

ONLINE FIRST

Anterior Cingulate Cortex γ -Aminobutyric Acid in Depressed Adolescents

Relationship to Anhedonia

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Context: Anhedonia, a core symptom of major depressive disorder (MDD) and highly variable among adolescents with MDD, may involve alterations in the major inhibitory amino acid neurotransmitter system of γ -aminobutyric acid (GABA).

Objective: To test whether anterior cingulate cortex (ACC) GABA levels, measured by proton magnetic resonance spectroscopy, are decreased in adolescents with MDD. The associations of GABA alterations with the presence and severity of anhedonia were explored.

Design: Case-control, cross-sectional study using single-voxel proton magnetic resonance spectroscopy at 3 T.

Setting: Two clinical research divisions at 2 teaching hospitals.

Participants: Twenty psychotropic medication-free adolescents with MDD (10 anhedonic, 12 female, aged 12-19 years) with episode duration of 8 weeks or more and 21 control subjects group matched for sex and age.

Main Outcome Measures: Anterior cingulate cortex GABA levels expressed as ratios relative to unsuppressed voxel tissue water (w) and anhedonia scores expressed as a continuous variable.

Results: Compared with control subjects, adolescents with MDD had significantly decreased ACC GABA/w ($t=3.2$; $P<.003$). When subjects with MDD were categorized based on the presence of anhedonia, only anhedonic patients had decreased GABA/w levels compared with control subjects ($t=4.08$; $P<.001$; $P_{\text{Tukey}}<.001$). Anterior cingulate cortex GABA/w levels were negatively correlated with anhedonia scores for the whole MDD group ($r=-0.50$; $P=.02$), as well as for the entire participant sample including the control subjects ($r=-0.54$; $P<.001$). Anterior cingulate cortex white matter was also significantly decreased in adolescents with MDD compared with controls ($P=.04$).

Conclusions: These findings suggest that GABA, the major inhibitory neurotransmitter in the brain, may be implicated in adolescent MDD and, more specifically, in those with anhedonia. In addition, use of a continuous rather than categorical scale of anhedonia, as in the present study, may permit greater specificity in evaluating this important clinical feature.

Arch Gen Psychiatry. 2012;69(2):139-149.

Published online October 3, 2011.

doi:10.1001/archgenpsychiatry.2011.131

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ADOLESCENT MAJOR DEPRESSIVE disorder (MDD), a severe neuropsychiatric illness that is associated with significant morbidity and heightened suicide risk, remains poorly understood because of its inherent heterogeneity and symptomatic overlap with other psychopathologies.^{1,2} Therefore, investigating specific symptoms of the disorder has the potential to advance our understanding of the neurobiology of MDD. An ideal candidate for such a focused investigation is *anhedonia*: the reduced capacity to expe-

rience pleasure and a core symptom of MDD that is prevalent and highly variable in adolescents with MDD.^{3,4} Among depressed adolescents, up to 59% are reported to present with significant anhedonia.⁵ Anhedonia has been associated with dysfunction of the dopaminergic brain system and the neural circuitry of reward.⁶ However, the direct mechanisms have not been determined. Growing preclinical and clinical evidence has associated dysregulation of both γ -aminobutyric acid (GABA) and glutamate, respectively the major inhibitory and excitatory amino acid neurotransmitters,

with anhedonia and MDD pathophysiology.⁷⁻¹⁰ In support of this view are clinical studies that have reported decreased cerebrospinal fluid and blood GABA levels in melancholic MDD, in which the presence of anhedonia is essential for diagnosis.^{11,12} In addition, proton magnetic resonance spectroscopy (¹H MRS) studies in adults have documented decreases of cortical GABA in melancholic MDD¹³ and decreases of glutamine (the molecular precursor of GABA and glutamate) in anhedonic depressed patients,¹⁴ further implicating these neurotransmitter systems in anhedonia. To the best of our knowledge, no studies to date have investigated brain GABA alterations in adolescents with MDD.

In the present study, we aimed to extend prior work in adults to adolescents and assess GABA levels in the anterior cingulate cortex (ACC), a brain region strongly implicated in MDD.¹⁵ Structurally, both postmortem and magnetic resonance imaging (MRI) studies of MDD have shown a reduction in volume in the subgenual region of the ACC.^{16,17} Multiple functional neuroimaging^{15,18} and ¹H MRS studies in adult¹⁹⁻²² and pediatric^{23,24} populations have supported a role for the ACC in MDD, and as a region that is part of the reward neural circuitry,²⁵⁻²⁷ the ACC is highly relevant to anhedonia.

Based on prior ¹H MRS data in adult MDD,^{22,28} we hypothesized that ACC GABA levels would be decreased in psychotropic medication-free adolescents with MDD compared with group-matched healthy controls (HCs). In secondary analyses and by analogy to melancholic MDD data in adults,¹¹⁻¹³ we also postulated that the subset of patients categorized as anhedonic would have decreased ACC GABA levels compared with both nonanhedonic patients and HCs and that ACC GABA levels would negatively correlate with severity of MDD episodes and with anhedonia scores. In light of evidence linking metabolic dysregulation of glutamate and glutamine to MDD and anhedonia,^{13,14,22} we explored potential group differences in Glx (combined resonances of glutamate and glutamine).

METHODS

STUDY SUBJECTS

The population of subjects enrolled into this study consisted of 20 adolescents with MDD (aged 12-19 years; mean [SD] age, 16.7 [2.7] years; 12 female) and 21 HCs (aged 13-19 years; mean [SD] age, 16.2 [1.6] years; 15 female), who were group matched for age and sex. The subjects with MDD were recruited through the New York University Child Study Center, the Bellevue Hospital Department of Psychiatry, and local advertisements in the New York, New York, metropolitan area. The HC subjects were recruited through local advertisements in the greater New York metropolitan area and from families of New York University staff. The study was approved by the New York University School of Medicine institutional review board and the institutional review board of Weill Cornell Medical College, where the neuroimaging scans were performed. Prior to clinical evaluation, study procedures were explained to the subjects and the parents. Participants 18 years and older provided signed informed consent; those younger than 18 years provided signed assent and a parent provided signed informed consent.

Inclusion and Exclusion Criteria

Exclusion criteria for all subjects included the presence of a significant medical or neurological disorder, IQ less than 80, claustrophobia, MRI contraindication as assessed by a standard safety screening form, positive urine toxicology test results, and in females, a positive pregnancy test result.

