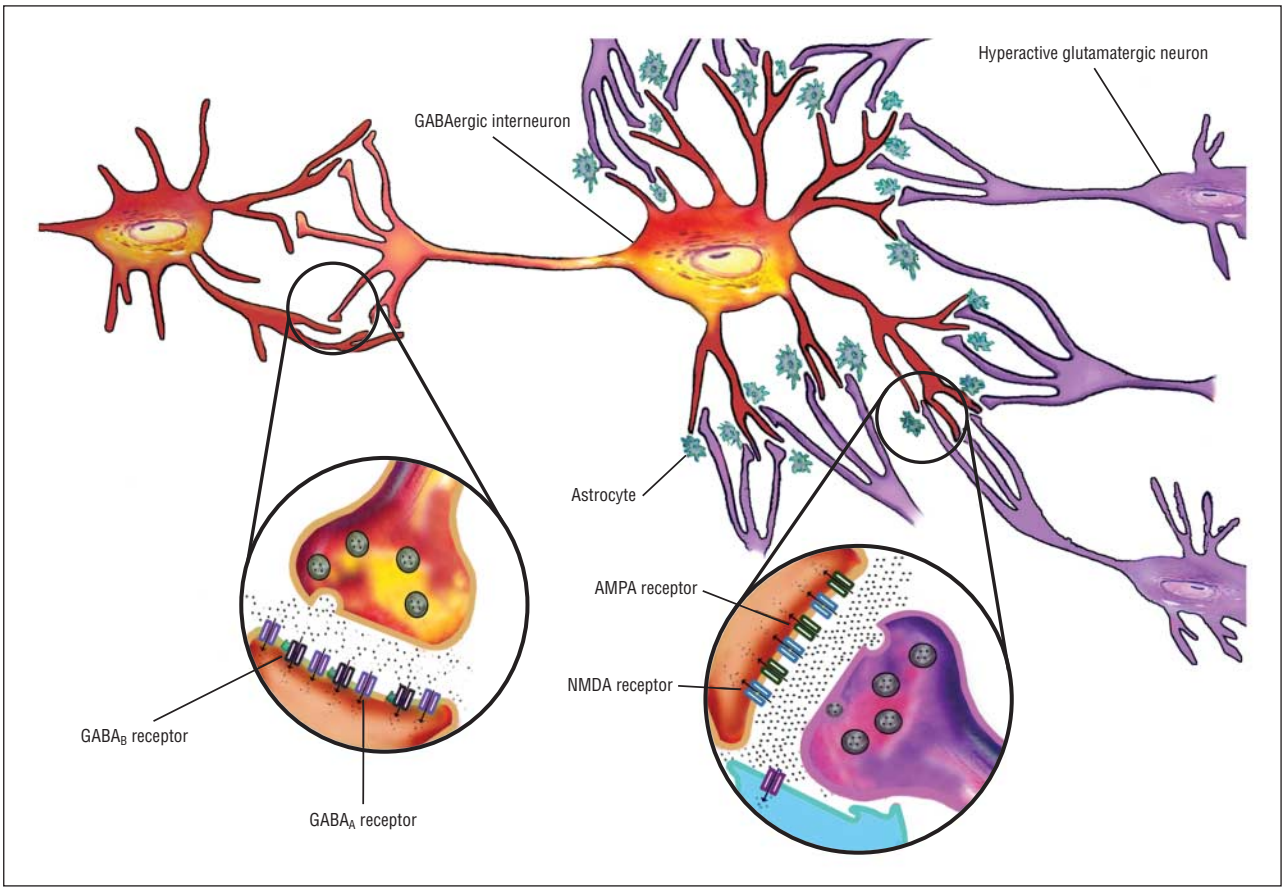

Figure 2. Increased cortical excitability in childhood depression. This illustration presents a theoretical model regarding the impact of increased glutamatergic intracortical facilitation in child and adolescent depression. Increased intracortical facilitation in depressed children and adolescents indicates increased cortical glutamatergic *N*-methyl-D-aspartate (NMDA) receptor functioning. At this stage, γ-aminobutyric acid (GABA) ionotropic receptor family A (GABA$_A$) receptor–mediated and GABA metabotropic receptor family B (GABA$_B$) receptor–mediated neurotransmission is unchanged because GABAergic interneurons are functioning normally. However, increased NMDA activity is thought to play a key role in excitotoxic effects. The impact is likely widespread and could subsequently impair or damage GABAergic interneurons. AMPA indicates α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid.