All adolescents with MDD met the *DSM-IV-TR* diagnosis of MDD with current episode duration of 8 weeks or more, severity score of 38 or more on the Children's Depression Rating Scale-Revised (CDRS-R), and psychotropic medication-free status for 3 months or more. Stimulant treatment for attention-deficit/hyperactivity disorder was discontinued at least 24 hours prior to scan. Exclusionary diagnoses included a lifetime psychiatric history of bipolar disorder, schizophrenia, pervasive developmental disorder, obsessive-compulsive disorder, Tourette disorder, panic disorder, conduct disorder, and a substance-related disorder in the past 12 months. A current diagnosis of posttraumatic stress disorder and an eating disorder were also exclusionary.

All enrolled HC subjects did not meet the criteria for any current or past *DSM-IV-TR* diagnoses and had never received psychotropic medication.

Clinical Assessments

All subjects were assessed by a trained child and adolescent psychiatrist (V.G. and C.M.A.) at the New York University Child Study Center. Diagnoses were established using the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version,²⁹ a semistructured diagnostic interview completed by both the subjects and the parents. Additional assessments included the CDRS-R and the Beck Depression Inventory, second edition.³⁰ IQ was estimated with the Kaufman Brief Intelligence Test or the Wechsler Abbreviated Scale of Intelligence. A urine toxicology test and a urine pregnancy test for females were administered the day of the scan.

Anhedonia

To classify a subject categorically as anhedonic, we required the presence of both anhedonia and lack of mood reactivity based on semistructured interviews with the adolescent and parent for subjects younger than 18 years. The designation of anhedonia was made by consensus by the 2 clinicians (child and adolescent psychiatrist) without knowledge of the biological data. Quantitatively, anhedonia scores were computed by summing the responses associated with anhedonia on (1) the self-rated Beck Depression Inventory, second edition (total score 0-6, with each item rated on a scale of 0-3; item 4: "loss of pleasure" and item 12: "loss of interest"), and (2) the clinician-rated CDRS-R (item rated on a scale of 1-7; item 2: "difficulty having fun"). Thus, the clinician- and self-rated assessments each contributed equally to the computed anhedonia score. Such an approach has been used in several investigations that assessed anhedonia severity,^{25,31} and scores were shown to correlate with other anhedonia assessments (eg, Snaith-Hamilton Pleasure Scale).³²

Severity of MDD Episode

Severity of MDD episode was determined from the CDRS-R score obtained without the anhedonia question.

MDD With Melancholic Features

Subjects with MDD were classified as melancholic based on *DSM-IV* criteria.

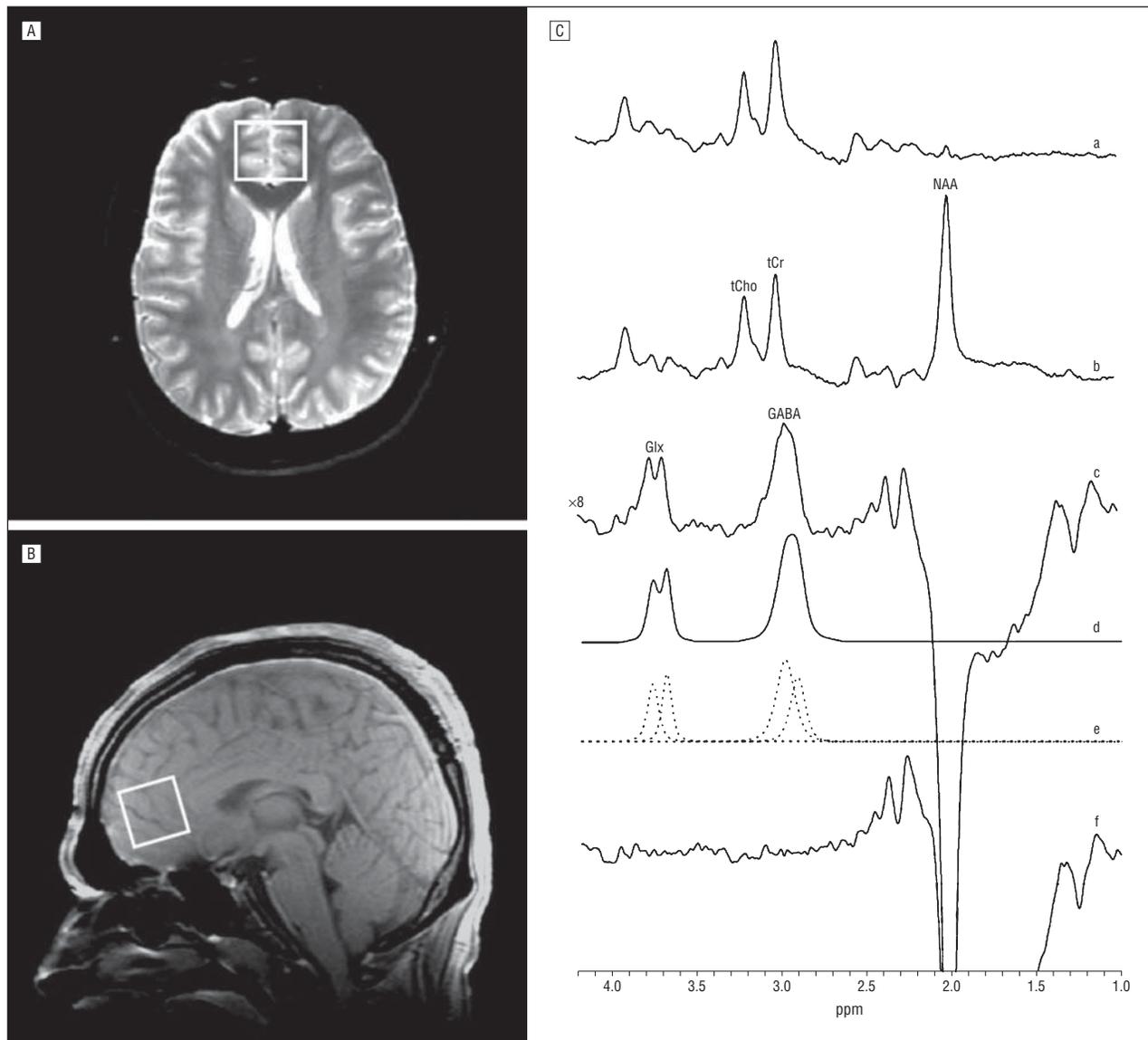


Figure 1. Axial (A) and sagittal (B) images showing anterior cingulate cortex voxel size and location and volume-selective point-resolved spectroscopy proton magnetic resonance spectra (C) with the editing radiofrequency pulse on (a) and off (b). With the editing pulse off (b), a standard point-resolved spectroscopy spectrum is obtained, which yields high-quality spectra for *N*-acetylaspartate (NAA), total creatine (tCr), and total choline (tCho) in the anterior cingulate cortex. The difference of the spectra in parts a and b showing the edited γ -aminobutyric acid (GABA) and combined resonances of glutamate and glutamine (Glx) peaks (c), with the best-fit model curve of the edited spectrum (d), the individual components of the best-fit curve (e), and the residuals of the difference between the edited (c) and best-fit (d) spectra (f). The data were acquired in 15 minutes from a $2.5 \times 2.5 \times 3.0\text{-cm}^3$ voxel using a repetition time of 1500 milliseconds, echo time of 68 milliseconds, and 290 interleaved excitations (total, 580) with the editing pulse on or off.

NEUROIMAGING DATA ACQUISITION AND PROCESSING

All neuroimaging studies were conducted on a research-dedicated General Electric 3-T Excite MRI system (GE Medical Systems, Milwaukee, Wisconsin) in the Citigroup Biomedical Imaging Center at Weill Cornell Medical College.

Structural MRI

A 3-plane, low-resolution, high-speed scout imaging series was obtained, followed by a series of high-resolution scans, consisting of standard axial, coronal, and sagittal T1-, T2-, and spin density-weighted scans that were appropriately obliqued for prescribing the ^1H MRS voxel. In addition, a T1-weighted spoiled gradient-recalled echo volumetric scan (repetition time=30 mil-

liseconds, echo time=8 milliseconds; flip angle, 45° ; field of view, 24 cm; 256×256 matrix; 124 coronal slices; and a slice thickness of 3.0 mm) and an axial fast fluid-attenuated inversion recovery scan were performed for brain tissue segmentation and detection of exclusionary focal brain lesions, respectively.

^1H MRS GABA Acquisition Methods

The GABA-edited ^1H MRS spectra were acquired using the standard J-editing difference method,³³ as modified by Sailasuta et al³⁴ and recently fully described,³⁵ with an 8-channel phased-array head coil for signal detection. The scanner's body coil was used to excite all the ^1H signals (580 excitations) in a single $2.5 \times 2.5 \times 3.0\text{-cm}^3$ voxel in the ACC (**Figure 1** A and B). Using the standard J-editing technique,³³ GABA detection is achieved by applying a frequency-selective inversion pulse that avoids

excitation of the GABA C-3 peak at 1.9 ppm on alternate scans (Figure 1C, parts a and b), with a repetition time of 1500 milliseconds and an echo time of 68 milliseconds. This results in selective inversion of the outer lines of the C-4 GABA triplet resonance at 3.0 ppm on alternate scans by, respectively, inhibiting and allowing its J-modulation. Subtracting the 2 subspectra thus acquired yields the GABA difference spectrum, consisting of the outer lines of the GABA C-4 triplet at 3.0 ppm, while the much stronger overlapping total creatine resonance, a singlet that is not J-modulated, is eliminated (Figure 1C, part c). While this pulse sequence is optimized for GABA detection, it also achieves detection of the combined resonances of Glx at 3.7 ppm (Figure 1C, part c) because of their high structural, chemical, and magnetic similarities with GABA.

Eight-Channel GABA ¹H MRS Data Processing and Quantitation

The 8-channel phased-array coil editing data recorded as described were combined into a single regular time-domain free-induction decay signal, using a C language computer program that implemented a previously published algorithm,³⁶ in which the simultaneously acquired unsuppressed voxel tissue water signal from each receiver coil element is used to derive the required relative array coil sensitivities. The 2 resulting free-induction decay signals were subtracted in the time domain to yield the difference signal, which was Fourier transformed to obtain the edited GABA and Glx spectra. Metabolite peak areas were obtained using a robust and highly optimized public-domain Levenberg-Marquardt nonlinear least-squares IDL minimization routine³⁷ to model the resonance peaks in the frequency domain as a linear combination of pseudo-Voigt functions, which enable more precise analysis of line shapes that consist of mixtures of Lorentzian and gaussian functions,³⁸ as is often the case for in vivo spectra (Figure 1C, parts d-f). To enable groupwise comparisons, the GABA and Glx peak areas were expressed as ratios relative to the area of the unsuppressed ACC voxel tissue water (w).

Macromolecule Contamination of Detected GABA

We previously assessed the degree to which the detected GABA resonance is contaminated by the known coresonant and coedited mobile macromolecules.^{39,40} These mobile macromolecule contributions were evaluated for voxels in the dorsolateral prefrontal cortex, medial prefrontal cortex, ACC, and occipital cortex. The contribution of mobile macromolecules to total GABA was relatively constant across the 3 voxels, ranging from 41% to 49%, a nonsignificant regional variation.

Assessment of Voxel Tissue Heterogeneity

Because GABA and Glx concentration differences have been reported between the gray matter and white matter (WM),^{41,42} we implemented volumetric MRI-based tissue segmentation to correct the ¹H MRS-derived levels of these neurotransmitters for brain matter heterogeneity in our relatively large ACC voxel. This was accomplished by (1) acquiring high-resolution volumetric MRI data that included the ACC; (2) using the commercial software MEDx (Medical Numerics, Sterling, Virginia) to segment and classify tissue types; and (3) entering voxel tissue type as a covariate in the statistical analysis if the proportions of tissue types differed significantly between the diagnostic groups. In practice, the MEDx software was used to

segment the brain tissue based on the signal-intensity histogram of each subject's volumetric (spoiled gradient-recalled echo) MRI. From the histogram, a segmentation mask of the ACC voxel was generated and the proportions of gray matter, WM, and cerebrospinal fluid for the voxel were computed. These were then compared between groups and adjusted for statistically in case of significant differences.

All imaging analyses were done while blinded to the clinical status of the subject.

STATISTICAL ANALYSIS

Unequal-variance *t* tests were used to compare the subject groups for severity, age, ethnicity, and IQ. Since brain structure differs with age and sex, brain tissue heterogeneity between the groups was compared by using analysis of covariance controlling for age and sex. Analysis of covariance was used to test our hypotheses and compare mean GABA/w and Glx/w levels between the subject groups, controlling for age and sex and any additional factor that was statistically significant ($P \leq .05$) or trended toward a significant difference between the groups ($P < .10$). A Tukey multiple hypothesis correction was applied to the 3 group comparisons. The associations of GABA/w with severity and anhedonia scores were characterized using Pearson correlations. All reported *P* values are 2-sided. SAS version 9.0 (SAS Institute, Cary, North Carolina) was used for all statistical computations.