increased glutamatergic neurotransmission. The ICF paradigm is a direct measure of "active" NMDA neurotransmission rather than of free glutamate in the brain. Therefore, low concentrations of glutamate as identified by MRS may suggest increased glutamate expenditure or turnover from hyperactive, excitatory neurotransmission. Another important consideration is that reduced glutamate measured by MRS may be a consequence of glial abnormalities in MDD. One could postulate that a glial abnormality that reduces the glutamate availability from glial cells might result in compensatory upregulation of NMDA neurotransmission. In vivo studies of glutamate neurotransmission throughout development are crucial in advancing knowledge in this area. Ideal future efforts would involve complementary studies with MRS and TMS or interleaved experiments.

It is intriguing that our CSP and ICI findings did not vary significantly among the depressed participants and the healthy controls. Previously, CSP and ICI deficits in adults with MDD have been a consistent finding.[43,44] The glutamate and GABA systems have a complex relationship across development that serves to regulate both excitatory and inhibitory functions. Prior work suggests that, compared with adults, children and adolescents may have less cortical inhibition (CSP and ICI deficits) and GABAergic inhibitory functioning.[55] Although CSP measures can be produced reliably as early as age 5 years, no systematic studies to our knowledge have examined the developmental course or the impact of age on this marker of GABA$_B$ activity.[56] Our results show a trend in depressed children and adolescents toward hemispheric differences in CSP and GABA$_B$ functioning, with a decrease in the left hemisphere as compared with the right. Prior work has identified electroencephalographic hemispheric coherence abnormalities in at-risk adults and depressed children and adolescents.[57,58] As with these findings, hemispheric differences in CSP may reflect a perturbation in physiological regulatory systems that warrants further study as a potential marker of vulnerability or disease burden. As with CSP measures and GABA$_B$ functioning, changes in ICI measures of GABA$_A$ across development are poorly understood. However, 4 decades of preclinical work have shown that excessive glutamate and NMDA activity is neurotoxic in the central nervous system.[59]

Animal models suggest that glutamate-mediated toxic effects may be more profound in children and adolescents because of developmental differences in the excitatory-inhibitory balance.[60,61] Hence, it might be predicted that excessive glutamatergic functioning in