RESULTS

SUBJECTS

Clinical and demographic characteristics of the MDD and HC groups are summarized in **Table 1**. Of the 20 subjects with MDD, 10 were categorically identified as anhedonic. At the time of scan, 15 adolescents with MDD were antidepressant medication naive and 5 were medication free for at least 3 months. Ethnic distribution was significantly different between the groups; therefore, ethnicity was entered as a covariate in the hypothesis-testing analyses.

CLINICAL COMPARISON BETWEEN THE MDD SUBGROUPS

The anhedonic MDD group had significantly higher disease severity scores than the nonanhedonic subgroup (mean [SD], 52.3 [6.5] vs 44.4 [5.3]; $t = -2.98$; $P = .008$). However, the 2 MDD subgroups did not differ on their anhedonia scores. Three anhedonic adolescents with MDD met full criteria for the melancholic subtype. The groups did not differ in comorbid attention-deficit/hyperactivity disorder and anxiety disorders.

¹H MRS VOXEL TISSUE HETEROGENEITY

Table 2 provides the proportions of gray matter, WM, and cerebrospinal fluid in the ACC voxel for each group as determined by tissue segmentation, as well as the mean unsuppressed voxel tissue water signal for each group. Using analysis of variance with age and sex as covariates, we detected a significant difference in percentage of WM between the MDD group and HC group (mean

Table 1. Demographic and Clinical Characteristics of Adolescents With MDD and Healthy Controls

Characteristic	Mean (SD) [Range]			
	All MDD (n = 20)	Anhedonic MDD (n = 10)	Nonanhedonic MDD (n = 10)	Healthy Controls (n = 21)
Age, y	16.7 (2.7) [12-19]	18.0 (1.9) [14-19] ^a	15.3 (2.7) [12-19] ^a	16.2 (1.6) [13-19]
Sex, No. (%)				
M	8 (40)	4 (40)	4 (40)	6 (32)
F	12 (60)	6 (60)	6 (60)	15 (68)
IQ	106.2 (11.3)	109.8 (10.7) ^a	102.5 (11.2) ^a	110.0 (11.6)
Ethnicity, No. (%)				
White	8 (40)	5 (50)	3 (30)	9 (43)
African American	2 (10)	0	2 (20)	7 (33)
Hispanic	8 (40)	3 (30)	5 (50)	0
Asian	0	0	0	3 (14)
Other	2 (10)	2 (20)	0	2 (10)
Illness history				
Episode duration, mo	11.7 (8.6) [2-36]	13.1 (10.7) [4-36]	10.2 (6.0) [2-21]	0
No. of episodes	1.4 (0.6) [1-3]	1.6 (0.7) [1-3]	1.2 (0.4) [1-2]	0
Suicide attempts	0.3 (0.6) [0-2]	0.1 (0.3) [0-1]	0.4 (0.7) [0-2]	0
Medication naive, No. (%)	15 (75)	6 (60)	9/1 (90)	21 (100)
Medication free, No. (%)	5 (25)	4 (40)	1 (10)	0
Melancholic subtype, No. (%)	3 (15)	3 (30)	0	0
CDRS-R score	52.6 (7.5) [38-68]	56.9 (6.9) [43-68] ^a	48.3 (5.5) [38-58] ^a	18.8 (1.1) [17-21]
BDI-II score	30.1 (12.7) [12-51]	31.8 (13.6) [17-51]	28.4 (12.2) [12-47]	2.6 (3.0) [0-10]
Anhedonia score	7.7 (2.5) [3-11]	8.3 (2.2) [5-11]	7.0 (2.6) [3-11]	1.3 (0.6) [1-3]
Current comorbidity, No. (%)				
ADHD	4 (20)	2 (20)	2 (20)	0
Any anxiety disorder	11 (55)	6 (60)	5 (50)	0
GAD	11 (55)	6 (60)	5 (50)	0

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; BDI-II; Beck Depression Inventory, second edition; CDRS-R; Children's Depression Rating Scale-Revised; GAD, generalized anxiety disorder; MDD, major depressive disorder.

^aAnhedonic and nonanhedonic MDD subgroups differed significantly with respect to age ($P = .02$), ethnicity, and MDD severity ($P = .007$).

Table 2. GABA/w, Water, Gray Matter, White Matter, and CSF in the ACC of Adolescents With MDD and Healthy Controls

Measure	All MDD (n = 20)	Anhedonic MDD (n = 10)	Nonanhedonic MDD (n = 10)	Healthy Controls (n = 21)
ACC GABA/w, mean (SD)	2.37×10^{-3} (0.46×10^{-3}) ^a	2.14×10^{-3} (0.37×10^{-3}) ^{b,c}	2.60×10^{-3} (0.43×10^{-3})	2.68×10^{-3} (0.27×10^{-3})
ACC water, mean (SD)	1.58×10^{12} (0.40×10^{12})	1.47×10^{12} (0.24×10^{12})	1.69×10^{12} (0.50×10^{12})	1.65×10^{12} (0.32×10^{12})
Gray matter, %	57.4 (7.0)	57.4 (6.7)	57.5 (7.5)	54.6 (5.4)
White matter, %	35.9 (7.4) ^d	36.2 (6.6)	35.6 (8.4)	39.8 (5.8)
CSF, %	6.7 (3.1)	6.5 (2.9)	6.9 (3.5)	5.6 (3.4)

Abbreviations: ACC, anterior cingulate cortex; CSF, cerebrospinal fluid; GABA/w, γ -aminobutyric acid level relative to unsuppressed voxel tissue water; MDD, major depressive disorder.

^aSignificantly decreased ACC GABA/w in whole MDD group compared with healthy controls ($P < .003$).

^bSignificantly decreased ACC GABA/w in anhedonic MDD subgroup compared with healthy controls ($P < .001$; $P_{\text{Tukey}} < .001$).

^cSignificantly decreased ACC GABA/w in anhedonic MDD subgroup compared with nonanhedonic MDD subgroup ($P = .02$; $P_{\text{Tukey}} = .06$).

^dSignificantly decreased ACC white matter in whole MDD group compared with healthy controls ($P = .04$).