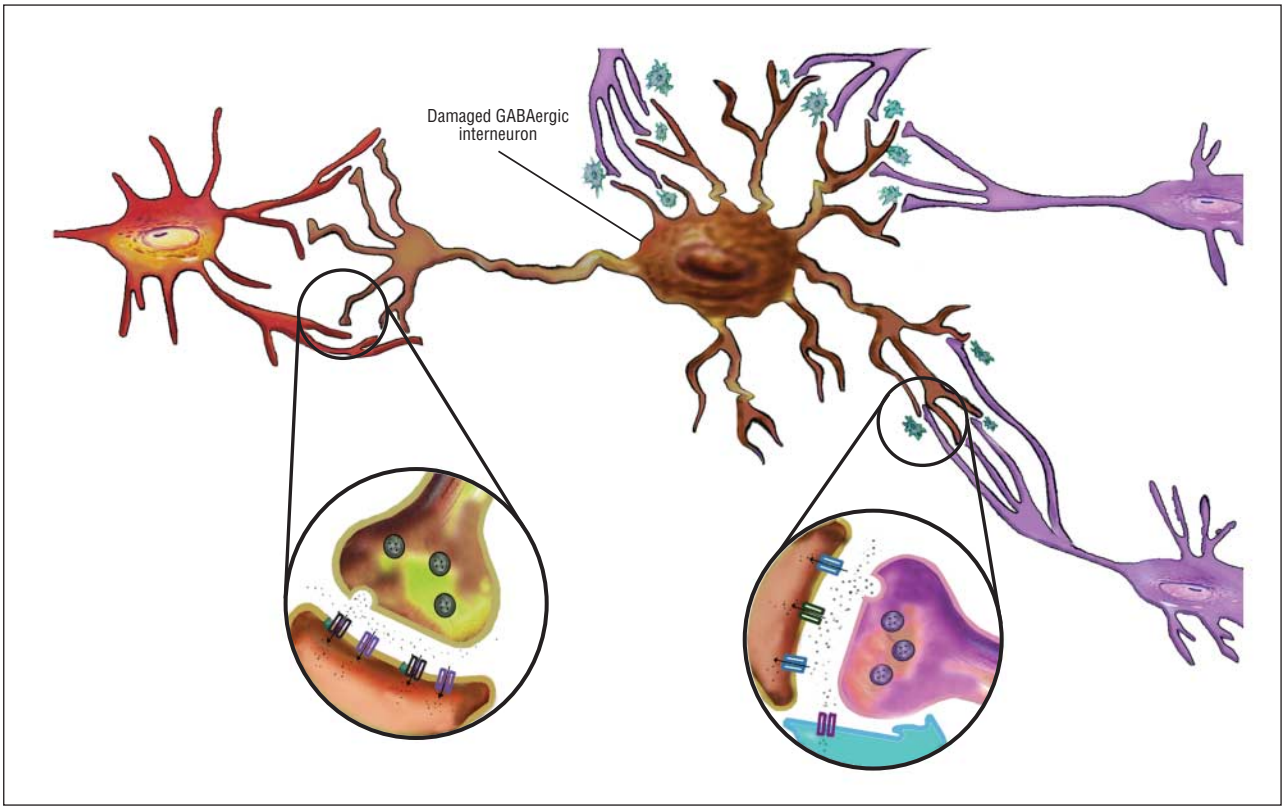

Figure 3. Adult consequences of glutamatergic excitotoxic effects. This illustration presents a theoretical model that attempts to reconcile divergent findings in transcranial magnetic stimulation neurophysiological studies of childhood and adult depression. Our findings suggest that depressed children and adolescents have increased cortical glutamatergic *N*-methyl-D-aspartate receptor functioning. Across the lifespan, this excess *N*-methyl-D-aspartate activity could eventually impair or damage γ-aminobutyric acid (GABA)–ergic interneuronal functioning through excitotoxic effects. Hence, adults with depression might be expected to have deficits in GABAergic functioning as demonstrated in previous neurophysiological research.

depressed children and adolescents (**Figure 2**) leads to excitotoxic damage to GABAergic interneurons, with resultant GABAergic deficits in adulthood (**Figure 3**). Future longitudinal studies of these neurotransmitter systems and neurophysiological measures across wide age ranges would be important in future work. Another consideration regarding the current findings is that a more treatment-refractory and homogeneous sample of adolescents might have yielded findings more in line with those of prior studies of CSP and ICI in adults with MDD. Recent work with healthy controls suggests that children in general have less cortical inhibition than adults, thereby presenting a possible floor effect in our sample.[62]

This study had several limitations that provide a context in which to consider the findings. First, the sample size was small. Second, this initial study included participants with a broad range of ages and with various clinical presentations. In comparison with adult MDD, childhood and adolescent MDD is much more heterogeneous, and this sample may be more diverse than those in prior adult studies.[63] Future efforts might involve more restricted age ranges and use improved selection for disease severity or type on the basis of genetic, clinical, or physiological factors. Third, we did not control for menstrual cycle. Cortical excitability measures can vary across the menstrual cycle.[64] Fourth, the depressed and healthy control groups differed in ethnicity. This is a potential limitation, but ethnicity has not been demonstrated to affect cortical excitability measures.[65] Fifth, this investigation involved measures of cortical excitability and inhibition of the motor cortex. Although the paradigms that were used are affected by relevant afferent pathways, the direct study of other brain structures such as the dorsolateral prefrontal cortex would be ideal. Future studies of child and adolescent MDD might take into consideration the recent findings of studies that combined TMS paradigms with electroencephalography.[66] Finally, we evaluated patients with MDD and healthy controls at just 1 point in time. Longitudinal studies could better examine the impact of neurodevelopment and treatment on cortical excitability and inhibition measures.

In conclusion, to our knowledge, this examination is the first to investigate cortical excitability and inhibition measures in children and adolescents with MDD. Our findings demonstrate that these measures can be used in the investigation of mood disorders during development. They provide evidence that children and adolescents with MDD have increased ICF and cortical glutamatergic activity. Future work might evaluate these paradigms as biomarkers and further elucidate the neurophysiology of psychiatric disorders in children and adolescents. Longitudinal studies with larger, well-defined samples are warranted to better examine the trajectories of these neurotransmitter systems and the impact of treatment. Further complementary and correlational studies of TMS and MRS would also advance understanding of the glutamate system and its role in the pathophysiology of adult and childhood mood disorders.

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