[SD], 35.9% [7.4%] vs 39.8% [5.8%]; $t = 2.10$; $P = .04$). Additionally, we detected a trend toward a significant difference in percentage of WM between the anhedonic MDD subgroup and HCs (mean [SD], 36.2% [6.6%] vs 39.8% [5.8%]; $t = 1.82$; $P = .08$). There were no significant differences or trends with respect to tissue proportions of gray matter, cerebrospinal fluid, or unsuppressed voxel tissue water between any of the groups. Consequently, percentage of WM was added as a covariate to all pairwise comparisons of the groups.

PRIMARY HYPOTHESIS TESTING: ACC GABA GROUP COMPARISONS

All comparisons were made controlling for age, sex, ethnicity, and WM as covariates.

Full MDD Group vs HC Group

Mean (SD) ACC GABA/w in the full MDD group was significantly decreased compared with the HC group

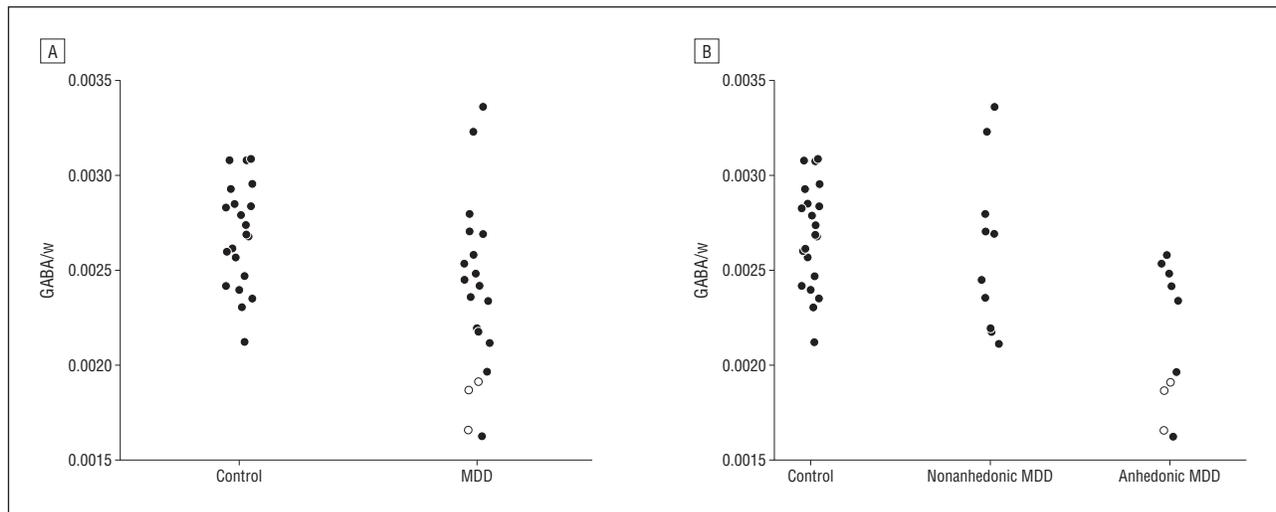


Figure 2. Scatterplots of raw data of anterior cingulate cortex γ -aminobutyric acid relative to unsuppressed voxel tissue water concentrations (GABA/w) in healthy controls and adolescents with major depressive disorder (MDD) (A) and healthy controls, nonanhedonic adolescents with MDD, and anhedonic adolescents with MDD (B). Open circles represent subjects with melancholic MDD.

$(2.37 \times 10^{-3} [0.46 \times 10^{-3}]$ vs $2.68 \times 10^{-3} [0.27 \times 10^{-3}]$; $df=34$; $t=3.2$; $P<.003$).

Comparisons Between MDD Subgroups and HC Group

Mean (SD) ACC GABA/w was decreased in anhedonic adolescents with MDD compared with the HC group ($2.14 \times 10^{-3} [0.37 \times 10^{-3}]$ vs $2.68 \times 10^{-3} [0.27 \times 10^{-3}]$; $df=33$; $t=4.08$; $P<.001$; $P_{\text{Tukey}}<.001$) and, notably, compared with depressed nonanhedonic adolescents with MDD ($2.14 \times 10^{-3} [0.37 \times 10^{-3}]$ vs $2.60 \times 10^{-3} [0.43 \times 10^{-3}]$; $df=33$; $t=2.35$; $P=.02$; $P_{\text{Tukey}}=.06$). There were no significant differences between nonanhedonic adolescents with MDD and HCs. The GABA/w values for each group are provided in Table 2 and are graphically compared in **Figure 2** as scatterplots. Ratios of GABA to total creatine and *N*-acetylaspartate are available in the eTable (<http://www.archgenpsychiatry.com>).

To ensure that controlling for WM did not result in significant differences, we repeated all analyses while not controlling for WM, with findings remaining the same. Specifically, GABA/w was still significantly lower in the full MDD group compared with the HC group ($df=35$; $t=3.24$; $P<.003$), in the anhedonic MDD subgroup compared with the HC group ($df=34$; $t=4.05$; $P<.001$; $P_{\text{Tukey}}<.001$), and in the anhedonic MDD subgroup compared with the nonanhedonic MDD subgroup ($df=34$; $t=2.34$; $P<.03$; $P_{\text{Tukey}}=.06$).

ASSOCIATIONS OF ACC GABA WITH CLINICAL VARIABLES

GABA/w and MDD Severity

There was a negative association between ACC GABA/w levels and severity of depressive symptoms as measured by CDRS-R scores minus the anhedonia question ($r=-0.46$; $P=.04$) (**Figure 3A**).

GABA/w and Anhedonia Scores

There were negative associations between GABA/w and anhedonia scores in the anhedonic MDD subgroup ($r=-0.69$; $P<.03$) (Figure 3B), in the full MDD group ($r=-0.50$; $P<.03$) (Figure 3C), and in the combined sample of MDD and HC subjects ($r=-0.54$; $P<.001$) (Figure 3D). There was no significant correlation between GABA/w and anhedonia scores in the nonanhedonic group ($r=-0.26$; $P=.47$). Analyses were repeated while controlling for MDD severity, with similar results: negative associations were found between GABA/w and anhedonia scores in the anhedonic MDD subgroup ($r=-0.67$; $P<.03$) and in the full MDD group ($r=-0.43$; $P=.06$) but not in the nonanhedonic group ($r=-0.24$; $P=.50$).

Added Value of Categorical Anhedonia Assessment

Since we did not find significant differences in anhedonia scores between the 2 anhedonic subgroups, we tested the added value of the categorical assessment of anhedonia for the prediction of GABA/w. To do so, we fit a model to predict GABA/w with the continuous assessment of anhedonia, which revealed an R^2 of 25.2% ($P<.02$). When the model was augmented to include the categorical assessment of anhedonia, the model R^2 increased substantially to 41.4%, with a trend increment toward significance ($P=.059$). This suggests that the categorical assessment of anhedonia has an added value.

Association Between MDD Severity and Anhedonia Scores

To examine whether the significant relationships between GABA/w and anhedonia scores were confounded by MDD severity, we examined the association between anhedonia and severity scores. The correlation was not significant ($r=0.30$; $P=.20$).

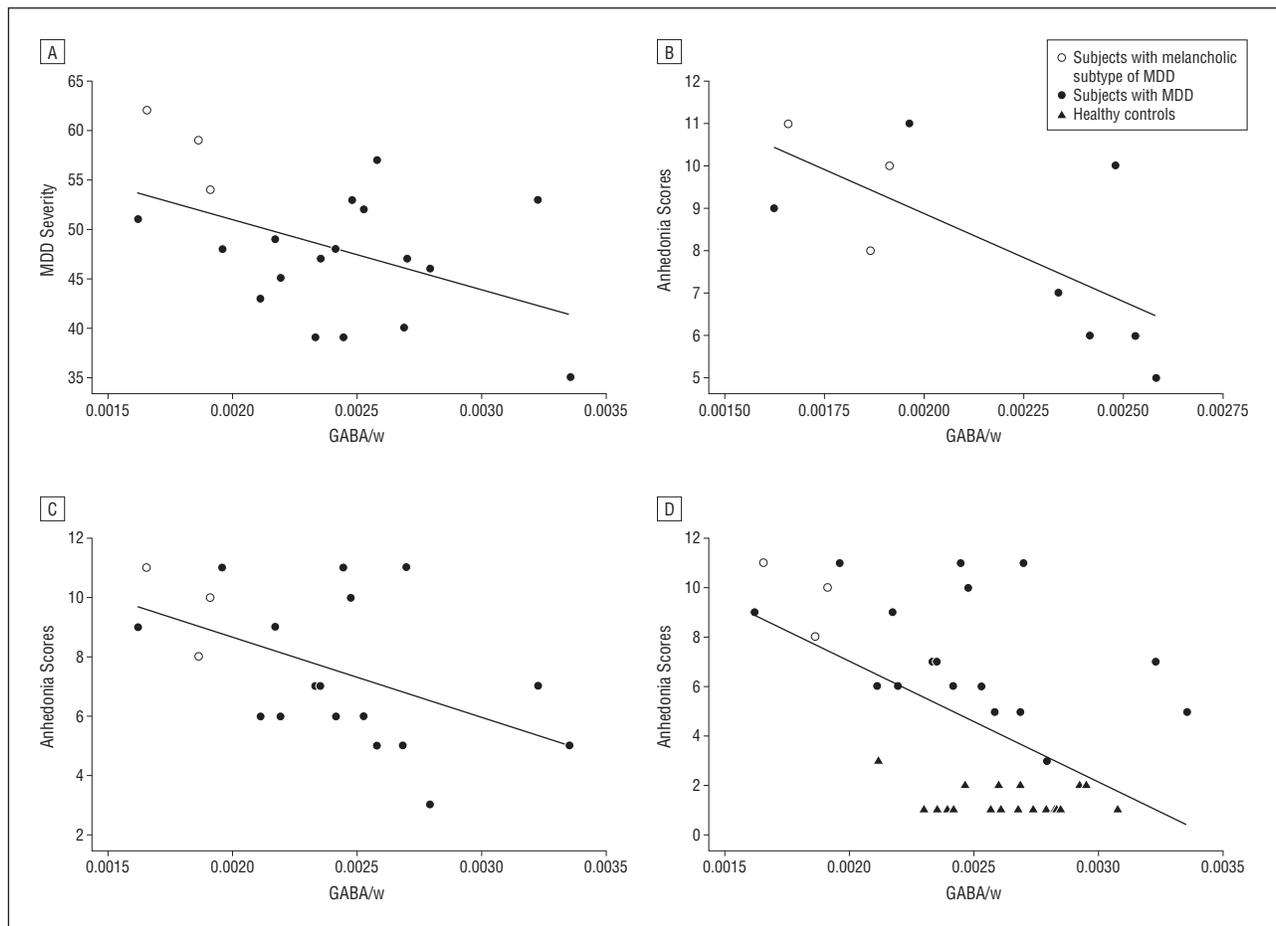


Figure 3. Scatterplots with regression lines characterizing associations of γ -aminobutyric acid level relative to unsuppressed voxel tissue water (GABA/w) values and major depressive disorder (MDD) severity scores in all 20 subjects with MDD (A); anhedonia scores in the 10 subjects with MDD categorized as anhedonic (B); anhedonia scores in all 20 subjects with MDD (C); and anhedonia scores in the combined group of subjects with MDD and healthy controls (D).

EXPLORATORY HYPOTHESIS: ACC Glx GROUP COMPARISONS AND ASSOCIATIONS WITH CLINICAL VARIABLES

The Glx/w values were not significantly different between the groups and were not associated with severity of MDD episodes or anhedonia scores.

COMMENT

In this first study, to our knowledge, to examine brain GABA/w levels in adolescents with MDD, we found significant decreases of this inhibitory neurotransmitter in the ACC of adolescents with MDD compared with HC subjects. In addition, we found significant differences in ACC GABA/w between adolescents with anhedonia and both nonanhedonic depressed adolescents and HCs but no differences between the latter 2 groups. There were negative associations between ACC GABA/w and severity of depressive episodes, as well as between ACC GABA/w and anhedonia scores in the full MDD group and in the combined sample of adolescents with MDD and HCs. However, we did not detect differences in Glx/w between adolescents with MDD and controls or in any of the subgroups nor did we detect an association between Glx/w and severity of MDD

symptoms. An incidental finding is the significantly decreased WM fraction in the ACC voxel of adolescents with MDD compared with controls.

The present finding of decreased ACC GABA/w in adolescents with MDD is consistent with previous ^1H MRS studies in adult MDD, which reported decreased GABA in the ACC and occipital cortex.^{22,28,43} Postmortem human brain studies further implicate this neurotransmitter system, with findings of reduced GABAergic neuronal density and decreased activity of the GABA-synthesizing enzyme, glutamic acid decarboxylase (GAD 67), in the prefrontal cortex of patients with MDD.⁴⁴⁻⁴⁶ The similarity of these findings between adolescent and adult MDD and their consistency seems to implicate cortical GABA abnormalities early in the course of illness.

One of the key findings of the present study is the association between decreased ACC GABA/w and the clinical classification of anhedonia. Supporting this result are reports of decreased cerebrospinal fluid and brain GABA concentrations in subjects with melancholic MDD, in which anhedonia is an essential symptom.^{11,13} It can be argued that GABA plays a role in the melancholic MDD subtype. In fact, GABA levels of the 3 adolescents with MDD who met criteria for melancholic MDD were within the lower end of the GABA range that

we measured in this study. Further support derives from preclinical and clinical studies, which have reported associations between GABA dysfunction and negative symptoms in schizophrenia that also overlap clinically with anhedonia.^{47,48}

Additional evidence for the role of GABA dysfunction in anhedonia is the significant negative correlation between ACC GABA/w and our quantitative measures of anhedonia severity in the full MDD group, as well as in the combined sample of depressed adolescents and HCs. This association between ¹H MRS measures of ACC GABA and anhedonia, using quantitative scores of symptom severity, demonstrates the potential for achieving continuous neurochemical correlations with depressive symptoms (eg, anhedonia) that span the entire continuum from normal to abnormal, rather than using the traditional binary classification system in which the disease or a symptom is considered present or absent. As such, this study is in line with recent recommendations promoting the use of dimensional rather than categorical scoring systems in neurobiological research on mental disorders.⁴⁹

A surprising finding of this study is that the MDD subgroups did not significantly differ in their anhedonia scores. This is most likely due to the inherently continuous rather than categorical nature of anhedonia, emphasizing a potential limitation of categorical assessments in evaluating neuropsychiatric disorders.

Our finding of altered GABA levels in anhedonia may be related to the increased severity of MDD episodes in subjects with anhedonia and/or melancholic depression, akin to our finding of decreased GABA levels in adults with treatment-resistant MDD.⁵⁰ However, the non-significant and weak correlation between MDD severity and anhedonia scores in the full MDD group suggests that the association between anhedonia and GABA is independent of disease severity.

Our GABA findings in adolescents with MDD and in anhedonic patients do not agree with 1 prior study by Walter et al¹⁴ that also assessed GABA and glutamatergic compounds in anhedonic adults with MDD using ¹H MRS. In that study, neither cortical GABA nor glutamate differed between patients with MDD, anhedonic or otherwise, and matching control groups. Rather, the only abnormality found was a decreased glutamine level in 5 anhedonic patients compared with 14 controls. However, GABA data were available for only 4 anhedonic patients compared with 11 controls, suggesting that the study might have been too underpowered to detect significant differences. In addition, the obvious population age difference may have contributed to the discrepant findings between the 2 studies. Another source of discrepancy could be methodological. Whereas our study used J-editing to cleanly isolate GABA and Glx (combined glutamate and glutamine) and found no Glx/w differences, Walter et al¹⁴ had attempted to separate glutamate and glutamine. However, reliable discrimination of glutamine and glutamate is highly challenging and prone to errors with most existing methods at 3 T or lower fields.⁵¹ While it might be possible for altered glutamine or glutamate levels to exist in our sample of adolescents with MDD, availability of methods for achieving reliable glutamine or glutamate discrimination would be required

to establish whether these glutamatergic compounds are altered in adolescents with MDD.

Although there is evidence implicating the glutamatergic system in MDD, several ¹H MRS investigations, including 1 study from our group, did not find Glx differences between adults with MDD and controls.^{7,50,52}

While this study focuses on GABA, the possible role of glutamate has also been strongly implicated in MDD, with evidence to suggest that GABA and glutamate transmitter systems may be linked.⁵³ As **Figure 4** indicates, synthesis of GABA and glutamate occurs through closely related metabolic pathways.⁵⁴ Preclinical data have implicated the glutamate/glutamine cycle and GABA/glutamine cycle in models of depression and anhedonia. Blockade of glutamate uptake by astrocytes triggers anhedonia in mice,⁵⁵ and mice with impaired glutamatergic transport also exhibit anhedonic behavior.⁹ Moreover, antidepressant treatment reversed alterations in glutamate levels induced by chronic mild stress, a common animal model for depression and anhedonia.⁵⁶ The neurobiological pathways linking GABA and glutamate to anhedonia may act via the tight regulation of dopamine by these neurotransmitters.⁵⁷ Circadian fluctuations in GABA, glutamate, and dopamine are closely linked in the nucleus accumbens,^{58,59} a key region implicated in reward and anhedonia.⁶⁰ Further studies have demonstrated glutamine alterations in mice bred to lack the type 1 dopamine receptor.⁶¹

This study is also the first to report, to our knowledge, a relationship between severity of MDD episodes and cortical GABA concentrations in addition to group differences specifically in adolescents. Research focusing on this population is highly significant for understanding the neurobiology of MDD, as it avoids the confounding effects of chronicity and psychotropic medication use.

While the region of interest for this study was the ACC, converging evidence has implicated diffuse GABA alterations in MDD. Postmortem studies have documented significantly altered distributions of neurons expressing GAD 65/67 (the key enzymes of GABA synthesis) in the dorsolateral prefrontal cortex, orbitofrontal cortex, superior temporal cortex, and hippocampus of subjects with MDD.⁶² Proton MRS studies also demonstrated GABA alterations in the dorsomedial/dorsal anterolateral prefrontal cortex and occipital regions in addition to the ACC.^{13,22,28,43,63} These findings suggest that the GABAergic alterations associated with MDD are not limited to 1 region but rather manifest in several areas throughout the brain.

An incidental finding of our study is that adolescents with MDD had significantly decreased WM fractions in the examined ACC voxel, with a similar trend found between the anhedonic MDD and HC groups. This finding is consistent with multiple structural and diffusion tensor imaging studies in pediatric and adult populations with MDD, as well as healthy participants with a familial history of MDD.⁶⁴⁻⁶⁶ Postmortem studies have further highlighted the pathology of WM in the prefrontal cortex in MDD with reports of decreased oligodendrocyte density.⁶⁷ Related to our finding in anhedonic patients, Korgaonkar et al⁶⁸ also reported WM

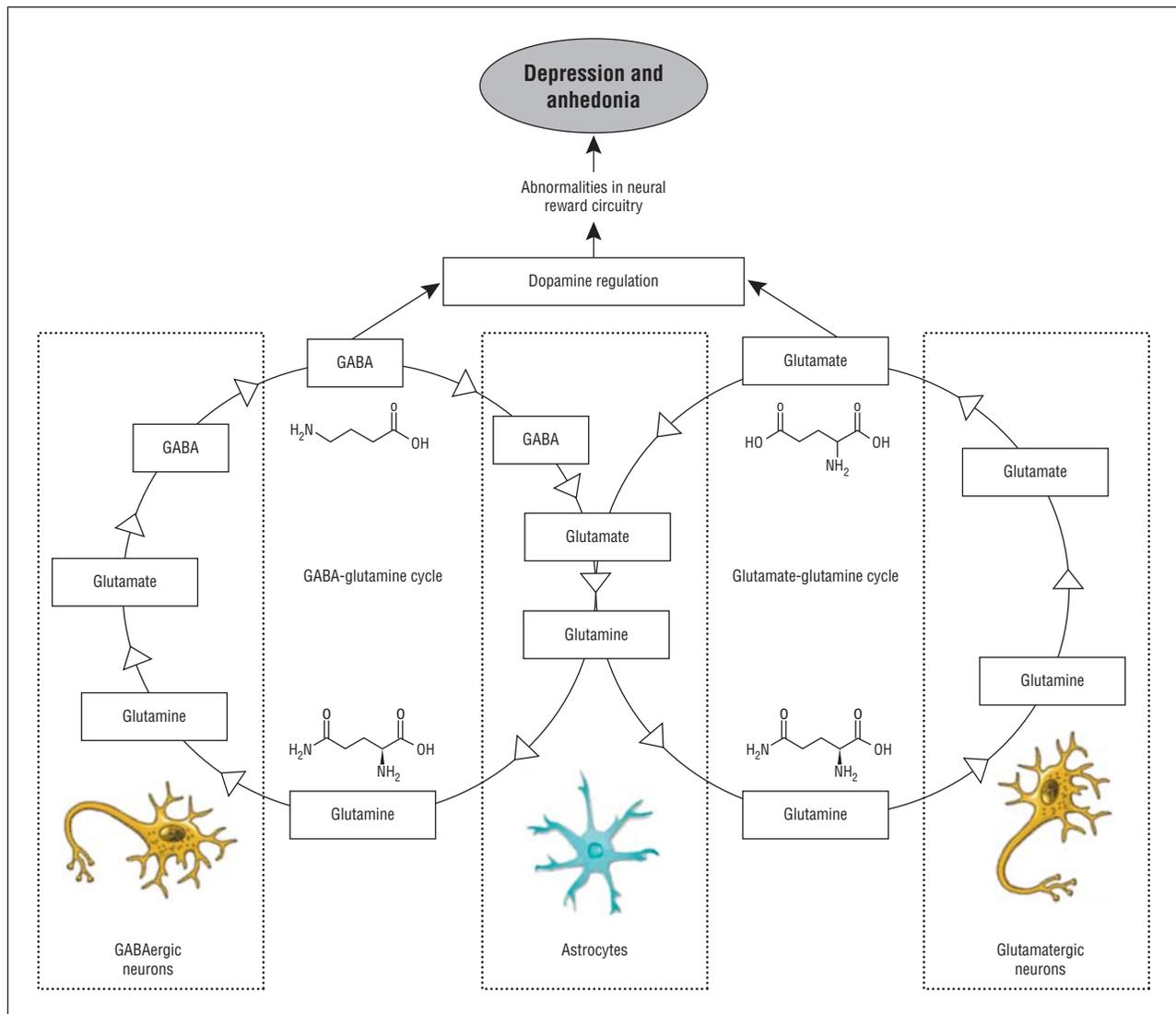


Figure 4. Illustration of the γ -aminobutyric acid (GABA)–glutamine and glutamate–glutamine cycles. Astrocytes release glutamine, which is absorbed by GABAergic and glutamatergic neurons, where it is metabolized into GABA and glutamate, respectively. These neurotransmitters regulate dopaminergic neurons; preclinical and clinical evidence suggest that alterations in GABA and glutamate signaling may induce major depressive disorder and anhedonia symptoms via this mechanism. Following release, GABA and glutamate molecules are absorbed by astrocytes and converted back into glutamine. H₂N indicates primary amine group; HO, hydroxyl group; NH₂, primary amine group; O, oxygen; OH, hydroxyl group.

deficits in melancholic patients with MDD compared with controls.

A limitation of this study is that, while comparable with similar imaging studies in MDD, our sample size of 20 subjects per group was too modest to enable reliable assessment of within- as well as between-group correlations in outcome variables and clinical status. Therefore, our results remain to be replicated in similar or larger cohorts before they can be generalized. Another methodological limitation was that our ¹H MRS sequence did not allow the discrimination of glutamate from glutamine resonances and that our detected GABA contained up to 50% contribution from mobile macromolecules.

In summary, the results of the present study support a pathophysiological role for GABA, the major inhibitory neurotransmitter in the brain, in the ACC in adolescent MDD and, specifically, in anhedonia. Future

larger studies, replicating our findings as well as investigating GABA, glutamate, and glutamine in other reward-related brain regions (eg, the striatum), appear to be warranted.

Submitted for Publication: March 17, 2011; final revision received July 13, 2011; accepted August 11, 2011.

Published Online: October 3, 2011. doi:10.1001/archgenpsychiatry.2011.131

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Author Contributions: Dr Gabbay had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Financial Disclosure: Dr Gabbay reports serving as a subinvestigator on studies sponsored by Otsuka, Biovail, Shire Pharmaceuticals, Bristol-Myers Squibb, and Boehringer Ingelheim but has received no compensation for this work.

Funding/Support: This study was supported by National Institutes of Health grants AT002395, AT004576, MH077072, MH077072-03S1, and MH075895; the Chrissy Rossi National Alliance for Research on Schizophrenia and Depression Award; and generous gifts from the Leon Levy and Anita Saltz foundations.

Role of the Sponsors: These sources of funding had no further role in study design and conduct; the collection, management, analysis, and interpretation of data; and preparation, review, and approval of this manuscript.

Online-Only Material: The eTable is available at <http://www.archgenpsychiatry.com>.

Additional Contributions: We thank Donald F. Klein, MD, DSc, for contributions to the scientific content of this work.

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